AN INVESTIGATION OF THE INFLUENCE OF OVARIAN HORMONAL AGENTS UPON THE CARDIOVASCULAR SYSTEM OF THE RAT

By

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To try to determine whether ovarian hormonal agent-induced hypertension is mediated by potentiation of cardiovascular responsiveness, the effect of short-term changes in the plasma concentration of ovarian hormonal agents was examined upon responses of the cardiovascular system of female rats.

Resting systolic blood pressure, heart rate, pressor responses to exogenous noradrenaline and depressor responses and positive chronotropic responses to isoprenaline were reduced in pithed female rats following constant release of 10 $\mu g.day^{-1}$ 17β-oestradiol for ten days from osmotic minipumps.

Contractile responses of isolated aortic strips, portal veins and paired atria were examined following nine daily subcutaneous injections to female rats of ethinyloestradiol or norethisterone acetate.

Following ethinyloestradiol pre-treatment, the potencies of noradrenaline and angiotensin II and maximal fast and slow aortic strip contractile responses to noradrenaline were increased. Spontaneous mechanical activity of portal veins was better maintained and the potencies of exogenous noradrenaline and angiotensin II were increased following ethinyloestradiol pre-treatment. Inotropic responses, but not positive chronotropic responses, to noradrenaline and to isoprenaline were increased following ethinyloestradiol pre-treatment.

The potency of angiotensin II was increased in isolated aortic strips and portal veins and there was a wide spread in inotropic responses, without change in positive chronotropic responses, to noradrenaline in isolated atria following norethisterone acetate pre-treatment.

The similarity between the effect of constant 17β-oestradiol administration to female rats and human pregnancy upon the cardiovascular system is discussed. Daily subcutaneous administration of ethinyloestradiol and norethisterone acetate to female rats appeared to induce changes in the cardiovascular system which may elevate blood pressure. The possibility that the effect of ovarian hormonal agents upon the cardiovascular system is dependent upon the mode of administration is discussed, together with the problems involved in controlling ovarian hormone levels.

Key words: ovarian hormonal agents, blood pressure, cardiovascular responsiveness, oral contraceptives.

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CONTENTS

INTRODUCTION

Continued . . . . /

INTRODUCTION

METHODS

Continued . . . . /

METHODS

CHAPTER 1.

The Effect of Changes in the Plasma Concentration of Ovarian Hormones upon Cardiovascular Responses to Noradrenaline, Angiotensin II, Vasopressin, Oxytocin, Isoprenaline and Electrical Stimulation in the Pithed Female Rat.

CHAPTER 1.

CHAPTER 2.

The Effect of Pre-treatment of Female Rats with (17α) Ethinyloestradiol and Norethisterone Acetate upon the Responses of Isolated Aortic Strips, Portal Veins and Paired Atria to Several Spasmogens.

CHAPTER 2.

FIGURES

FIGURES

FIGURES

FIGURES

FIGURES

FIGURES

FIGURES

FIGURES

TABLES

TABLES

TABLES

INTRODUCTION

1. General Introduction

Administration of oral contraceptives may elevate the blood pressure of susceptible women (see reviews by Mackay, Khoo & Shah, 1973; Fregly & Fregly, 1977). Since it has been shown that potentiation of cardiac and vascular contractile responsiveness may also lead to a chronic elevation of blood pressure (see reviews by Webb & Bohr, 1981; Weidmann, 1981), present studies were undertaken to determine whether oral contraceptives and other ovarian hormonal agents may elevate blood pressure by potentiating cardiac and vascular contractile responsiveness.

Although potentiation of cardiac and vascular responsiveness may lead to elevated blood pressure, structural changes may occur in the vasculature, subsequent to a chronic elevation of blood pressure, and these changes also modify vascular contractility (see reviews by Folkow, 1978; Webb & Bohr, 1981). Present studies were carried out to determine whether changes in the plasma concentration of ovarian hormonal agents induce changes in cardiac or vascular responsiveness prior to elevation of blood pressure, with a view to extending the understanding of oral contraceptive-induced hypertension.

By examining the effects of short-term changes in the plasma concentration of ovarian hormonal agents, it was hoped to identify any changes in cardiovascular responses which occur prior to the establishment of chronically-elevated blood pressure. Since administration of oral contraceptive preparations has been found to elevate the blood pressure of female rats (Fregly, 1974), studies have been concerned with an examination of the effects of short-term elevations in the plasma concentration of ovarian hormonal agents

upon cardiac and vascular contractile responsiveness in female rats.

In initial experiments, the effect of short-term changes in the plasma concentration of 17β-oestradiol upon positive chronotropic and blood pressure responses was examined in the pithed female rat. These studies were extended to an examination of the effect of short-term administration of oral contraceptive agents to female rats upon the contractile responses of isolated cardiac and vascular tissue.

2. Literature Review

Introduction to the Literature Review

The present study has involved an investigation of the effect of alterations in the plasma concentrations of ovarian hormones upon cardiac and vascular responsiveness, with a view to extending the understanding of oral contraceptive-induced hypertension. It is therefore appropriate to review our knowledge of hypertension and the means by which blood pressure may be elevated. The first part of the literature review is therefore presented in the following sections:

(A) Hypertension

(1) Blood Pressure Control Systems

(a) Involvement of the Kidney in Blood Pressure Control

(b) Involvement of the Nervous System in Blood Pressure Control

(c) Involvement of Cardiac Output in Blood Pressure Control

(d) Involvement of Vascular Contractility in Blood Pressure Control.

The second part of the literature review is concerned with evidence for oral contraceptive-induced hypertension and the ways in which derangements in the renal, nervous, cardiac and vascular blood pressure control systems may be involved in the aetiology of oral contraceptive-induced hypertension. Particular attention is given to the involvement of altered vascular contractility in the aetiology of oral contraceptive-induced hypertension. This second part of the

literature review is therefore presented in the following sections:

(B) Oral Contraceptives and Hypertension

(1) Control of Blood Pressure during Ovarian Hormonal Therapy

(a) Effect of Ovarian Hormonal Agents upon the Renal Blood Pressure Control System

(b) Effect of Ovarian Hormonal Agents upon Nervous Control of Blood Pressure

(c) Effect of Ovarian Hormonal Agents upon Cardiac Output

(d) Effect of Ovarian Hormonal Agents upon Vascular Contractility

(i) Amount of Vasoconstrictor Agent at Receptor Site

(ii) Smooth Muscle Membrane Function

(iii) Agonist/Receptor Interaction

(iv) Smooth Muscle Calcium Content and Contraction System

(v) Smooth Muscle Cyclic Nucleotides.

The final part of the literature review is concerned with evidence for potentiation of vascular contractile responses in established hypertension and is entitled:

(C) Vascular Contractility in Established Hypertension.

(A) Hypertension

It has been known at least since Janeway's studies in 1913 that patients with elevated blood pressure tend to die prematurely. In his recent review, Grollman (1980) has discussed the way in which the tendency toward elevated blood pressure is genetically inherited and how the condition progresses from being symptomless to fatal. The principal organs affected by elevated blood pressure are the heart and arterial blood vessels. The former leads to cardiac failure and the latter to strokes and to kidney failure (Grollman, 1980).

Grollman (1980) has described hypertension as a condition in which the blood pressure is chronically elevated above an arbitrary level which is considered normal. Hereafter, this will be taken as the definition of hypertension. Despite the arbitrary nature of this definition however, mortality has been shown to increase with increasing arterial pressure and this is true from the lowest to the highest pressures (see review by Paul, 1980). In this literature review, therefore, reference will be made, whenever possible, to changes in blood pressure, rather than to normotensive or hypertensive conditions.

(1) Blood Pressure Control Systems

In 1949 Page first proposed the mosaic theory of blood pressure control in which a number of systems control blood pressure. Blood pressure in any vessel is dependent upon the intravascular blood volume and peripheral resistance (Ferrario & Page, 1978). Over the whole circulatory system therefore, blood pressure is dependent upon

the cardiac output and total peripheral resistance. The principal systems controlling cardiac output and total peripheral resistance are renal, nervous, cardiac and vascular in origin and a derangement in any of these systems may disturb the blood pressure equilibrium, resulting in hypertension (Weidmann, 1981). The ways in which these systems are involved in the control of blood pressure are briefly discussed below.

(a) Involvement of the Kidney in Blood Pressure Control

Laragh (1972) has summarised the means by which a renal system appears to be involved in the control of blood pressure. Whenever renal perfusion is reduced by a fall in arterial pressure induced by any stimulus which reduces the 'effective blood volume', the kidney secretes renin into the blood stream. Renin acts enzymatically on a plasma globulin to release angiotensin I. Angiotensin I is rapidly hydrolysed to angiotensin II by a converting enzyme found in vascular endothelium (Caldwell, Seegal, Hsu, Das & Soffer, 1976) and in circulating blood, especially in transit across the lungs (Semple, 1977). Together with its potent vasoconstrictor action, angiotensin II also increases the release of catecholamines, potentiates the activity of the sympathetic nervous system (Regoli, 1979) and stimulates aldosterone secretion from the adrenal glands. Aldosterone acts on the kidney to induce sodium retention and expansion of intravascular fluid (Laragh, 1972). Angiotensin II and aldosterone therefore act together to raise arterial pressure and restore renal perfusion, thereby compensating the system and shutting off the initial signal for renin release. Retained salt may bind in blood

vessel walls and lead to osmotic trapping of water (Wolinsky, 1971), a condition known as 'water logging' of the vascular walls. This water logging may reduce the vascular calibre and so increase the resistance to blood flow (Lim & Walters, 1970) which also restores arterial pressure.

McGiff & Quilley (1981) in their review have cited evidence which indicates that the kidneys of many species, including man, produce prostaglandins which may enhance the vasodilator and depressor effects of kinins. McGiff & Quilley (1981) have also cited evidence which indicates that prostaglandins produced by the rat kidney have pressor, rather than depressor, actions. In this respect, therefore, the rat cannot be taken to be an accurate model for blood pressure control in man.

(b) Involvement of the Nervous System in Blood Pressure Control

There is considerable evidence indicating the importance of nervous regulation of blood pressure (see review by Zanchetti, 1979). Changes in central and peripheral nervous systems may also initiate derangements in the circulation which may then lead to arterial hypertension (Zanchetti & Bartorelli, 1977). It has been postulated by Zanchetti (1979) that primary sympathetic hyperactivity can give rise to stable hypertension which outlasts the stimulus and leads to secondary vascular changes. Sivertsson (1970) and Weiss (1974) have shown that sympathetic hyperactivity is most likely to be intermittent in nature and occur during alerting situations. Other studies have demonstrated changes in peripheral nerve storage, release and metabolism of noradrenaline which may lead to the establishment of a

hypertensive condition (Ayitey-Smith & Varma, 1970; Lightman & Iversen, 1969; Kalsner, 1969). Also, it is now evident that the baroreceptor mechanisms for central nervous control of arterial pressure are reset in both experimental (McCubbin, Greene & Page, 1956) and clinical (Folkow, 1978; Zanchetti, Marcia & Malliani, 1980) hypertension. Records of activity from carotid sinus nerves are shifted to higher blood pressure levels in hypertensive conditions so that baroreceptor reflexes act to maintain, rather than to reduce, the elevated blood pressure (Doyle, Mendelsohn & Morgan, 1980). Alterations in the baroreceptor reflex may also act to elevate the blood pressure (Hilton & Spyer, 1980).

(c) Involvement of Cardiac Output in Blood Pressure Control

Tarazi (1977) and Günther (1980) have reviewed the evidence which indicates involvement of the heart in the development of hypertension. Increases in myocardial contractility may increase cardiac output which could lead to the development of hypertension (Albrecht, 1974) and an increase in cardiac output without any observable increase in peripheral resistance is a common feature in early hypertensive disease (Folkow, 1971; Weiss, 1974; Ferrario & Page, 1978).

(d) Involvement of Vascular Contractility in Blood Pressure Control

Arteries and arterioles have medial layers consisting of smooth muscle arranged concentrically with supporting connective tissue (Devine, 1978). Peripheral resistance, a primary determinant of blood pressure, is a function of the contractile state of arterial

smooth muscle cells (Hudgins, 1969). Webb & Bohr (1981) and Weidmann (1981) have reviewed in depth the manner in which potentiated responses of blood vessels to circulating or to nervously-released constrictor agents may lead to a hypertensive condition. In their review, Webb & Bohr (1981) have summarised evidence which suggests that changes in vascular responsiveness may be due to changes in either cell membrane function, agonist/receptor interactions, calcium content and vascular contraction system or in vascular smooth muscle cyclic nucleotides. The contractile state of vascular smooth muscle may also be altered by changes in the plasma concentration of vasoconstrictor agents (Weiss, 1974).

Veins are not subjected to the elevated blood pressure of hypertensive conditions (see review by Greenberg, 1980). However, changes in venous distensibility affect blood pressure by altering cardiac output (Ulyrich, Frohlich, Tarazi, Dustan & Page, 1969).

(B) Oral Contraceptives and Hypertension

Throughout this literature review, the term 'oral contraceptive' is applied to combined oestrogen-progestogen preparations which are administered orally to women.

Prior to 1967 there were only two case reports in the literature relating hypertension to the use of steroidal contraceptives. In 1962 Brownrigg described a 37 year-old patient with endometriosis who presented with severe headache, generalised oedema and rapid body weight gain. Although her blood pressure in two previous pregnancies had been normal, a rise from 120/80 to 200/110 mm Hg was observed after six months of treatment with norethynodrel (10 mg increasing to 40 $mg.day^{-1}$). Her blood pressure returned to 110/80 mm Hg four months after cessation of treatment. Four years later, a similar case report of a patient using norethynodrel for contraception was described by Owen (1966) who speculated that the reaction was due to pseudotoxaemia.

Since 1967 a number of other investigators have reported an association between ingestion of oral contraceptives and elevation of blood pressure in some women (Laragh, Sealey, Ledingham & Newton, 1967; Woods, 1967; Tyson, 1968; Weinberger, Collins, Dowdy, Nokes & Luetscher, 1969; Saruta, Saade & Kaplan, 1970; Weir, Briggs, Mack, Taylor, Browning, Naismith & Wilson, 1971) and in 1971 Wallace observed that the incidence of oral contraceptive use among women aged 20-45 years admitted to hospital with hypertension was almost twice that expected in the general population.

Wide variations now exist in the reported incidences of hypertension in women taking oral contraceptives; from 0 (Mackay,

Khoo & Adam, 1971; Weir et al., 1971) up to 18 % or more (Chernick, 1968; Saruta et al., 1970). The high estimates result largely from the reporting of small increases in blood pressure, sometimes within the pressure range considered normotensive (Laragh, 1974). In most women, rises in blood pressure due to oral contraceptives are minimal (Kaplan, 1978). However, in some women, severe and rapidly accelerating hypertension may cause irreversible renal damage (Zacherle & Richardson, 1972; Schoolwerth, Sandler, Klahr & Kissane, 1976). These findings indicate that some women may have a particular predisposition toward an oral contraceptive-induced elevation of blood pressure.

Race is a factor which may influence the development of oral contraceptive-induced hypertension. It is interesting that the study conducted by Saruta and co-workers (1970) which reported one of the highest incidences of initially normotensive women who developed hypertension when on oral contraceptive therapy (10 of 56), also included one of the highest percentages of black somen (17.8 %). These findings have been taken by Saruta and co-workers (1970) to suggest that some women may have a genetic predisposition toward the development of oral contraceptive-induced hypertension.

Elevations of systolic and diastolic pressure following oral contraceptive therapy appear to be readily reversible in most women. Blood pressure frequently returns to pre-treatment or lower levels (Wallace, 1971) within one to three months after stopping therapy (Laragh et al., 1967; Stokes, 1976) and it has even been reported that malignant hypertension has been reversed by cessation of oral contraceptive therapy (Smith, 1972). Woods, Algary & Stier (1974) however, found that, of a group of women who became hypertensive while

taking an oral contraceptive, half later became hypertensive again after stopping the therapy. Woods and co-workers (1974) found that when two women who became normotensive after stopping treatment were later challenged with another oral contraceptive, their hypertension recurred. Some women may therefore be susceptible to oral contraceptive-induced hypertension, whereas a tendency toward other forms of hypertension may be 'revealed' by administration of oral contraceptives to some women.

Crane, Harris & Winsor (1971) have shown that some women develop hypertension within one to three weeks after they start taking an oral contraceptive and that the same women became normotensive during that part of the cycle during which they are not taking the therapy. Other women demonstrate a persistent elevation of blood pressure within three to four months after oral contraceptive therapy began (Clezy, Foy, Hodge & Lumbers, 1972) while some women may not develop oral contraceptive-induced hypertension until they have taken the therapy for up to eight years (Weinberger, 1977).

Susceptible women taking oestrogen-progestogen oral contraceptive therapy tend to demonstrate elevations of systolic and mean arterial pressure in excess of any increase in diastolic blood pressure (Walters & Lim, 1970; Clezy et al., 1972; Weir, Briggs, Mack, Naismith, Taylor & Wilson, 1974; Fischer & Swain, 1980). Systolic pressure also appears to increase at a faster rate than diastolic pressure (Weir, Fraser, McElwee, Morton, Tree & Young, 1974). This increase in systolic pressure may be due to the oestrogenic component of the preparation, since Lim, Lumbers, Walters & Whelan (1970) have observed that intravenous injection of oestrone or 17β-oestradiol induces a significant increase in systolic blood pressure in women. Similarly, Spellacy & Birk (1972) reported incidences of hypertension

in groups of women taking mestranol, ethinyloestradiol and combined and sequential types of oral contraceptives although hypertension was not found in women using intrauterine devices (controls) or in women taking either progestogens or conjugated oestrogens. Crane & Harris (1978) observed that administration of conjugated oestrogens, diethylstilboestrol or oral contraceptives to some subjects was associated with hypertension that was reversible by cessation of treatment. Both oestrogen treatment of prostatic cancer (Veterans Administration Co-operative Urological Research Group, 1970) and conjugated oestrogen treatment in the menopause (Crane et al., 1971) have also been associated with the development of hypertension in some individuals. High levels of progesterone in early pregnancy, however, may be related to reduction in blood pressure observed at this time (MacGillivray, 1974). Similarly, progesterone administered to men and women with essential hypertension has been shown to have an antihypertensive effect (De Soldati, De Forteza, Pelligata & Cammerota, 1966). These findings again indicate that it may be the oestrogenic component of oral contraceptives which is primarily responsible for elevation of blood pressure in susceptible women. In order for oestrogens to increase blood pressure, however, intact and functioning ovaries may be necessary, since Pfeffer, Kurosaki & Charlton (1979) have found that post-menopausal women taking oestrogens had lower systolic pressures than non-users, although users and non-users had similar diastolic pressures. Original reports by Brownrigg (1962) and Owen (1966), however, indicate that administration of norethynodrel, a progestogen, led to the development of hypertension in two susceptible women. These latter findings indicate that, although the oestrogenic component of oral contraceptives is generally believed to be responsible for the

elevation of blood pressure, this hypothesis has not yet been adequately substantiated.

The incidence of development of hypertension during ingestion of oral contraceptives has been observed by Fregly & Fregly (1977) to increase with increase in age of the user. Women in the age range 40 to 44 using oral contraceptives have been shown to be at approximately five times the risk of developing hypertension as those using oral contraceptives in the age range 30 to 34 and at approximately ten times the risk as those using oral contraceptives in the age range 25 to 29 (Ramcharan, Pellegrin & Hoag, 1974).

Other factors may exist which influence the development of hypertension in users of oral contraceptives (see review by Fregly & Fregly, 1977). Some investigators, for example, believe that a correlation may exist between development of hypertension following oral contraceptive ingestion and a history of pregnancy-induced hypertension (PIH)(Carmichael, Taylor & Ayers, 1970, Saruta et al., 1970; Clezy et al., 1972). The assumptions are that the hormonal environment present during pregnancy is similar to that during oral contraceptive therapy (Fregly & Fregly, 1977) and that some women respond to a particular hormone profile with an elevation of blood pressure. However, this correlation between PIH and development of hypertension during oral contraceptive therapy has not been substantiated by others (Chidell, 1970; Laragh, 1972).

Another factor which has been suggested to predispose individuals to the hypertensive effects of oral contraceptives is obesity (Stokes, 1976).

The effect of administration of oral contraceptives to women with established hypertension remains controversial. Saruta and co-

workers (1970) have suggested that oral contraceptives may aggrevate a hypertensive condition, while Spellacy & Birk (1974) observed that oral contraceptive administration lowered blood pressure in hypertensive women.

Administration of oral contraceptives has been shown to increase the clotting factors in blood (see review by Kushner, 1970). Since increases in blood pressure may damage the vascular intima, hypertension and alterations in the clotting mechanism may act to predispose the individual to thromboembolic disease (Egeberg & Owren, 1963). There are reports suggesting that women using oral contraceptives have a greater risk of strokes than non-users (Collaberative Group for the Study of Stroke in Young Women, 1973, 1975; Royal College of General Practitioners' Oral Contraceptive Study, 1977; Vessey, McPherson & Johnsson, 1977) although this phenomenon has not yet been shown to be definately related to an increase in blood pressure (Laragh, 1972).

Oral contraceptives, and especially the oestrogenic component thereof, have therefore been shown to increase the blood pressure of susceptible women. Since any increase in blood pressure increases mortality (Lew, 1974; Paul, 1980) and some women demonstrate irreversible renal damage due to oral contraceptive-induced increases in blood pressure (Zacherle & Richardson, 1972), it is of clinical importance to determine the mechanisms involved in the aetiology and development of oral contraceptive-induced hypertension. With an understanding of the aetiology and development of oral contraceptive-induced hypertension, it may ultimately be possible both to predetermine those women who will develop this form of hypertension and to establish effective means of prevention and/or treatment of

the condition.

Some women develop a pregnancy-induced hypertension (PIH) (see review by Welt & Crenshaw, 1978). It has been postulated that, since plasma concentrations of oestrogens and progesterone are elevated during pregnancy (Challis, 1980), the development of PIH may be similar to that of oral contraceptive-induced hypertension (Fregly & Fregly, 1977). While such a relationship has not been conclusively demonstrated (Chidell, 1970; Laragh, 1972), many studies conducted to determine the aetiology of ovarian hormonal agent-induced hypertension have been performed on pregnant women.

Also, because of the difficulty of studying the mechanisms involved in oral contraceptive-induced hypertension in women, some studies have been performed on experimental animals. That results of experiments performed on experimental rats may be similar to those in the human situation is demonstrated in that administration of combined oestrogen-progestogen preparations to female rats (Fregly, 1974; Lew, 1975) and administration of oestrogenic agents to both male (Saruta, Ozawa & Asano, 1972) and female rats (Lew, 1975; Fischer & Swain, 1980) have been shown to result in an increase in blood pressure.

(1) Control of Blood Pressure during Ovarian Hormonal Therapy

Oral contraceptives and ovarian hormones would be expected to elevate blood pressure by causing derangements in one or more of the systems which control blood pressure, in particular the renal, nervous, cardiac or vascular control systems (see pages 5-10). The ways in which ovarian hormonal agents may affect these control

systems and so alter blood pressure are briefly discussed below.

(a) Effect of Ovarian Hormonal Agents upon the Renal Blood Pressure Control System

Several workers have found derangements in the renin-angiotensin-aldosterone (RAA) system in women who became hypertensive whilst taking oral contraceptives (Laragh et al., 1967; Skinner, Lumbers & Symonds, 1969; Weinberger et al., 1969; Saruta et al., 1970; Cain, Walters & Catt, 1971; Kaplan, 1974). Changes observed during these investigations have included measurable increases in plasma renin activity and plasma aldosterone concentration while plasma renin concentration (PRC) was decreased. These observations led Saruta and co-workers (1970) to suggest that those women who become hypertensive during ingestion of an oral contraceptive may have a defective angiotensin II feedback mechanism. When functioning properly, increasing plasma concentration of angiotensin II acts to reduce PRC which, in turn, reduces the plasma concentration of angiotensin II (see page 6). The hypertension which develops could then be attributed to the elevated plasma concentration of angiotensin II, acting principally as a vasoconstrictor agent (Bohr, 1974). Alternatively, Fregly & Fregly (1977) have suggested that alterations in the RAA system may be accounted for if the rate of metabolism of angiotensin II by angiotensinase increases only in women who remain normotensive whilst ingesting oral contraceptives. However, no evidence has yet been presented which indicates that angiotensin II metabolism is defective in women who become hypertensive whilst taking oral contraceptives.

Another hypothesis which makes a causative association

between the aetiology of oral contraceptive-induced bypertension and derangements in the RAA system has been proposed by Crane & Harris (1974a). They suggested that when an oral contraceptive is given, the oestrogenic component produces a mild to moderate hyperaldosterone state which is accompanied by mild sodium retention. Sodium retention then leads to plasma volume expansion and, therefore, increased cardiac output. The output of natural progesterone which would induce a sodium diuresis (Landau & Lugibihl, 1961) is suppressed by oestrogenic feedback on pituitary gonadotrophins. Since synthetic progestogenic agents with an α-side chain at carbon-17 administered in oral contraceptives also cause direct sodium retention by a mineralocorticoid-like action (Landau, Lugibihl & Dimick, 1958), there would be an additional increase in plasma volume which would tend to elevate the blood pressure. Crane & Harris (1974a) have suggested that those individuals who are sensitive to mineralocorticoids will eventually exhibit an increase in blood pressure. Evidence to support this hypothesis has been provided by Walters & Lim (1969) and Littler, Borjorges-Bueno & Banks (1974). Walters & Lim (1969) and Littler and co-workers (1974) have shown that combined oestrogen-progestogen oral contraceptives may increase cardiac output in women and Littler and co-workers (1974) have demonstrated that it is the oestrogenic component of the preparation which is responsible for any increase in cardiac output. An increase in cardiac output accompanying administration of oral contraceptives to women suggests a reason why most studies have found more prominent elevations of systolic than diastolic pressure in oral contraceptive users (Walters & Lim, 1970; Clezy et al., 1972; Weir et al., 1974; Fischer & Swain, 1980). Beckerhoff, Vetter, Armbruster, Luetscher &

Siegenthaler (1973) however, have demonstrated that women who did not become hypertensive on oral contraceptives had similar derangements of the RAA system as those who became hypertensive. Therefore, although the RAA system has been implicated in the aetiology of oral contraceptive-induced hypertension, a condition which may also be exacerbated by pre-existing renal disease (Harris, 1969; Weinberger et al., 1969; Crawford & Palmer, 1973), present evidence does not ascribe a major role to it.

(b) Effect of Ovarian Hormonal Agents upon Nervous Control of Blood Pressure

Side effects of oral contraceptive therapy such as headache, migraine, altered visual function, increased appetite, convulsions and depression indicate that oral contraceptives may affect the hypothalamus and other centres of the brain (El-Sherif, El-Said, Kamal, Hefnawi, Younis, Ghoneim & Talaat, 1969). Since the hypothalamus may play a role in the baroreceptor reflex (Hilton & Spyer, 1980), oral contraceptives may alter the arterial pressure by modifying the baroreceptor reflex.

Results from experiments performed by Assali, Lieutenant, Vergon, Tada & Garba (1952) indicate that pregnancy may be associated with markedly increased sympathetic activity which may act to elevate arterial pressure.

(c) Effect of Ovarian Hormonal Agents upon Cardiac Output

Since several workers (Walters & Lim, 1970; Clezy et al., 1972; Weir et al., 1974; Fischer & Swain, 1980) have shown that

systolic pressure elevations were greater than elevations of diastolic pressure in oral contraceptive users, and since systolic hypertension may be due to an increase in cardiac output (Walters & Lim, 1969), it is possible that oral contraceptives may increase the cardiac output in susceptible women. Walters & Lim (1969) initially proposed that oral contraceptives increase the cardiac output by potentiating myocardial contractility. Several workers have examined the effects of oestrogenic agents upon the cardiac contractile system of experimental animals. The results of these experiments provide evidence to support the hypothesis that the oestrogenic component of oral contraceptives increases cardiac output by potentiating myocardial contractility.

In 1977 Stumpf, Sar & Aumüller demonstrated that 17β-oestradiol is accumulated throughout rat atrial contractile tissue. King, Whitehorn, Reeves & Kubota (1959) have shown that ovariectomy reduced the strength of isolated ventricular muscle contraction and that treatment of ovariectomised rats with α-oestradiol raised the force developed by isolated ventricular muscle to the level observed prior to ovariectomy. In similar studies, administration of adreno-corticotrophic hormone to ovariectomised rats did not have this effect (Ullrick, Brennan & Whitehorn, 1955). King and co-workers (1959) have also shown that α-oestradiol pre-treatment increases the actomyosin content of rat hearts.

Rubin & Salter (1950) studied the isolated frog heart in vitro. Conjugated and unconjugated steroids of the oestrogen and pregnane series were added to the heart after it had been made hypodynamic by reducing by one half the concentration of calcium in the surrounding physiological saline solution. Only sodium oestrone

augmented the amplitude of contraction. Loynes & Gowdey (1952) described similar effects on the isolated frog heart and both oestrone and 17β-oestradiol were shown to augment the amplitude of contraction. However, these workers (Loynes & Gowdey, 1952) did not observe any effect of either ethinyloestradiol or progesterone upon cardiac function.

Lim & Walters (1970) in their review, however, have summarised evidence indicating that it is not certain whether oestrogenic agents have any effect upon the excitability and refractoriness of cardiac muscle. Also, since several workers (Walters & Lim, 1970; Crane & Harris, 1974b; Lehtovirta, 1974) have shown that oral contraceptives may increase the plasma concentration of aldosterone in women, any increase in cardiac output in women may be due to plasma volume expansion, rather than to an increase in myocardial contractility.

(d) Effect of Ovarian Hormonal Agents upon Vascular Contractility

Webb & Bohr (1981) have summarised evidence which indicates that potentiated vascular smooth muscle contractile responsiveness may lead to the establishment of a hypertensive condition. Experiments designed to determine whether ovarian hormonal agents increase blood pressure by potentiating vasoconstrictor responses have included clinical studies, studies on the pressor responses of whole animals and studies of the properties of isolated blood vessels.

Lloyd and co-workers carried out a series of experiments between 1959 and 1968 to determine whether changes in the plasma concentration of ovarian hormonal agents alter pressor responses in anaesthetised rats.

Lloyd (1959a, b) demonstrated that the pressor action of intravenously-administered vasopressin was greater in oestrus compared with dioestrus and was potentiated during the second half of pregnancy compared with the first. The pressor actions of vasopressin was also temporarily potentiated after administration of the synthetic oestrogenic agent, stilboestrol dipropionate, or progesterone to male or female rats (Lloyd, 1959a). These experiments indicate that increasing the plasma concentration of ovarian hormonal agents increases pressor responses to vasopressin in rats. The converse is also true, since Lloyd (1959a) demonstrated that, after ovariectomy, the pressor action of vasopressin was reduced.

Lloyd (1959a) has shown that, whereas oxytocin had no effect upon blood pressure in anaesthetised rats, in oestrous rats it was pressor. Administration of stilboestrol diproprionate or progesterone also caused oxytocin to become a pressor agent in anaesthetised male rats (Lloyd, 1959a).

In 1968 Hettiaratchi & Pickford provided evidence that the stage of the oestrous cycle and administration of oestrogen did not affect the pressor responses of anaesthetised rats to intravenously-administered angiotensin II, although pregnancy and administration of progesterone reduced pressor responses to angiotensin II. Hettiaratchi & Pickford (1968) concluded that refractoriness to the pressor action of angiotensin II was due to the elevation of plasma progesterone during pregnancy in the rat. Normal human pregnancy is associated with elevation of the plasma concentrations of both progesterone and oestrogens (Challis, 1980) and also considerable refractoriness to the pressor effects of intravenously infused angiotensin II (Abdul-Karim & Assali, 1961; Lim & Walters, 1970).

Pregnant women who are destined to develop PIH, however, lose this refractoriness several weeks prior to the onset of hypertension (Everett, Worley, MacDonald & Gant, 1978). It was found that vascular refractoriness in normotensive pregnant women could be reduced by administration of prostaglandin synthetase inhibitors and that refractoriness could subsequently be restored by an intravenous infusion of the progesterone metabolite, 5α-pregnane-3, 20-dione (Everett et al., 1978). The findings of Everett and co-workers (1978) indicate that the progesterone metabolite may induce refractoriness to the pressor action of angiotensin II by stimulating the synthesis of prostaglandins. However, a direct effect of the progesterone metabolite upon vascular smooth muscle cannot yet be excluded as the mechanism of vascular refractoriness (see review by Gant, Worley, Everett & MacDonald, 1980).

McGiff & Quilley (1981) have reviewed the evidence which indicates that renal prostaglandins act to lower blood pressure in man by augmenting the vasodilatation and diuretic-natriuretic actions of the kinins. Progestogens may not, however, reduce blood pressure by increasing the renal secretion of prostaglandins in the rat, since, in the rat, renal prostaglandins have been shown to increase the blood pressure (McGiff & Quilley, 1981).

The above observations are consistent with a view that one or more progestogen metabolite may be important in the maintenance of normal (low) blood pressure during human pregnancy. Also, changes in responses to pressor agents during human pregnancy appear to be selective for particular pressor agents, since Chesley, Talledo, Bohler & Zuspan (1965) have shown that pressor responses to noradrenaline are normal during late pregnancy.

The influence of sex hormones, oral contraceptives and pregnancy upon isolated vascular muscle at rest and during responses to constrictor agents has been reviewed by Altura & Altura (1977).

Some studies indicate that the tone of arterial blood vessels may be increased by exposure to ovarian hormones (see review by Altura & Altura, 1977). Keates & FitzGerald (1975) have observed monthly cyclical changes in the calf blood flow of both users and non-users of oral contraceptives. These changes may have been due to changes in peripheral resistance resulting from elevation of vasoconstrictor tone, since Keates & FitzGerald (1975) observed no changes either in blood pressure or in pulse rate. Keates & FitzGerald (1975) have suggested that these changes in blood flow may have been due to cyclical changes in the plasma concentration of ovarian hormones during the menstrual cycle. When Strömberg & Westin (1972) injected 1 µg 17β-oestradiol subcutaneously into ovariectomised rats they observed increased vascular tone in some arteries. Similarly, Gabrielsen & Greitz (1970) have shown that the cerebral arteries of women are relatively more narrow than those in men. Since the plasma concentration of ovarian hormones is higher in women than in men (Martin, 1976), the findings of Gabrielsen & Greitz (1970) indicate that circulating ovarian hormones may increase the tone of vascular smooth muscle.

Potentiation of vasoconstrictor responses may be induced by an increase in the amount of vasoconstrictor agent present (Weiss, 1974) or by alterations in either cell membrane function, agonist/receptor interactions, the calcium content and vascular contraction

system or in vascular smooth muscle nucleotides (Webb & Bohr, 1981). Evidence for the involvement of these factors in the aetiology of oral contraceptive-induced hypertension is presented below.

(i) Amount of Vasoconstrictor Agent at Receptor Site

Zakheim, Molteni, Mattioli & Mullis (1976) have shown that plasma concentrations of angiotensin II are increased in women following administration of an oestrogen-progestogen oral contraceptive. Fregly & Fregly (1977) have suggested that an increase in the plasma concentration of angiotensin II may be due to reduced breakdown by angiotensinase and that it may lead to hypertension in some women. However, Devynck & Meyer (1976) in their review have summarised evidence which indicates that the number of angiotensin II receptors is inversely related to the plasma concentration of the agent. If, therefore, the plasma concentration of angiotensin II is elevated during oral contraceptive administration, it is unlikely to alter the vasoconstrictor tone or the blood pressure.

Green & Miller (1966) and Nagle & Rosner (1976) have shown that, in rats, plasma concentrations of noradrenaline and adrenaline alter during the course of the oestrous cycle and pregnancy, such that the concentrations of catecholamines may be related to the plasma concentration of ovarian hormones.

In 1969 Lightman & Iversen demonstrated that, in arteries where few smooth muscle cells are in close proximity to adrenergic nerves, extraneuronal accumulation and metabolism of noradrenaline may serve as an important means of inactivation. Also, Nicol & Rae (1972) have shown that addition of 17β-oestradiol in the perfusate of rabbit

ear artery may produce a concentration-dependent inhibition of smooth muscle accumulation of adrenaline and noradrenaline. However, this does not necessarily indicate that constrictor responses will be amplified by the administration of the oestrogen, since Avakian & Gillespie (1967) have presented evidence which indicates that uptake of catecholamines into vascular smooth muscle may lead to their re-release and, therefore, potentiation of vasoconstrictor responses. By inhibiting extraneuronal uptake of noradrenaline and adrenaline, therefore, 17β-oestradiol may inhibit constrictor responses to circulating and nervously-released catecholamines.

In 1969 Kalsner administered either 17β-oestradiol or progesterone directly to rabbit aortic strips. Both hormones potentiated contractile responses of the tissue to catecholamines. The response-potentiating effect of the ovarian hormones was inhibited by prior administration of known inhibitors of catechol-o-methyltransferase (COMT). Kalsner (1969) therefore, proposed that potentiation of catecholamine responses by the ovarian hormones was due to inhibition of COMT, an enzymatic pathway for inactivation of catecholamines (Trendelenburg, 1977). In this way, therefore, ovarian hormones may elevate the amount of catecholamines present at vascular smooth muscle receptor sites.

(ii) Smooth Muscle Membrane Function

Administration of oestrogen to rats has been shown to increase the membrane potential of uterine smooth muscle in vivo (Marshall, 1962). Results of work carried out by McCalden (1975) indicate that oestrogenic hormones may also alter the membrane potential of vascular

smooth muscle. He showed that one low concentration (0.1 $\mu g.ml^{-1}$) of 17β-oestradiol added directly to isolated rat portal vein amplified the spontaneous mechanical activity, whereas higher concentrations ()1.0 $\mu g.ml^{-1}$) of 17β-oestradiol attenuated the spontaneous mechanical activity. McCalden (1975) proposed that low concentrations of the oestrogen moved the vascular smooth muscle membrane potential into a more excitable range, whereas higher concentrations hyperpolarised the membrane out of the contraction firing range.

(iii) Agonist/Receptor Interaction

In 1975 Altura studied the effect of changes in the plasma concentration of ovarian hormones upon responses of isolated mesenteric arteries. Constrictor dose-response curves to noradrenaline, adrenaline, vasopressin and oxytocin were all shifted to the left in a parallel manner in arteries from female rats compared with those from male rats. Pre-treatment of male rats with 17β-oestradiol also enhanced constrictor responses to noradrenaline and adrenaline. Dose-response curves to angiotensin II or dopamine, however, were not affected by sex or oestrogen pre-treatment of male rats. Altura (1975) concluded that oestrogenic hormones selectively enhance α-adrenoreceptor-mediated vasoconstrictor responses and vasoconstrictor responses to posterior pituitary hormones.

Although the plasma concentration of angiotensin II may be elevated by administration of combined oestrogen-progestogen oral contraceptives (Zakheim et al., 1976) and this may lead to reduction in the number of available angiotensin II receptors (Devynck & Meyer, 1976), there is no evidence that alterations in the plasma

concentration of angiotensin II alter agonist-receptor interactions (see review by Devynck & Meyer, 1976).

(iv) Smooth Muscle Calcium Content and Contraction System

Since the penultimate event in the cascade which induces vascular smooth muscle contraction or relaxation is mobilisation of calcium (Ca^{2+})(Hurwitz & Suria, 1971), alterations in the calcium mobilisation, content or distribution in vascular smooth muscle may alter the contractile state of the vessel.

In 1973 DeFelice & Joiner demonstrated that aortae from male rats were more responsive to the vasoconstrictor effect of high potassium concentrations (K^{+}) than to adrenaline, wheras aortae from female rats were more responsive to adrenaline than to high potassium concentrations. It has been suggested that adrenaline, like noradrenaline, elicits α-adrenoreceptor-mediated responses (Vanhoutte, 1978) principally by mobilising intracellular calcium, whereas potassium-induced contractions may be dependent upon calcium located on the cell membrane (Paiva, Paiva, Miyamoto & Nakaie, 1977). The findings of DeFelice & Joiner (1973) indicate that the calcium in aortae from male and female rats may be located at different sites. It is therefore possible that ovarian hormones alter the distribution of vascular smooth muscle stores of calcium.

Csapo (1950) has shown that ovariectomy decreased, and subsequent administration of oestrogen increased, the actomyosin content of rat uterine smooth muscle. King and co-workers (1959) have demonstrated that ovariectomy decreased, and administration of α-oestradiol increased, the actomyosin content of rat cardiac muscle.

These findings indicate that oestrogenic agents may stimulate actomyosin production in all muscular beds. If this is true, then oestrogenic agents may increase the actomyosin content of vascular smooth muscle and the constrictor tone of arterial blood vessels. This in turn may lead to arterial hypertension (Hudgins, 1969).

(v) Smooth Muscle Cyclic Nucleotides

Williams-Ashman & Reddi (1971) have reviewed the evidence which indicates that oestrogens may be taken upon into intact cells and that they have intracellular actions. There is some evidence that administration of the oestrogens 17β-oestradiol and stilboestrol induces a rapid increase in the cyclic adenosine monophosphate (cAMP) content of the uterus of ovariectomised rats, and it has been suggested that this increase may be related to the oestrogen-induced proliferation of rat uterine smooth muscle (Williams-Ashman & Reddi, 1971). It is therefore possible that cyclic nucleotides mediate an oestrogen-induced proliferation of vascular smooth muscle which would both potentiate vascular contractile responsiveness and increase arterial blood pressure (Folkow, 1978; Webb & Bohr, 1981).

Veins are not subjected to the elevated blood pressure of hypertensive states (Greenberg, 1980). However, changes in venous distensibility and tone affect blood pressure by altering cardiac output (Ulyrich et al., 1969). Fogarty, in 1971, performed experiments on spirally-cut strips of human and dog saphenous vein. She administered progesterone in polyethylene glycol directly to the

isolated tissues and concluded that progesterone acts on venous smooth muscle as a non-competitive inhibitor of noradrenaline- and acetylcholine-induced contractions.

Using plethysmographic techniques, McCausland, Hyman, Winsor & Trotter (1961) have shown that venous distensibility is increased during pregnancy, and Goodrich & Wood (1964) have shown that oral contraceptive administration may also increase venous distensibility in women. Since Goodrich & Wood (1966) demonstrated an increase in peripheral venous distensibility in human leg veins after administration of 17β-oestradiol, it appears that it is the rise in plasma concentration of 17β-oestradiol which is responsible for the increase in venous distensibility observed during pregnancy. Decrease in venous distensibility may lead to elevation of cardiac output and arterial pressure (Greenberg, 1980). It therefore appears unlikely that increase in venous distensibility induced by administration of ovarian hormones would lead to elevation of blood pressure.

(C) Vascular Contractility in Established Hypertension

Folkow (1978) and Webb & Bohr (1981) have reviewed evidence indicating that, although potentiated vascular responses to endogenous vasoconstrictor agents may lead to the establishment of a hypertensive condition, structural changes may also occur in the vasculature subsequent to a sustained increase in blood pressure. This principally takes the form of smooth muscle hypertrophy which is a result of the increased load against which the vascular muscle exerts its force (Folkow, 1978). Hypertrophied blood vessel walls may encroach upon the lumen and account for the increase in total peripheral resistance to flow at complete muscular relaxation (Folkow, 1971). The increased wall:lumen ratio due to structural changes in the vessel may also potentiate vascular contractile responses for purely geometrical reasons without any altered sensitivity of the contractile elements (Folkow, 1971, 1978). Similarly, water logging of vascular smooth muscle could also increase the wall thickness which augments total peripheral resistance (Tobian, Olson & Chesley, 1969; Lim & Walters, 1970) and potentiates vascular responsiveness (Johansson & Jonsson, 1968). Water logging may result from increased fluid retention by hypersecretion of aldosterone (Lim & Walters, 1970) or it may be another aspect of morphological changes seen in blood vessels following sustained hypertension (Sivertsson, 1970; Greenberg, 1980). Potentiation of vascular responsiveness may therefore be causative of, or the result of, elevated blood pressure.

In a study to determine whether oral contraceptives and other ovarian hormonal agents induce hypertension in susceptible individuals by potentiating vascular responsiveness, it is important to distinguish

between smooth muscle supersensitivity prior to, and after, the establishment of hypertension (Weiss, 1974). Present studies were undertaken to examine the effects of ovarian hormonal agents upon cardiovascular responsiveness prior to the establishment of a hypertensive condition. To this end, therefore, the effects of short-term changes in the plasma concentration of ovarian hormonal agents upon cardiovascular responses were examined in the rat.

METHODS

1: ANIMAL HUSBANDRY

All experimental rats were Wistar females. They were housed at $21 \pm 1^{\circ}C$ in daylight and allowed to feed on Hewgate's breeding diet and tap water ad libitum.

(i) Vaginal Smearing

Cell samples were obtained from the inner surface of the vagina using a glass rod moistened with saline (0.9 g.100 ml^{-1} sodium chloride) solution, suspended in saline solution on a glass slide, and examined under a light microscope. When Wang (1923) examined the vaginal smears of rats in this way, he observed signs of pseudo-pregnancy. However, no such signs were observed in present experiments. The stages of the oestrous cycle were designated as follows:

Dioestrus: The smear consisted mainly of leucocytes.

Proestrus: The smear consisted of a few nucleated epithelial cells.

Oestrus: The smear consisted of a large number of cornified epithelial cells.

Metoestrus: The smear consisted of many leucocytes with some residual epithelial cells.

(ii) Study of the Effect of Housing Female Rats Separately and Together

The following study was undertaken to determine the oestrous cycle length and growth rate of female rats housed individually and whether they differed compared to those of rats housed in groups.

Group 1: Six female rats were held together in a cage measuring 50 cm x 30 cm.

Group 2: Six female rats were held in individual cages measuring 40 cm x 25 cm.

All rats were marked for identification. The experiment commenced 1 week after their reception at the animal house, when the rats weighed 180-210 g. Daily at 10 a.m., vaginal smears from all rats were examined and once weekly all rats were weighed.

Oestrous cycle length was taken as the time it took a rat to proceed through the various stages of the cycle between one oestrus and the next. Oestrus was chosen because it is the most easily identifiable stage of the cycle and may be characterised both by the vaginal smear and by the marked increase in spontaneous locomotor activity which occurs at this time (Wang, 1923). The average oestrous cycle length was calculated for each rat, and for each group a stastical mean was determined. Mean weight was calculated for each group of rats at weekly intervals.

2: PRACTICAL PROCEDURES

(i) Pithed Rat Preparation

Adult female rats (180-280 g) were weighed and the vaginal smears were examined to determine the stage of the oestrous cycle (see page 33). The rats were anaesthetised with intraperitoneal sodium pentobarbitone (60 mg.kg body weight^{-1}). Following tracheal intubation, the rats were pithed with a 0.1 cm diameter, 20 cm long, steel rod via the right eye orbit by the method of Shipley & Tilden (1947).

Immediately after pithing, the rats were artificially respired with a Palmer 'Miniature Ideal' stroke pump. This reciprocal pump operated at 80 strokes per minute and at a volume sufficient to give adequate chest expansion (5-6 ml). The rate used was higher than that employed by Shipley & Tilden (1947). It was found that increasing the rate made possible a reduction in the inspiration volume and this minimised the influence of respiration upon the blood pressure recording.

The left carotid artery and the right jugular vein were cannulated with polypropylene tubing (pp 25). The cannulae were previously filled with saline solution (0.9 g.100 ml^{-1} sodium chloride) containing heparin (20 iu.ml^{-1}) to prevent blood from clotting. Arterial blood pressure was measured by means of a Bell & Howell pressure transducer (type 4/422/0001). All drugs were injected intravenously and each injection was followed by an injection of heparinised saline solution ('flush') to ensure that all of the dose was administered to the animal. Total injection volume (drug plus

flush) was maintained constant at 0.3 ml. Despite the close proximity of the sites for blood pressure recording and injection, no appreciable bolus effect was observed in the recordings. The pithed rat was initially injected with atropine sulphate (1 mg.kg body weight^{-1}) to prevent asphyxiation by muscarinic-stimulated bronchial secretions (Innes & Nickerson, 1975).

In order to maintain the temperature of the preparation at approximately 37°C, the animal was mounted on a heated table (Palmer).

Wounds were covered with saline solution-soaked cotton wool to prevent dehydration.

After cannulation, all animals were left for 30 minutes before the start of the experiment. This was in order to achieve a consistent baseline blood pressure.

Pithed rats were killed at the end of experiments.

(a) Electrocardiogram (ECG)

The electrocardiogram was recorded from a Devices clinical ECG. The potentials recorded from rats are small when compared with those from humans and this necessitated modification in the use of the apparatus. Various electrode positions were investigated before chest/left leg was found to give the strongest and most reproducible signal. That is, with the indifferent electrode in the left hind-limb and another electrode, which normally records the right arm potential, recording the chest potential. Both of these electrodes, together with a third electrode in the left fore-limb to 'earth' the animal, were attached to steel needles which were implanted subcutaneously. Recordings were made on a Devices M2 recorder.

(b) Drug Administration

In order to provide the most reproducible results, the times for response to the agonists were as follows:

Angiotensin II: 5 min.

Isoprenaline: 15 min.

Noradrenaline: 5 min.

Oxytocin: 8 min.

Vasopressin: 10 min.

Isoprenaline was found to alter cardiovascular responses to other drugs and was therefore administered at the end of an experiment.

(c) Electrical Stimulation

Stimulation of intact nerves synapsing in the spinal cord was achieved using a square-wave stimulator (Scientific and Research Instruments Ltd.). The positive pole was connected to the shaft of the pithing rod and the indifferent electrode, a steel hypodermic needle, was inserted subcutaneously into the left hind-limb. Since nerve stimulation leads to muscle contraction, which causes the animal to jerk, and to present altered blood pressure responses, D-tubocurarine (3 mg.kg body weight^{-1}), a competitive blocker of the nerve/skeletal muscle junction (Standaert, 1964), was injected intravenously to act as a muscle relaxant.

The rat was stimulated electrically every 5 minutes at a constant frequency of 10 s^{-1} until peak systolic pressure was achieved. Voltage was varied between 20 and 60 V at fixed pulse widths (200, 400, 600 and 800 μs). In this way it was hoped to determine the

effect of alterations in the plasma concentration of ovarian hormones upon recruitment of sympathetic nerve fibres. An alternative method of stimulation, in which the frequency is raised at supramaximal voltage, was not employed, since Gillespie & Muir (1967a, b) have presented evidence which suggests that responses to electrical stimulation do not accurately reflect the amount of noradrenaline released by sympathetic nerves at frequencies above 1-4 s^{-1}.

No ECG recordings could be made during electrical stimulation because of interference.

Prior to some pithed rat experiments, the following surgical procedures were carried out:

Bilateral ovariectomy (p. 45);
Sham bilateral ovariectomy (p. 46);
Implantation of osmotic minipumps containing 17β-oestradiol in polyethylene glycol (PEG) 300 (p. 47).

(ii) Isolated Organ Preparations

Female rats (180-280 g) were stunned with a blow on the head and killed by cervical dislocation.

Tissues were suspended in jacketed organ baths containing freshly prepared physiological saline solution (PSS) and bubbled with 95 % O_2 and 5 % CO_2. The composition of the PSS was as follows: NaCl; 94 mM, KCl; 4.7 mM, $MgSO_4.7H_2O$; 0.45 mM, $KH_2PO_4.2H_2O$; 1.2 mM, $NaHCO_3$; 25 mM, $CaCl_2.2H_2O$; 2.4 mM, glucose; 11.1 mM. Temperatures were kept constant by circulating water from a Churchill pump.

(a) Spiral-Cut Strip of Rat Thoracic Aorta

The descending thoracic aorta was excised and placed in a Petri dish containing PSS at room temperature. The excised vessel was then cleaned and surrounding connective tissue was removed. The aorta was then cut helically by placing it over polypropylene tubing (pp 25) and turning the tissue while an incision was made at one end with sharp-pointed scissors in a procedure similar to that outlined by Furchgott (1960). During this procedure, the tissue was kept moist with PSS. The dimensions of the resulting strip were approximately 20 mm x 2 mm.

Preparations were suspended in 10 ml organ baths and maintained at $37 \pm 0.5^{\circ}C$. The strips were mounted vertically under isometric conditions, exerting a force of 9.8 mN for 1 hour, during which the tissue was washed 3 times. Drug injection volumes were consistently 0.1 ml and contact times were as follows:

Angiotensin II, 25 min &
Noradrenaline, 20 min.

Aortic strips were washed several times between the attainment of maximal force and administration of the subsequent concentration.

(b) Hepatic Portal Vein of the Rat

The portal vein was ligated near the hepatic hilum and near the junction of the mesenteric veins. Surrounding connective tissue was removed and the vessel was excised. During this procedure, the vein was kept moist with PSS at room temperature.

The excised vessel was mounted between 'field' stimulating platinum electrodes (figure 1, p. 44) and placed in a 40 ml organ bath, maintained at 37±0.5°C and under a resting force of 4.9 mN. Drug administration commenced after a 1 hour recovery period during which the tissue was washed every twenty minutes. Concentration-response curves to noradrenaline, angiotensin II and potassium chloride were cumulative and injection volumes were constant at 0.4 ml. The portal vein was also stimulated electrically in a cumulative manner by a square-wave stimulator (Scientific and Research Instruments Ltd.)(S.R.I.).

(c) Separated Atria of the Rat

It was hoped that this preparation would make possible a determination of the inotropic and chronotropic responses of isolated atria without their mutual interference. This preparation, in which the right atrium beats spontaneously and the left atrium is stimulated electrically, is similar to that described by Broadley (1974) who used guinea-pig atria. However, when stimulated by noradrenaline or isoprenaline, the right atrium gave a maximal response at the primary dose, regardless of the concentration. Sensitivity to subsequent doses was diminished and it was difficult to obtain consistent results. Ectopic beats frequently arose in the left atrium at the parameters of 2.5 Hz, 5 ms pulse width and threshold voltage plus 50 % which were recommended by Broadley (1974) for isolated guinea-pig left atrium.

In an attempt to overcome the above problems, a number of changes were made in the protocol:

a) stepwise increases in resting force, from 4.9 mN to 9.8 mN for the right atrium and from 9.8 mN to 19.6 mN for the left atrium, were investigated,

b) a range of organ bath temperatures above and below 32°C, which was suggested by Broadley (personal communication, 1979), were examined,

c) in order to determine whether lack of energy was the limiting factor, glucose concentration in the PSS was doubled to 22.2 mN, and

d) the voltage was raised above threshold plus 50 %.

After extensive investigation, no means was found of overcoming the above problems and so use of the preparation was suspended. The problems arising may have been due to the use of rat, rather than guinea-pig, tissue or to the small size of the tissue.

(d) Paired Atria of the Rat

The heart was rapidly excised and transfered to a beaker containing chilled PSS. Ventricular tissue was trimmed away from the paired atria. A cotton thread was passed through the margin of 1 atrium and tied to the wire loop on a tissue holder (figure 2, p. 44). A second thread was attached to the margin of the other atrium for connection with an isometric transducer.

The holder was immersed in a 40 ml organ bath incorporating a sintered glass aerator and containing PSS at 32°C. A force of 9.8 mN was exerted by the atria which were allowed to stabilise for 30-60 minutes, until a consistent spontaneous beat was established. During this period the bathing medium was changed 2 or 3 times.

Both noradrenaline and isoprenaline were administered cumulatively and all doses were given in a constant volume of 0.4 ml.

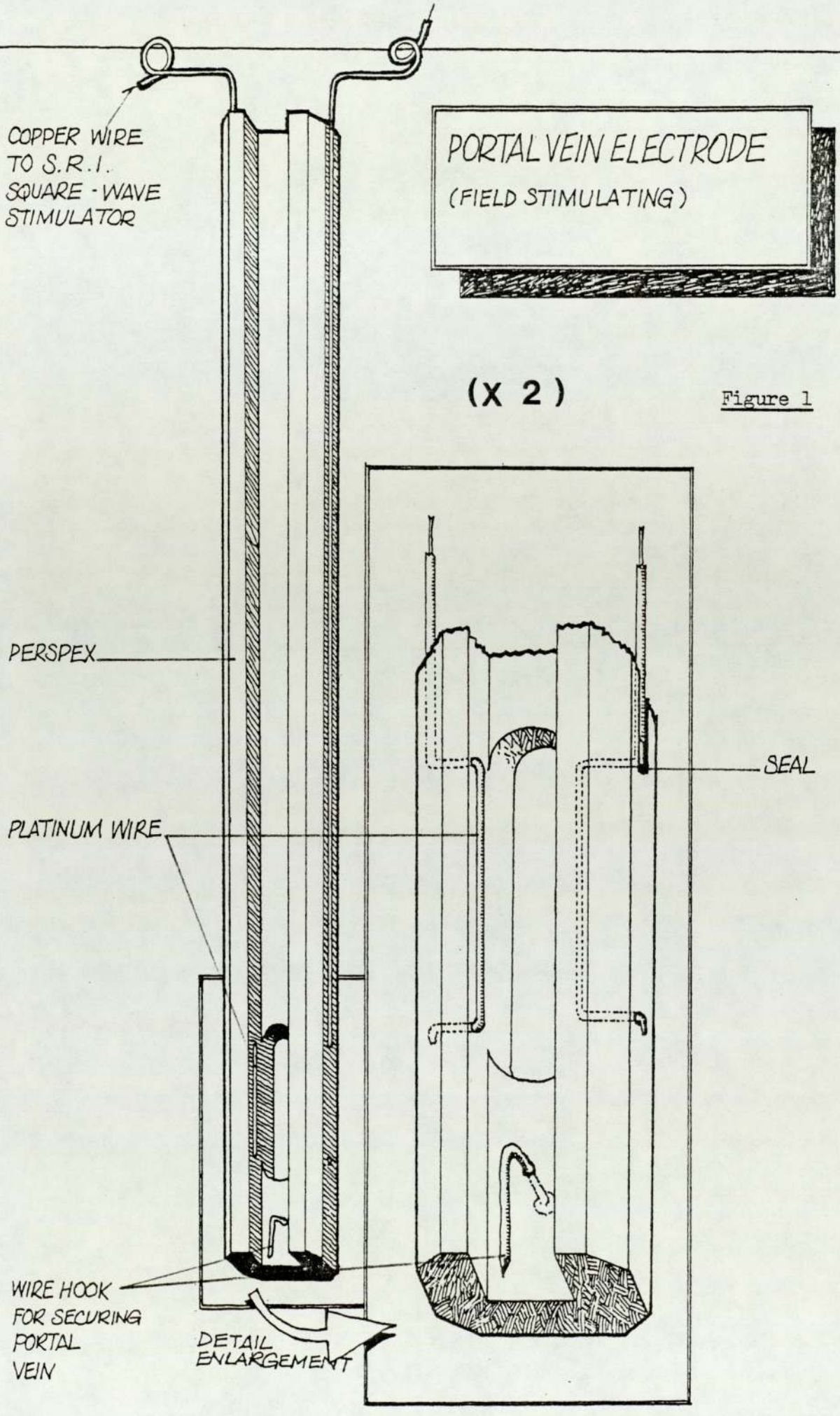
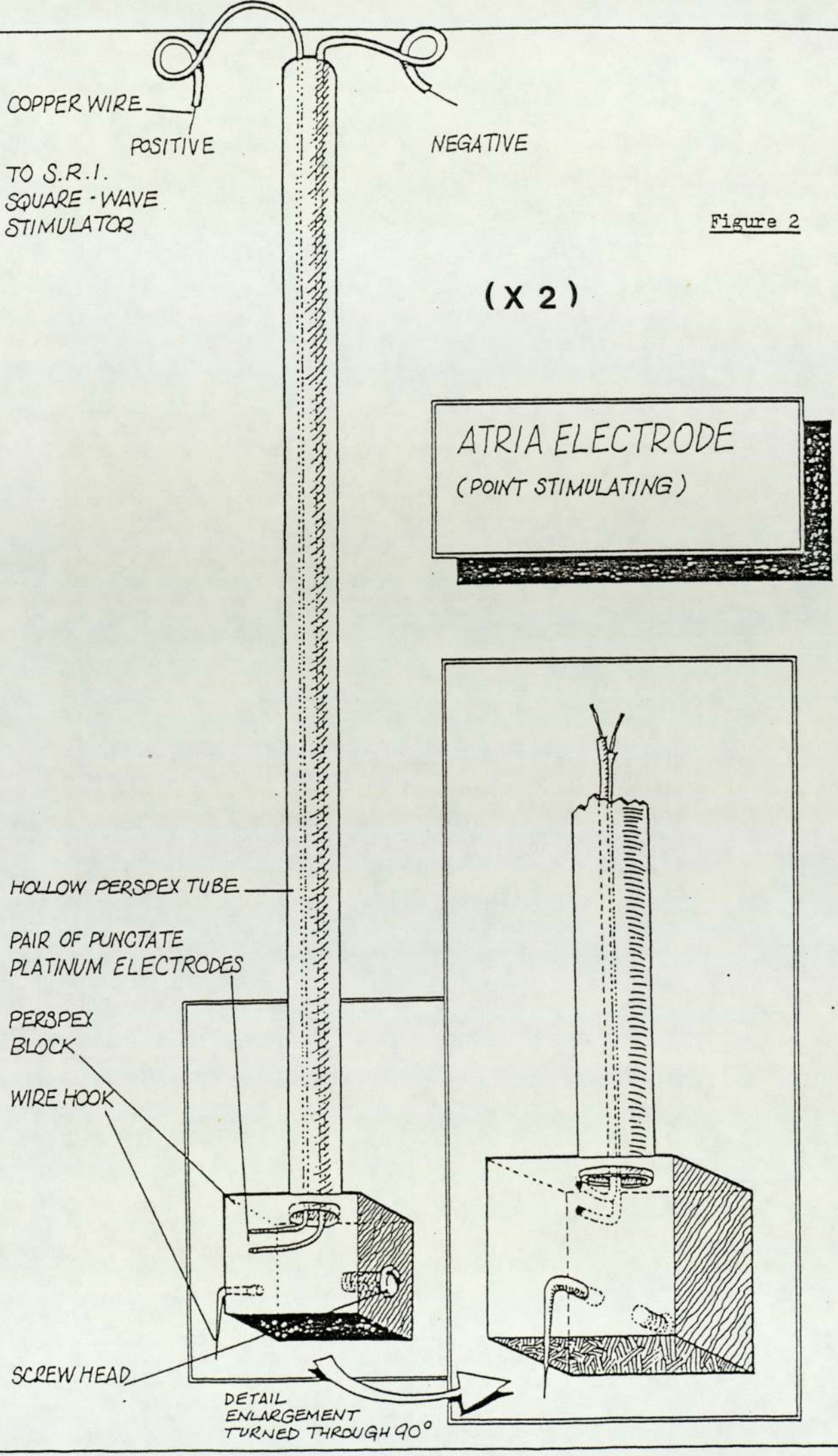

COPPER WIRE TO S.R.I. SQUARE - WAVE STIMULATOR
PORTAL VEIN ELECTRODE
(FIELD STIMULATING)
(X 2)
PERSPEX
SEAL
PLATINUM WIRE
WIRE HOOK FOR SECURING PORTAL VEIN
DETAIL ENLARGEMENT

Figure 1


COPPER WIRE
POSITIVE
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TO S.R.I.
SQUARE - WAVE
STIMULATOR
(X 2)
ATRIA ELECTRODE
(POINT STIMULATING)
HOLLOW PERSPEX TUBE
PAIR OF PUNCTATE
PLATINUM ELECTRODES
PERSPEX
BLOCK
WIRE HOOK
SCREW HEAD
DETAIL
ENLARGEMENT
TURNED THROUGH 90°

Figure 2

3: SURGICAL PROCEDURES

(i) Bilateral Ovariectomy

Female rats with normal oestrous cycles were ovariectomised under near sterile conditions. Surgical instruments were soaked in a solution of aqueous chlorhexidine gluconate in 70 % ethyl alcohol and rinsed in double distilled water prior to use.

Anaesthesia was induced with 3 % halothane and maintained with nitrous oxide (750 $ml.min^{-1}$) and oxygen (200 $ml.min^{-1}$) using Boyle's apparatus.

The mid-lateral area was shaved and washed with aqueous chlorhexidine gluconate and the abdomen was opened on each side with 2 small incisions. The fallopian tubes were ligated close to the ovaries and the ovaries were removed. The muscle wall was sutured using non-capillary braided thread (Arbralon, 6/6) and a round-bodied curved needle. The skin was clipped together with surgical clips (Michel's suture clips, 12 mm). The area was subsequently washed with aqueous chlorhexidine gluconate and sprayed with plastic dressing (Nobecutane, Parke-Davis) to prevent the entry of foreign particles into the wound.

Following surgery, animals were housed separately. At least 6 weeks elapsed between the time of the operation and the day in which the animals were pithed.

Successful removal of the ovaries was verified by post-mortem examination.

(ii) Sham Bilateral Ovariectomy

The procedure for bilateral ovariectomy was carried out with the exception that the ovaries were not removed, but returned to the abdominal cavity.

After 5 weeks, vaginal smears were examined daily until the animals were pithed. Only sham-operated rats with normal oestrous cycles were pithed and used as controls.

(iii) Hormone Pre-treatment by Injection

The vaginal smears of rats were examined daily (see page 33). Only those rats with normal oestrous cycle length and in dioestrus at 10 a.m. were selected for daily hormone injections.

The selected rat was weighed and the volume of drug to be injected was calculated. Both ethinyloestradiol and norethisterone acetate were prepared in polyethylene glycol (PEG) 300 and the concentration of solutions for injection were: ethinyloestradiol, 1 $mg.ml^{-1}$; norethisterone acetate, 81 $mg.ml^{-1}$.

The same hormone at the same dose was administered at 10.30 a.m. from day 1 to day 9 inclusive (day 1 = dioestrus). On day 10, the animal was sacrificed for examination of the responses of isolated cardiac or vascular tissue (see pages 38 - 42). Injections were made subcutaneously in the scruff of the neck from a Hamilton 25 µl microsyringe with a luer fitting and via a 25 guage hypodermic needle (Gillette Sabre).

All rats were housed individually and vaginal smears were examined daily from day 1 to day 10, inclusive. Prior to sacrifice, animals were weighed and at the end of each experiment, a gross post-mortem examination was carried out.

(iv) Implantation of Osmotic Minipumps

(For theory of operation, see page 50).

The vaginal smears of rats were examined daily (see page 33). Only those rats with normal oestrous cycle length and in dioestrus at 10 a.m. were selected for minipump implantation. The selected rat was weighed and the pump was filled with either 17β-oestradiol in PEG 300 (758 $\mu g.ml^{-1}$) or PEG 300 alone (control).

The pumps were implanted at 11 a.m. on day 1. Surgical instruments were soaked in a solution of aqueous chlorhexidine gluconate in 70 % ethyl alcohol and rinsed in double distilled water prior to use.

Anaesthesia was induced with 3 % halothane and maintained with nitrous oxide (750 $ml.min^{-1}$) and oxygen (200 $ml.min^{-1}$) using Boyle's apparatus.

The scapula region was shaved and washed with aqueous chlorhexidine gluconate and a 1 cm long incision was made. The skin was eased away from the underlying connective tissue to make a pocket for the minipump, then the filled minipump was inserted, delivery portal first. The wound was sutured using non-capillary braided thread and a round-bodied curved needle. The area was subsequently washed with aqueous chlorhexidine gluconate.

Following surgery, the animals were housed individually and vaginal smears were examined daily. On day 10, the rats were weighed and pithed (see page 35). At the end of an experiment, the pump was examined to determine its integrity. A gross post-mortem examination was also carried out, during which particular attention was taken to determine whether the pump had moved or whether the pump had induced

any local inflammatory reactions. The macroscopic appearance of the uterus was also examined.

CROSS SECTION OF FUNCTIONING MINIPUMP (X 5)

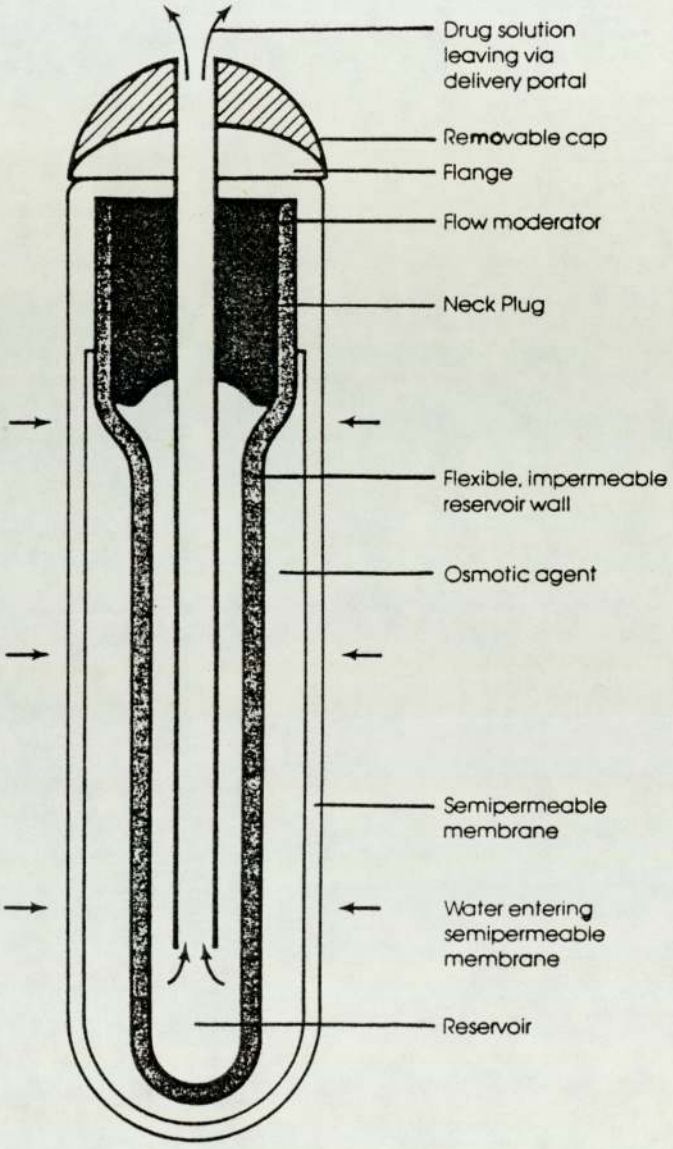


Figure 3

4: THE ALZET* OSMOTIC MINIPUMP

A limitation of conventional dosage forms is that they release drugs at time-varying rates (Struyker-Boudier & Smits, 1978). However, steady-state distribution of a drug can be achieved if it is administered from an Alzet* osmotic minipump which may deliver a variety of solutions at zero-order rates for periods varying from hours to several weeks (Theeuwes, 1975; Capozza, Eckenhoff & Yum, 1977; Theeuwes, 1981).

The minipump, which is illustrated in figure 3 (p. 49), consists of a cylindrical collapsible reservoir, surrounded by a sealed layer containing an osmotic agent which, in turn, is encapsulated by a semi-permeable membrane. When the filled minipump is placed in an aqueous environment, in vitro or in vivo, there is a net flux across the membrane into the compartment that contains the osmotic agent. The rate of flux is controlled by the water-permeable membrane; hydrostatic pressure is exerted on the collapsible lining of the reservoir and its contents are displaced through the delivery portal. The outside walls of the pump, including the membrane, are rigid and so, because of the incompressibility of water, the volume of water entering the system is equal to the volume of drug solution that is pumped out (Theeuwes & Yum, 1977).

During present experiments 17β-oestradiol was administered from Alzet* osmotic minipumps implanted subcutaneously. Butcher, Inskeep & Pope (1978) have recently demonstrated that plasma levels of 17β-oestradiol are constant when administered in this way. Since not all solvents are compatible with the interior of the Alzet* osmotic minipump (Alza Co. literature), 17β-oestradiol was administered in PEG 300.

The model used throughout this study was number 1702, which released at a rate of 0.55 $\mu l.hr^{-1}$ for 10 days. In order to have uninterupted release of a drug from an osmotic minipump, the drug solution must be free from air bubbles. In model 1702, a drug volume of less than 156 μl would indicate the presence of air bubbles. For this reason, the volume of drug in the filled minipump was estimated. If it was less than 156 μl, it would be emptied and refilled.

The unused minipumps were stored below $5^{o}C$ in a dessicator. Pumps were removed from the refridgerator 2 hours prior to use, in order to raise their temperatures to near body temperature.

* Alzet is a registered trademark of the Alza Corporation, Palo Alto, California 94304 U.S.A.

5: RECORDING METHODS

(i) Pithed Rat Preparation

Blood pressure was measured by means of a blood pressure transducer (Bell & Howell, type 4/422/0001) connected to a Lectromed MX212 recorder, calibrated to give a full scale deflection of 100 mm Hg pressure. In some experiments, heart rate was measured by means of a Devices Instantaneous Ratemeter (type 2751) triggered from the blood pressure signal. During other experiments, in which an ECG was recorded, heart rate was measured by means of a Devices Instantaneous Ratemeter (type 2751) triggered from the ECG signal. The heart rate recordings were displayed on a Lectromed MX212 recorder.

(ii) Isolated Organ Preparations

Isometric contractions were measured by means of a transducer (Pi-Odin, type UF 1, 50 g sensitivity range) connected to a Lectromed MX412 or MX212 recorder.

In experiments on the portal vein, the force recorded was integrated by a Lectromed Integrator (type 3630) in order to reduce irregularities in recording due to spontaneous mechanical activity.

In experiments on isolated atria, the rate of atrial beating was measured by means of a Devices Instantaneous Ratemeter (type 2751) or a Lectromed Ratemeter (type 4522 with trigger adjustment) triggered by the signal from the force recording. In order to record the rate of change of force, the force signal was differentiated by a Lectromed Differentiator (type 3642).

6: UNITS OF MEASUREMENT

The Système international d'Unités (SI) has been adopted as recommended by the thirtieth World Health Assembly (WHA). In accordance with the WHA, however, arterial pressure has been recorded in mm Hg and not in the SI unit of the kilopascal. The committee is of the opinion that, because of its universal acceptance, the millimetre of mercury (mm Hg) is a more appropriate unit of measurement.

7: EXPRESSION OF RESULTS

Weight

The weight of animals was recorded to the nearest 10 g.

(i) Pithed Rat Preparation

(a) Blood Pressure

Blood pressure recordings were made following both atropine sulphate and D-tubocurarine administration. An indication of mean arterial pressure was calculated as diastolic pressure plus one third of the pulse pressure, which, in turn, was taken to be the difference between systolic and diastolic pressures. Pressor responses to noradrenaline, angiotensin II, vasopressin, oxytocin and electrical stimulation were recorded as the maximal sustained systolic pressure. In order to determine the influence of the resting pressure upon the maximal sustained systolic pressure response, the increase in systolic pressure was also recorded.

Since diastolic pressure could be measured more accurately than systolic pressure during responses to isoprenaline, depressor responses to isoprenaline were recorded as the minimal sustained diastolic pressure. Also, in order to determine the influence of the resting blood pressure upon the minimal sustained diastolic pressure, the decrease in diastolic pressure was also recorded.

Two concentration-response curves to each agent or three stimulus-response curves to electrical stimulation were constructed during an experiment. The mean values for each parameter recorded during an experiment were then calculated.

(b) Heart Rate

Basal heart rate was recorded following both atropine sulphate and D-tubocurarine administration. Following stimulation, the maximal heart rate was recorded, together with the increase in rate induced by the stimulation. The mean values for these measures were then calculated.

(c) ECG

Figure 22 (p. 109) shows a typical ECG trace. The PQ interval was measured as the time taken to pass from the beginning of the P wave to the beginning of the Q wave. In cases where the Q wave was not sufficiently distinct, the PR interval was recorded instead. This was taken to be the atrial conduction time. The QT interval was measured from the beginning of the Q wave to the end of the T wave and was taken to be the ventricular contraction period (Guyton, 1981). During an experiment, an ECG was run prior to, and during, administration of agents in the first concentration-response curves. Two traces were chosen for each concentration and the mean values for the PQ and QT intervals were calculated.

(ii) Isolated Aortic Strip

Figures 49 and 54 (pp. 177 and 185) show typical aortic strip responses to noradrenaline and angiotensin II. The sustained increase in force above basal (set) force was recorded, together with the initial increase in force, recorded 20 seconds after administration of the agent. Three concentration-response curves to either noradrenaline

or angiotensin II were constructed during an experiment and the mean values for these measures of contraction were recorded at each concentration of agonist.

(iii) Isolated Portal Vein

Force of contraction of the portal vein was integrated automatically with a resistance-capacitor operational amplifier. The output of the integrator was balanced so that the resting force exerted by the preparation did not affect the integral. The capacitor was automatically discharged every 20 seconds. Responses were measured as the total integrator peak height recorded for 2 minutes after stimulation. Two concentration-response curves were constructed to each agonist and to electrical stimulation and the mean values for the responses were recorded.

(iv) Isolated Paired Atria

Basal measurements were made of the force (f), together with the rate of change of force (df/dt) and the rate of beating (r)(see figure 63, p. 210). Following administration of noradrenaline or isoprenaline, the increase in 'systolic' force was recorded (c), together with the rate of change of force (df/dt) and the sustained rate of beating (r). Three concentration-response curves were constructed for each agonist and the mean values for these measures of contraction were recorded at each concentration of agonist.

8: EVALUATION OF RESULTS

The effect of alteration in the plasma concentration of ovarian hormonal agents was determined, wherever possible, in two ways. Comparisons were made of responses at standard concentrations of vasoactive agent. Also, the ratio of the mean of the concentrations of vasoactive agent required to elicit a standard response was calculated. Where maximal responses were obtained, the $E.C._{50}$ was taken to be the standard response. Pre-treatment of rats with ovarian hormonal agents may alter the contractile mechanism and, therefore, the maximal contractile response of cardiovascular tissue. The $E.C._{50}$ was therefore calculated as the concentration of vaso-active agent required to elicit 50 % of the maximal contractile response of each individual tissue. Although $E.C._{50}$ values would ideally be quoted for all preparations, where maximal responses were not obtained, this was not possible. Where maximal responses were not obtained, therefore, the concentration-ratio was calculated at a point on the linear portion of the concentration-response curve, approximating to 50 % of the maximal response. When expressing differences in sensitivity to vasoactive agents in the whole animal by means of a shift along the horizontal axis, only the change in blood pressure or heart rate were examined. A left shift in the concentration-response curve to an agent was taken to indicate an increase in the potency of the agent. Conversely, a right shift was taken to indicate a decrease in the potency of the agent. When an individual isolated preparation did not produce the 'standard' response, the concentration-response curve was extended to give an estimation of the concentration required to elicit the standard response.

When comparing responses to, or concentrations of, vaso-active agents, results were characterised by a mean$\pm$standard error of the mean (s.e. mean) and analysed by Student's 't' test (2 tailed). Probability (P) of < 0.05 was taken as sufficient to confirm significant difference.

Where X is each individual observation,

$\bar{X}$ is the mean value and

N is the number of observations;

$$\text{s.e. mean} = \sqrt{\frac{\sum(X - \bar{X})^2}{N - 1}} \times \frac{1}{\sqrt{N - 1}}$$

't' between $\bar{X}_1 \pm$ s.e. mean_1 and $\bar{X}_2 \pm$ s.e. mean_2 was calculated as follows:

$$t = \frac{\bar{X}_1 - \bar{X}_2}{\text{s.e. } (1 - 2)}$$

Where s.e. (1-2) is the standard error of the difference between the means. Where the groups 1 of 2 are of equal size:

$$t = \frac{X_1 - X_2}{\sqrt{\text{s.e. mean}_1^2 + \text{s.e. mean}_2^2}}$$

The Mann-Whitney Test

Where results are normally-distributed, the Student's 't' test is a suitable test of significance. However, when there is some reason to believe that the results may not be normally-distributed, the Mann-Whitney test for small samples is a more suitable test of significance. For this reason, the effect of 17β-oestradiol administration upon pressor responses to electrical stimulation and the effect of ethinyloestradiol and norethisterone acetate administration upon the inotropic responses of isolated atria to noradrenaline were anaylsed using the Mann-Whitney test (2 tailed). Probability (P) of <0.05 was taken as sufficient to confirm significant difference.

Notes

n_1 is the number of scores in group 1.

n_2 is the number of scores in group 2.

$n_1 \leqslant n_2$.

Procedure

1. Rank all scores, irrespective of group, from 1 to $n_1 + n_2$.
2. Sum the ranks (R) of group 1.
3. Calculate R' such that $R' = n_1 (n_1 + n_2 + 1) - R$.
4. Consult the Mann-Whitney table, using the smaller of the values, R and R'.

9: DRUGS AND CHEMICALS

All salts were of an analytical grade of purity and supplied by BDH. All drugs were stored on ice for the duration of an experiment. Commercial drug preparations are listed below according to the supplier. Registered trade names are shown in parentheses.

BDH

Atropine sulphate:	Supplied as a solid. Prepared as a sterile solution (1 $mg.ml^{-1}$) in double distilled water and frozen in 1 ml ampules.

British Oxygen Corporation

Nitrous oxide,

Oxygen.

Calmic Medical Devision/The Wellcome Foundation

D-tubocurarine: (Tubarine, miscible)	Supplied as a solution in sealed ampules. Stored below 25°C and protected from light.

Ciba

$Asp(NH_2)^1$-Val^5-Angiotensin II: (Hypertensin)	Supplied as an 83.4 % pure freeze-dried solid. Prepared as a sterile solution (10^{-6} M) in double distilled water and frozen in 1 ml ampules.

Duncan, Flockhart and Co. Ltd.

Heparin sodium (injection) (with chlorcresol, 0.15 $g.100\ ml^{-1}$, as preservative)	Supplied as a solution. Stored below 25°C.

I.C.I.	
Chlorhexidine gluconate: (Hibitane)	Supplied as a 5 % aqueous solution. Prepared in double distilled water prior to use.
Halothane: (Fluothane)	Stored in a refridgerator at 0-5°C to protect it from heat and light.
May & Baker	
Sodium pentobarbitone: (Sagital, Vetinary)	Supplied as a solution (60 $mg.ml^{-1}$) and stored protected from light.
Parke-Davis	
Oxytocin: (synthetic, with chlorbutol, 0.5 $g.100\ ml^{-1}$, as preservative) (Pitocin)	Supplied as a solution. Stored at 0-5°C and prepared immediately prior to use.
Sigma	
(17α) Ethinyloestradiol: (17αEthynyl Estradiol)	Supplied as a solid. Prepared in PEG 300 (1 $mg.ml^{-1}$) and stored frozen.
Isoprenaline bitartrate (L(-)-Isoproterenol D Bitartrate)	Supplied as a solid. Prepared as a sterile solution (1 $mg.ml^{-1}$) in double distilled water with ascorbic acid ($\leq 10^{-4}$ M)(pH~6.7) to prevent oxidation, and stored frozen.
Noradrenaline bitartrate (L-Arterenol Bitartrate)	Supplied as a solid. Prepared as a sterile solution (1 $mg.ml^{-1}$) in double distilled water with ascorbic acid ($\leq 10^{-4}$ M)(pH~6.7) to prevent oxidation, and stored frozen.

Sigma

Norethisterone acetate: (Norethindrone Acetate)	Supplied as a solid. Prepared in PEG 300 (81 $mg.ml^{-1}$) and stored frozen.
Polyethylene glycol (PEG) 300:	Stored frozen for control experiments.
17β-Oestradiol: (β-Estradiol)	Supplied as a solid. Solutions in PEG 300 (758 $\mu g.ml^{-1}$) were prepared prior to filling of minipumps for implantation.
Arginine Vasopressin: (synthetic)	Supplied as a solution. Prepared as a stock solution in double distilled water (5 $iu.ml^{-1}$) and stored at 0-5°C in 1 ml ampules.

Where drugs are referred to by weight, this weight is given in terms of the salt.

C H A P T E R 1.

THE EFFECT OF CHANGES IN THE PLASMA CONCENTRATION OF OVARIAN HORMONES UPON CARDIOVASCULAR RESPONSES TO NORADRENALINE, ANGIOTENSIN II, VASOPRESSIN, OXYTOCIN, ISOPRENALINE AND ELECTRICAL STIMULATION IN THE PITHED FEMALE RAT.

Chapter 1

General Introduction

The first evidence to suggest that pressor responses may be modified by changes in the plasma concentration of ovarian hormones was provided in 1938 by Byrom who demonstrated that administration of the oestrogenic agent, oestradiol benzoate, potentiated the renal-damaging effect of very high doses of vasopressin. Since Byrom (1938) believed that such vaso-renal lesions were the result of excessive vasoconstriction, he proposed that oestrogenic agents potentiate vascular spasms induced by vasopressin. Lloyd and co-workers (Lloyd, 1959a, b; Lloyd & Pickford, 1961; Hettiaratchi & Pickford, 1968) have carried out a series of experiments which demonstrate that changes in the plasma concentration of ovarian hormones may alter pressor responses to vasopressin, oxytocin and angiotensin II in anaesthetised rats. In order to extend the studies of Lloyd and co-workers (Lloyd, 1959a, b; Lloyd & Pickford, 1961; Hettiaratchi & Pickford, 1968), the effect of changes in the plasma concentration of ovarian hormones upon cardiovascular responsiveness was examined in female rats pithed by the method of Shipley & Tilden (1947).

It was useful to use a pithed animal since this preparation gives information on integrated responses of the cardiovascular system without the large, nervously mediated, reflex changes which accompany administration of pressor and depressor agents in conscious and anaesthetised animals (Price, 1960; Gillespie & Muir, 1967b). Removal of the nervous innervation of blood vessels by pithing also reduces vascular smooth muscle tone (Gillespie & Muir, 1967b).

Pithing therefore made possible an examination of the direct effect of pressor and depressor agents upon the cardiovascular system.

Two methods of recording pressor responses have been presented. The maximal sustained systolic blood pressure has been recorded to indicate the extent to which the cardiovascular system can respond to pressor agents. The increase (incremental rise) in systolic pressure has also been recorded to determine the influence of changes in systolic pressure prior to stimulation upon the magnitude of the stimulus-evoked response. Similarly, depressor responses to isoprenaline have been recorded both as the minimal sustained diastolic blood pressure and also as the decrease in diastolic pressure. Positive chronotropic responses have been recorded both as the maximal sustained heart rate and also as the increase in heart rate.

The influence of ovarian hormones upon blood pressure responses and positive chronotropic responses has been recorded in two ways. The responses of pithed female rats have been compared at standard stimulus strength. Also, the ratio of the stimulus strengths required to elicit a standard change in blood pressure or heart rate was calculated, where the standard response lay on the linear portion of the stimulation-response curve. Ideally, the stimulation (or concentration)-ratio would have been recorded at the $E.C._{50}$. Unfortunately, insufficient data was available to determine the $E.C._{50}$s. Consequently, the standard response was recorded as a point on the stimulation-response curve believed to approximate to the $E.C._{50}$. A left shift in the stimulation-response curve was taken to indicate an increase in potency of the agent and a right shift was taken to indicate a decrease in agent potency.

The following experiments are presented in chronological order.

Experiment 1

Effect of different housing conditions upon the oestrous cycle lengths and body weights of rats

Introduction

Initial experiments were undertaken to determine whether changes in the plasma concentration of ovarian hormones during the oestrous cycle alters the responses to several pressor agents in pithed female rats. Since any conclusions drawn from the results of these experiments are dependent upon reliable oestrous cycles, a separate study was undertaken to assess the dependability of the length of oestrous cycles in experimental animals. Since some experiments were performed using rats which were housed separately, while other experiments were performed using rats which were housed together in groups of six, this study was also undertaken to determine whether the oestrous cycle lengths and body weights were different in rats housed under these different conditions.

Results

Table 1 (p. 67) records the oestrous cycle lengths of rats housed separately and together (6 per cage) over the 80 day study period. The oestrous cycle length of rats housed separately (4.0 ± 0.1 days, n=6) was not significantly different to that of rats held together (3.7 ± 0.1 days, n=6)($P > 0.05$).

Figure 4 (p. 68) shows the mean body weights, recorded over 80 days, of rats housed separately and together in a group. At no time was the difference between the groups significant ($P>0.1$ throughout).

TABLE 1 - Effect of different housing conditions upon the oestrous cycle lengths of rats.

Housing	Cycle length (days)	P
Separate	4.0 ± 0.1 (6)	0.05-0.1
Together	3.7 ± 0.1 (6)	i.e. n.s.

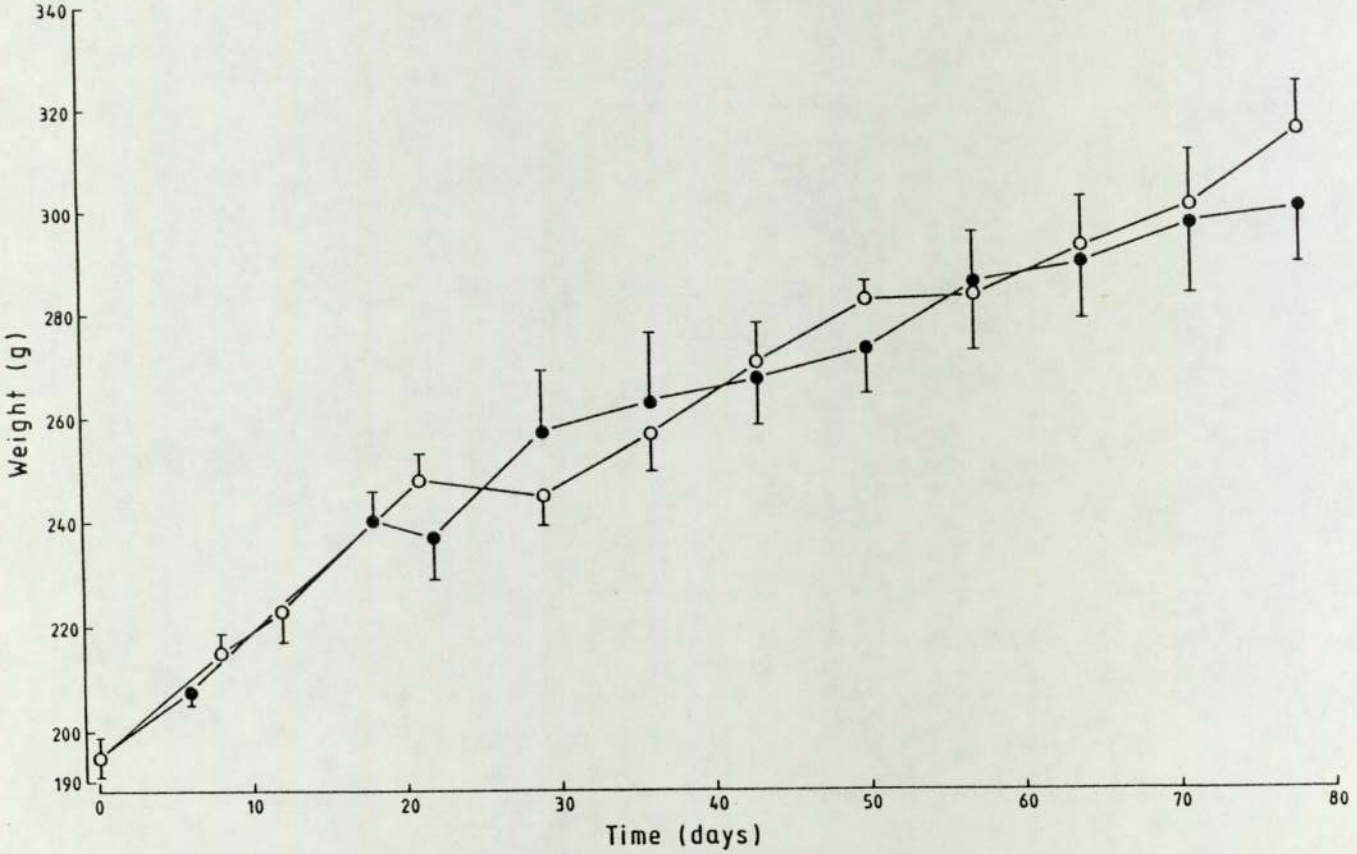
Number of observations in parentheses.

Results are mean $\pm$ s.e. mean.

n.s. - not significant.

Figure 4

Body weights of female rats housed separately (o) and together (•) from the time of the start of the study. Each point represents the mean, and vertical bars denote the s.e. mean (n=6).


340
320
300
280
260
240
220
200
190
Weight (g)
0
10
20
30
40
50
60
70
80
Time (days)

Discussion

All rats in this study had oestrous cycles of approximately four days in length. Yoshinaga, Hawkins & Stocker (1969) have shown that, in rats with a similar cycle length of approximately four days, plasma progesterone concentrations do not vary significantly between the oestrus and dioestrus stages of the cycle. However, although the plasma concentration of 17β-oestradiol was shown to be very low at dioestrus, it was shown to increase just prior to ovulation (oestrus) and this increase appeared to be responsible for vaginal cornification observed at oestrus (Yoshinaga et al., 1969). Responses to pressor agents were examined at oestrus and at dioestrus. Any differences in the responses of oestrous rats compared with those of dioestrous rats may therefore be due to the increase in the plasma concentration of 17β-oestradiol just prior to ovulation.

Experiment 2

Effect of the stage of the oestrous cycle upon cardiovascular responses to noradrenaline, angiotensin II, vasopressin and oxytocin

Introduction

Lloyd (1959a) examined pressor responses to vasopressin and to oxytocin in anaesthetised oestrous and dioestrous rats. Similarly, Hettiaratchi & Pickford (1968) compared pressor responses to angiotensin II in anaesthetised oestrous and dioestrous rats. To extend these studies, therefore, pressor responses to vasopressin, oxytocin and angiotensin II were examined in pithed oestrous and dioestrous rats. Since noradrenaline is an endogenous vasoconstrictor agent which is involved in the regulation of arterial pressure (Su, 1977; Vanhoutte, 1978a), pressor responses to noradrenaline were also examined.

Results

Oestrous and dioestrous rats were pithed and the resting blood pressure and heart rate were recorded. Table 2 (p. 74) shows the body weight, blood pressure and heart rate of pithed rats in oestrus and dioestrus. It can be seen that the systolic pressure in oestrous rats was significantly higher (74.4 ± 2.0 mm Hg, n=7) compared with the systolic pressure in dioestrous rats (61.3 ± 3.8 mm Hg, n=7)($P<0.02$). Resting diastolic pressure, pulse pressure and heart rate and body weight were not significantly different in oestrous, compared with dioestrous, rats ($P>0.05$ throughout).

Each rat was then injected intravenously with noradrenaline (25-200 $ng.kg^{-1}$), angiotensin II (6.25-50 $ng.kg^{-1}$), vasopressin (0.00125-0.01 $iu.kg^{-1}$) and oxytocin (0.2-0.8 $iu.kg^{-1}$) and the pressor responses and positive chronotropic responses were recorded. Figures 5, 6, 7 and 8 (pp. 76 and 77) show typical pressor responses to, respectively; noradrenaline, angiotensin II, vasopressin and oxytocin. Pressor responses were recorded both as the maximal sustained systolic pressure and also as the increase in systolic pressure. Figure 5 (p. 76) shows how these responses were measured. Positive chronotropic responses were recorded both as the maximal sustained heart rate and also as the increase in heart rate.

Figure 9 (p. 78) shows the maximal sustained systolic pressure induced by geometrically increasing concentrations of intravenously administered noradrenaline in oestrous and dioestrous rats. Similarly, figures 11 (p. 79), 13 (p. 80) and 15 (p. 81) show the maximal sustained systolic pressure induced by geometrically increasing concentrations of intravenously administered angiotensin II, vasopressin and oxytocin in oestrous and dioestrous rats.

The maximal sustained systolic pressure of oestrous rats was significantly higher compared with that of dioestrous rats at the two lowest concentrations of noradrenaline used (25 and 50 $ng.kg^{-1}$) ($P<0.025$). Differences in the maximal sustained systolic pressure between oestrous and dioestrous rats were not significant at any concentration of angiotensin II or oxytocin used ($P>0.05$ throughout). At all concentrations of vasopressin, however, the maximal sustained systolic pressure was significantly higher in oestrous rats compared with dioestrous rats ($P<0.05$ throughout).

Figure 10 (p. 78) shows pressor responses, expressed as the increase in systolic pressure, induced by geometrically increasing concentrations of intravenously administered noradrenaline in oestrous and dioestrous rats. At all but one concentration of noradrenaline ($50\ ng.kg^{-1}$), pressor responses in oestrous and dioestrous rats were not significantly different when responses were expressed as the increase in systolic pressure ($P>0.05$ throughout). Similarly, figures 12 (p. 79), 14 (p. 80) and 16 (p. 81) show pressor responses, expressed as the increase in systolic pressure, induced by geometrically increasing concentrations of intravenously administered angiotensin II, vasopressin and oxytocin in oestrous and dioestrous rats. Differences in pressor responses between oestrous and dioestrous rats were not significant at any concentration of angiotensin II or oxytocin used when responses were expressed as the increase in systolic pressure ($P>0.2$ throughout). When expressed as the increase in systolic pressure, pressor responses were significantly higher in oestrous rats compared with dioestrous rats at the two highest concentrations of vasopressin used (0.005 and $0.01\ iu.kg^{-1}$)($P<0.05$).

Since an increase in systolic pressure of 30 mm Hg lay on the linear portion of each concentration-response curve (see figures 10, 12, 14 and 16), the concentration of each agent required to elicit a rise of 30 mm Hg was compared in oestrous and dioestrous rats. These concentrations and concentration-ratios are shown on table 3 (p. 75). Oestrous rats required a significantly lower concentration of vasopressin compared with dioestrous rats to elicit this standard pressor response ($P<0.05$). Oestrous and dioestrous rats required similar concentrations of noradrenaline, angiotensin II and oxytocin to elicit this same pressor response ($P>0.05$ throughout).

When positive chronotropic responses were expressed either as the maximal sustained heart rate or as the increase in heart rate, differences between responses of oestrous and dioestrous rats were not significant at any concentration of noradrenaline, angiotensin II, vasopressin or oxytocin used ($P > 0.05$ throughout).

Positive chronotropic responses to angiotensin II, vasopressin and oxytocin lay in the threshold region of the concentration-response curve and so horizontal shifts could be measured. Positive chronotropic responses to noradrenaline in oestrous rats appeared to lie in the threshold region and at the start of the linear portion of the concentration-response curve (see figure 17, p. 82). Although the horizontal shift could not be measured from figure 17, it appears that oestrous rats may have required a lower concentration of noradrenaline to elicit the same increase in heart rate compared with that required by control rats.

TABLE 2 - Recordings of body weight, blood pressure and heart rate in pithed oestrous and dioestrous rats following atropine sulphate administration.

Parameter	Oestrus (7)	Dioestrus (7)	P
Body weight (g)	224.0±5.6	209.0± 9.7	0.20-0.30
Systolic b.p. (mm Hg)	74.4±2.0	61.3± 3.8	0.01-0.02*
Diastolic b.p. (mm Hg)	62.4±2.6	54.1± 3.8	0.05-0.10
Pulse pressure (mm Hg)	12.0±1.7	9.0± 1.7	0.20-0.30
M.A.P. (mm Hg)	66.3±2.3	57.1± 3.8	0.05-0.10
Heart rate (min^{-1})	276.0±8.6	254.0±17.0	0.20-0.30

Number of observations in parentheses.

Results are mean±s.e. mean.

* Indicates statistical significant difference.

b.p. - Blood pressure.

M.A.P. - Mean arterial pressure.

TABLE 3 - Concentration of agents required to elicit an increase in systolic blood pressure of 30 mm Hg in pithed oestrous and dioestrous rats.

Agent	Oestrus (7)	Dioestrus (7)	P	Concn-ratio
NA ($ng.kg^{-1}$)	73.57 ± 17.47	139.50 ± 32.03	0.05 -0.1	1.89
A II ($ng.kg^{-1}$)	20.75 ± 4.69	45.71 ± 15.80	0.1 -0.2	2.20
VP ($iu.kg^{-1}$)	0.0045 ± 0.0004	0.0072 ± 0.0010	0.025-0.05*	1.60
OXY ($iu.kg^{-1}$)	0.51 ± 0.06	0.36 ± 0.07	0.1 -0.2	1.42

Number of observations in parentheses.

Results are mean $\pm$ s.e mean.

* Indicates statistical significant difference.

Conc-ratio is the ratio of the mean concentrations in oestrous and dioestrous rats.

NA - Noradrenaline.

A II - Angiotensin II.

VP - Vasopressin.

OXY - Oxytocin.

Figure 5

A typical trace of pressor responses to intravenously administered noradrenaline in the pithed female rat.

1 = 25 $ng.kg^{-1}$
2 = 50 $ng.kg^{-1}$
3 = 100 $ng.kg^{-1}$
4 = 200 $ng.kg^{-1}$

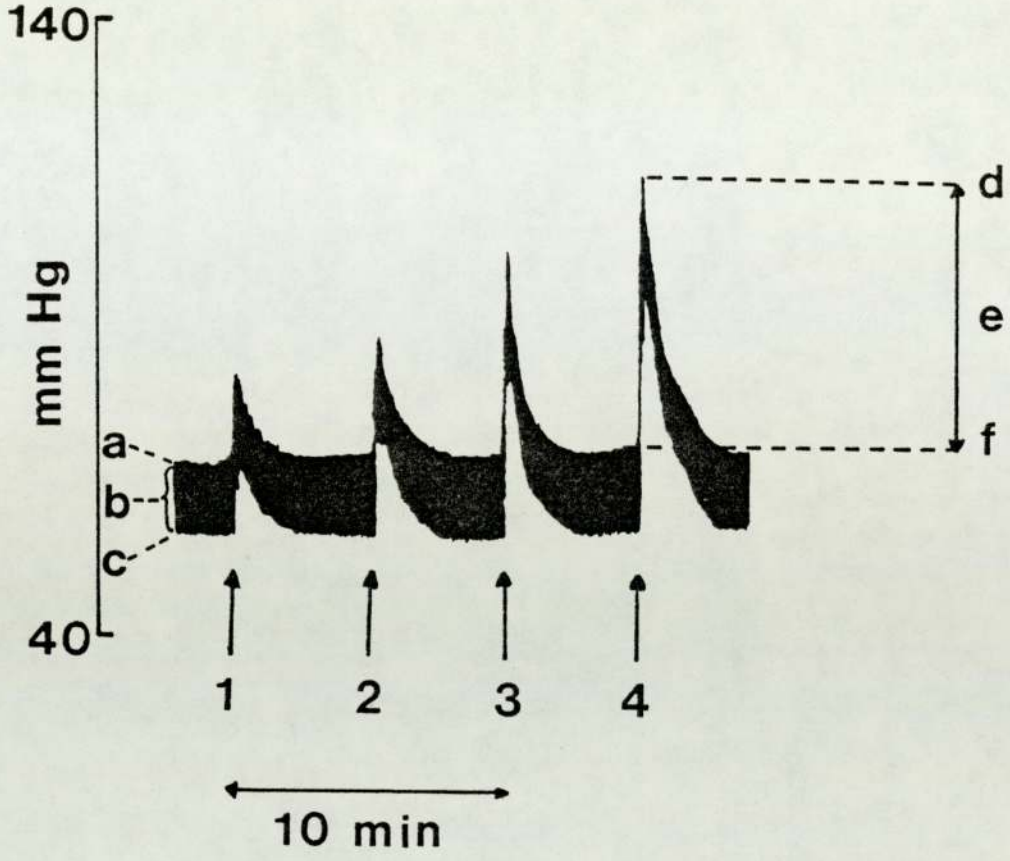
a = Resting systolic pressure.
b = Resting pulse pressure. b = a-c
c = Resting diastolic pressure.

d = Maximal sustained systolic pressure.
e = Increase in systolic pressure. e = d-f
f = Systolic pressure prior to stimulation.

Figure 6

A typical trace of pressor responses to intravenously administered angiotensin II in the pithed female rat.

1 = 6.25 $ng.kg^{-1}$
2 = 12.5 $ng.kg^{-1}$
3 = 25.0 $ng.kg^{-1}$
4 = 50.0 $ng.kg^{-1}$


140
mm Hg
40
a
b
c
d
e
f
1
2
3
4
10 min

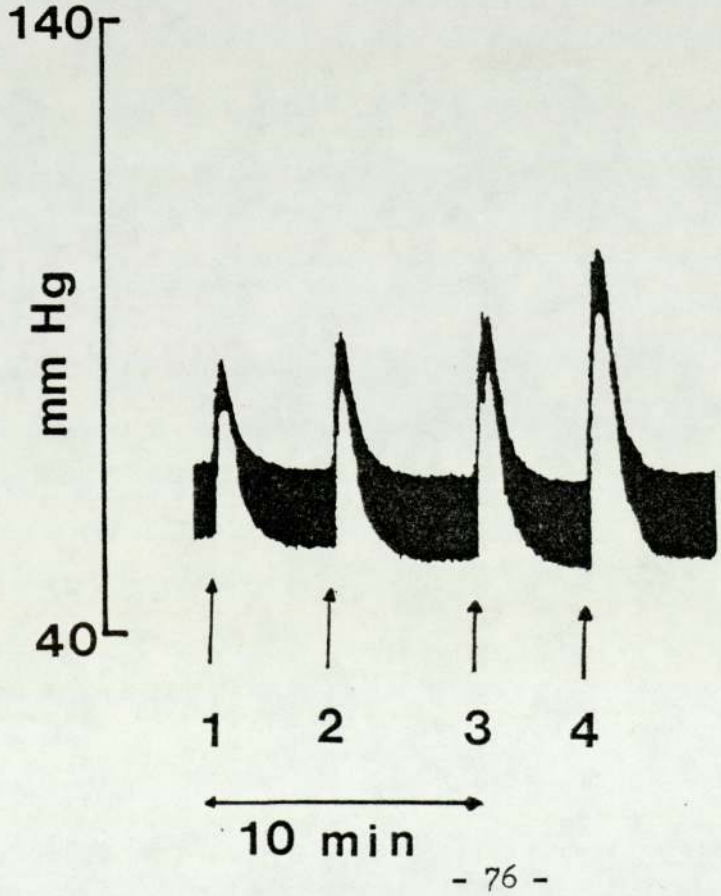

140
mm Hg
40
1
2
3
4
10 min

Figure 7

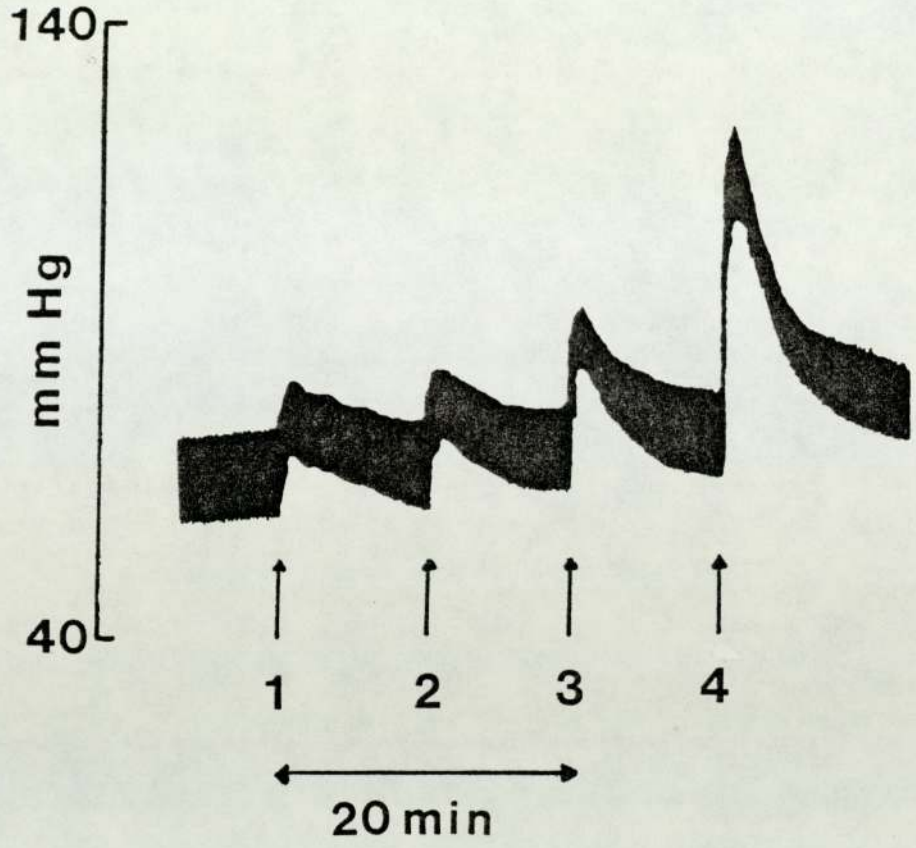
A typical trace of pressor responses to intravenously administered vasopressin in the pithed female rat.

1 = 0.00125 $iu.kg^{-1}$
2 = 0.0025 $iu.kg^{-1}$
3 = 0.005 $iu.kg^{-1}$
4 = 0.01 $iu.kg^{-1}$

Figure 8

A typical trace of pressor responses to intravenously administered oxytocin in the pithed female rat.

1 = 0.2 $iu.kg^{-1}$
2 = 0.4 $iu.kg^{-1}$
3 = 0.8 $iu.kg^{-1}$


140
mm Hg
40
1
2
3
4
20 min

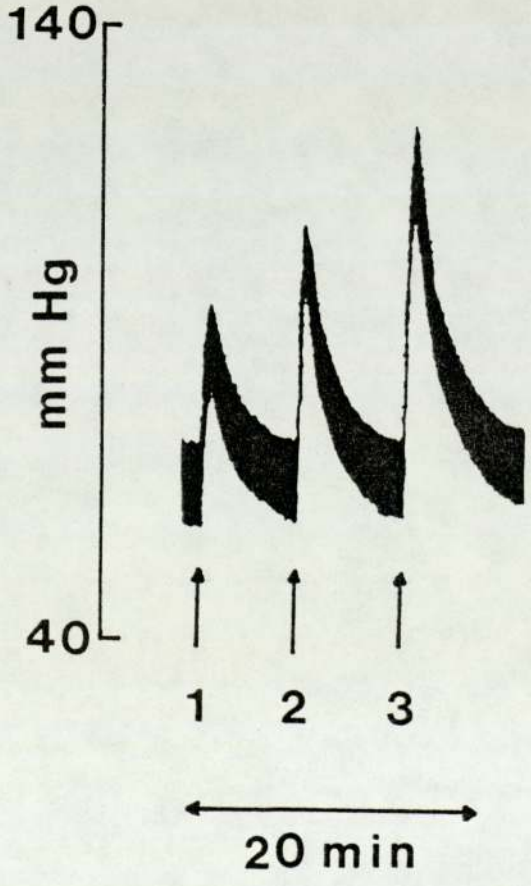

140
mm Hg
40
1
2
3
20 min

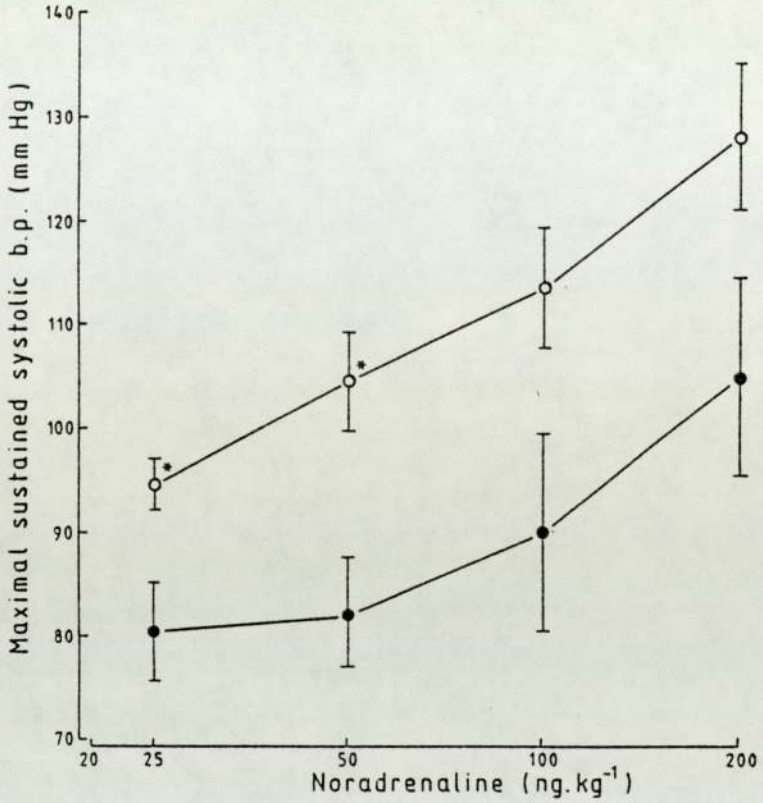
Figure 9

Maximal sustained systolic blood pressure induced by intravenously administered noradrenaline in pithed oestrous (o) and dioestrous (•) rats. Each point represents the mean, and vertical bars denote the s.e. mean (n=7).

* Indicates statistical significant difference.

Figure 10

Increase in systolic blood pressure induced by intravenously administered noradrenaline in pithed oestrous (o) and dioestrous (•) rats. Each point represents the mean, and vertical bars denote the s.e. mean (n=7).


Maximal sustained systolic b.p. (mm Hg)
140
130
120
110
100
90
80
70
20
25
50
100
200
Noradrenaline (ng.kg⁻¹)

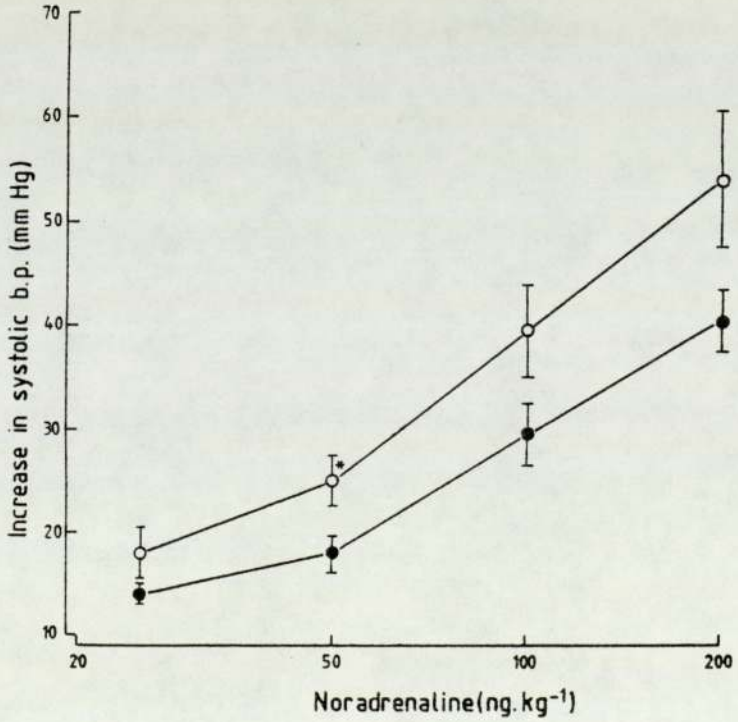
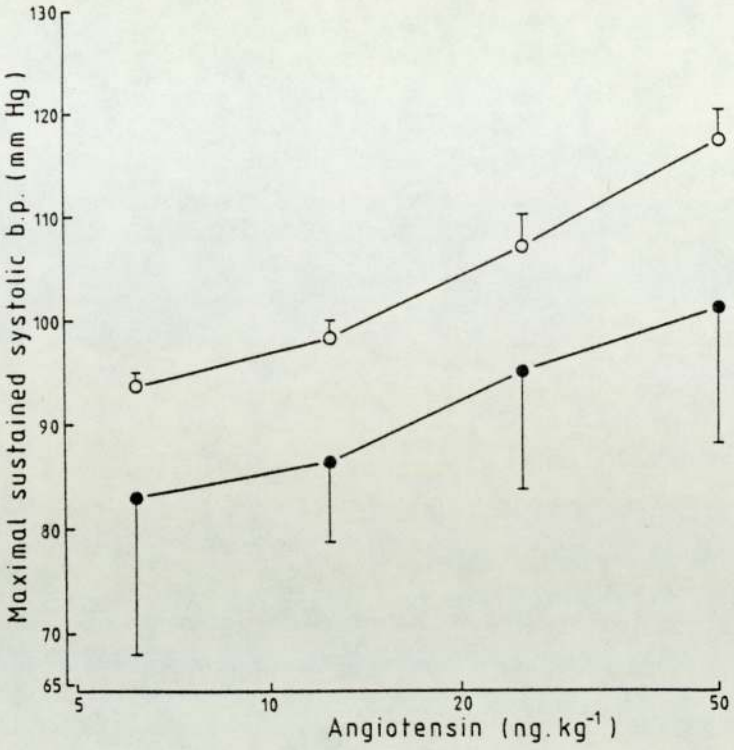

Increase in systolic b.p. (mm Hg)
70
60
50
40
30
20
10
20
50
100
200
Noradrenaline(ng.kg⁻¹)

Figure 11

Maximal sustained systolic blood pressure induced by intravenously administered angiotensin II in pithed oestrous (o) and dioestrous (•) rats. Each point represents the mean, and vertical bars denote the s.e. mean (n=7).

Figure 12

Increase in systolic blood pressure induced by intravenously administered angiotensin II in pithed oestrous (o) and dioestrous (•) rats. Each point represents the mean, and vertical bars denote the s.e. mean (n=7).


Maximal sustained systolic b.p. (mm Hg)
130
120
110
100
90
80
70
65
5
10
20
50
Angiotensin (ng.kg^{-1})

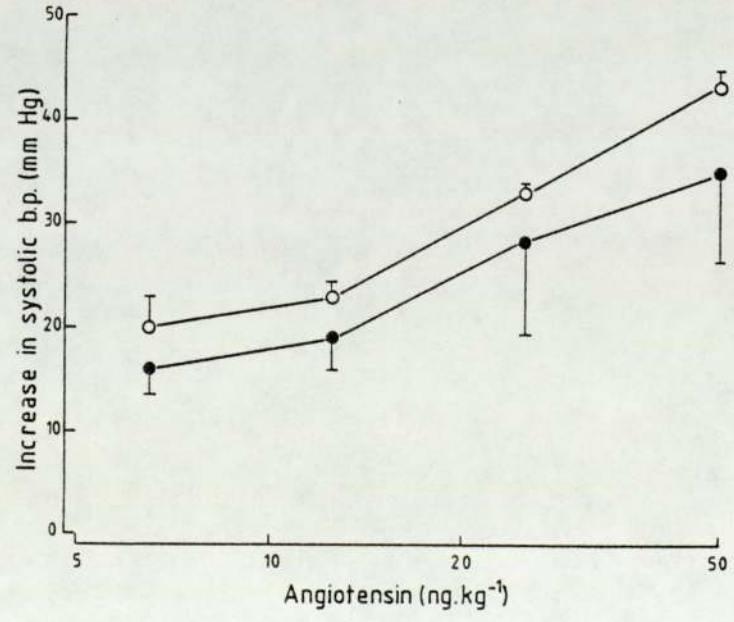

Increase in systolic b.p. (mm Hg)
50
40
30
20
10
0
5
10
20
50
Angiotensin (ng.kg^{-1})

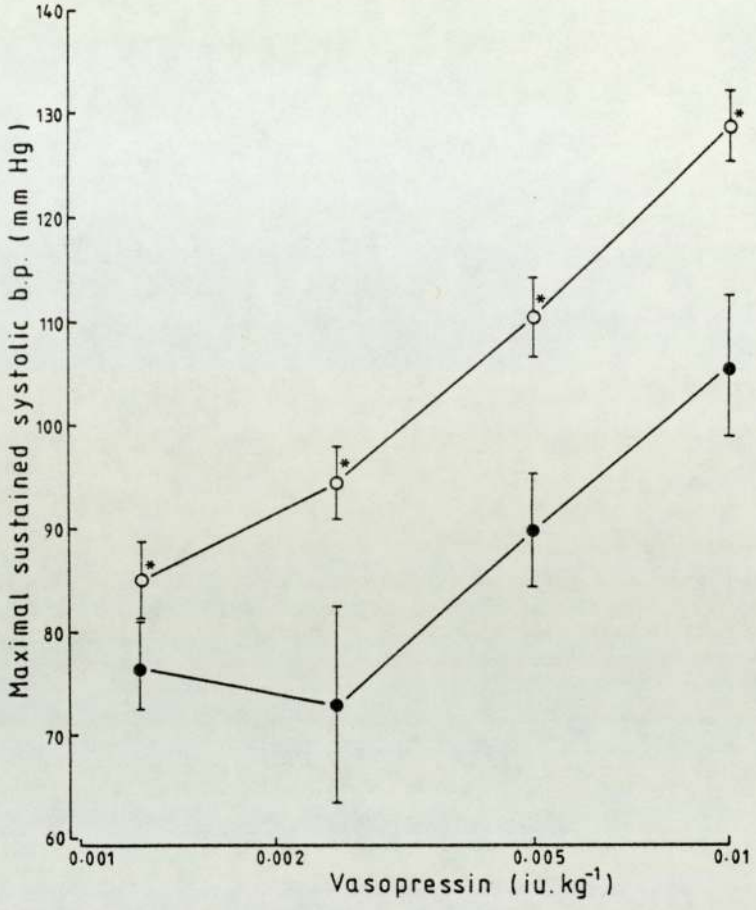
Figure 13

Maximal sustained systolic blood pressure induced by intravenously administered vasopressin in pithed oestrous (o) and dioestrous (•) rats. Each point represents the mean, and vertical bars denote the s.e. mean (n=7).

* Indicates statistical significant difference.

Figure 14

Increase in systolic blood pressure induced by intravenously administered vasopressin in pithed oestrous (o) and dioestrous (•) rats. Each point represents the mean, and vertical bars denote the s.e. mean (n=7).


Maximal sustained systolic b.p. (mm Hg)
140
130
120
110
100
90
80
70
60
0·001
0·002
0·005
0·01
Vasopressin (iu.kg⁻¹)

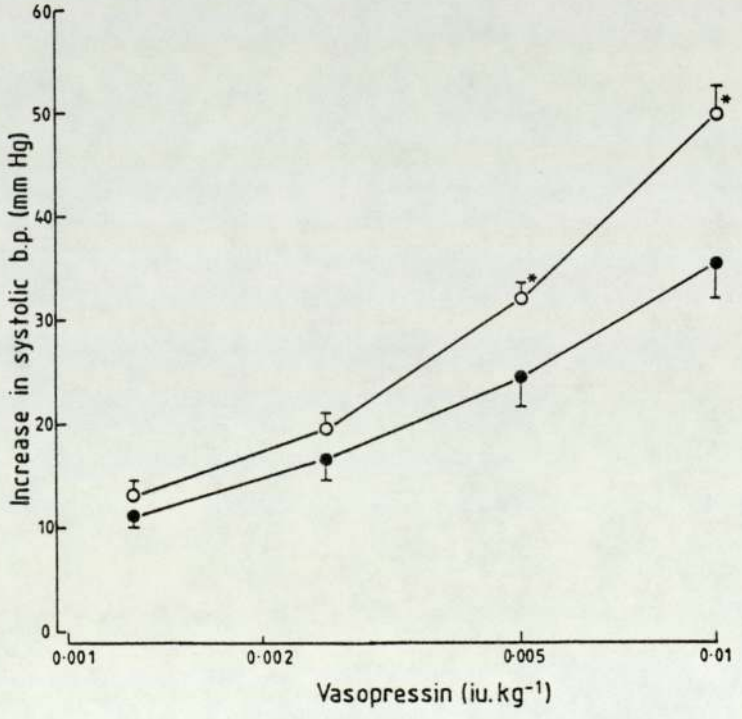
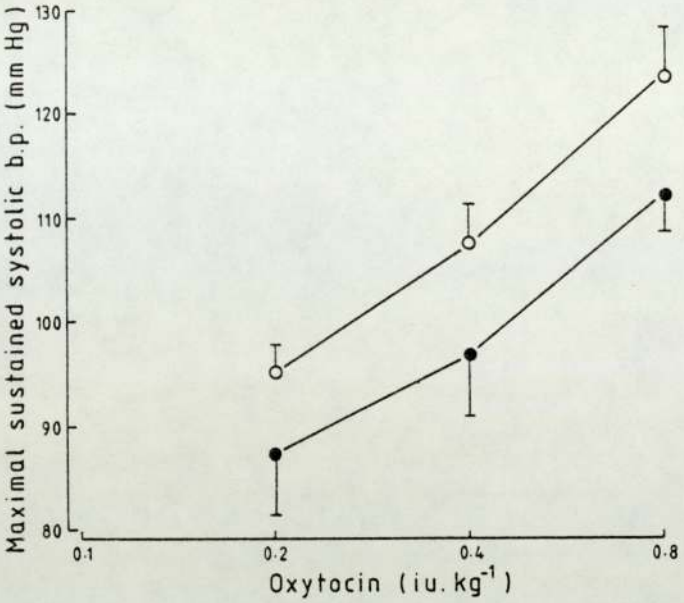

Increase in systolic b.p. (mm Hg)
60
50
40
30
20
10
0
0·001
0·002
0·005
0·01
Vasopressin (iu.kg⁻¹)

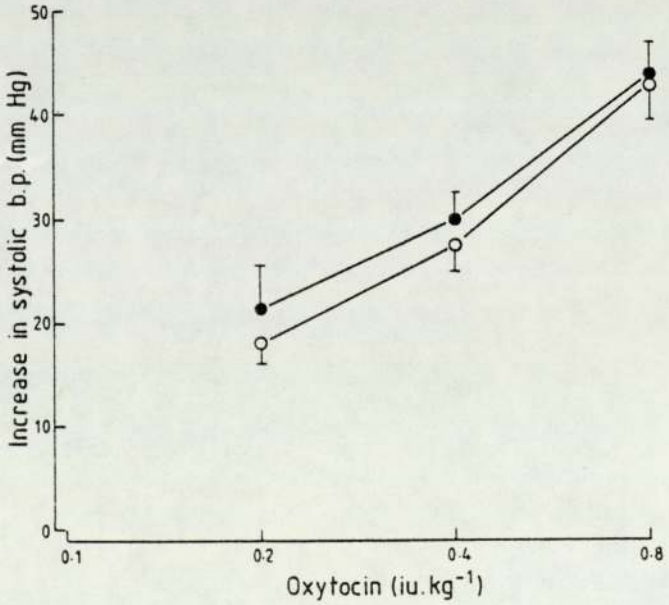
Figure 15

Maximal sustained systolic blood pressure induced by intravenously administered oxytocin in pithed oestrous (o) and dioestrous (•) rats. Each point represents the mean, and vertical bars denote the s.e. mean (n=7).

Figure 16

Increase in systolic blood pressure induced by intravenously administered oxytocin in pithed oestrous (o) and dioestrous (•) rats. Each point represents the mean, and vertical bars denote the s.e. mean (n=7).


Maximal sustained systolic b.p. (mm Hg)
130
120
110
100
90
80
0·1
0·2
0·4
0·8
Oxytocin (iu.kg^{-1})


Increase in systolic b.p. (mm Hg)
50
40
30
20
10
0
0·1
0·2
0·4
0·8
Oxytocin (iu.kg^{-1})

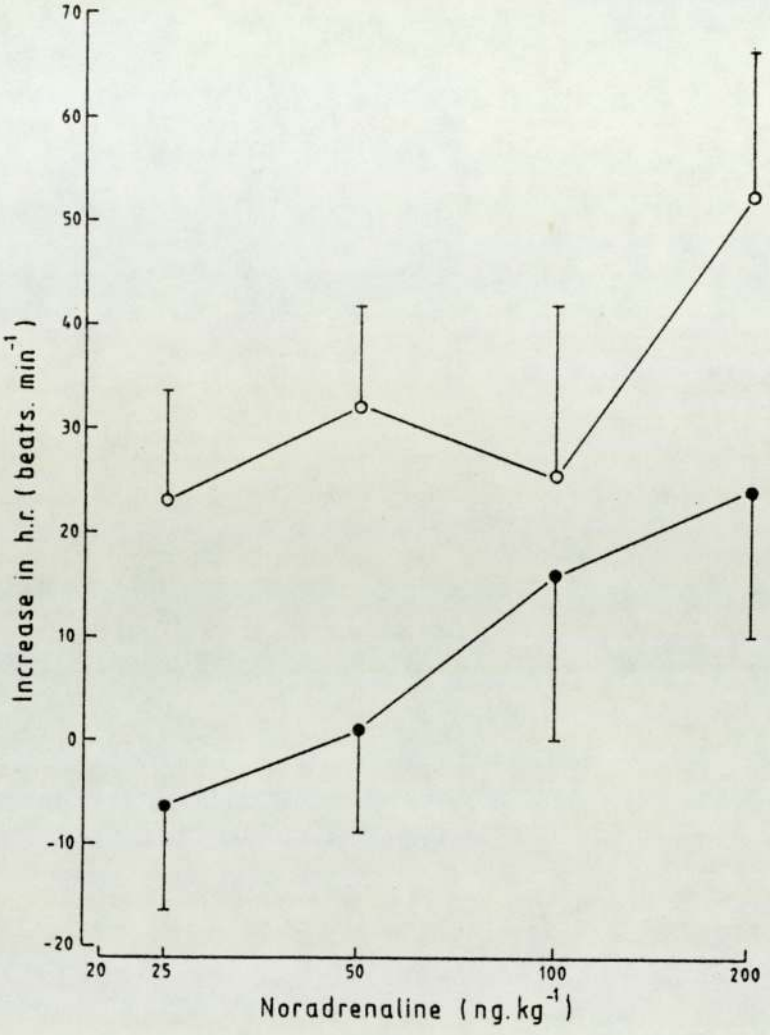


Figure 17

Increase in heart rate induced by intravenously administered noradrenaline in pithed oestrous (o) and dioestrous (•) rats. Each point represents the mean, and vertical bars denote the s.e. mean (n=7).

Discussion

Resting systolic blood pressure was elevated in oestrous rats compared with dioestrous rats, although there was no significant difference in resting diastolic pressure. Koch-Weser (1974) has suggested that this alteration in blood pressure profile may be due to an increase in cardiac output. Since cardiac output is the product of heart rate and stroke volume (Metcalf, McAnulty & Ueland, 1981) and since heart rate was not significantly different in oestrous rats compared with dioestrous rats, an increase in plasma 17β-oestradiol at oestrus (Yoshinaga et al., 1969) may be associated with an elevation in stroke volume as a result of an increase in cardiac contractility. This suggestion is consistent with the observation that there was no significant difference between positive chronotropic responses to noradrenaline, angiotensin II, vasopressin or oxytocin in oestrous, compared with dioestrous, rats.

Other workers have provided evidence which suggests that administration of ovarian hormonal agents augment cardiac contractility: an increase in myocardial contractility in women taking oral contraceptives (Walters & Lim, 1969), an increase in force developed to ATP in isolated rat ventricular columnae following in vivo administration of α-oestradiol to ovariectomised rats (King et al., 1959), an increase in amplitude of contraction of hypodynamic isolated frog heart following in vitro administration of sodium oestrone or 17β-oestradiol (Rubin & Salter, 1950; Loynes & Gowdey, 1952). Present and published findings therefore indicate that in vivo and in vitro administration of both natural and synthetic oestrogenic agents increases cardiac contractility in several different species.

Pressor responses to two concentrations of noradrenaline and to all concentrations of vasopressin were potentiated in oestrous, compared with dioestrous, rats, when responses were expressed as the maximal sustained systolic pressure. To determine whether this potentiation of pressor responses was due to the elevation in resting systolic pressure observed in oestrous rats, pressor responses were also expressed as the incremental rise in systolic pressure. The incremental rise in systolic pressure was significantly greater in oestrous rats compared with dioestrous rats at only one concentration of noradrenaline and at two concentrations of vasopressin. Elevation of systolic pressure during oestrus therefore appeared to be responsible in part for the increase in pressor responses to noradrenaline and to vasopressin.

Since the concentration of noradrenaline required to elicit a standard pressor response was similar in pithed oestrous and dioestrous rats, it appeared that oestrus is not associated with an alteration in the potency of exogenous noradrenaline.

A lower concentration of vasopressin was required to elicit a standard pressor response in oestrous, compared with dioestrous, rats. This indicates an increase in potency of vasopressin in oestrous rats. This finding is similar to that of Lloyd (1959a), since Lloyd (1959a) observed that anaesthetised rats in oestrus required a lower concentration of vasopressin to elicit a standard pressor response compared with dioestrous rats. However, it is possible that Lloyd (1959a) compared concentrations required to elicit a response in the threshold region of the concentration-response curve, since she recorded a standard pressor response of 10 mm Hg and, in present experiments, this response lay in the threshold region of the curve.

It is unfortunate that Lloyd (1959a) did not clarify this point, since conclusions drawn from comparison of concentrations required to elicit a standard response depend upon the part of the concentration-response curve at which the response is measured.

The concentration of angiotensin II required to elicit a standard pressor response was similar in oestrous and dioestrous rats. Also, pressor responses to angiotensin II, expressed as the increase in systolic pressure or as the maximal sustained systolic pressure, were not significantly different in oestrous, compared with dioestrous, rats. Similarly, Hettiaratchi & Pickford (1968) observed no alteration in pressor responses to angiotensin II in anaesthetised oestrous and dioestrous rats, when they compared the incremental rise in blood pressure induced by three standard concentrations of angiotensin II. Since angiotensin II may induce part of its vasoconstrictor action by release of intramural noradrenaline (Khairallah, Irvine, Bumpus & Türker, 1966), it would be interesting to examine the effect of the oestrous cycle upon the direct pressor action of angiotensin II. To do this, present experiments would be repeated following chemical sympathectomy or concurrent α- and β-adrenoreceptor blockade.

Pressor responses to oxytocin, expressed either as the increase in systolic blood pressure or as the maximal sustained systolic pressure, were not significantly different in oestrous, compared with dioestrous, rats. Also, the concentration of oxytocin required to elicit a standard pressor response was similar in oestrous and dioestrous rats. Lloyd (1959a) observed that oxytocin had no effect upon blood pressure in anaesthetised dioestrous rats, whereas oxytocin was pressor in anaesthetised oestrous rats. As in present experiments, Lloyd & Pickford (1961) observed that oxytocin gave pressor responses

in both oestrous and dioestrous pithed rats. Unfortunately, however, Lloyd & Pickford (1961) did not compare the responses to oxytocin in pithed oestrous and dioestrous rats.

Since pressor responses to noradrenaline, angiotensin II, vasopressin and oxytocin were of a similar magnitude in present experiments, it seemed reasonable to compare the effect of the oestrous cycle upon cardiovascular responses to these agents. It appears from present findings that an increase in plasma 17β-oestradiol at oestrus has variable effects upon responses to pressor agents. Changes in responses are therefore unlikely to be due to vascular structural alterations. This is consistent with the view that hypertrophy is unlikely to take place during oestrus and be reversed during dioestrus.

Experimental work carried out by others have shown that oestrogenic agents may increase vascular responsiveness by:

a) an alteration in elimination or metabolism of the vasoconstrictor agent,

b) an effect at the receptor,

c) an increase in calcium mobilisation,

d) an increase in energy availability or

e) an alteration in vascular muscle contraction mechanism.

Since most published studies have been concerned with an examination of the effect of oestrogenic agents upon in vitro tissue preparations (see pages 24-29), it was not possible to estimate the mechanism of potentiation of pressor responses by reference to the results of previous studies.

Although pressor responses to noradrenaline, angiotensin II, vasopressin and oxytocin appeared to lie in the linear portion of the concentration-response curves, it appeared that positive chronotropic

responses lay in the threshold region of the concentration-response curves. The pithed rat therefore seemed to be more responsive to the pressor action than to the positive chronotropic action of noradrenaline, angiotensin II, vasopressin and oxytocin.

Oestrus appeared to induce no changes in positive chronotropic responses to noradrenaline, angiotensin II, vasopressin and oxytocin. However, it appears from figure 17 (p. 82) that, in the linear portion of the concentration-response curve, oestrous rats may require a higher concentration of noradrenaline to elicit a standard positive chronotropic response compared with dioestrous rats. If this is true, then, since there is some evidence for an increase in pressor responses to noradrenaline during oestrus, and since oestrus does not appear to induce vascular structural changes, it is possible that oestrus is associated with potentiation of all cardiovascular responses to exogenous noradrenaline. Such potentiation may be due to an alteration in metabolism or elimination of exogenous noradrenaline. An increase in the concentration of the agents used in present experiments may induce positive chronotropic responses in the linear portion of the concentration-response curve. Production of such responses would help in the understanding of the potentiation of both noradrenaline- and vasopressin-induced pressor responses at oestrus.

Experiment 3

Effect of the stage of the oestrous cycle upon cardiovascular responses to electrical stimulation

Introduction

The results of experiment 2 indicated that alterations in the plasma concentration of ovarian hormones during the oestrous cycle (Yoshinaga et al., 1969) are associated with increases in systolic blood pressure and in pressor responses to exogenous noradrenaline. To extend this study, experiments were undertaken to determine whether responses to endogenously-released noradrenaline are altered by changes in the plasma concentration of ovarian hormones.

Nervous release of noradrenaline was induced by electrical stimulation of the sympathetic nervous system by a method similar to that described by Gillespie & Muir (1967a). Since nerve stimulation leads to muscle contraction, which causes the animal to jerk and to present altered pressor responses, D-tubocurarine, a competitive blocker of nerve/skeletal muscle junctions (Standaert, 1964), was injected intravenously to act as a muscle relaxant. Gillespie & Muir (1967a) have shown that D-tubocurarine administration does not alter pressor responses to sympathetic nerve stimulation and that D-tubocurarine does not have a ganglion-blocking effect.

Results

Oestrous and dioestrous rats were pithed and the resting blood pressure and heart rate were recorded, both before and after intravenous administration of D-tubocurarine. Table 4a (p. 92) shows the body weight and table 4b (p. 92) shows the resting blood pressure and heart rate before and after D-tubocurarine administration to oestrous and dioestrous rats. No significant difference in body weight, systolic pressure, diastolic pressure, pulse pressure or heart rate was recorded between oestrous and dioestrous rats either before or after D-tubocurarine administration ($P>0.1$ throughout).

The sympathetic spinal outflow was then stimulated electrically by the method outlined on page 37 by varying the voltage (20-60 V) at fixed frequency (10 s^{-1}) and fixed pulse widths (200, 400, 600 or 800 μs). Pressor responses and positive chronotropic responses were recorded. Pressor responses were recorded both as the maximal sustained systolic pressure and also as the increase in systolic pressure. Positive chronotropic responses were recorded both as the maximal sustained heart rate and also as the increase in heart rate. Figure 18 (p. 93) shows typical pressor responses to electrical stimulation and how these responses were measured.

Figure 19 (p. 94) shows the maximal sustained systolic pressure during electrical stimulation of 10 s^{-1} at a fixed pulse width of 200 μs and variable voltage (20-60 V) in oestrous and dioestrous rats. No significant differences were recorded between the maximal sustained systolic pressure of oestrous and dioestrous rats ($P>0.1$ throughout). Similarly, at all other stimulation parameters, no significant differences in maximal sustained systolic pressure were recorded between oestrous and dioestrous rats ($P>0.6$ throughout).

No signficant differences were recorded between the pressor responses of oestrous and dioestrous rats when responses were expressed as the increase in systolic pressure ($P>0.5$ throughout). This is demonstrated in figure 20 (p. 94) which shows pressor responses, expressed as the increase in systolic pressure, during electrical stimulation of fixed frequency (10 s^{-1}), fixed pulse width (200 μs) and variable voltage (20-60 V) in oestrous and dioestrous rats.

The voltage required to elicit a standard pressor response was not significantly different in oestrous, compared with dioestrous, rats. For example, during electrical stimulation at 10 s^{-1} and 200 μs pulse width, the voltage required to elicit an increase in systolic pressure of 30 mm Hg in oestrous rats was 47.28 ± 1.90 V ($n=6$) and that required by dioestrous rats was 51.17 ± 3.67 V ($n=6$)($P>0.3$). The stimulation-ratio was 1.08.

No significant differences were recorded between the positive chronotropic responses, recorded either as the maximal sustained heart rate or as the increase in heart rate, of oestrous and dioestrous rats at any of the electrical stimulation parameters used ($P>0.05$ throughout).

Figure 21 (p. 95) shows the increase in heart rate during electrical stimulation of fixed frequency (10 s^{-1}), fixed pulse width (200 μs) and variable voltage (20-60 V) in oestrous and dioestrous rats. This figure appears to show the threshold and start of the linear portion of the stimulation-response curves. During electrical stimulation at 10 s^{-1} and 200 μs pulse width, the voltage required to elicit an increase in heart rate of 15 beats.min^{-1} was not significantly different in oestrous rats (49.48 ± 8.64 V, $n=6$) compared with dioestrous rats (40.65 ± 4.00 V, $n=6$)($P>0.3$), such that the

stimulation-ratio was 1.22. However, it appears from figure 21 (p. 95) that in the linear portion of the stimulation-response curve, oestrous rats may have required a higher voltage to elicit the same increase in heart rate compared with that required by dioestrous rats.

TABLE 4 - Recordings of body weight, blood pressure and heart rate in pithed oestrous and dioestrous rats.

	Parameter	Oestrus (6)	Dioestrus (6)	P
a)	Body weight (g)	235.0 ± 5.5	241.7 ± 6.5	0.4-0.5
b)	After pithing			
	Following atropine sulphate administration			
	Systolic b.p. (mm Hg)	54.8 ± 3.5	59.5 ± 3.4	0.3-0.4
	Diastolic b.p. (mm Hg)	43.7 ± 0.9	47.2 ± 2.3	0.1-0.2
	Pulse pressure (mm Hg)	11.2 ± 3.2	12.3 ± 1.2	0.7-0.8
	M.A.P. (mm Hg)	47.4 ± 1.6	51.3 ± 2.6	0.2-0.3
	Heart rate (min^{-1})	302.0 ± 20.2	311.7 ± 17.4	0.7-0.8
	Following atropine sulphate and D-tubocurarine administration			
	Systolic b.p. (mm Hg)	53.8 ± 2.6	58.3 ± 3.6	0.3-0.4
	Diastolic b.p. (mm Hg)	43.0 ± 1.3	45.8 ± 2.9	0.3-0.4
	Pulse pressure (mm Hg)	10.8 ± 3.3	12.5 ± 1.5	0.6-0.7
	M.A.P. (mm Hg)	46.6 ± 1.0	50.0 ± 3.1	0.3-0.4
	Heart rate (min^{-1})	303.0 ± 19.4	290.0 ± 10.9	0.5-0.6

Number of observations in parentheses.

Results are mean ± s.e. mean.

b.p. - Blood pressure.

M.A.P. - Mean arterial pressure.

Figure 18

A typical trace of pressor responses to electrical stimulation, of fixed frequency and fixed pulse width, of the sympathetic spinal outflow in the pithed female rat.

1 = 20 V
2 = 40 V
3 = 60 V

a = Resting systolic pressure.
b = Resting pulse pressure. b = a-c
c = Resting diastolic pressure.

d = Maximal sustained systolic pressure.
e = Increase in systolic pressure. e = d-f
f = Systolic pressure prior to stimulation.

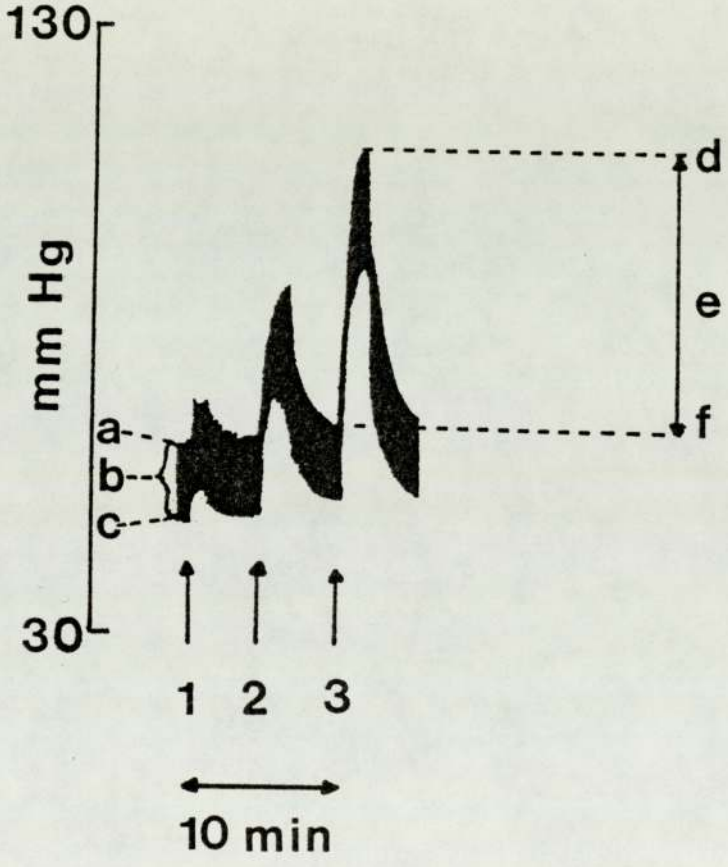
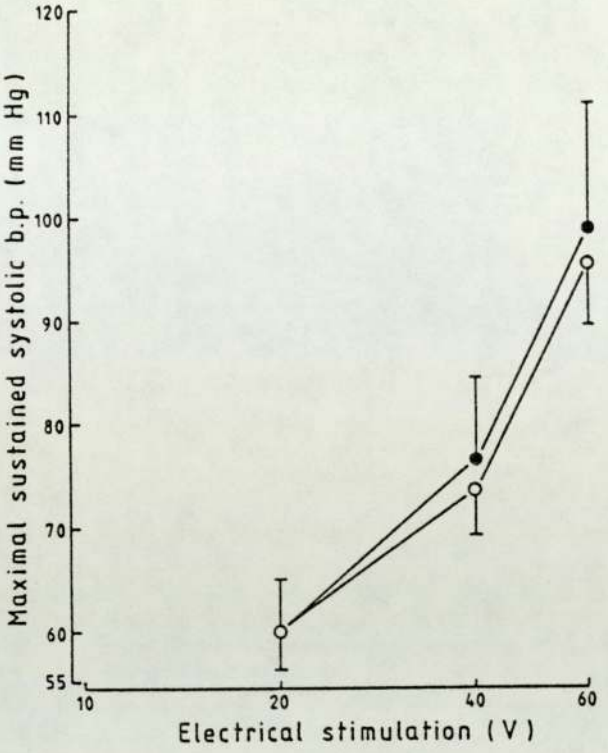

130
mm Hg
30
a
b
c
d
e
f
1
2
3
10 min

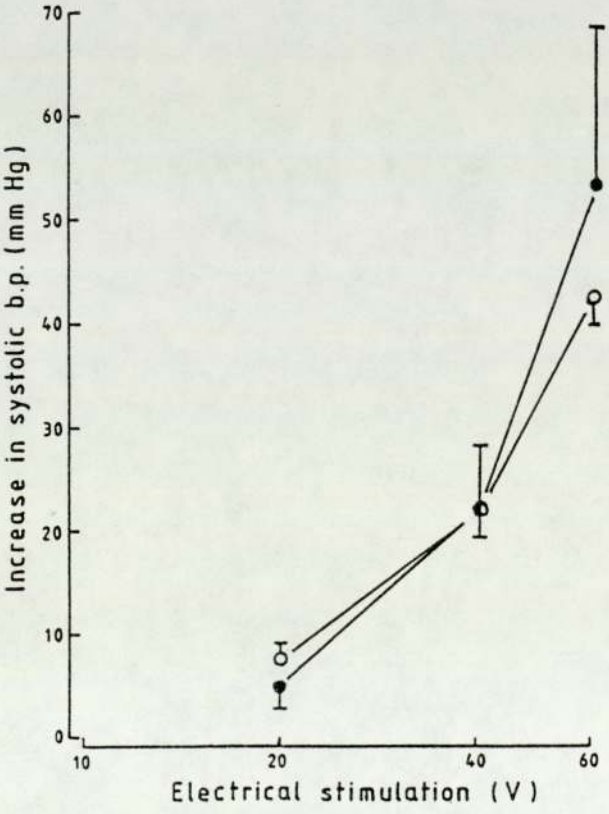
Figure 19

Maximal sustained systolic blood pressure during electrical stimulation of fixed frequency (10 s^{-1}), fixed pulse width (200 μs) and variable voltage of the sympathetic spinal outflow in pithed oestrous (o) and dioestrous (•) rats. Each point represents the mean, and vertical bars denote the s.e. mean (n=6).

Figure 20

Increase in systolic blood pressure during electrical stimulation of fixed frequency (10 s^{-1}), fixed pulse width (200 μs) and variable voltage of the sympathetic spinal outflow in pithed oestrous (o) and dioestrous (•) rats. Each point represents the mean, and vertical bars denote the s.e. mean (n=6).


Maximal sustained systolic b.p. (mm Hg)
120
110
100
90
80
70
60
55
10
20
40
60
Electrical stimulation (V)


Increase in systolic b.p. (mm Hg)
70
60
50
40
30
20
10
0
10
20
40
60
Electrical stimulation (V)

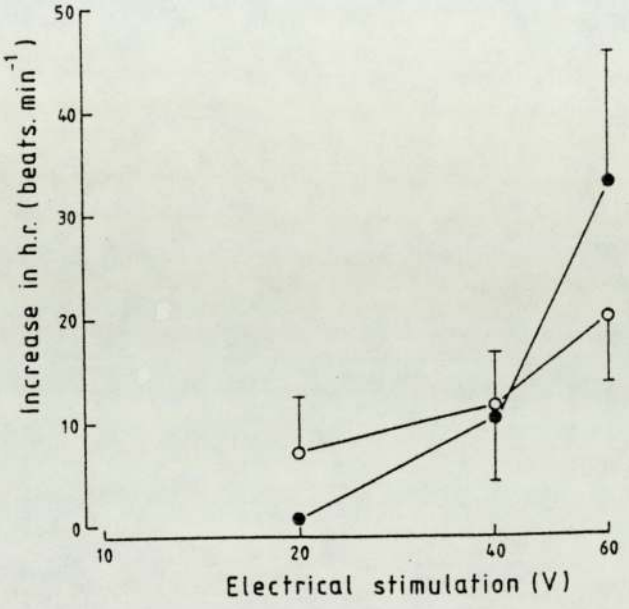


Figure 21

Increase in heart rate during electrical stimulation of fixed frequency (10 s^{-1}), fixed pulse width (200 μs) and variable voltage of the sympathetic spinal outflow in pithed oestrous (o) and dioestrous (•) rats. Each point represents the mean, and vertical bars denote the s.e. mean (n=6).

Discussion

Cardiovascular responses were elicited by increasing the voltage between 20 and 60 V at fixed frequency of 10 s^{-1} and at a fixed pulse width of 200, 400, 600 or 800 μs. Stein (1974) has shown that electrical stimulation leads to release of noradrenaline from nerve endings. Gillespie & Muir (1967a) have shown that the threshold for pressor responses is 5-15 V and that the supramaximal voltage is in the region of 80 V. Present experimental findings confirm the observations of Gillespie & Muir (1967a) that increasing the voltage between 20 and 60 V gives graded pressor responses. However, pressor responses induced by constant stimulation as high as 10 s^{-1} were shown by Gillespie & Muir (1967a) to be diminished after reaching a maximal response. Gillespie & Muir (1967a) have suggested that this phenomenon may be due to expenditure of available noradrenaline stores. However, no such diminution in response was observed in present experiments during electrical stimulation at 10 s^{-1}. It therefore appears reasonable to assume that the increase in pressor responses observed whilst augmenting the voltage in present experiments is associated with potentiation of noradrenaline release from sympathetic nerves.

Pressor responses in this experiment and in experiment 2 were of the same order of magnitude. It therefore seemed reasonable to compare the findings of experiments 2 and 3.

When pressor responses were recorded as the incremental rise in systolic pressure, there were no differences in response to electrical stimulation in oestrous, compared with dioestrous, pithed rats. Since Folkow (1978) has shown that potentiation of the incremental rise in

blood pressure is due to vascular hypertrophic structural changes, this finding is consistent with the suggestion (see page 86) that oestrus is not associated with alterations in vascular wall structure. There appeared to be no difference in the potency of endogenously-released noradrenaline in oestrous, compared with dioestrous, rats. This is similar to the findings in experiment 2 (see page 84) that there is no alteration in the potency of exogenous noradrenaline during the oestrous cycle.

The findings discussed above indicated similarities in the results of experiments 2 and 3. However, at one concentration of exogenous noradrenaline, the incremental rise in systolic pressure was greater in oestrous rats compared with that in dioestrous rats. It is therefore possible that changes in the plasma concentration of ovarian hormones during the oestrous cycle may have different effects upon pressor reponses to exogenous and endogenous noradrenaline. Since exogenous noradrenaline is eliminated primarily by extraneuronal uptake and by metabolism with catechol-o-methyltransferase (COMT) (Trendelenburg, 1977; Vanhoutte, 1978a), whereas endogenous noradrenaline is eliminated primarily by metabolism with monoamine oxidase (MAO)(Vanhoutte, 1978a), it is possible that oestrus is associated with a decrease in the activity of COMT and/or extra-neuronal uptake. Present findings also indicate that changes in circulating ovarian hormones during the oestrous cycle may have a greater effect upon the metabolism of circulating noradrenaline than upon noradrenaline metabolism or elimination at the synapse.

Another explanation for the difference between the results of experiments 2 and 3 is that the rats used in experiments 2 and 3 are fundamentally different. The observation that the systolic and

diastolic blood pressure of oestrous rats in experiment 3 are significantly reduced compared with those of oestrous rats in experiment 2 (see table 6, p. 107) is consistent with this possibility. Also, in experiment 2 oestrous rats demonstrated an increase in resting systolic pressure compared with dioestrous rats, whereas in experiment 3 the systolic blood pressure of oestrous rats and dioestrous rats was not significantly different. It is possible that potentiation of the incremental rise in systolic pressure to one concentration of exogenous noradrenaline in oestrous rats in experiment 2 is related to this increase in resting systolic pressure, such that the magnitude of pressor responses is dependent upon the resting systolic blood pressure. This possibility is particularly interesting, since pressor responses were recorded as incremental rise in systolic pressure to minimise the influence of the resting systolic pressure upon responses.

There is also evidence for a difference between positive chronotropic responses to noradrenaline in experiments 2 and 3. In experiment 2, the concentration-response curve to noradrenaline appeared to be shifted to the left in oestrus, whereas the concentration-response curve to endogenous noradrenaline appeared to be unaltered or shifted to the right during oestrus in experiment 3. This finding is consistent with the view that there is a fundamental difference between the rats used in experiments 2 and 3.

Experiment 4

Effect of bilateral ovariectomy upon cardiovascular responses to noradrenaline, angiotensin II, vasopressin and isoprenaline

Introduction

The findings of experiments 2 and 3, which were undertaken to determine whether changes in circulating ovarian hormones during the oestrous cycle induce alterations in cardiovascular responsiveness, were difficult to interpret. This may be because changes in the concentration of circulating ovarian hormones during the oestrous cycle, are not great, and are of short duration (Yoshinaga et al., 1969).

It was proposed that an examination of the influence of changes in circulating ovarian hormones greater than, and of a longer duration than, those which take place during the oestrous cycle, would help in the understanding of the findings of experiments 2 and 3.

Bilateral ovariectomy is associated with plasma concentrations of 17β-oestradiol which are substantially lower than those found during the normal oestrous cycle (Rabii & Ganong, 1976). Also, bilateral ovariectomy has the advantage of inducing long-term changes in plasma ovarian hormones without the problems involved with administration of ovarian hormones. For these reasons, the influence of bilateral ovariectomy was examined upon cardiovascular responses.

Responses of bilaterally ovariectomised rats were compared with those of sham-operated rats in dioestrus, since dioestrous rats were used as controls throughout all present pithed rat experiments. Also, since the effect of the oestrous cycle upon the resting blood pressure

profile was different in experiments 2 and 3, an additional comparison was made of the resting cardiovascular parameters and responses in oestrous and dioestrous rats which had undergone sham bilateral ovariectomy.

As in experiment 2, cardiovascular responses to noradrenaline, angiotensin II and vasopressin were examined. Responses to oxytocin were examined in experiment 2 to determine whether the findings of Lloyd (1959a) could be repeated in pithed rats. However, Nakano (1973) has shown that the oxytocin preparation used in present experiments, pitocin, may be contaminated by vasopressin. In present experiments, therefore, cardiovascular responses to oxytocin were not examined. Findings from experiment 2 indicated that cardiac contractility may be altered by a change in the plasma concentration of ovarian hormones (see page 83). To examine this possibility, the electrocardiogram (ECG) was recorded. In accordance with the view of Guyton (1981), the PQ interval was taken to be a measure of the atrial conduction time and the QT interval was taken to be a measure of the ventricular contraction period. In addition, the effect of bilateral ovariectomy was examined upon cardiovascular responses to the β-adrenoreceptor agonist, isoprenaline (Fleisch, 1977).

Figure 22 (p. 109) shows a typical ECG of the pithed rat prior to stimulation and how the PQ and QT intervals were recorded. The height of the ECG wave did not change following injection of noradrenaline, angiotensin II, vasopressin or isoprenaline.

Isoprenaline reduced systolic and diastolic blood pressures below resting levels. The minimal diastolic pressure following injection of isoprenaline was therefore recorded and taken as an indication of the absolute ability of the cardiovascular system to respond to isoprenaline. The decrease in diastolic pressure was also

recorded and taken as a measure of the depressor action of isoprenaline, independent of the resting pressure. Figure 23 (p. 110) shows typical depressor and positive chronotropic responses to isoprenaline and indicates where responses were measured. Typical pressor responses to noradrenaline, angiotensin II and vasopressin have previously been shown in figures 5, 6 and 7 (pp. 76 and 77). Pressor responses to noradrenaline, angiotensin II and vasopressin were recorded both as the maximal sustained systolic pressure and also as the increase in systolic pressure. Positive chronotropic responses were recorded both as the maximal sustained heart rate and also as the increase in heart rate.

Results

Ovariectomised and control (dioestrous sham-operated) rats were pithed and the resting blood pressure, heart rate and PQ and QT intervals were recorded. Table 5 (p. 106) shows the body weight, blood pressure, heart rate and PQ and QT intervals of ovariectomised and control rats. No significant differences were recorded between ovariectomised and control rats for any of these parameters ($P>0.1$ throughout).

Each rat was then injected intravenously with noradrenaline (25-400 $ng.kg^{-1}$), angiotensin II (6.25-100 $ng.kg^{-1}$), vasopressin (0.00125-0.02 $iu.kg^{-1}$) and isoprenaline (25-200 $ng.kg^{-1}$) and the blood pressure responses, positive chronotropic responses and PQ and QT intervals were recorded.

Figure 24 (p. 111) shows the maximal sustained systolic pressure induced by geometrically increasing concentrations of

noradrenaline in ovariectomised and control rats. No significant differences were recorded between the maximal sustained systolic pressures of ovariectomised and control rats ($P>0.4$ throughout). Similarly, at all concentrations of angiotensin II and vasopressin, no significant differences were recorded between the maximal sustained systolic pressure of ovariectomised and control rats ($P>0.1$ throughout) (see figures 26 and 28, pp. 112 and 113).

Also, at all concentrations of isoprenaline, no significant differences were recorded between the minimal sustained diastolic pressure of ovariectomised and control rats ($P>0.3$ throughout)(see figure 30, p. 114).

Figure 25 (p. 111) shows pressor responses, expressed as the increase in systolic pressure, induced by geometrically increasing concentrations of intravenously administered noradrenaline, in ovariectomised and control rats. No significant differences were recorded between the pressor responses to noradrenaline of ovariectomised and control rats when responses were expressed as the increase in systolic pressure ($P>0.05$ throughout). Similarly, pressor responses to intravenously administered angiotensin II did not differ significantly between ovariectomised and control rats when responses were expressed as the increase in systolic pressure ($P>0.4$ throughout) (see figure 27, p. 112).

Since an increase in systolic pressure of 10 mm Hg lay on the linear portion of the concentration-response curves to noradrenaline and angiotensin II, the concentration of noradrenaline and angiotensin II required to elicit a rise of 10 mm Hg was compared in ovariectomised and control rats. These concentrations and concentration-ratios are shown in table 7 (p. 108). The concentrations of noradrenaline and

angiotensin II required to elicit a standard incremental rise in systolic pressure were not significantly different in bilaterally ovariectomised, compared with control, rats ($P>0.1$ throughout).

Figure 29 (p. 113) shows pressor responses, expressed as the increase in systolic pressure, induced by geometrically increasing concentrations of intravenously administered vasopressin, in ovariectomised and control rats. At all but one concentration of vasopressin ($0.01\ iu.kg^{-1}$) there were no significant differences in pressor responses of ovariectomised and control rats, when responses were expressed as the increase in systolic pressure ($P>0.05$ throughout). Pressor responses to vasopressin, especially in ovariectomised rats, appeared to lie in the threshold region of the concentration-response curve. For this reason, no comparison could be made of the concentrations required to elicit a standard pressor response in ovariectomised and control rats. However, it seems that ovariectomised rats may have required a higher concentration of vasopressin, compared with that required by control rats, to elicit a standard pressor response on the linear portion of the concentration-response curve.

Depressor responses to intravenously administered isoprenaline did not differ significantly between ovariectomised and control rats when responses were expressed as the decrease in diastolic pressure ($P>0.6$ throughout)(see figure 31, p. 114). Depressor responses to isoprenaline appeared to lie in the maximal region of the concentration-response curve (see figure 23, p. 110). For this reason, no comparison was made of the concentrations required to elicit a standard depressor response in ovariectomised and control rats.

At all concentrations of noradrenaline, angiotensin II, vasopressin and isoprenaline, positive chronotropic responses, expressed either as the maximal sustained heart rate or as the increase in heart rate, did not differ significantly between ovariectomised and control rats ($P>0.1$ throughout).

Positive chronotropic responses to angiotensin II and vasopressin appeared to lie in the threshold region of the concentration-response curve. Since any horizontal shift in this region would appear to be disproportionally large, concentration-ratios were not recorded. Figure 32 (p. 115) shows the increase in heart rate induced by geometrically increasing concentrations of noradrenaline in ovariectomised and control rats. Responses to noradrenaline appeared to lie in the threshold region and at the start of the linear portion of the curve. Horizontal shift in the concentration-response curve was therefore not measured. However, it appears that, following ovariectomy, rats may have required a higher concentration of noradrenaline to elicit a standard positive chronotropic response in the linear portion of the curve. Figure 33 (p. 115) shows the increase in heart rate induced by geometrically increasing concentrations of isoprenaline in ovariectomised and control rats. It was difficult to determine the part of the concentration-response curve in which positive chronotropic responses to isoprenaline lay (see figure 33). The concentration of isoprenaline required to induce the same increase in heart rate of 82.5 beats.min^{-1} was not significantly different in ovariectomised rats (57.17 ± 20.19 ng.kg^{-1}, n=6) compared with control rats (48.60 ± 10.21 ng.kg^{-1}, n=5) ($P>0.7$), such that the concentration-ratio was 1.18. However, positive chronotropic responses of control rats may have lain in the maximal region of the curve. It is therefore possible that the maximal

positive chronotropic response was increased following bilateral ovariectomy.

Following injection of all concentrations of vasopressin and isoprenaline and all but one concentration of angiotensin II (25 $ng.kg^{-1}$) and all but one concentration of noradrenaline (200 $ng.kg^{-1}$), the PQ interval did not differ significantly between ovariectomised and control rats ($P>0.05$ throughout).

Following injection of all concentrations of noradrenaline, vasopressin and isoprenaline and all but two concentrations of angiotensin II (12.5 and 25 $ng.kg^{-1}$), the QT interval did not differ significantly between ovariectomised and control rats ($P>0.05$ throughout).

There were no significant differences in the resting heart rate, PQ or QT interval, systolic, diastolic or pulse pressure or in the blood pressure or positive chronotropic responses to noradrenaline, angiotensin II, vasopressin or isoprenaline between oestrous and dioestrous (control) sham-operated rats ($P>0.05$ throughout). Also, there were no significant differences in the resting heart rate, QT interval, systolic, diastolic or pulse pressure or in the blood pressure or positive chronotropic responses to noradrenaline, angiotensin II, vasopressin or isoprenaline between oestrous sham-operated rats and ovariectomised rats ($P>0.05$ throughout). However, the PQ interval of oestrous sham-operated rats prior to stimulation was shown to be significantly longer (51.1 ± 1.0 ms, $n=6$) compared with that of ovariectomised rats (45.0 ± 2.2 ms, $n=6$)($P<0.05$).

TABLE 5 - Recordings of body weight, blood pressure, heart rate and PQ and QT intervals in pithed ovariectomised and control rats following atropine sulphate administration.

Parameters	Control (5)	Ovariectomised (6)	P
Days after surgery	49.2± 4.0	46.0± 2.0	0.4-0.5
Body weight (g)	278.0±11.1	280.0± 7.9	0.8-0.9
Systolic b.p. (mm Hg)	47.8± 5.6	53.7± 2.4	0.3-0.4
Diastolic b.p. (mm Hg)	42.6± 6.2	46.8± 2.4	0.5-0.6
Pulse pressure (mm Hg)	5.2± 1.3	6.8± 1.1	0.3-0.4
M.A.P. (mm Hg)	43.4± 5.6	49.1± 2.3	0.3-0.4
Heart rate (min^{-1})	229.0±27.9	282.0± 9.2	0.1-0.2
PQ interval (ms)	48.0± 5.0	45.0± 2.2	0.5-0.6
QT interval (ms)	109.0± 8.0	92.0± 8.0	0.1-0.2

Number of observations in parentheses.

Results are mean±s.e. mean.

b.p. - Blood pressure.

M.A.P. - Mean arterial pressure.

TABLE 6 - Recordings of blood pressure in pithed oestrous and dioestrous rats following atropine sulphate administration.

Oestrous Rats

Parameter	Expt 2 (7)	Expt 3 (6)	P	Expt 4 (6)	P
Systolic b.p. (mm Hg)	74.4±2.0	54.8±3.5	<0.001*	46.7±4.1	<0.001*
Diastolic b.p. (mm Hg)	62.4±2.6	43.7±0.9	<0.001*	40.7±4.1	<0.001*

Dioestrous Rats

Parameter	Expt 2 (7)	Expt 3 (6)	P	Expt 4 (5)	P
Systolic b.p. (mm Hg)	61.3±3.8	59.5±3.4	0.7-0.8	47.8±5.6	0.05-0.1
Diastolic b.p. (mm Hg)	54.1±3.8	47.2±2.3	0.1-0.2	42.6±6.2	0.1 -0.2

Number of observations in parentheses.

Results are mean±s.e. mean.

P values are with reference to rats used in experiment 2.

* Indicates statistical significant difference.

b.p. - Blood pressure.

TABLE 7 - Concentration of agents required to elicit an increase in systolic blood pressure of 10 mm Hg in pithed ovariectomised and control rats.

Agent	Control (5)	Ovariectomised (6)	P	Concn-ratio
NA ($ng.kg^{-1}$)	104.00 ± 26.74	60.50 ± 6.83	0.1-0.2	1.72
A II ($ng.kg^{-1}$)	66.67 ± 23.50	23.60 ± 8.55	0.1-0.2	2.83

Number of observations in parentheses.

Results are mean $\pm$ s.e. mean.

Concn-ratio is the ratio of the mean concentrations in ovariectomised and control rats.

NA - Noradrenaline.

A II - Angiotensin II.

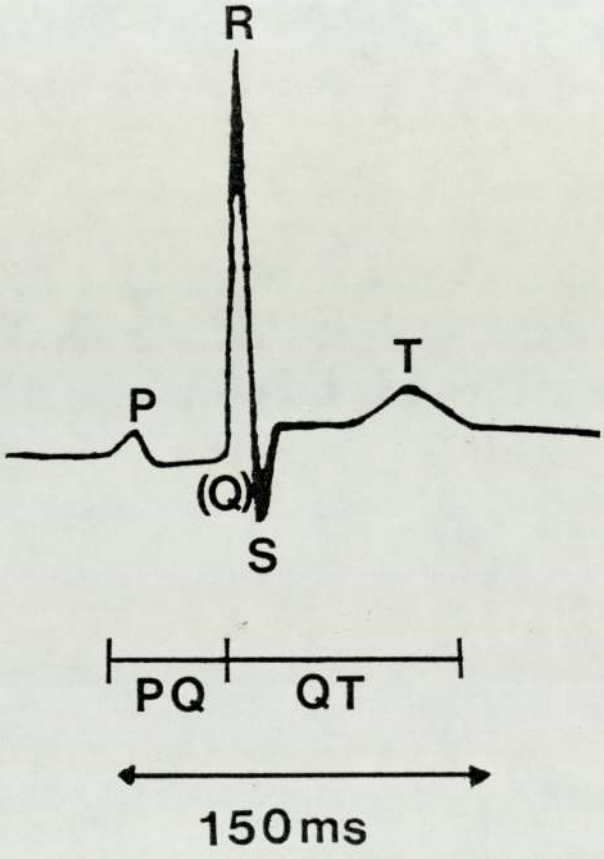


Figure 22

A typical trace of the ECG, prior to stimulation, of the pithed female rat.

Figure 23

A typical trace of depressor responses and positive chronotropic responses to intravenously administered isoprenaline in the pithed female rat.

1 = 25 $ng.kg^{-1}$
2 = 50 $ng.kg^{-1}$
3 = 100 $ng.kg^{-1}$
4 = 200 $ng.kg^{-1}$

a = Diastolic pressure prior to stimulation.
b = Decrease in diastolic pressure. b = a-c
c = Minimal sustained diastolic pressure.

d = Maximal sustained heart rate.
e = Increase in heart rate. e = d-f
f = Heart rate prior to stimulation.

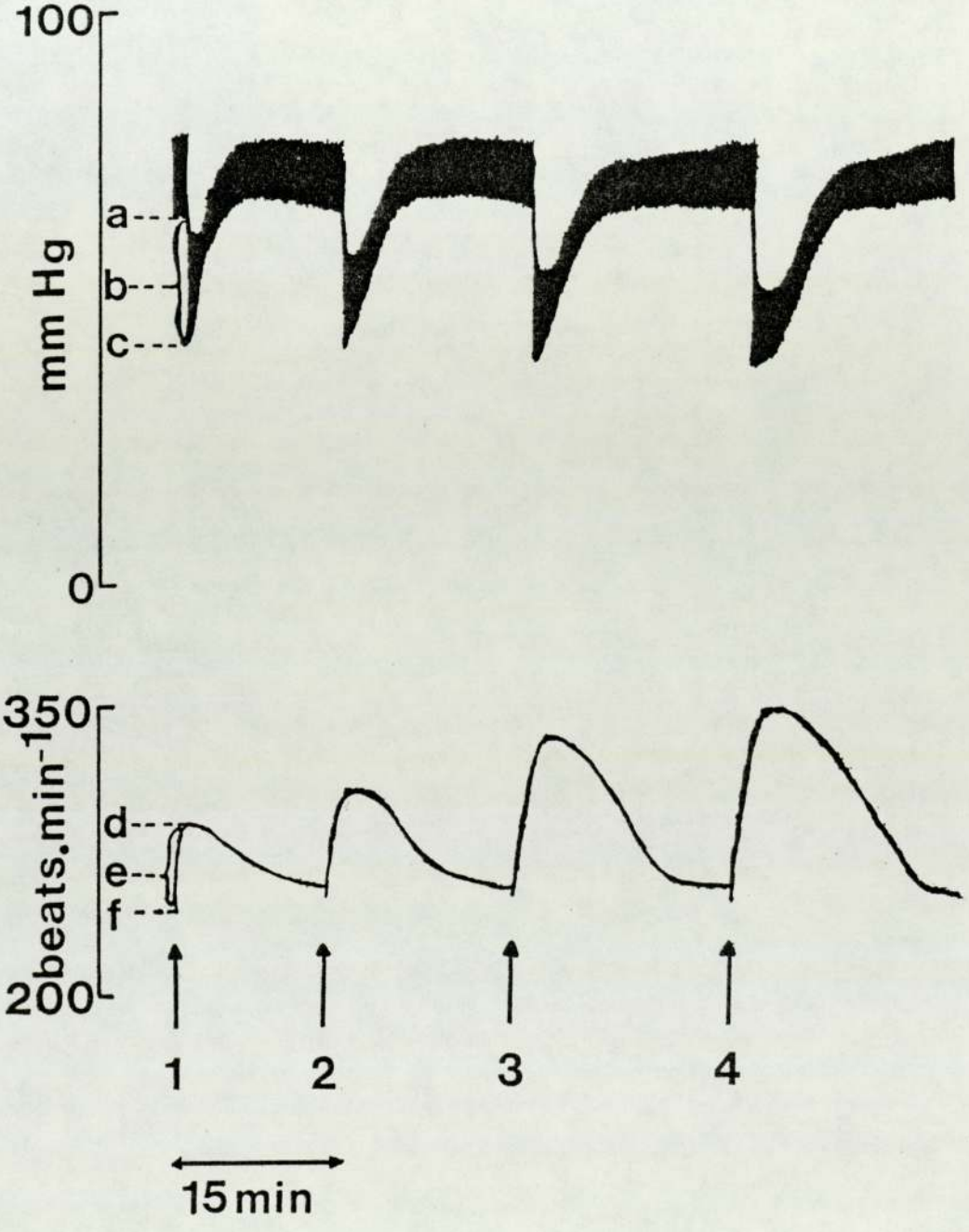
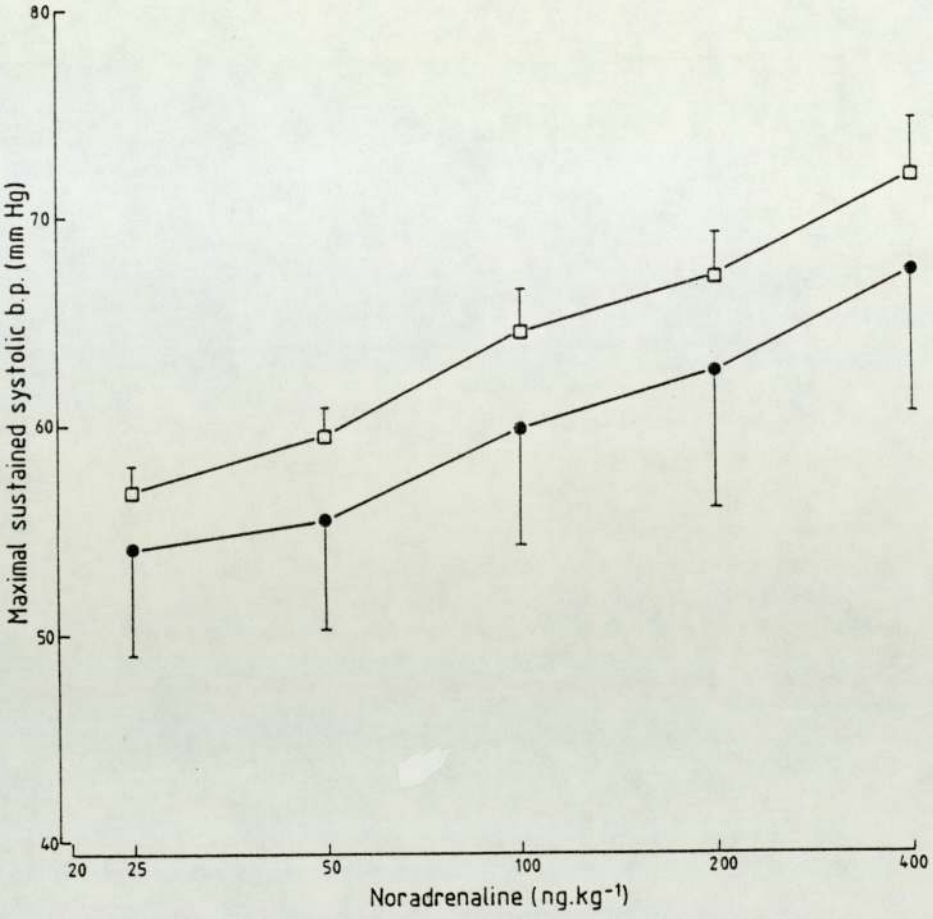

100
mm Hg
0
a
b
c
350
beats.min-1
200
d
e
f
1
2
3
4
15 min

Figure 24

Maximal sustained systolic blood pressure induced by intravenously administered noradrenaline in pithed bilaterally ovariectomised (□) and control (•) rats. Each point represents the mean, and vertical bars denote the s.e. mean (ovariectomised, n=6; control, n=5).

Figure 25

Increase in systolic blood pressure induced by intravenously administered noradrenaline in pithed bilaterally ovariectomised (□) and control (•) rats. Each point represents the mean, and vertical bars denote the s.e. mean (ovariectomised, n=6; control, n=5).


Maximal sustained systolic b.p. (mm Hg)
80
70
60
50
40
20
25
50
100
200
400
Noradrenaline (ng.kg⁻¹)

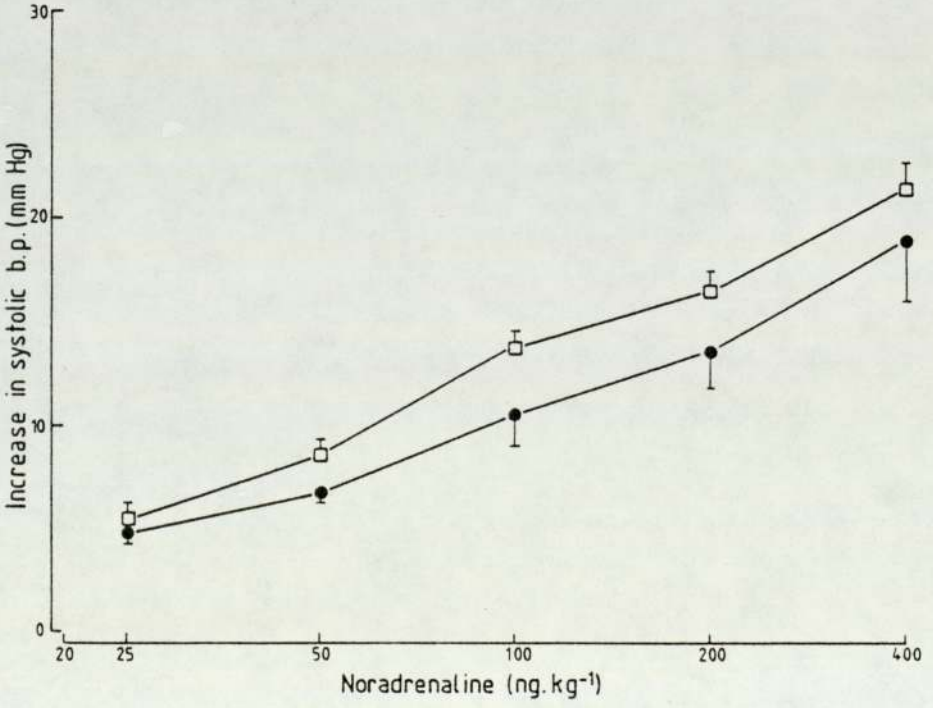
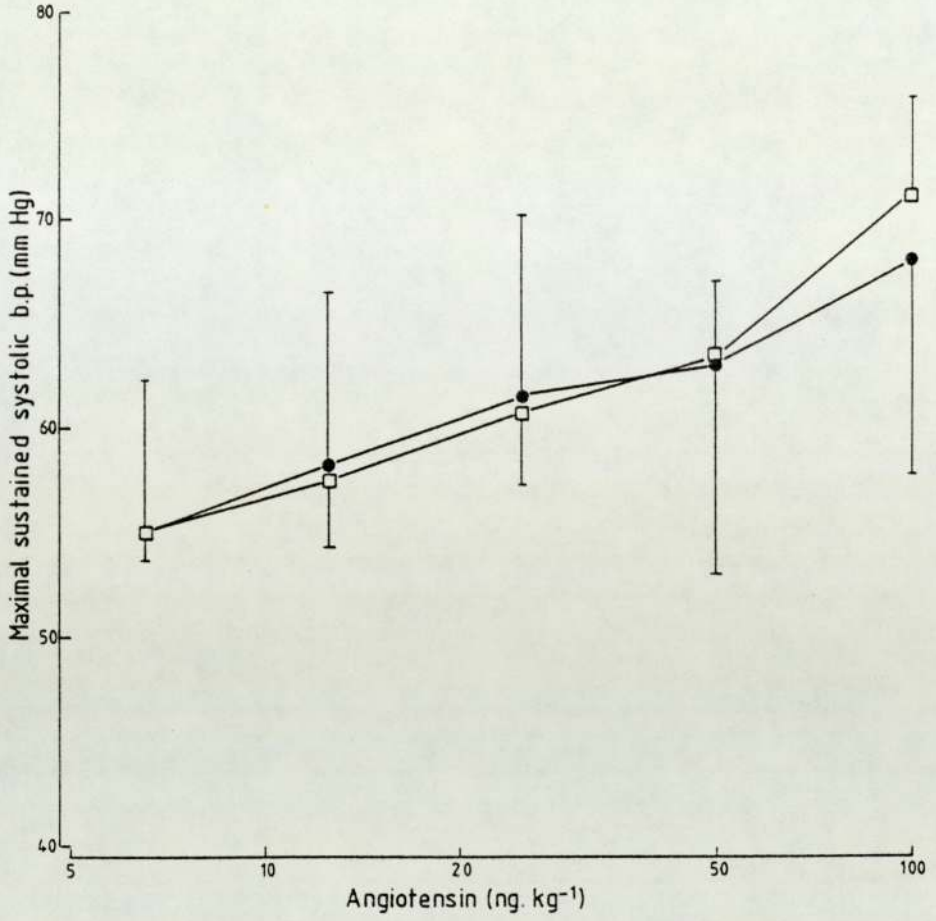

Increase in systolic b.p. (mm Hg)
30
20
10
0
20
25
50
100
200
400
Noradrenaline (ng.kg⁻¹)

Figure 26

Maximal sustained systolic blood pressure induced by intravenously administered angiotensin II in pithed bilaterally ovariectomised (□) and control (•) rats. Each point represents the mean, and vertical bars denote the s.e. mean (ovariectomised, n=6; control, n=5).

Figure 27

Increase in systolic blood pressure induced by intravenously administered angiotensin II in pithed bilaterally ovariectomised (□) and control (•) rats. Each point represents the mean, and vertical bars denote the s.e. mean (ovariectomised, n=6; control, n=5).


Maximal sustained systolic b.p.(mm Hg)
80
70
60
50
40
5
10
20
50
100
Angiotensin (ng. kg⁻¹)

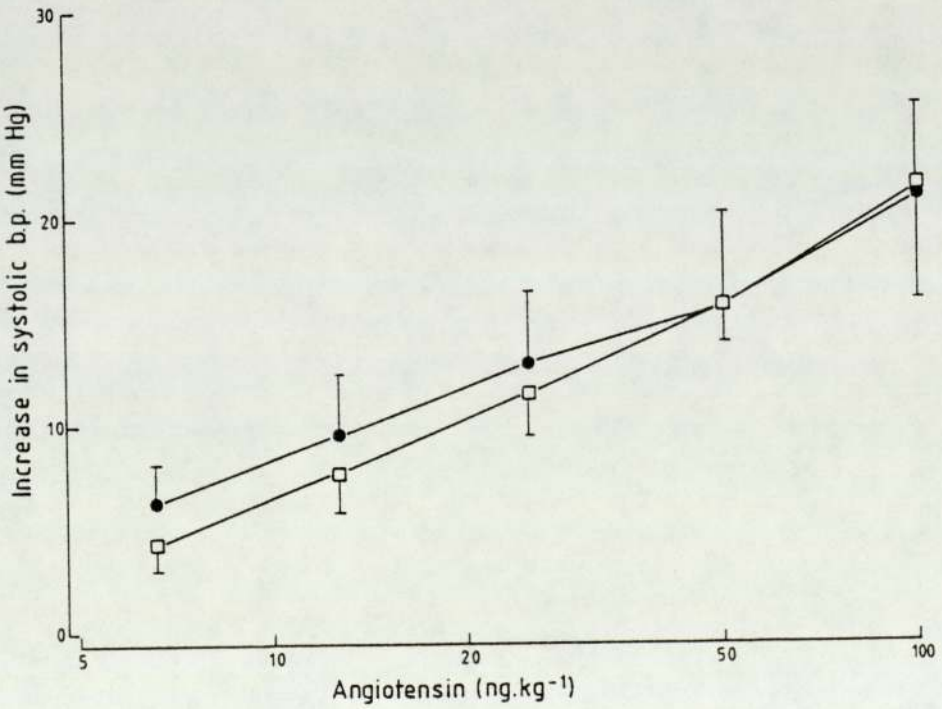

Increase in systolic b.p. (mm Hg)
30
20
10
0
5
10
20
50
100
Angiotensin (ng.kg⁻¹)

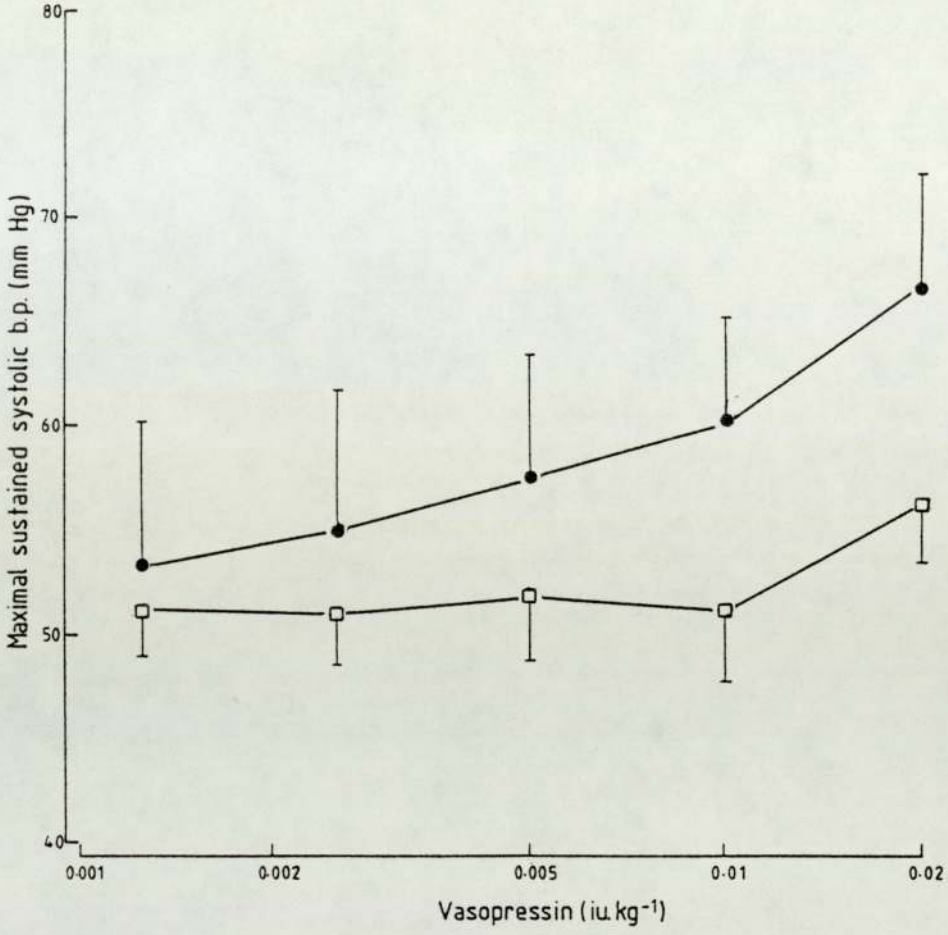
Figure 28

Maximal sustained systolic blood pressure induced by intravenously administered vasopressin in pithed bilaterally ovariectomised (□) and control (•) rats. Each point represents the mean, and vertical bars denote the s.e. mean (ovariectomised, n=6; control, n=5).

Figure 29

Increase in systolic blood pressure induced by intravenously administered vasopressin in pithed bilaterally ovariectomised (□) and control (•) rats. Each point represents the mean, and vertical bars denote the s.e. mean (ovariectomised, n=6; control, n=5).

* Indicates statistical significant difference.


Maximal sustained systolic b.p. (mm Hg)
80
70
60
50
40
0·001
0·002
0·005
0·01
0·02
Vasopressin (iu.kg⁻¹)

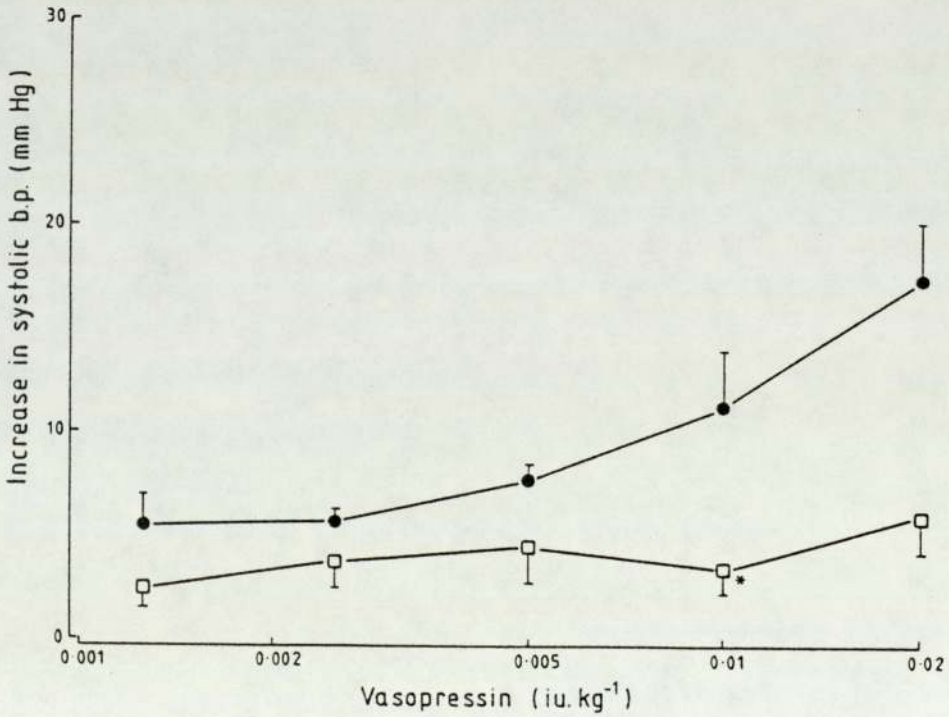
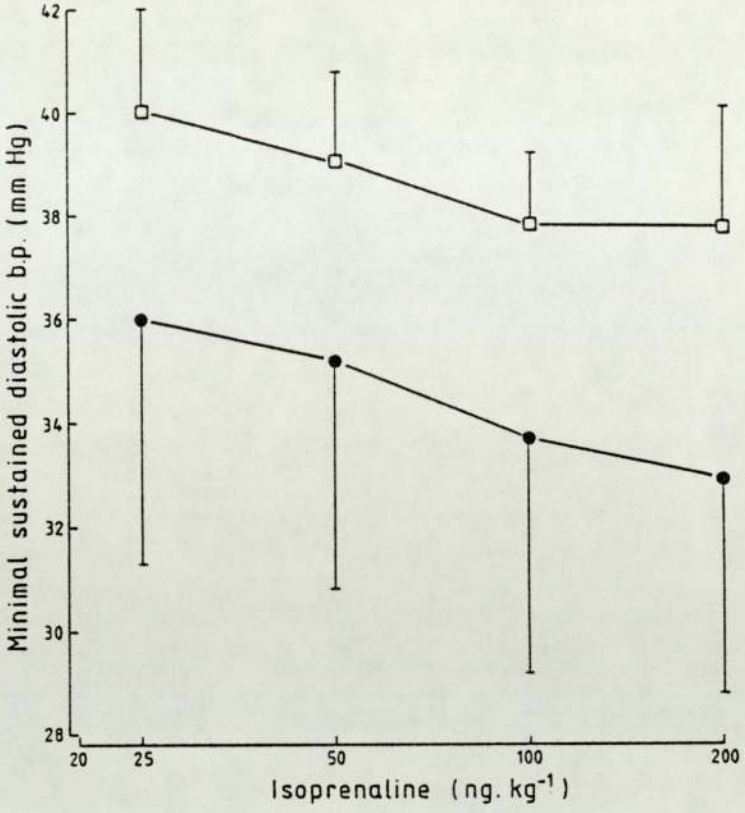

Increase in systolic b.p. (mm Hg)
30
20
10
0
0·001
0·002
0·005
0·01
0·02
*
Vasopressin (iu.kg⁻¹)

Figure 30

Minimal sustained diastolic blood pressure induced by intravenously administered isoprenaline in pithed bilaterally ovariectomised (▫) and control (•) rats. Each point represents the mean, and vertical bars denote the s.e. mean (ovariectomised, n=6; control, n=5).

Figure 31

Decrease in diastolic blood pressure induced by intravenously administered isoprenaline in pithed bilaterally ovariectomised (▫) and control (•) rats. Each point represents the mean, and vertical bars denote the s.e. mean (ovariectomised, n=6; control, n=5).


Minimal sustained diastolic b.p. (mm Hg)
42
40
38
36
34
32
30
28
20
25
50
100
200
Isoprenaline (ng. kg^{-1})

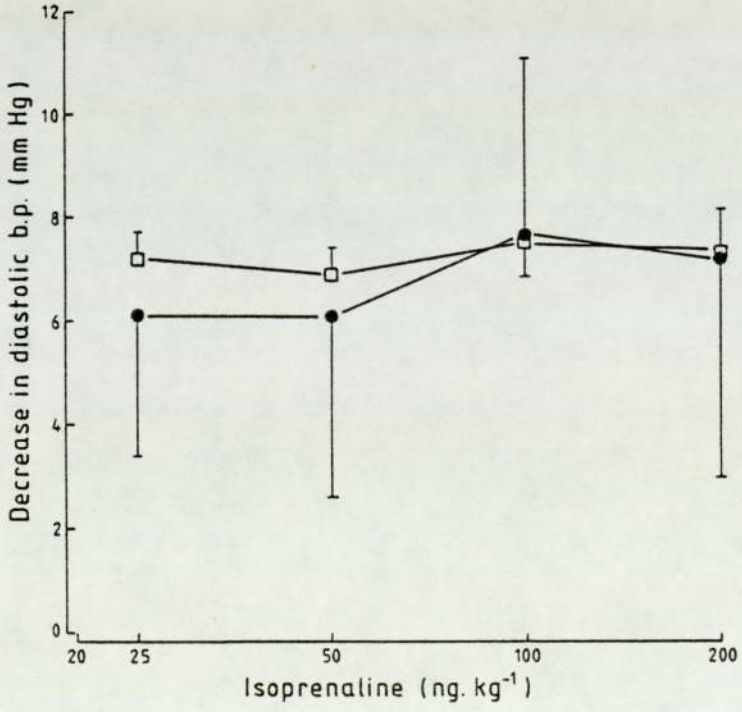
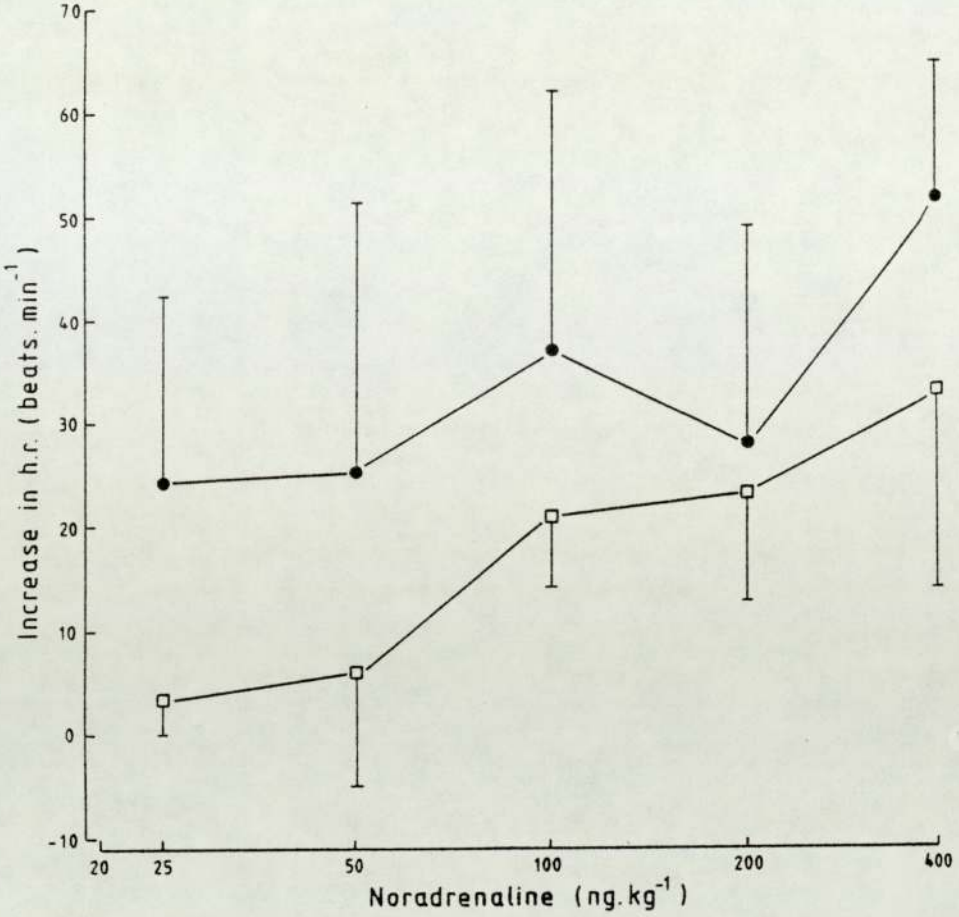

Decrease in diastolic b.p. (mm Hg)
12
10
8
6
4
2
0
20
25
50
100
200
Isoprenaline (ng. kg^{-1})

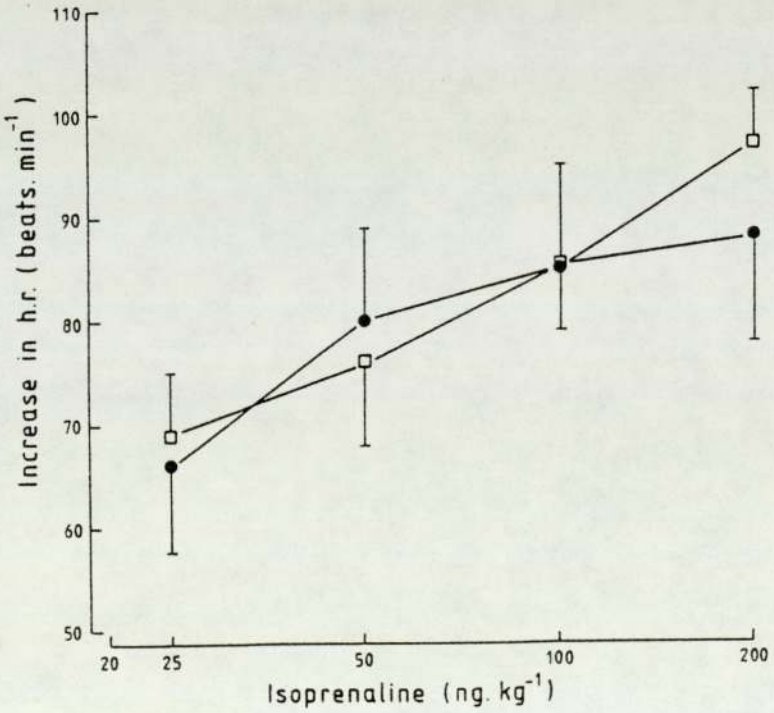
Figure 32

Increase in heart rate induced by intravenously administered noradrenaline in pithed bilaterally ovariectomised (□) and control (•) rats. Each point represents the mean, and vertical bars denote the s.e. mean (ovariectomised, n=6; control, n=5).

Figure 33

Increase in heart rate induced by intravenously administered isoprenaline in pithed bilaterally ovariectomised (□) and control (•) rats. Each point represents the mean, and vertical bars denote the s.e. mean (ovariectomised, n=6; control, n=5).


Increase in h.r. (beats. min^{-1})
70
60
50
40
30
20
10
0
-10
20
25
50
100
200
400
Noradrenaline (ng. kg^{-1})


Increase in h.r. (beats. min^{-1})
110
100
90
80
70
60
50
20
25
50
100
200
Isoprenaline (ng. kg^{-1})

Discussion

Prior to any discussion of the influence of bilateral ovariectomy upon cardiovascular responses, it is necessary to sound a note of caution with regard to interpretation of the findings.

The systolic and diastolic blood pressures of sham-operated oestrous rats were not significantly different to those of sham-operated dioestrous rats. This is similar to the findings of experiment 3, but not experiment 2. Also, the systolic and diastolic blood pressures of oestrous rats were significantly reduced compared with those of oestrous rats in experiment 2, but not significantly different compared with those of oestrous rats in experiment 3 (see table 6, p. 107). It is therefore possible that there is a fundamental difference between sham-operated rats in experiment 4 and rats in experiment 2.

Another difference between the rats used in experiments 2 and 4 was the magnitude of pressor responses. Pressor responses to noradrenaline, angiotensin II and vasopressin were of a similar magnitude in all rats used in experiment 4 and of a lower magnitude compared with those in rats used in experiment 2. Since pressor responses in dioestrous sham-operated rats were of a lower magnitude compared with those of dioestrous rats used in experiment 2 (see table 6, p. 107), although there was no significant difference in resting systolic blood pressure between the two groups of animals, present findings do not support the earlier suggestion (see pages 84 and 98) that the magnitude of pressor responses is dependent upon the resting systolic pressure.

Concentration-response curves to noradrenaline, angiotensin II, and vasopressin were also flatter in experiment 4 compared with

responses in experiment 2 and responses appeared to lie in the threshold region of the curve. The reason for the different observations in experiments 2 and 4 is, as yet, unknown.

There was no significant difference in the concentrations of noradrenaline and angiotensin II required to induce a standard incremental rise in systolic pressure following ovariectomy. Since responses appeared to lie in the threshold region of the curve, it was not possible to determine whether there was any shift in the linear portion of the concentration-response curve to vasopressin following ovariectomy. However, figure 29 (p. 113) indicates that ovariectomised rats may have required a higher concentration of vasopressin, compared with that required by control rats, to elicit a standard pressor response on the linear portion of the concentration-response curve. Bilateral ovariectomy may therefore reduce vasopressin potency. However, it is not possible from present findings to determine whether bilateral ovariectomy has variable effects upon responses to pressor agents. It would be valuable to repeat the present experimental protocol using higher concentrations of pressor agents, to determine whether bilateral ovariectomy alters maximal pressor responses or induces a horizontal shift in the linear portion of the concentration-response curve to vasopressin.

In 1959 (a) Lloyd examined the effect of bilateral ovariectomy upon pressor responses to vasopressin. Lloyd (1959a) also examined the concentration of vasopressin required to elicit an increase in blood pressure of 10 mm Hg. The protocol employed by Lloyd (1959a) was different to that used in present experiments, since she recorded pressor responses for the first eleven days after ovariectomy in anaesthetised rats. During this period, Lloyd (1959a) demonstrated that the concentration of vasopressin required to elicit this standard

pressor response was increased in bilaterally ovariectomised rats. However, Lloyd (1959a) observed that the concentration required to elicit this response returned to pre-ovariectomy levels seven days after surgery. Decrease in the potency of vasopressin observed by Lloyd (1959a) may therefore have been due to the stress of surgery, and not to any alteration in circulating ovarian hormone levels after ovariectomy. Since Lloyd (1959a) did not examine the effect of sham bilateral ovariectomy upon pressor responses to vasopressin, this proposition cannot be verified.

The incremental rise in systolic pressure induced by noradrenaline and angiotensin II were not significantly different in bilaterally ovariectomised, compared with control, rats. Folkow (1971, 1978) has demonstrated that change in the incremental rise in blood pressure is due to alteration in vascular wall structure. Present findings therefore indicate that bilateral ovariectomy does not induce vascular structural alterations.

Administration of isoprenaline to both intact and ovariectomised pithed female rats elicited depressor responses; a finding which indicates that pithing does not lead to complete vascular smooth muscle relaxation in female rats. However, since depressor responses appeared to lie in the maximal region of the concentration-response curve, whereas positive chronotropic responses to isoprenaline may have lain in the linear portion of the curve, the pithed female rat may be more responsive to the depressor, than to the positive chronotropic, action of isoprenaline. Alternatively, it is possible that vascular muscle relaxation is sufficiently extensive after pithing that only a low concentration of isoprenaline is necessary to induce complete vascular smooth muscle relaxation. When isoprenaline was administered to pithed male rats, they showed no change in blood pressure. This may indicate

that pithing leads to complete vascular smooth muscle relaxation in male rats or that male rats are unresponsive to the depressor action of isoprenaline. Since the plasma concentration of ovarian hormones is reduced following bilateral ovariectomy (Rabii & Ganong, 1976), this difference between the responses of male and female rats is not likely to be due to differences in plasma ovarian hormone levels. It would be interesting to investigate the reason for this apparent sex-related difference in depressor responses.

Depressor responses and positive chronotropic responses to isoprenaline were not altered following ovariectomy. These findings indicate that ovariectomy, which is associated with reduced plasma concentrations of 17β-oestradiol (Rabii & Ganong, 1976), does not induce changes in β-adrenoreceptor-mediated responses. Other workers have noted alterations in β-adrenoreceptor-mediated responses subsequent to increases in the plasma concentration of oestrogenic agents in rats. For example, Boyle & Digges (1980) demonstrated reduction in β-adrenoreceptor-mediated responses in pregnant rat uterus and Fregly & Thrasher (1977) observed reduction in positive chronotropic responses to isoprenaline following subcutaneous implantation of ethinyloestradiol in female rats. Also, Ćirić & Sušić (1980) found that, following daily intramuscular injections of 17β-oestradiol, positive chronotropic and depressor responses to isoprenaline were increased. It is therefore possible that only elevation, but not depression, of the normal plasma concentration of oestrogenic agents induces changes in β-adrenoreceptor-mediated responses.

It is interesting to note that the maximal positive chronotropic to isoprenaline may have been increased following bilateral ovariectomy.

Resting heart rate, PQ and QT intervals and blood pressure values were not significantly different following ovariectomy. According to the reasoning of Koch-Weser (1974), this indicates that cardiac output was not altered following ovariectomy. Positive chronotropic responses to noradrenaline, angiotensin II and vasopressin appeared to lie in the threshold region of the concentration-response curve. There was some indication that, following ovariectomy, rats may have required a higher concentration of noradrenaline to elicit a standard positive chronotropic response on the linear portion of the curve. If the same experimental protocol was repeated using higher concentrations of noradrenaline, it may be possible to determine whether bilateral ovariectomy alters maximal positive chronotropic responses to noradrenaline or induces a horizontal shift in the concentration-response curve.

The resting heart rate and PQ and QT intervals were not significantly different in oestrous sham-operated rats compared with dioestrous sham-operated rats. However, the resting PQ interval of oestrous sham-operated rats was significantly longer compared with that of ovariectomised rats although the resting QT interval and heart rate were not significantly different. This finding indicates that the atrial conduction time was increased and the repolarisation time was decreased in oestrous, compared with ovariectomised, rats. Since bilateral ovariectomy is associated with a decrease in the plasma concentration of 17β-oestradiol compared with oestrous rats (Yoshinaga et al., 1969; Rabii & Ganong, 1976), this finding is consistent with the view that the plasma concentration of 17β-oestradiol is inversely related to the speed of atrial conduction and directly related to the speed of cardiac repolarisation.

Experiment 5

Effect of constant administration of 17β-oestradiol upon cardiovascular responses to noradrenaline, angiotensin II and isoprenaline

Introduction

Experiments 2 and 3 were undertaken to determine the influence of short-term, small changes in the plasma concentration of ovarian hormones upon cardiovascular responsiveness. To extend the understanding of the influence of ovarian hormones upon cardiovascular responsiveness, it was decided to examine the effect of changes in the plasma concentration of ovarian hormones which are greater, and of a longer duration, than those which take place during the oestrous cycle. Experiment 4 was therefore performed to determine the effect of bilateral ovariectomy, which is associated with long-term reduction in plasma 17β-oestradiol (Rabii & Ganong, 1976), upon cardiovascular responsiveness. This study has been extended to an examination of the effect of a long-term increase in plasma ovarian hormones upon cardiovascular responsiveness.

Prior to this examination, several decisions had to be made. These included:

a) whether to administer a natural or synthetic hormone,

b) which hormone to administer,

c) the route of hormone administration,

d) the concentration of hormone to be administered and

e) the length of treatment.

Any one of these factors may influence the outcome of the experiment.

a) and b) Since the difference between cardiovascular responses in oestrous and dioestrous rats in experiment 2 may have been due to an alteration in circulating levels of 17β-oestradiol (see page 69) it was decided to administer this natural oestrogenic hormone.

c) It was decided to induce an elevation in plasma 17β-oestradiol which was constant. For this reason, the hormone was administered at zero-order rate from an osmotic minipump implanted subcutaneously.

d) It was decided to raise the plasma concentration of 17β-oestradiol to a level substantially higher than that found during the normal oestrous cycle. Butcher, Collins & Fugo (1974) have shown that the peak concentration of plasma 17β-oestradiol during the rat oestrous cycle is 88 ± 2 pg.ml^{-1}. The plasma concentration of 17β-oestradiol following implantation of one osmotic minipump which administered 1 μg 17β-oestradiol per day into ovariectomised rats has been shown to be 38 ± 3 pg.ml^{-1} (Butcher et al., 1978). Butcher and co-workers (1978) have also shown that administration of twice this amount of 17β-oestradiol (2 μg.day^{-1}) resulted in doubling of the plasma concentration of 17β-oestradiol (84 ± 6 pg.ml^{-1}). In order to raise the plasma concentration of 17β-oestradiol to a level higher than that found during the oestrous cycle, therefore, a concentration of 17β-oestradiol in PEG 300 (758 μg.ml^{-1}) was chosen such that pumps delivered 10 μg 17β-oestradiol per day.

e) The aim of the present study was to determine whether long-term alterations in the plasma concentration of ovarian

e) Cont. hormones induce changes in cardiovascular responsiveness prior to any elevation in blood pressure. In 1976 Gammal showed that treatment of rats with ethinyloestradiol for thirty days induced hypertophic changes in blood vessel walls which may have been the result of hypertension. In order to examine the effects of long-term elevation in plasma 17β-oestradiol upon cardiovascular responses prior to induction of hypertrophic vascular structural changes, 17β-oestradiol was therefore administered for ten days.

Together with constant release of 17β-oestradiol from osmotic minipumps, other methods of administration of oestrogenic agents were attempted. Ethinyloestradiol or 17β-oestradiol were injected subcutaneously for ten days. Unlike administration from minipumps, the pharmacokinetic profile induced by this mode of administration involves variation in the plasma concentration of the administered agent (Butcher et al., 1978). Subcutaneous administration of these oestrogenic agents appeared to alter the responses of female rats to genereal anaesthesia induced prior to pithing. Spinal reflexes in rats anaesthetised with either sodium pentobarbitone, urethane or halothane were depressed to a lesser extent in rats pre-treated with oestrogenic agents compared with controls. In order to induce general anaesthesia at a stage suitable for pithing, therefore, it was necessary to administer higher concentrations of the anaesthetic agent. However, pre-treatment with oestrogenic agents was associated with greater depression in respiration following administration of anaesthetic agents compared with controls. For this reason, administration of a higher concentration of anaesthetic agent frequently led to death in the pre-treated rats. Present experiments were possible since alterations in

response to general anaesthesia were not so apparent when 17β-oestradiol was administered from osmotic minipumps.

Rats may not have survived the entire length of an experiment if cardiovascular responses to noradrenaline, angiotensin II, vasopressin and isoprenaline were examined. Because of the expense and difficulty in obtaining osmotic minipumps, it was decided to examine the effects of pre-treatment with 17β-oestradiol upon responses to three agents. Since the pressor action of vasopressin is not physiologically significant (Nakano, 1973), its use was suspended in present experiments.

Results

Rats in dioestrus were weighed and implanted with an Alzet osmotic minipump containing either 17β-oestradiol in PEG 300 (758 $\mu g.ml^{-1}$) or PEG 300 alone (control). After 10 days the rats were weighed again and pithed. The blood pressure, heart rate and PQ and QT intervals were then recorded. Table 8a (p. 130) shows the body weight of rats before implantation of minipumps, the weight of rats prior to pithing and the increases in weight during the 10 day implantation period for both 17β-oestradiol pre-treated and control rats. It may be seen that the body weight of rats before implantation, just prior to pithing, and the increases in weight during the implantation period were not significantly different in 17β-oestradiol pre-treated rats compared with control rats ($P>0.05$ throughout). Table 8b (p. 130) shows the resting blood pressure, heart rate and also the PQ and QT intervals in 17β-oestradiol pre-treated and control rats. Resting systolic blood pressure was significantly lower in rats pre-treated

with 17β-oestradiol (34.2 ± 2.5 mm Hg, n=6) compared with control rats (48.2 ± 5.1 mm Hg, n=6)($P<0.05$). Differences in diastolic pressure and pulse pressure between 17β-oestradiol pre-treated and control rats were not significant ($P>0.05$ throughout). The resting heart rate of rats pre-treated with 17β-oestradiol was significantly lower (236.7 ± 12.3 min^{-1}, n=6) compared with the heart rate of control rats (291.7 ± 16.8 min^{-1}, n=6)($P<0.025$). The difference in resting PQ interval between 17β-oestradiol pre-treated and control rats was not significant ($P>0.8$). Similarly, the difference in resting QT interval between 17β-oestradiol pre-treated and control rats was not significant ($P>0.8$).

Each rat was then injected intravenously with noradrenaline (25-400 ng.kg^{-1}), angiotensin II (6.25-200 ng.kg^{-1}) and isoprenaline (25-200 ng.kg^{-1}) and the blood pressure responses, positive chronotropic responses and PQ and QT intervals were recorded. Pressor responses to noradrenaline and angiotensin II were recorded both as the maximal sustained systolic pressure and also as the increase in systolic pressure. Depressor responses to isoprenaline were recorded both as the minimal sustained diastolic pressure and also as the decrease in diastolic pressure. Positive chronotropic responses were recorded both as the maximal sustained heart rate and also as the increase in heart rate.

Figure 34 (p. 132) shows the maximal sustained systolic blood pressure induced by geometrically increasing concentrations of intravenously administered noradrenaline in 17β-oestradiol pre-treated and control rats. At all concentrations of noradrenaline, the maximal sustained systolic pressure was significantly lower in rats pre-treated with 17β-oestradiol compared with control rats ($P<0.025$

throughout). Similarly, figure 36 (p. 133) shows the maximal sustained systolic blood pressure induced by geometrically increasing concentrations of intravenously administered angiotensin II in 17β-oestradiol pre-treated and control rats. With the exception of the lowest and highest concentrations of angiotensin II (6.25 and 200 $ng.kg^{-1}$), the maximal sustained systolic pressure was significantly lower in rats pre-treated with 17β-oestradiol compared with control rats ($P<0.05$ throughout).

Figure 35 (p. 132) shows pressor responses, expressed as the increase in systolic pressure, induced by geometrically increasing concentrations of intravenously administered noradrenaline, in 17β-oestradiol pre-treated and control rats. With the exception of the lowest concentration of noradrenaline (25 $ng.kg^{-1}$), pressor responses were significantly lower in 17β-oestradiol pre-treated rats compared with control rats when responses were expressed as the increase in systolic pressure ($P<0.025$ throughout). Similarly, figure 37 (p. 133) shows pressor responses, expressed as the increase in systolic pressure, induced by geometrically increasing concentrations of intravenously administered angiotensin II, in 17β-oestradiol pre-treated and control rats. No significant differences were recorded between the pressor responses of 17β-oestradiol pre-treated and control rats to angiotensin II when responses were expressed as the increase in systolic pressure ($P>0.5$ throughout).

An increase in systolic pressure of 7.5 mm Hg lay on the concentration-response curves to noradrenaline and to angiotensin II. The concentrations of noradrenaline and angiotensin II required to elicit this standard rise in systolic pressure were compared in 17β-oestradiol pre-treated and control rats. These concentrations and

concentration-ratios are shown in table 9 (p. 131). The concentration of noradrenaline required to elicit this standard pressor response was significantly higher in 17β-oestradiol pre-treated rats (283.67 ± 55.67 $ng.kg^{-1}$, n=6) compared with control rats (86.50 ± 15.80 $ng.kg^{-1}$, n=6) ($P<0.01$). However, the concentration of angiotensin II required by 17β-oestradiol pre-treated rats and control rats to elicit this standard pressor response were not significantly different ($P>0.1$). It is possible that an increase of 7.5 mm Hg was not at the $E.C._{50}$ level. However, it was not possible to calculate the concentration-ratio at a greater response level. It is possible that any right shift in the concentration-response curves to noradrenaline and to angiotensin II following 17β-oestradiol pre-treatment would be more apparent at a greater response level.

Figure 38 (p. 134) shows the depressor responses, expressed as the minimal sustained diastolic pressure, induced by geometrically increasing concentrations of intravenously administered isoprenaline in 17β-oestradiol pre-treated and control rats. At the two lowest concentrations of isoprenaline (25 and 50 $ng.kg^{-1}$), the minimal sustained diastolic pressure was significantly lower in rats pre-treated with 17β-oestradiol compared with control rats ($P<0.05$). Figure 39 (p. 134) shows the depressor responses, expressed as the decrease in diastolic pressure, induced by geometrically increasing concentrations of isoprenaline in 17β-oestradiol pre-treated and control rats. At the two lowest concentrations of isoprenaline (25 and 50 $ng.kg^{-1}$), the depressor responses, expressed as the decrease in diastolic pressure, were significantly lower in rats pre-treated with 17β-oestradiol compared with control rats ($P<0.05$). Depressor responses to isoprenaline appeared to lie in the maximal region of the

concentration-response curve. For this reason, no comparison was made of the concentrations of isoprenaline required to elicit the same depressor response in 17β-oestradiol pre-treated and control rats.

At all concentrations of noradrenaline and angiotensin II, positive chronotropic responses, expressed as the maximal sustained heart rate and as the increase in heart rate, did not differ significantly between 17β-oestradiol pre-treated and control rats ($P>0.05$ throughout). Figure 40 (p. 135) shows the increase in heart rate, induced by geometrically increasing concentrations of noradrenaline, in 17β-oestradiol pre-treated and control rats. Positive chronotropic responses to noradrenaline and angiotensin II lay in the threshold region of the concentration-response curves. Horizontal shifts in the concentration-response curves to noradrenaline and angiotensin II were therefore not measured.

At all concentrations of isoprenaline, positive chronotropic responses, expressed as the maximal sustained heart rate and as the increase in heart rate, were significantly lower in rats pre-treated with 17β-oestradiol compared with control rats ($P<0.05$ throughout). This is illustrated in figure 41 (p. 135) which shows the increase in heart rate, induced by geometrically increasing concentrations of isoprenaline, in 17β-oestradiol pre-treated and control rats. Positive chronotropic responses to isoprenaline appeared to lie in the maximal region of the concentration-response curve. For this reason, no comparison was made of the concentrations of isoprenaline required to elicit the same positive chronotropic response in 17β-oestradiol pre-treated and control rats.

At all concentrations of noradrenaline and angiotensin II, the PQ intervals of 17β-oestradiol pre-treated rats and of control rats

were not significantly different ($P>0.1$ throughout). Following injections of 50 and 100 $ng.kg^{-1}$ isoprenaline, the PQ intervals of rats pre-treated with 17β-oestradiol were significantly longer than those of control rats ($P<0.05$ throughout).

At all concentrations of noradrenaline, angiotensin II and isoprenaline, the QT intervals of 17β-oestradiol pre-treated rats and of control rats were not significantly different ($P>0.1$ throughout).

TABLE 8 - The body weight of rats prior to minipump implantation, together with recordings of body weight, blood pressure, heart rate and PQ and QT intervals in pithed female rats following 10 days' minipump implantation.

Parameter	Minipump Contents: PEG 300 (6)	17βE (6)	P
a) Weight			
Day 1 (g)	221.7± 9.6	203.3± 2.5	0.05-0.1
Day 10 (g)	236.7±10.5	213.3± 6.1	0.05-0.1
Increase (g)	15.0± 6.2	11.7± 3.4	0.6 -0.7
b) After pithing and atropine sulphate administration			
Systolic b.p. (mm Hg)	48.2± 5.1	34.2± 2.5	0.025-0.05*
Diastolic b.p. (mm Hg)	37.8± 4.2	28.0± 2.9	0.05-0.1
Pulse pressure (mm Hg)	10.3± 1.4	6.2± 1.4	0.05-0.1
M.A.P. (mm Hg)	41.3± 4.5	30.1± 2.7	0.05-0.1
Heart rate (min^{-1})	291.7±16.8	236.7±12.3	0.02 -0.025*
PQ interval (ms)	35.0± 2.0	35.8± 4.1	0.8 -0.9
QT interval (ms)	92.5± 3.4	95.0±11.8	0.8-0.9

Number of observations in parentheses.

Results are mean±s.e. mean.

* Indicates statistical significant difference.

E - Oestradiol.

Day 1 - Day of minipump implantation.

Day 10 - Day of sacrifice.

b.p. - Blood pressure.

M.A.P. - Mean arterial pressure.

TABLE 9 - Concentration of agents required to elicit an increase in systolic blood pressure of 7.5 mm Hg in pithed female rats following 10 days' minipump implantation.

	Minipump Contents			
Agent	PEG 300 (6)	17βE (6)	P	Concn-ratio
NA ($ng.kg^{-1}$)	86.50 ± 15.80	283.67 ± 55.67	0.005-0.01*	3.28
A II ($ng.kg^{-1}$)	52.50 ± 24.32	115.67 ± 28.60	0.1 -0.2	2.20

Number of observations in parentheses.

Results are mean $\pm$ s.e. mean.

* Indicates statistical significant difference.

Concn-ratio is the ratio of the mean concentrations in 17β-oestradiol pre-treated and control rats.

E - Oestradiol.

NA - Noradrenaline.

A II - Angiotensin II.

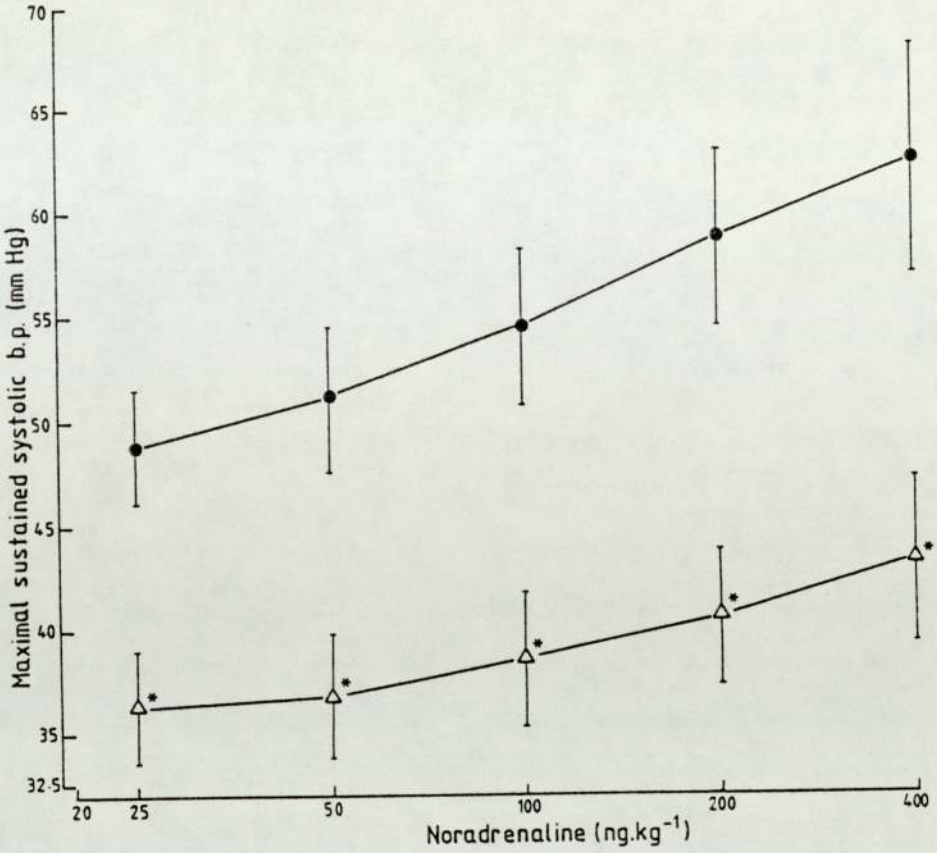
Figure 34

Maximal sustained systolic blood pressure induced by intravenously administered noradrenaline in pithed female rats implanted with minipumps containing either 17β-oestradiol in PEG 300 (758 $\mu g.ml^{-1}$)(Δ) or PEG 300 alone (control)($\bullet$). Each point represents the mean, and vertical bars denote the s.e. mean (n=6).

* Indicates statistical significant difference.

Figure 35

Increase in systolic blood pressure induced by intravenously administered noradrenaline in pithed female rats implanted with minipumps containing either 17β-oestradiol in PEG 300 (758 $\mu g.ml^{-1}$)(Δ) or PEG 300 alone (control)($\bullet$). Each point represents the mean, and vertical bars denote the s.e. mean (n=6).


Maximal sustained systolic b.p. (mm Hg)
70
65
60
55
50
45
40
35
32·5
20
25
50
100
200
400
Noradrenaline (ng.kg⁻¹)

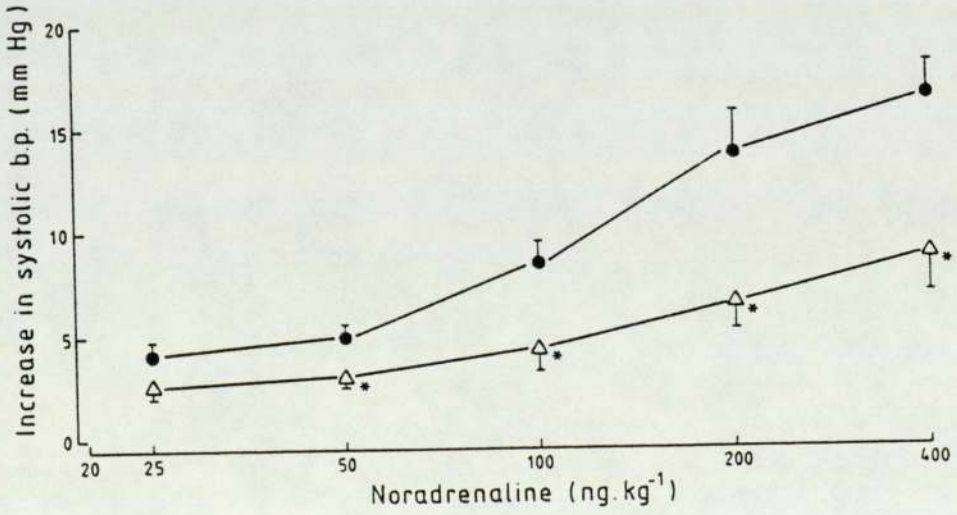

Increase in systolic b.p. (mm Hg)
20
15
10
5
0
20
25
50
100
200
400
Noradrenaline (ng.kg⁻¹)

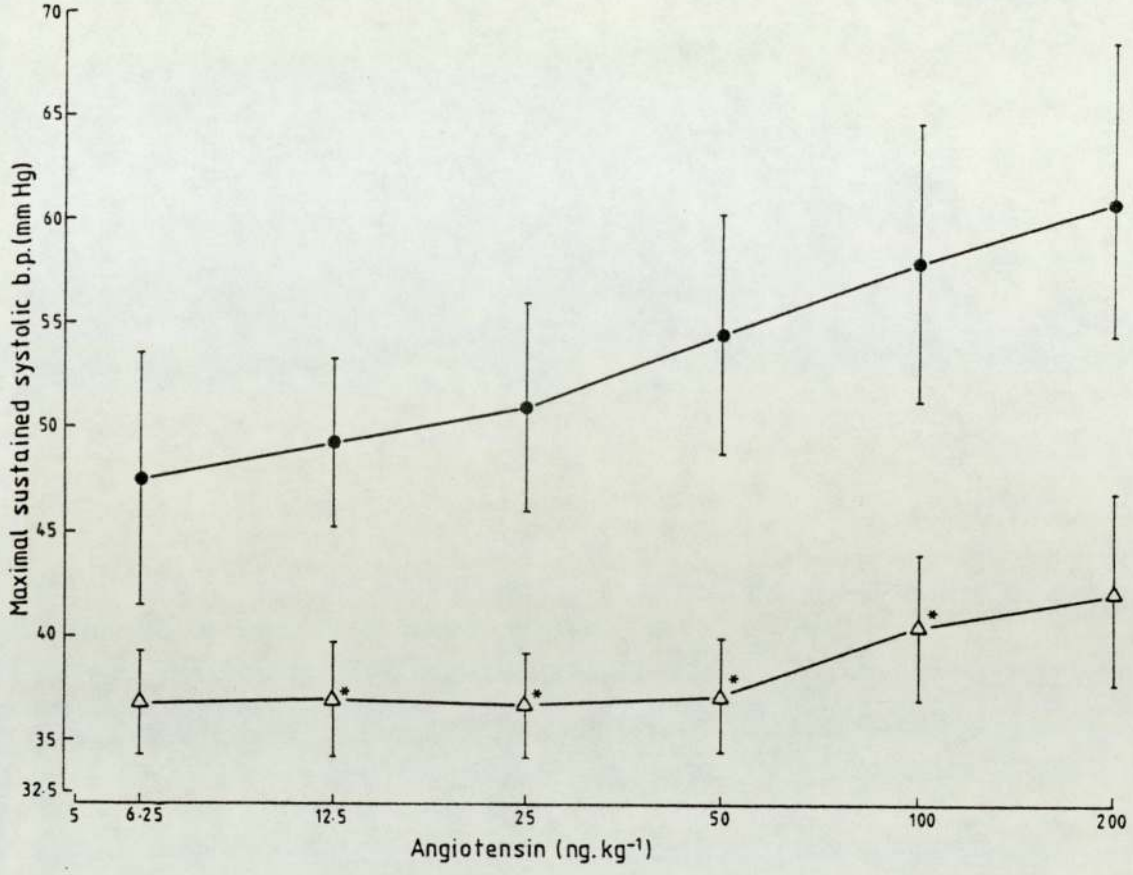
Figure 36

Maximal sustained systolic blood pressure induced by intravenously administered angiotensin II in pithed female rats implanted with minipumps containing either 17β-oestradiol in PEG 300 (758 $\mu g.ml^{-1}$)(Δ) or PEG 300 alone (control)(•). Each point represents the mean, and vertical bars denote the s.e. mean (n=6).

* Indicates statistical significant difference.

Figure 37

Increase in systolic blood pressure induced by intravenously administered angiotensin II in pithed female rats implanted with minipumps containing either 17β-oestradiol in PEG 300 (758 $\mu g.ml^{-1}$)(Δ) or PEG 300 alone (control)(•). Each point represents the mean, and vertical bars denote the s.e. mean (n=6).


Maximal sustained systolic b.p.(mm Hg)
70
65
60
55
50
45
40
35
32·5
5
6·25
12·5
25
50
100
200
Angiotensin (ng.kg⁻¹)
*
*
*
*

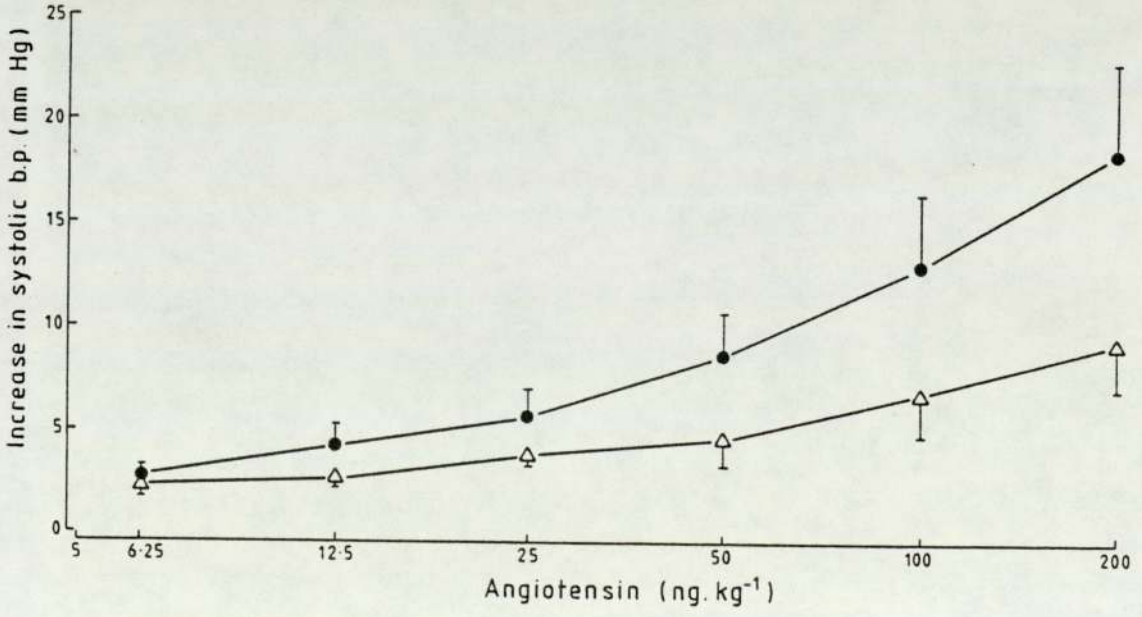

Increase in systolic b.p. (mm Hg)
25
20
15
10
5
0
5
6·25
12·5
25
50
100
200
Angiotensin (ng.kg⁻¹)

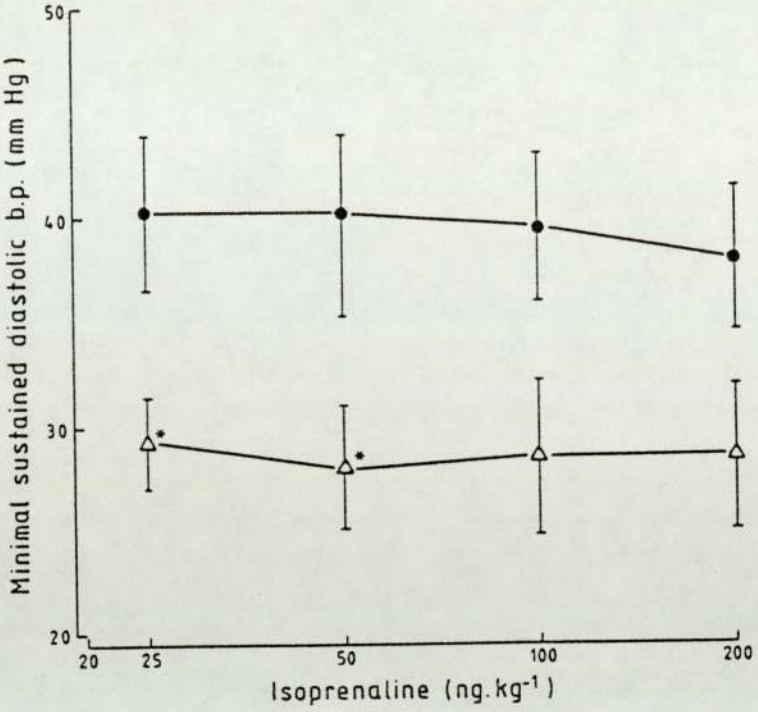
Figure 38

Minimal sustained diastolic blood pressure induced by intravenously administered isoprenaline in pithed female rats implanted with minipumps containing either 17β-oestradiol in PEG 300 (758 $\mu g.ml^{-1}$)(Δ) or PEG 300 alone (control)(•). Each point represents the mean, and vertical bars denote the s.e. mean (n=6).

* Indicates statistical significant difference.

Figure 39

Decrease in diastolic blood pressure induced by intravenously administered isoprenaline in pithed female rats implanted with minipumps containing either 17β-oestradiol in PEG 300 (758 $\mu g.ml^{-1}$)(Δ) or PEG 300 alone (control)(•). Each point represents the mean, and vertical bars denote the s.e. mean (n=6).


Minimal sustained diastolic b.p. (mm Hg)
50
40
30
20
20
25
50
100
200
Isoprenaline (ng.kg^{-1})

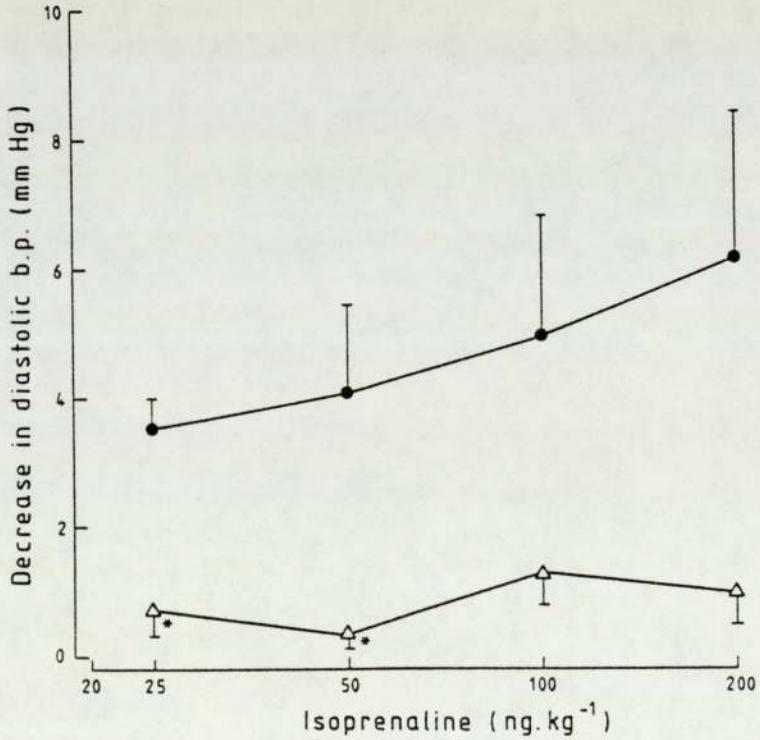

Decrease in diastolic b.p. (mm Hg)
10
8
6
4
2
0
20
25
50
100
200
Isoprenaline (ng.kg^{-1})

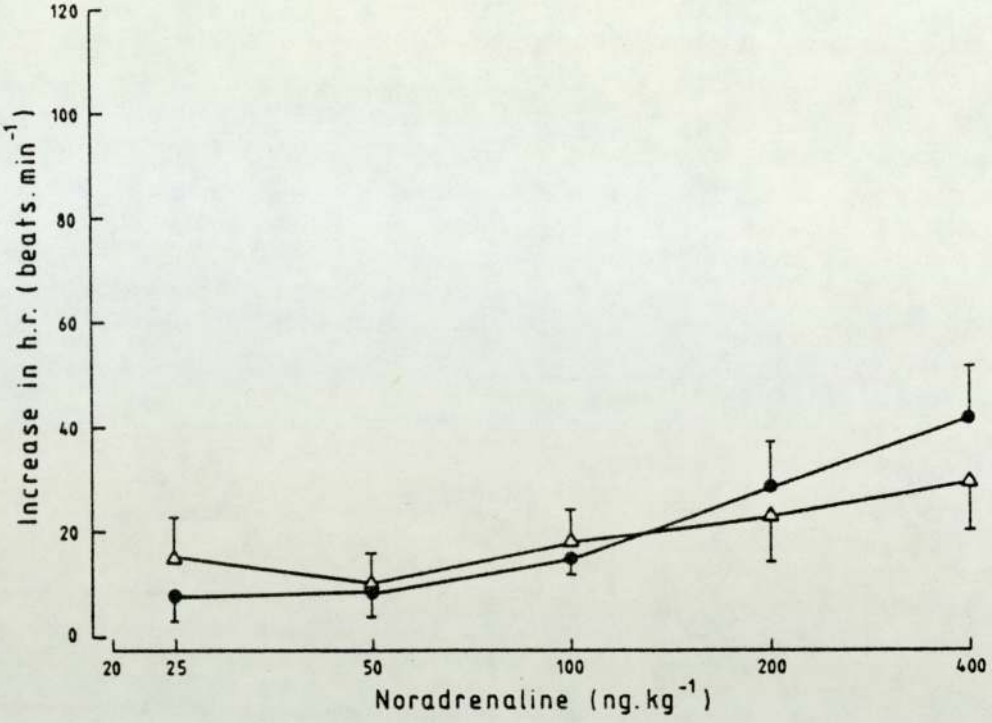
Figure 40

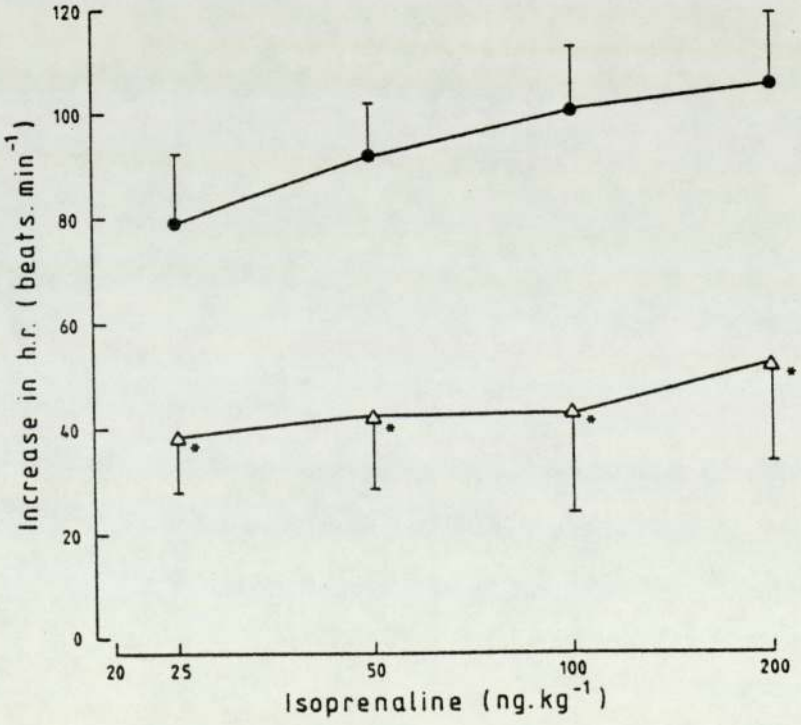
Increase in heart rate induced by intravenously administered noradrenaline in pithed female rats implanted with minipumps containing either 17β-oestradiol in PEG 300 (758 $\mu g.ml^{-1}$) (Δ) or PEG 300 alone (control)($\bullet$). Each point represents the mean, and vertical bars denote the s.e. mean (n=6).

Figure 41

Increase in heart rate induced by intravenously administered isoprenaline in pithed female rats implanted with minipumps containing either 17β-oestradiol in PEG 300 (758 $\mu g.ml^{-1}$) (Δ) or PEG 300 alone (control)($\bullet$). Each point represents the mean, and vertical bars denote the s.e. mean (n=6).

* Indicates statistical significant difference.


Increase in h.r. (beats.min^{-1})
120
100
80
60
40
20
0
20
25
50
100
200
400
Noradrenaline (ng.kg^{-1})


Increase in h.r. (beats.min^{-1})
120
100
80
60
40
20
0
20
25
50
100
200
Isoprenaline (ng.kg^{-1})

Discussion

Present experiments were performed to examine the possibility that elevation of plasma 17β-oestradiol was responsible for the increase in systolic blood pressure and potentiation of pressor responses observed during oestrus in experiment 2. However, there is some reason to believe that rats used in experiments 2 and 5 may be fundamentally different, since the resting blood pressure was lower in rats in experiment 5 compared with rats in experiment 2. Control rats in present experiments were pre-treated with PEG 300; some were in oestrus and some were in dioestrus. The systolic blood pressure was significantly higher in oestrus rats in experiment 2 compared with the systolic blood pressure in control rats in experiment 5. Also, diastolic blood pressure was significantly higher in oestrous and dioestrous rats in experiment 2 compared with the diastolic blood pressure in control rats in experiment 5. For this reason, the results of experiments 2 and 5 were not compared.

Following administration of 17β-oestradiol from osmotic minipumps, the systolic blood pressure of pithed female rats was reduced. However, when Fischer & Swain (1980) injected 10 μg 17β-oestradiol intramuscularly at weekly intervals into ovariectomised rats, systolic pressure was raised. Reduction in systolic pressure, observed in present experiments, cannot be attributed to the particular oestrogenic agent administered, since Fischer & Swain (1980) also administered 17β-oestradiol. Present findings may, therefore, be ascribed to the very large amount of agent administered in present experiments (10 $\mu g.day^{-1}$), since Fischer & Swain (1980) gave only 10 μg 17β-oestradiol per week. Alternatively, it is possible that

the difference between present experimental findings and those of Fischer & Swain (1980) is due to the fact that present experimental rats were pithed, while those used by Fischer & Swain (1980) were conscious. However, it appears unlikely that pithing could account for the reduction in systolic pressure observed in present experiments. The observations of Fischer & Swain (1980) may also be attributable to the mode of hormone administration.

Several other studies have shown that the systolic blood pressure of female rats may be elevated or maintained following administration of oestrogenic agents. When Lew (1975) administered seven daily intraperitoneal injections of either diethylstilboestrol (0.25 mg.100 g body weight^{-1}) or mestranol (5 µg.100 g body weight^{-1}) to female rats he observed an increase in systolic pressure. Hoeg, Willis & Weinberger (1977) were not able to demonstrate any alteration in the blood pressure of normotensive rats following daily subcutaneous injections of 0.05 mg mestranol. Similarly, Douglas (1974) observed no change in the resting systolic pressure of female rats administered mestranol in the diet. Administration of oestrogenic agents from osmotic minipumps has been shown to induce constant plasma concentrations of the agent (Theeuwes & Yum, 1977; Butcher et al., 1978), whereas other routes of administration induce a different pharmacokinetic profile characterised by variation in plasma concentration of the administered agent (Butcher et al., 1978; Stuyker-Boudier & Smits, 1978; Theeuwes, 1981). It is therefore possible that the effect of an administered oestrogenic agent upon the blood pressure of rats is dependent upon the pharmacokinetic profile induced by the mode of administration. Thus variation in plasma concentration may be associated with increase or no change in systolic

pressure and constant high plasma concentrations may be associated with reduction in systolic pressure. This would provide an explanation for the reduction in systolic pressure observed in present experiments and the elevation in systolic pressure observed by Fischer & Swain (1980) may have been the result of variation in plasma 17β-oestradiol levels following intramuscular administration.

The systolic blood pressure of rats pre-treated with 17β-oestradiol was lower than that of control rats. However, there was no alteration in diastolic pressure following administration of 17β-oestradiol. Koch-Weser (1974) has suggested that elevation of systolic blood pressure without change in diastolic pressure may be due to an increase in cardiac output. By inference, therefore, decrease in systolic pressure without change in diastolic pressure may be due to reduction in cardiac output. Since heart rate was also decreased in 17β-oestradiol pre-treated rats, there is no direct evidence that stroke volume is reduced following pre-treatment. However, this possibility cannot be precluded.

In present experiments, pressor responses, expressed as the incremental rise in systolic pressure, to noradrenaline and to angiotensin II, appeared to lie in the threshold region of the concentration-response curve in control, and 17β-oestradiol pre-treated, rats. However, pressor responses, expressed as the incremental rise in systolic pressure, to noradrenaline and to angiotensin II, appeared to lie in the linear portion of the concentration-response curves following bilateral ovariectomy and sham bilateral ovariectomy. Since the resting systolic pressure was not significantly different in PEG 300 pre-treated (control) rats compared with sham bilaterally ovariectomised (control) rats, this finding is consistent with the view (see page 116) that incremental rise in systolic pressure is not dependent upon the

resting systolic pressure.

The incremental rise in systolic blood pressure to all but the lowest concentration of noradrenaline was reduced in 17β-oestradiol pre-treated rats compared with control rats. Also, the concentration of noradrenaline required to elicit a standard pressor response was elevated following 17β-oestradiol pre-treatment. However, there was no difference in the incremental rise in systolic pressure induced by angiotensin II in 17β-oestradiol pre-treated and control rats. Similarly, there was no alteration in the concentration of angiotensin II required to elicit a standard pressor response following 17β-oestradiol pre-treatment. Since these findings appear to indicate variety in the effects of 17β-oestradiol upon pressor responses, there is some reason to believe that 17β-oestradiol administration does not have a generalised effect upon blood vessel walls. If this is true, pre-treatment with 17β-oestradiol may alter the elimination or metabolism of noradrenaline or affect noradrenaline/receptor interaction.

It is possible that 17β-oestradiol administration increases the activity of COMT, since this enzyme is responsible for metabolism of extraneuronal noradrenaline (Trendelenburg, 1977; Vanhoutte, 1978a). Alternatively, the α-/β_2-adrenoreceptor ratio may be decreased by an increase in the plasma concentration of ovarian hormones in the rat. Evidence that an alteration in the plasma concentration of ovarian hormones induces a change in smooth muscle α-/β-adrenoreceptor ratio has been provided by Boyle & Digges (1980) who suggested that pregnancy is associated with an increase in α-/β-adrenoreceptor ratio in rat uterus. However, it may not be reasonable to compare the present findings with those of Boyle & Digges (1980) since the plasma profile of ovarian hormones induced by constant 17β-oestradiol administration is unlikely

to be the same as that induced by pregnancy. Also, it is possible that rat uterus and vascular smooth muscle respond differently to changes in plasma ovarian hormones. To examine the possibility that the ratio of α- and β_2-adrenoreceptors is altered following 17β-oestradiol pre-treatment, it would be necessary to selectively block α- and then β_2-adrenoreceptors. Unfortunately, insufficient minipumps were available to determine the effect upon α- and β_2-adrenoreceptor-mediated responses of an increase in the concentration of 17β-oestradiol to consistent and high levels. It is also unfortunate that the results of experiments designed to determine the action of the oestrous cycle and ovariectomy upon pressor responses to noradrenaline after β_2-adreno-receptor blockade with propranolol were difficult to interpret.

Present findings appear to be similar to those of Hettiaratchi & Pickford (1968). Hettiaratchi & Pickford (1968) observed no change in the incremental rise in blood pressure produced by three standard concentrations of angiotensin II twenty four hours after subcutaneous administration of 0.15 µg oestradiol. However, pressor responses recorded in present experiments were in the threshold region of the curve and it appears from figure 37 (p. 133) that there may have been a reduction in response to angiotensin II in the linear portion of the curve following 17β-oestradiol pre-treatment. It also seems possible that the potency of angiotensin II may have been reduced in the linear portion of the curve.

Interpretation of present findings is complicated by the view that there is an inverse relationship between the plasma concentration of angiotensin II and pressor responses to exogenous angiotensin II (Devynck & Meyer, 1976), since it is generally believed that the plasma concentration of angiotensin II may be elevated by administration of

oestrogenic agents (see review by Fregly & Fregly, 1977). In order, therefore, to examine the effect of alteration in the plasma concentration of ovarian hormones upon pressor responses to angiotensin II without the influence of endogenous angiotensin II, the converting enzyme inhibitor, SQ 20881, was administered. Unfortunately, results were difficult to interpret, since this compound has a half life of only nine minutes. In future it may be possible to repeat these experiments with one of the more modern, and longer lasting, converting enzyme inhibitors, for example, Captopril.

To summarise the present findings, it appears that constant 17β-oestradiol administration does not induce a generalised reduction in vascular responsiveness. However, since pressor responses were not recorded in the linear portion of the concentration-response curve, this possibility cannot be precluded.

Depressor responses to isoprenaline appeared to lie in the maximal region of the curve and the decrease in diastolic pressure induced by isoprenaline was reduced following 17β-oestradiol pre-treatment. It is therefore possible that 17β-oestradiol may reduce β_2-adrenoreceptor-mediated responses. However, this seems unlikely, since such a reduction would have led to potentiation of noradrenaline-induced pressor responses. The minimal sustained diastolic pressure to isoprenaline was also reduced following 17β-oestradiol pre-treatment. Since the responses appeared to lie in the maximal region of the curve, this finding indicates that 17β-oestradiol may have induced vascular structural alterations. Another possibility, suggested above, is that 17β-oestradiol administration may lead to a generalised reduction in vascular responsiveness. Such a reduction in responsiveness may be the result of an increase in membrane stabilisation. However, the

effect of 17β-oestradiol pre-treatment upon noradrenaline- and angiotensin II-induced pressor responses is debated and it is interesting to speculate whether the magnitude of depressor responses is related to the resting systolic pressure, since this was also reduced following 17β-oestradiol pre-treatment.

Positive chronotropic responses to noradrenaline, recorded either as the maximal sustained heart rate or as the increase in heart rate, were not significantly different in 17β-oestradiol pre-treated rats compared with control rats. Unfortunately, positive chronotropic responses to noradrenaline lay in the threshold region of the concentration-response curve and it was not possible to determine whether there was a horizontal shift in the concentration-response curve following pre-treatment with 17β-oestradiol.

Positive chronotropic responses to isoprenaline appeared to lie in the maximal region of the concentration-response curve and they were reduced in 17β-oestradiol pre-treated rats compared with control rats. These findings may be explained by: a) reduction in the speed of cardiac conduction or contraction or b) attenuation in β_1-adreno-receptor-mediated responses.

Electrocardiogram recordings gave some indication of a reduction in the speed of atrial conduction following 17β-oestradiol pre-treatment. Although the QT interval was not altered following 17β-oestradiol pre-treatment, the PQ interval was shown to be longer in 17β-oestradiol pre-treated rats compared with control rats at two concentrations of isoprenaline. Pre-treatment with 17β-oestradiol therefore appeared to reduce the atrial conduction rate. This alteration in atrial conduction rate was not observed following

noradrenaline administration. However, it is possible that this change is not observed in the threshold region of the concentration-response curve.

Together with constant release of 17β-oestradiol from osmotic minipumps, other methods of administration of oestrogenic agents were attempted (see page 123). However, although most rats pre-treated with 17β-oestradiol by daily subcutaneous injections died following anaesthesia, many which survived demonstrated atrio-ventricular heart block after pithing. This is identified by lengthening or removal of the PQ interval (Macruz, Perloff & Case, 1958) and in several rats this led to death. It is interesting to note that the findings of experiment 4 also indicate that an increase in the plasma concentration of 17β-oestradiol may be associated with lengthening of the PQ interval (see page 120). Macruz and co-workers (1958) have shown that prolongation of the PQ interval may be evidence of right atrial enlargement. However, since resting systolic pressure was reduced following 17β-oestradiol pre-treatment, it appears unlikely that lengthening of the PQ interval is due to atrial enlargement. Evidence that atria may be particularly susceptible to 17β-oestradiol has been provided by Stumpf and co-workers (1977) who observed that the atria of male and female rats concentrate this oestrogen, whereas ventricles do not. To summarise, constant 17β-oestradiol administration may reduce atrial conduction speed, which may lead to reduction in positive chronotropic responses to isoprenaline.

Alternatively, 17β-oestradiol may reduce positive chronotropic responses to isoprenaline by attenuating β_1-adrenoreceptor-mediated responses. Further support for the suggestion that constant and high plasma concentrations of an oestrogenic agent reduce β-adrenoreceptor-

mediated responses in female rats is provided by the findings of Fregly & Thrasher (1977) and Ćirić & Sušić (1980). Fregly & Thrasher (1977) observed reduction in positive chronotropic responses to isoprenaline following subcutaneous implantation of ethinyloestradiol in silastic tubing, a procedure which induces reasonably constant plasma concentrations of this oestrogenic agent (Fregly, Thrasher, MacArthur & Kelleher, 1978). However, administration of 17β-oestradiol by daily intramuscular injections, which is characterised by variation in the plasma concentration of the administered agent (Theeuwes, 1981), was found by Ćirić & Sušić (1980) to potentiate positive chronotropic and depressor responses to isoprenaline. These findings are consistent with the suggestion that constant elevation in the plasma concentration of oestrogenic agents induces a reduction in β-adrenoreceptor-mediated responses and that variation in the concentration in plasma oestrogenic agents induces elevation in β-adrenoreceptor-mediated responses.

Human pregnancy is another situation which is associated with reasonably constant, but elevated, concentrations of plasma 17β-oestradiol (Patrick, Challis, Natale & Richardson, 1979; Challis, 1980) together with reduced systolic pressure (MacGillivray, Rose & Rowe, 1969; Welt & Crenshaw, 1978) and reduced cardiac output (Goodrich & Wood, 1964, 1966). Administration of 17β-oestradiol from osmotic minipumps to female rats therefore appeared to induce similar changes in the cardiovascular system as those observed during human pregnancy. Since constant and high plasma concentrations of 17β-oestradiol may be responsible for reduction in systolic pressure and cardiac output in female rats, constant elevation in the plasma concentration of 17β-oestradiol may be responsible for reduction in systolic pressure and cardiac output during human pregnancy. However, results from this

study show that the incremental rise in blood pressure to exogenous noradrenaline is attenuated by constant administration of 17β-oestradiol to female rats, whereas Chesley and co-workers (1965) found no change in the incremental rise in blood pressure to exogenous noradrenaline in pregnant women compared with non-pregnant women. Since the plasma concentration of progesterone and 17β-oestradiol are elevated during pregnancy (Ryan, 1973; Challis, 1980), the lack of change in pressor responses to noradrenaline during pregnancy observed by Chesley and co-workers (1965) may have been due to this high concentration of progesterone.

Present findings indicate that the incremental rise in blood pressure induced by angiotensin II administration is not altered following 17β-oestradiol pre-treatment. Gant and co-workers (1980) have reviewed the evidence which suggests that the incremental rise in blood pressure induced by angiotensin II is attenuated in pregnant women. However, it is interesting to note that Everett and co-workers (1978) have provided evidence that it is the elevation in plasma progesterone which induces this refractoriness to the pressor action of angiotensin II during human pregnancy. It is therefore possible that concomitant administration of 17β-oestradiol and progesterone from osmotic minipumps would reduce pressor responses to angiotensin II in female rats. If this was the case, concomitant administration of 17β-oestradiol and progesterone would be a more suitable model for the effect of human pregnancy upon the cardiovascular system than administration of 17β-oestradiol alone.

Experiment 6

Effect of constant administration of 17β-oestradiol upon cardiovascular responses to electrical stimulation

Introduction

Constant release of 17β-oestradiol appeared to reduce the potency of intravenously-administered noradrenaline. To determine whether cardiovascular responses to endogenously-released noradrenaline are altered by changes in the plasma concentration of 17β-oestradiol, responses were recorded following electrical stimulation of the sympathetic nervous system in rats pre-treated with 17β-oestradiol.

Results

Female rats in dioestrus were weighed and implanted with an Alzet osmotic minipump containing either 17β-oestradiol in PEG 300 (758 $\mu g.kg^{-1}$) or PEG 300 alone (control). After 10 days the rats were weighed again and pithed. The resting blood pressure and heart rate were then recorded. Table 10a (p. 152) shows the body weight of rats before implantation of minipumps, the weight of rats prior to pithing and the increases in weight during the 10 day implantation period of 17β-oestradiol pre-treated and control rats. Prior to implantation of the minipumps, there was no significant difference in body weight between the two groups of rats. After 10 days' implantation of the minipumps, rats receiving 17β-oestradiol weighed

significantly less (210.0 ± 5.0 g, n=5) compared with control rats (251.7 ± 10.7 g, n=6)($P<0.01$). The increase in weight over the implantation period was significantly lower in rats pre-treated with 17β-oestradiol (12.0 ± 4.2 g, n=5) compared with controls (26.7 ± 3.7 g, n=6)($P<0.05$). Table 10b (p. 152) shows the resting blood pressure and heart rate before and after intravenous administration of D-tubocurarine. Similar findings were observed both before and after D-tubocurarine administration. In order to simplify presentation, therefore, only recordings made after atropine sulphate administration alone will be discussed. It may be seen in table 10b that systolic pressure in 17β-oestradiol pre-treated rats was significantly lower (42.4 ± 3.6 mm Hg, n=5) compared with control rats (53.7 ± 1.5 mm Hg, n=6) ($P<0.02$). Similarly, pulse pressure in 17β-oestradiol pre-treated rats was significantly lower (3.4 ± 0.4 mm Hg, n=5) compared with control rats (9.0 ± 0.9 mm Hg, n=6)($P<0.001$). The difference in diastolic pressure between 17β-oestradiol pre-treated (39.0 ± 3.8 mm Hg, n=5) and control rats (44.7 ± 2.0 mm Hg, n=6) was not significant ($P>0.2$). The resting heart rate of rats pre-treated with 17β-oestradiol was significantly lower (223.1 ± 17.1 min^{-1}, n=5) compared with the heart rate of control rats (282.5 ± 15.3 min^{-1}, n=6)($P<0.05$).

The sympathetic spinal outflow was then stimulated electrically by varying the voltage (20-60 V) at fixed frequency (10 s^{-1}) and fixed pulse widths (200, 400, 600 or 800 μs). Pressor responses and positive chronotropic responses were recorded. Pressor responses were recorded both as the maximal sustained systolic pressure and also as the increase in systolic pressure. Positive chronotropic responses were recorded both as the maximal sustained heart rate and also as the increase in heart rate. Figure 18 (p. 93) shows typical pressor

responses to electrical stimulation and how responses were measured.

Figure 42 (p. 153) shows the maximal sustained systolic pressure during electrical stimulation of 10 s^{-1} at a fixed pulse width of 200 μs and variable voltage (20-60 V) in 17β-oestradiol pre-treated and control rats. No significant differences were recorded between the maximal sustained systolic pressure of 17β-oestradiol pre-treated rats compared with that of control rats ($P > 0.2$ throughout). Similarly, at all other stimulation parameters, no significant differences in maximal sustained systolic pressure were recorded between 17β-oestradiol pre-treated and control rats ($P > 0.2$ throughout).

No significant differences were recorded between the pressor responses of 17β-oestradiol pre-treated rats and of control rats to all electrical stimulation parameters when responses were expressed as the increase in systolic pressure ($P > 0.1$ throughout). This is demonstrated in figure 43 (p. 153) which shows pressor responses, expressed as the increase in systolic pressure, induced by electrical stimulation of fixed frequency (10 s^{-1}), fixed pulse width (200 μs) and variable voltage (20-60 V) in 17β-oestradiol pre-treated and control rats.

Figure 44 (p. 154) shows the maximal sustained systolic pressure at fixed frequency (10 s^{-1}), fixed pulse width (200 μs) and variable voltage (20-60 V) in individual control rats. Similarly, figure 45 (p. 154) shows the maximal sustained systolic pressure at the same fixed frequency (10 s^{-1}), fixed pulse width (200 μs) and variable voltage (20-60 V) in individual 17β-oestradiol pre-treated rats. It may be seen that the stimulation-response curves for rats pre-treated with 17β-oestradiol are more wide spread than those for control rats.

Figure 46 (p. 155) shows pressor responses, expressed as the increase in systolic pressure, to electrical stimulation of fixed frequency (10 s^{-1}), fixed pulse width (200 μs) and variable voltage (20-60 V) in individual control rats. Figure 47 (p. 155) shows pressor responses, expressed as the increase in systolic pressure, to electrical stimulation of the same fixed frequency (10 s^{-1}), fixed pulse width (200 μs) and variable voltage (20-60 V) in individual 17β-oestradiol pre-treated rats. By comparing figures 46 and 47, it may be seen that the wide separation of responses in rats pre-treated with 17β-oestradiol compared with control rats was still evident when responses were expressed as the increase in systolic pressure. For this reason, it was not possible to record any horizontal shift in the stimulation-response curve following 17β-oestradiol pre-treatment. These findings gave some indication that pressor responses to electrical stimulation were not normally distributed. For this reason, the Mann-Whitney test was applied (see page 59). Application of this test indicated that there was no statistically significant difference between pressor responses, expressed as the incremental rise in systolic pressure, in 17β-oestradiol pre-treated and control rats ($P > 0.1$).

At some electrical stimulation parameters of low pulse width and voltage (200 μs at 20 and 40 V; 400 μs at 20 V; 600 μs at 20 V), the maximal sustained heart rate was significantly lower in rats pre-treated with 17β-oestradiol compared with control rats ($P < 0.05$ throughout). At all electrical stimulation parameters, there were no significant differences in the increase in heart rate between 17β-oestradiol pre-treated rats and control rats ($P > 0.2$ throughout).

Figure 48 (p. 156) shows the increase in heart rate during electrical stimulation of fixed frequency (10 s^{-1}), fixed pulse width (200 μs) and variable voltage (20-60 V) in 17β-oestradiol pre-treated and control rats. Responses appeared to lie at the start of the linear portion of the stimulation-response curve. There was no significant difference in the voltage required to induce a standard positive chronotropic response in 17β-oestradiol pre-treated, compared with control, rats. For example, to elicit an increase in heart rate of 30 beats.min^{-1} at 10 s^{-1} and 200 μs pulse width, 17β-oestradiol pre-treated rats required 58.60 ± 7.16 V (n=5) while control rats required 52.40 ± 6.12 V (n=6)($P > 0.5$), such that the stimulation-ratio was 1.12.

Oestrous cycle length and post-mortem examination findings

Oestrous cycle lengths were determined in each rat by daily examination of vaginal smears. Control rats and most 17β-oestradiol pre-treated rats demonstrated cycles of 3-4 days in length. Some 17β-oestradiol pre-treated rats, however, demonstrated an increase in the length of the oestrous stage of the cycle.

The uteri and ovaries of 17β-oestradiol pre-treated rats and control rats were not markedly different in gross appearance.

None of the minipumps appeared to induce local inflammatory reactions, a finding similar to that of Capozza and co-workers (1977). However, two minipumps had moved slightly from the implantation site.

TABLE 10

Number of observations in parentheses.

Results are mean $\pm$ s.e mean.

* Indicates statistical significant difference.

E - Oestradiol.

Day 1 - Day of minipump implantation.

Day 10 - Day of sacrifice.

b.p. - Blood pressure.

M.A.P. - Mean arterial pressure.

TABLE 10 - The body weight of rats prior to minipump implantation, together with recordings of body weight, blood pressure and heart rate in pithed female rats following 10 days' minipump implantation.

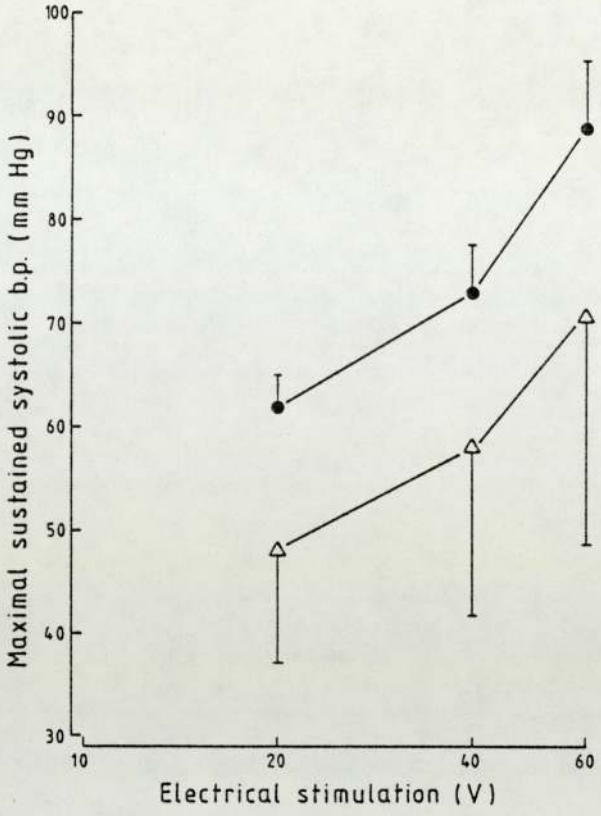
Parameter	Minipump Contents PEG 300 (6)	17βE (5)	P
a) Weight			
Day 1 (g)	225.0±10.1	202.0± 4.2	0.05-0.1
Day 10 (g)	251.7±10.7	210.0± 5.0	0.005-0.01*
Increase (g)	26.7± 3.7	12.0± 4.2	0.025-0.05*
b) After pithing			
Following atropine sulphate administration			
Systolic b.p. (mm Hg)	53.7± 1.5	42.4± 3.6	0.01-0.02*
Diastolic b.p. (mm Hg)	44.7± 2.0	39.0± 3.8	0.2 -0.3
Pulse pressure (mm Hg)	9.0± 0.9	3.4± 0.4	<0.001*
M.A.P. (mm Hg)	47.7± 1.8	40.1± 3.9	0.1 -0.2
Heart rate (min^{-1})	282.5±15.3	223.1±17.1	0.025-0.05*
Following atropine sulphate and D-tubocurarine administration			
Systolic b.p. (mm Hg)	52.8± 2.2	41.6± 2.5	0.005-0.01*
Diastolic b.p. (mm Hg)	43.8± 2.4	38.6± 2.8	0.1-0.2
Pulse pressure (mm Hg)	9.0± 0.8	2.8± 0.4	<0.001*
M.A.P. (mm Hg)	47.2± 2.4	39.5± 2.4	0.025-0.05*
Heart rate (min^{-1})	294.0± 8.9	240.9± 8.0	0.001-0.005*

Figure 42

Maximal sustained systolic blood pressure during electrical stimulation of fixed frequency (10 s^{-1}), fixed pulse width (200 μs) and variable voltage of the sympathetic spinal outflow in pithed female rats implanted with minipumps containing either 17β-oestradiol in PEG 300 (758 $\mu g.ml^{-1}$)(△) or PEG 300 alone (control)(•). Each point represents the mean, and vertical bars denote the s.e. mean. (17β-oestradiol pre-treated, n=5; control, n=6).

Figure 43

Increase in systolic blood pressure during electrical stimulation of fixed frequency (10 s^{-1}), fixed pulse width (200 μs) and variable voltage of the sympathetic spinal outflow in pithed female rats implanted with minipumps containing either 17β-oestradiol in PEG 300 (758 $\mu g.ml^{-1}$)(△) or PEG 300 alone (control)(•). Each point represents the mean, and vertical bars denote the s.e. mean (17β-oestradiol pre-treated, n=5; control, n=6).


Maximal sustained systolic b.p. (mm Hg)
100
90
80
70
60
50
40
30
10
20
40
60
Electrical stimulation (V)

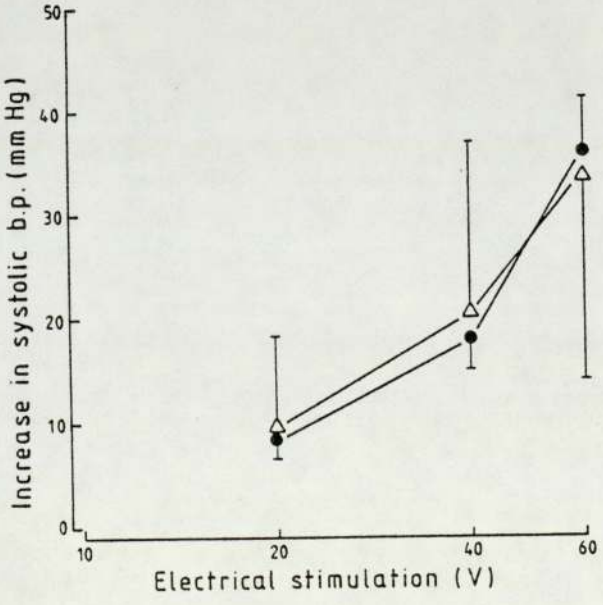
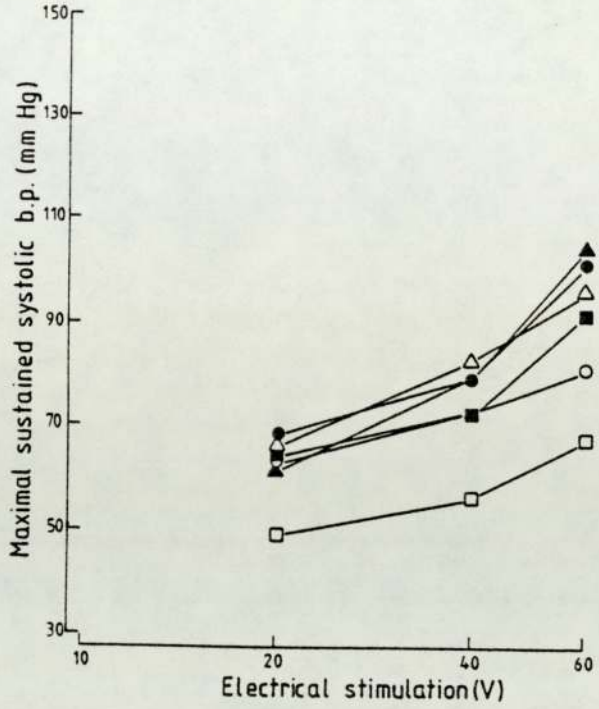

Increase in systolic b.p. (mm Hg)
50
40
30
20
10
0
10
20
40
60
Electrical stimulation (V)

Figure 44

Maximal sustained systolic blood pressure during electrical stimulation of fixed frequency (10 s^{-1}), fixed pulse width (200 μs) and variable voltage of the sympathetic spinal outflow in individual pithed female rats implanted with minipumps containing PEG 300 alone (controls).

Figure 45

Maximal sustained systolic blood pressure during electrical stimulation of fixed frequency (10 s^{-1}), fixed pulse width (200 μs) and variable voltage of the sympathetic spinal outflow in individual pithed female rats implanted with minipumps containing 17β-oestradiol in PEG 300 (758 $\mu g.ml^{-1}$).


Maximal sustained systolic b.p. (mm Hg)
150
130
110
90
70
50
30
10
20
40
60
Electrical stimulation (V)

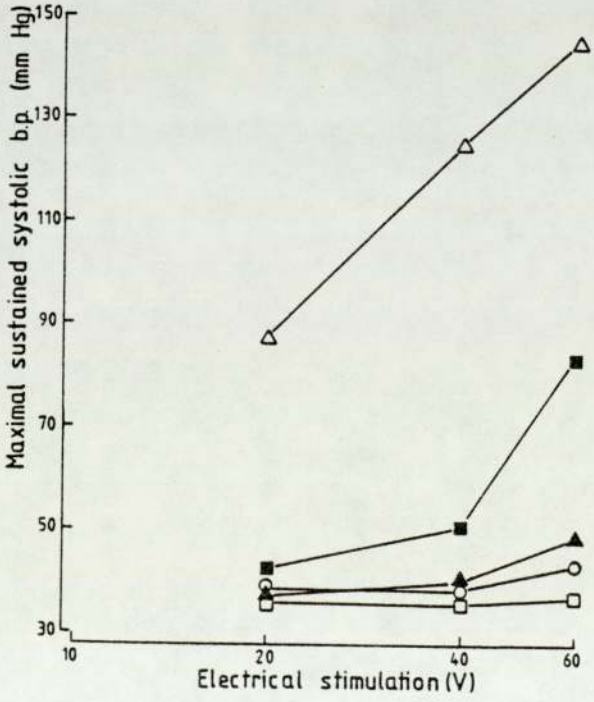
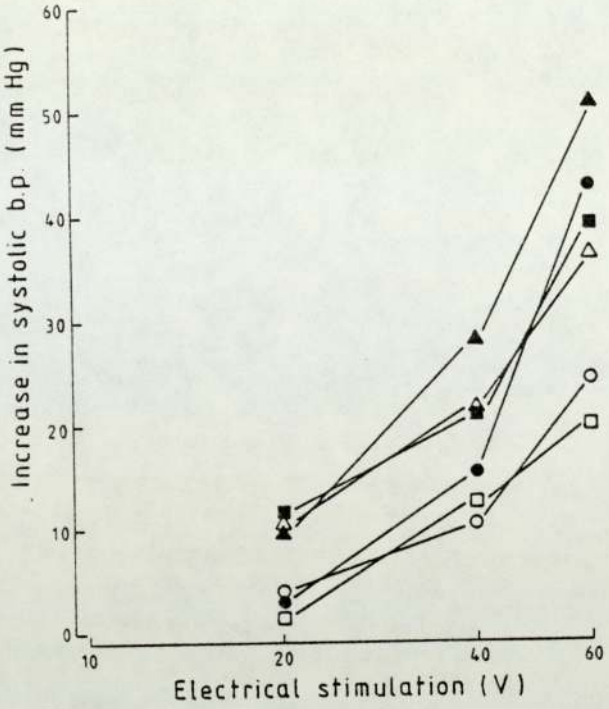

Maximal sustained systolic b.p. (mm Hg)
150
130
110
90
70
50
30
10
20
40
60
Electrical stimulation (V)

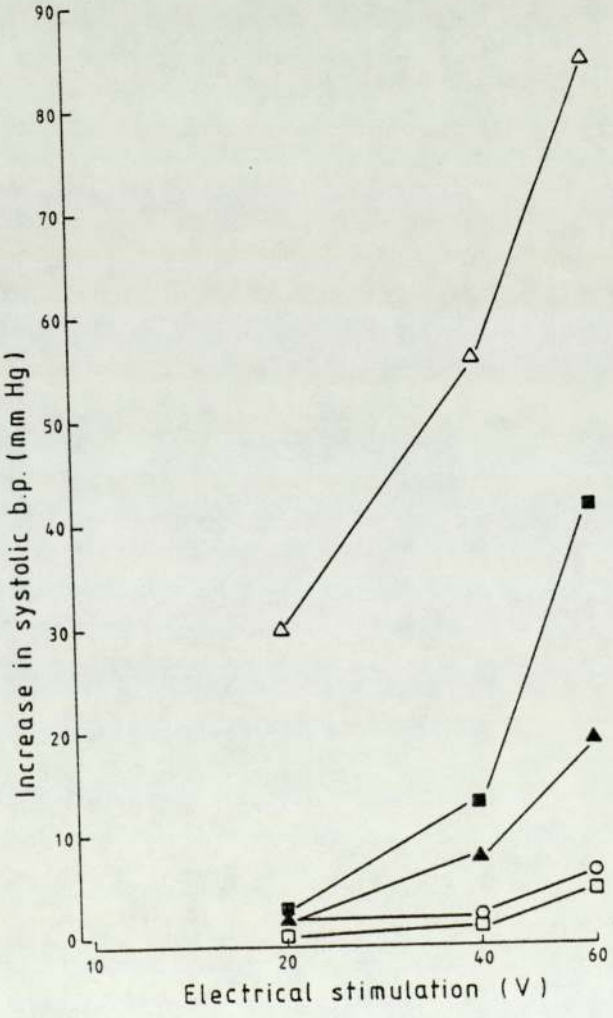
Figure 46

Increase in systolic blood pressure during electrical stimulation of fixed frequency (10 s^{-1}), fixed pulse width (200 μs) and variable voltage of the sympathetic spinal outflow in individual pithed female rats implanted with minipumps containing PEG 300 alone (controls).

Figure 47

Increase in systolic blood pressure during electrical stimulation of fixed frequency (10 s^{-1}), fixed pulse width (200 μs) and variable voltage of the sympathetic spinal outflow in individual pithed female rats implanted with minipumps containing 17β-oestradiol in PEG 300 (758 $\mu g.ml^{-1}$).


Increase in systolic b.p. (mm Hg)
0
10
20
30
40
50
60
10
20
40
60
Electrical stimulation (V)


Increase in systolic b.p. (mm Hg)
0
10
20
30
40
50
60
70
80
90
10
20
40
60
Electrical stimulation (V)

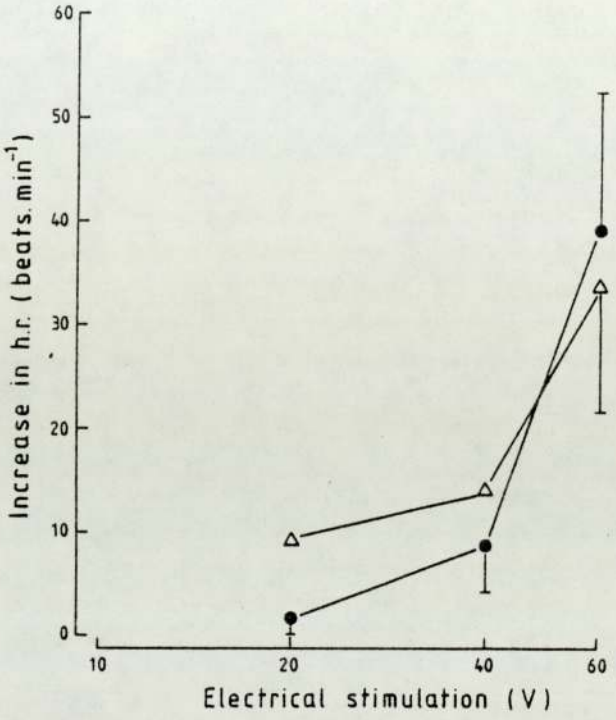


Figure 48

Increase in heart rate during electrical stimulation of fixed frequency (10 s^{-1}), fixed pulse width (200 µs) and variable voltage of the sympathetic spinal outflow in pithed female rats implanted with minipumps containing either 17B-oestradiol in PEG 300 (758 µg.ml^{-1})(△) or PEG 300 alone (control)(●). Each point represents the mean, and vertical bars denote the s.e. mean (17B-oestradiol pre-treated, n=5; control, n=6).

Discussion

Rats used in experiments 5 and 6 demonstrated reduction in systolic blood pressure and heart rate, with no change in diastolic blood pressure following 17β-oestradiol pre-treatment. It therefore seemed reasonable to compare the effects of 17β-oestradiol upon cardiovascular responses in experiments 5 and 6.

In experiment 6, the effect of 17β-oestradiol pre-treatment upon cardiovascular responses to electrical stimulation was examined, since Stein (1974) has shown that electrical stimulation leads to release of endogenous noradrenaline from nerve endings. In experiment 5, the effect of 17β-oestradiol pre-treatment upon cardiovascular responses to exogenous noradrenaline was examined.

There appeared to be a much wider variation in incremental rise in systolic pressure induced by electrical stimulation in individual 17β-oestradiol pre-treated rats compared with responses in control rats. Although application of the Mann-Whitney test indicated no significant difference between the groups, it seemed that, following 17β-oestradiol pre-treatment, pressor responses were reduced in a majority of rats, and potentiated in one rat. Pressor responses to exogenous noradrenaline in experiment 5 were attenuated following 17β-oestradiol and there was no wide variation in responses. It appears, therefore, that 17β-oestradiol pre-treatment attenuates pressor responses to exogenous and endogenous noradrenaline in a majority of rats, possibly by a common mechanism.

Alternatively, the mechanism of reduction in pressor responses to exogenous and endogenous noradrenaline following 17β-oestradiol pre-treatment may differ. Since exogenous noradrenaline is primarily

removed by extraneuronal uptake and by metabolism with COMT (Trendelenburg, 1977; Vanhoutte, 1978b), it is possible that an increase in extraneuronal uptake or enzymatic activity of COMT may account for reduction in pressor responses to exogenous noradrenaline in experiment 5. Since endogenous noradrenaline is primarily removed by neuronal uptake and by metabolism with MAO (Vanhoutte, 1978), it is possible that an increase in neuronal uptake or enzymatic activity of MAO may account for reduction in pressor responses to endogenous noradrenaline in experiment 6. Alternatively, since pressor responses to electrical stimulation appear to be dependent upon the number of conducting nerve fibres (see review by Stein, 1974), it is possible that pre-treatment with 17β-oestradiol reduces the number of sympathetic conducting nerve fibres in a majority of female rats.

Two possible mechanisms for potentiation of responses in one rat following 17β-oestradiol pre-treatment are suggested. Since endogenous noradrenaline is primarily removed by extraneuronal uptake and by metabolism with MAO (Vanhoutte, 1978b), neuronal uptake or enzymatic activity of MAO may be reduced following 17β-oestradiol pre-treatment. Alternatively, the number of sympathetic conducting nerve fibres may have been increased in the rat demonstrating potentiated pressor responses to endogenous noradrenaline following 17β-oestradiol pre-treatment.

Webb & Bohr (1981) and Weidmann (1981) have reviewed in depth the manner in which potentiated responses of blood vessels to constrictor agents may lead to a hypertensive condition. Also, Folkow (1975) and Zanchetti (1979) have reviewed the evidence which suggests that changes in nervous activity may be involved in the elevation of blood pressure in rats and in man. For this reason, it is interesting

to speculate whether the rat which demonstrated potentiation of pressor responses to electrical stimulation following 17β-oestradiol pre-treatment may be predisposed toward developing hypertension. Rats with reduced pressor responses to electrical stimulation, however, would not be so disposed.

Elevation in the plasma concentration of ovarian hormonal agents, during pregnancy and during administration of oral contraceptives, has been shown to be associated with an increase in blood pressure in a minority of women (Fregly & Fregly, 1977; Welt & Crenshaw, 1978). The rat with potentiated pressor responses to endogenous noradrenaline following 17β-oestradiol pre-treatment, may, therefore, be similar to those women who develop ovarian hormonal agent-induced hypertension. Saruta and co-workers (1970) have provided evidence which suggests that some women may have a particular genetic predisposition toward the development of this type of hypertension. There may be a similar genetic involvement in the elevation of blood pressure in rats, since selective breeding of rats with above average blood pressure has led to the development of a strain of spontaneously hypertensive rats (Okamoto & Aoki, 1963) and the ways in which certain genotypes may induce hypertension when exposed to various environmental stimuli has been reviewed by Cruz-Coke (1981).

Prolonged elevation of the plasma concentration of 17β-oestradiol, therefore, may potentiate pressor responses to electrical stimulation of the sympathetic spinal outflow in rats with a particular genetic constitution. The possibility that these rats have a greatly elevated plasma concentration of 17β-oestradiol following pre-treatment with this oestrogen may be excluded because Butcher and co-workers (1978) have shown that, when 17β-oestradiol is administered from an

osmotic minipump, a uniform plasma concentration of the oestrogen is achieved. Potentiation of pressor responses to electrical stimulation of the sympathetic spinal outflow is therefore unlikely to be due to a particularly high plasma concentration of 17β-oestradiol.

Positive chronotropic responses to electrical stimulation were not different in 17β-oestradiol pre-treated rats compared with control rats. Although responses lay at the base of the stimulation-response curve, it seemed unlikely that there would be a horizontal shift in the linear portion of the curve.

It was suggested previously that reduction in positive chronotropic responses to isoprenaline following 17β-oestradiol pre-treatment may have been due to an action at the β-adrenoreceptor (see page 142). However, the findings of experiments 5 and 6 show that the positive chronotropic responses to noradrenaline in the threshold portion of the concentration-response curve are unaltered following 17β-oestradiol pre-treatment and that they may not be different in the linear portion of the curve. Since positive chronotropic responses to isoprenaline appeared to lie in the maximal portion of the curve, it is possible that only maximal chronotropic responses are reduced following 17β-oestradiol pre-treatment. Since there is some evidence that the maximal positive chronotropic response to isoprenaline may be increased following bilateral ovariectomy (see page 119), it is possible that the plasma concentration of 17β-oestradiol is inversely related to the maximal positive chronotropic response. The plasma concentration of 17β-oestradiol also appears to be inversely related to the speed of of atrial conduction (see pages 120 and 143). It is possible that alteration in atrial conduction rate following a change in plasma 17β-oestradiol induces a change in the maximal positive chronotropic response.

Body weight gain

In present experiments, administration of 17β-oestradiol from an osmotic minipump appeared to reduce body weight gain in female rats. Similarly, Hoeg and co-workers (1977) found that administration of an oestrogenic agent, mestranol, to rats reduced normal body weight gain. Hoeg and co-workers (1977) have shown that administration of mestranol not only reduced body weight gain but also reduced the rise in systolic pressure of spontaneously hypertensive rats. Attenuation of the rise in systolic pressure in spontaneously hypertensive rats observed by Hoeg and co-workers (1977) does not appear to have been induced by reduction in body weight gain, since reduction in body weight gain was also observed in normotensive rats treated with mestranol, although the systolic pressure was not altered. It is unlikely, therefore, that reduced body weight gain observed in 17β-oestradiol pre-treated rats in present experiments led to the reduction in systolic pressure observed in these animals, although this possibility cannot be precluded.

Oestrous cycle length

The results of experiment 2 indicate that oestrus was associated with elevation of systolic pressure and potentiation of pressor responses to noradrenaline. It was proposed that this change in the cardiovascular system was due to raised plasma concentration of 17β-oestradiol at oestrus (see page 69). However, when 17β-oestradiol was administered at a constant rate, systolic blood pressure and pressor responses to noradrenaline were depressed. Although constant 17β-oestradiol administration did not reproduce the apparent effect of oestrus upon the cardiovascular system observed in experiment 2, a similar alteration in the oestrous cycle was observed in both groups of animals. A few oestrous rats in experiment 2 and a few rats pre-treated with 17β-oestradiol demonstrated lengthening of the oestrous stage of the cycle. It is therefore possible that a constant high plasma concentration of 17β-oestradiol induced the constant oestrus-like state observed in rats in experiment 2, but that another factor, not yet identified, was responsible for the elevation of systolic pressure and potentiation of pressor responses observed in oestrous rats in experiment 2.

Summary

Since Folkow (1978) had shown that an increase in cardiovascular responses may lead to an elevation of blood pressure and since oral contraceptive therapy is associated with raised blood pressure in some women (Fregly & Fregly, 1977), the present study was undertaken to determine whether ovarian hormones may induce an elevation in blood pressure by potentiating cardiovascular responsiveness. From 1959 until 1968 Lloyd and co-workers (Lloyd, 1959a, b; Lloyd & Pickford, 1961; Hettiaratchi & Pickford, 1968) published the findings of a series of studies concerned with the influence of circulating ovarian hormones upon pressor responses to several agents in the rat. The present study was therefore carried out to extend the findings of Lloyd and co-workers.

The findings of experiment 2 which showed that, in pithed rats, oestrus was associated with an increase in resting systolic pressure and an increase in pressor responses to noradrenaline and vasopressin but also showed that there was no change in pressor responses to angiotensin II compared with dioestrus, appeared to be consistent with the findings of Lloyd (1959a) and Hettiaratchi & Pickford (1968). However, in subsequent experiments oestrus was not associated with such changes, and the findings of experiment 2 could not be repeated. It has been suggested that the rats used in experiment 2 were fundamentally different to the rats used in other experiments. Constant administration of 17β-oestradiol induced similar changes in the oestrous cycle as those observed in oestrous rats in experiment 2. Oestrous rats in experiment 2 may therefore have had raised plasma concentrations of 17β-oestradiol. However, constant administration of

17β-oestradiol did not induce the type of changes in the cardiovascular system observed in oestrous rats in experiment 2. Constant high plasma concentrations of 17β-oestradiol did not therefore appear to have been responsible for the elevation of systolic pressure and potentiation of pressor responses observed in oestrous rats in experiment 2. However, since experiment 2 was performed in spring, whereas subsequent experiments were executed in late summer, it is possible that elevation of systolic blood pressure and potentiation of pressor repsonses is a seasonal effect. Unfortunately, insufficient time was available to examine this possibility, and further investigation will be necessary to elucidate the reasons for any season-dependent elevation in systolic pressure and potentiation of pressor responses.

One unexpected finding was the reduction in blood pressure, heart rate, pressor responses, depressor responses and positive chronotropic responses following constant administration of 17β-oestradiol. On, reflection, however, other studies which showed an increase or no change in blood pressure (Douglas, 1974; Lew, 1975; Hoeg et al., 1977; Fischer & Swain, 1980) or potentiation or no change in cardiovascular responses (Lloyd, 1959a; Hettiaratchi & Pickford, 1968) following administration of an oestrogenic agent to rats had delivered the agent at a discontinuous rate. Also, the study of Fregly & Thrasher (1977) showed that constant administration of ethinyloestradiol from implanted silastic tubing was associated with reduction in positive chronotropic responses to isoprenaline. Present and published studies, therefore, suggest the interesting possibility that the action of an administered oestrogenic agent is dependent upon its pharmacokinetic profile.

Although daily subcutaneous injections of 17β-oestradiol and ethinyloestradiol induced alterations in responses to general anaesthesia and many rats died, some survived to be pithed. However, those which were pithed died shortly afterwards and the ECG recording from these rats showed that atrio-ventricular heart block was the likely cause of death. It is interesting to speculate whether massive sympathetic outflow during pithing induced this cardiac effect. If sympathetic nervous outflow was raised in rats following administration of oestrogenic agents, these rats may have had elevated resting blood pressure. This would be similar to the observation of Lew (1975) of an increase in blood pressure following seven daily intraperitoneal injections of either diethylstilboestrol or mestranol to female rats and that of Fischer & Swain (1980) who observed an increase in blood pressure following weekly intramuscular injections of 17β-oestradiol in female rats. Since Folkow (1975) has shown how an increase in nervous activity may lead to an elevation in the blood pressure of rats, and since intraperitoneal and intramuscular administration of agents induce a pharmacokinetic profile characterised by variation in plasma concentrations of the administered agent (Struyker-Boudier & Smits, 1978; Theeuwes, 1981), it is possible that variation in the plasma concentration of oestrogenic agents leads to an increase in nervous activity, which leads to an elevation in blood pressure. In future it would be interesting to examine the proposition that variation in the plasma concentration of oestrogenic agents leads to elevation in blood pressure and potentiation of cardiovascular responses, whereas constant elevation of the circulating level of oestrogenic agents leads to reduction in blood pressure and cardiovascular responses.

Alternatively, reduction in cardiovascular parameters and responses observed in present experiments may be related to the very high concentration of 17β-oestradiol administered, 10 $\mu g.day^{-1}$, since when Fischer & Swain (1980) administered only 10 μg 17β-oestradiol per week, they observed an increase in systolic pressure.

An interesting finding in present experiments is the similarity between the effects of constant 17β-oestradiol administration to female rats and of human pregnancy upon the cardiovascular system. This similarity may be due to the fact that there is a massive increase in circulating 17β-oestradiol in both conditions (Butcher et al., 1974; Butcher et al., 1978; Challis, 1980) or to the fact that the increase is constant in both conditions. For whichever reason, this preparation may become a useful tool to understand the influence of ovarian hormones upon the cardiovascular system during human pregnancy. This model may be particularly useful, since one rat pre-treated with 17β-oestradiol demonstrated potentiated pressor responses to endogenous noradrenaline. Since potentiated pressor responses may lead to hypertension (Webb & Bohr, 1981), it is possible that this rat may be part of a minority of rats which will develop hypertension after long-term constant administration of 17β-oestradiol. Such a minority may be similar to the minority of women who develop pregnancy-induced hypertension (Welt & Crenshaw, 1978), in which case constant administration of 17β-oestradiol to female rats may also extend the understanding of pregnancy-induced hypertension in women.

In experiment 5, no examination was made of the effect of 17β-oestradiol pre-treatment upon cardiovascular responses to vasopressin. Since there was some indication that the potency of vasopressin may be reduced following bilateral ovariectomy, although responses to

noradrenaline and angiotensin II are unaltered, this omission seems to have been unfortunate. In retrospect, such an examination may have helped in the understanding of the effect of 17β-oestradiol administration upon the cardiovascular system.

Similarly, since there is some evidence that maximal positive chronotropic responses to isoprenaline may be reduced following 17β-oestradiol pre-treatment and increased following bilateral ovariectomy, it would have been reasonable to administer a greater spread in isoprenaline concentrations to determine the effect of changes in plasma 17β-oestradiol upon the entire concentration-response curve.

CHAPTER 2.

THE EFFECT OF PRE-TREATMENT OF FEMALE RATS WITH (17α) ETHINYLOESTRADIOL AND NORETHISTERONE ACETATE UPON THE RESPONSES OF ISOLATED AORTIC STRIPS, PORTAL VEINS AND PAIRED ATRIA TO SEVERAL SPASMOGENS.

Chapter 2

General Introduction

The experiments described in chapter 1 were undertaken to develop the understanding of oral contraceptive-induced hypertension. However, continuous administration of 17β-oestradiol appeared to reduce systolic blood pressure in pithed female rats. It was not possible to determine whether reduction in systolic pressure was due to the pharmacokinetic profile induced by this mode of administration, since rats which underwent daily subcutaneous injections of oestrogenic agents presented altered responses to anaesthetic agents and pre-treated rats which survived anaesthesia demonstrated atrio-ventricular heart block after pithing (see page 143). Since alteration in responses to anaesthetic agents was also observed following administration of ethinyloestradiol from osmotic minipumps, it was not possible to determine whether continuous administration of all oestrogenic agents leads to reduction in systolic pressure. To extend the findings of chapter 1, and to develop the understanding of oral contraceptive-induced hypertension, therefore, the effect of in vivo administration of oral contraceptive agents was examined upon the responsiveness of isolated cardiac and vascular tissue.

Female rats were given 9 daily subcutaneous injections of either ethinyloestradiol (822 $\mu g.kg^{-1}.day^{-1}$) or norethisterone acetate (67 $mg.kg^{-1}.day^{-1}$) in PEG 300. Control rats received PEG 300 alone. Twenty four hours after the last injection, animals were sacrificed and the aorta, portal vein or paired (joined) atria were excised for study.

The influence of oral contraceptives upon the responsiveness of isolated cardiac and vascular tissue has been recorded in two ways. Responses have been compared at standard concentration or stimulus strength. Also, the ratio of the concentration or stimulus strengths required to elicit a standard response was calculated. Where maximal responses were obtained, the mean of the concentrations of spasmogen required to elicit 50 % of the maximal contractile response of each individual tissue ($E.C._{50}$) was taken to be the standard response. Where maximal responses were not obtained, the concentration-ratio was calculated at a point on the linear portion of the concentration-response curve approximating to 50 % of the maximal response. A left shift in the concentration-response curve to an agent was taken to indicate an increase in agent potency and a right shift was taken to indicate a decrease in agent potency.

Vaginal smears were examined daily in experimental animals before, and during, the injection period. All rats were weighed immediately prior to the initial injection and just before sacrifice, and a gross post-mortem examination was carried out after removal of the organ under study.

Effect of hormone pre-treatment upon the body weight of rats

Table 11 (p. 171) shows the body weight of rats prior to the initial injection and the increase in body weight recorded during the treatment period. The body weight of rats prior to treatment with ethinyloestradiol (216.2 ± 5.4 g, n=26) or with norethisterone acetate (214.0 ± 3.4 g, n=25) were not significantly different to the body weight of control rats (206.4 ± 3.3 g, n=25)($P>0.1$ throughout).

There was also no significant difference in the increase in body weight recorded during the hormone treatment period between norethisterone acetate pre-treated (15.2 ± 2.8 g, n=25), compared with control (12.4 ± 2.4 g, n=25), rats ($P>0.4$). The increase in body weight of ethinyloestradiol pre-treated rats (1.2 ± 3.6 g, n=26) was significantly lower than that of control rats (12.4 ± 2.4 g, n=25) ($P<0.02$).

Oestrous cycle length and post-mortem examination findings

Oestrous cycle lengths were determined in each rat by daily examination of vaginal smears. Control rats and norethisterone acetate pre-treated rats demonstrated cycles of 3-4 days duration. After a few days of treatment with ethinyloestradiol, the vaginal smears of some rats were found to consist of only a few leucocytes and this type of smear was then observed until the animals were sacrificed. Also, several rats pre-treated with ethinyloestradiol were shown to have very vascular and fluid-filled uteri compared with those of norethisterone acetate pre-treated and control rats.

TABLE 11 - The body weight of female rats prior to hormone pre-treatment by injection, together with increases in body weight recorded during the injection period.

	Pre-treatment				
Weight (g)	EE (26)	P	NAc (25)	P	PEG 300 (25)
Day 1	216.2±5.4	0.1-0.2	214.0±3.4	0.1-0.2	206.4±3.3
Increase	1.2±3.6	0.01-0.02*	15.2±2.8	0.4-0.5	12.4±2.4

Number of observations in parentheses.

Results are mean±s.e. mean.

* Indicates statistical significant difference.

P values are with reference to PEG 300 pre-treated rats (controls).

EE - Ethinyloestradiol.

NAc - Norethisterone acetate.

Experiment 1

a) Effect of hormone pre-treatment upon contractile responses of isolated aortic strips to noradrenaline

After sacrifice, the aorta of each rat was excised, cut into a helical strip and mounted in an organ bath under a resting force of 9.8 mN. After one hour, a non-cumulative concentration-response curve to noradrenaline was constructed (concentration range, 10^{-13}-10^{-5} M). Figure 49 (p. 177) shows a typical response of the aortic strip to noradrenaline. All aortic strip responses to noradrenaline consisted of a fast and a slow phase. The sustained increase in force above basal (set) force was recorded, together with the initial increase in force. This was recorded 20 seconds after administration of noradrenaline. Figure 49 (p. 177) demonstrates how these phases of contraction were measured. Responses to noradrenaline were inconsistent if concentration-response curves were commenced at organ bath concentrations higher than 10^{-13} M.

Figure 50 (p. 178) shows the fast response to noradrenaline (10^{-13}-10^{-5} M) in aortic strips from control rats and from rats pre-treated with ethinyloestradiol or with norethisterone acetate. At all but three concentrations of noradrenaline (10^{-13}, 10^{-12} and 10^{-10} M), the fast response was significantly greater in aortic strips from ethinyloestradiol pre-treated rats compared with aortic strips from control rats ($P<0.05$). The maximal fast response to noradrenaline was also significantly greater in aortic strips from ethinyloestradiol pre-treated rats (2.70 ± 0.24 mN, n=7) compared with aortic strips from control rats (1.86 ± 0.16 mN, n=6)($P<0.02$). The fast response to all

concentrations of noradrenaline and the maximal fast response to noradrenaline were not significantly different in aortic strips from norethisterone acetate pre-treated rats compared with aortic strips from control rats ($P>0.05$ throughout).

Figure 51 (p. 179) shows responses of aortic strips to noradrenaline plotted as percentage of the maximal fast response. Table 12 (p. 175) shows the $E.C._{50}$ for fast responses to noradrenaline in aortic strips from control rats and from rats pre-treated with ethinyloestradiol or with norethisterone acetate. Following ethinyloestradiol pre-treatment, the $E.C._{50}$ for fast responses to noradrenaline was significantly reduced ($P<0.05$). There was no significant difference in the $E.C._{50}$ for fast responses to noradrenaline in aortic strips from norethisterone acetate pre-treated rats compared with aortic strips from control rats ($P>0.7$).

Figure 52 (p. 180) shows the slow responses to noradrenaline (10^{-13}-10^{-5} M) in aortic strips from control rats and from rats pre-treated with ethinyloestradiol or with norethisterone acetate. With the exception of two concentrations of noradrenaline (10^{-9} and 10^{-7} M), the slow response was not significantly different in aortic strips from ethinyloestradiol pre-treated rats compared with aortic strips from control rats ($P>0.05$ throughout). The maximal slow response to noradrenaline was significantly greater in aortic strips from ethinyloestradiol pre-treated rats (5.82 ± 0.56 mN, n=7) compared with aortic strips from control rats (3.92 ± 0.48 mN, n=6)($P<0.05$). The slow response to noradrenaline was not significantly different in aortic strips from norethisterone acetate pre-treated rats compared with aortic strips from control rats ($P>0.2$ throughout). Similarly, the maximal slow response to noradrenaline was not significantly different

in aortic strips from norethisterone acetate pre-treated rats compared with aortic strips from control rats ($P > 0.4$).

Figure 53 (p. 181) shows the responses of aortic strips to noradrenaline plotted as percentage of the maximal slow response. Table 13 (p. 176) shows the $E.C._{50}$ for slow responses to noradrenaline in aortic strips from control rats and from rats pre-treated with ethinyloestradiol or with norethisterone acetate. The $E.C._{50}$ was significantly reduced following ethinyloestradiol pre-treatment ($P < 0.05$). There was no significant difference in the $E.C._{50}$ for slow responses to noradrenaline in aortic strips from norethisterone acetate pre-treated rats compared with aortic strips from control rats ($P > 0.8$).

TABLE 12 - Concentration of noradrenaline required to elicit 50 % of maximal fast contractile responses in individual aortic strips taken from female rats pre-treated with hormonal agents and with PEG 300 (controls).

Agent		$E.C._{50}$ ($x10^{-9}$ M)	P	Concn-ratio
EE	(7)	1.14 ± 2.80	0.025-0.05*	8.34
NAc	(6)	8.24 ± 3.51	0.7 -0.8	1.15
PEG 300	(6)	9.51 ± 2.53		

Number of observations in parentheses.

Results are mean $\pm$ s.e. mean.

P values are with reference to aortic strips from PEG 300 pre-treated rats (controls).

* Indicates statistical significant difference.

Concn-ratio is the ratio of the mean concentrations in aortic strips from hormone pre-treated, compared with control, rats.

EE - Ethinyloestradiol.

NAc - Norethisterone acetate.

TABLE 13 - Concentration of noradrenaline required to elicit 50 % of maximal slow contractile responses in individual aortic strips taken from female rats pre-treated with hormonal agents and with PEG 300 (controls).

Agent		$E.C._{50}$ ($\times 10^{-10}$ M)	P	Concn-ratio
EE	(7)	1.59 ± 0.50	0.025-0.05*	14.03
Nac	(6)	25.03 ± 11.99	0.8 -0.9	1.12
PEG 300	(6)	22.30 ± 9.34		

Number of observations in parentheses.

Results are mean $\pm$ s.e. mean.

P values are with reference to aortic strips from PEG 300 pre-treated rats (controls).

* Indicates statistical significant difference.

Concn-ratio is the ratio of the mean concentrations in aortic strips from hormone pre-treated, compared with control, rats.

EE - Ethinyloestradiol.

NAc - Norethisterone acetate.

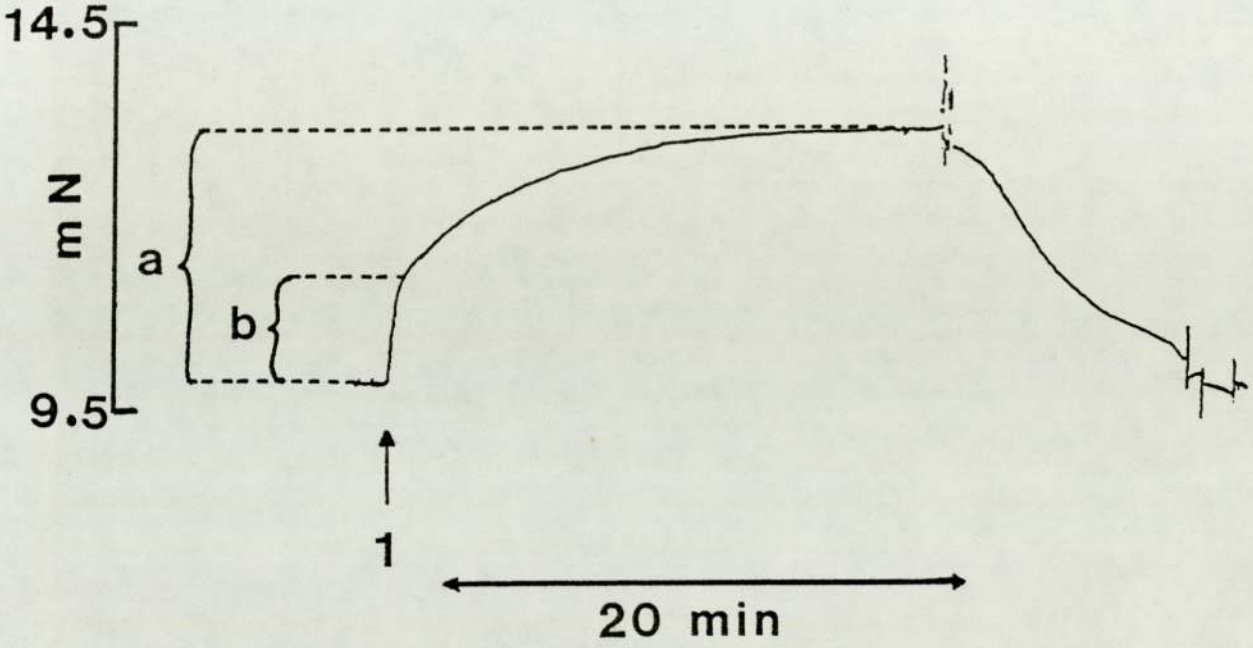


Figure 49

A typical trace of the response to noradrenaline of the isolated aortic strip of the rat.

1 = Point of administration of noradrenaline.

a = Increase in force due to the slow phase of contraction.
b = Increase in force due to the fast phase of contraction.

Figure 50

Initial increase in force induced by noradrenaline administration in isolated aortic strips from female rats pre-treated with ethinyloestradiol in PEG 300 (822 $\mu g.kg^{-1}.day^{-1}$)(o), norethisterone acetate in PEG 300 (67 $mg.kg^{-1}.day^{-1}$)(□) or PEG 300 alone (control)(●). Each point represents the mean, and vertical bars denote the s.e. mean (ethinyloestradiol, n=7; norethisterone acetate, n=6; PEG 300, n=6).

* Indicates statistical significant difference compared with control responses.

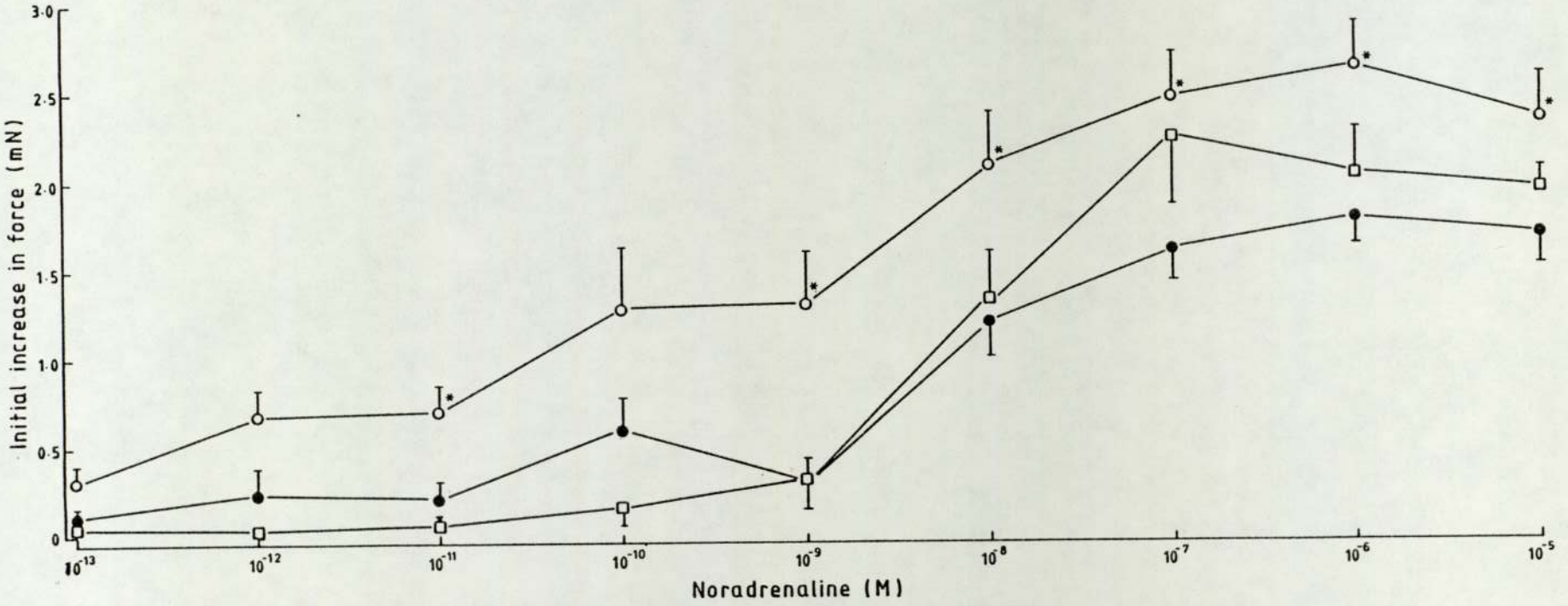

Initial increase in force (mN)
3·0
2·5
2·0
1·5
1·0
0·5
0
10⁻¹³
10⁻¹²
10⁻¹¹
10⁻¹⁰
10⁻⁹
10⁻⁸
10⁻⁷
10⁻⁶
10⁻⁵
Noradrenaline (M)

Figure 51

Percentage of the maximal initial increase in force induced by noradrenaline administration in isolated aortic strips from female rats pre-treated with ethinyloestradiol in PEG 300 (822 $\mu g.kg^{-1}.day^{-1}$)(○), norethisterone acetate in PEG 300 (67 $mg.kg^{-1}.day^{-1}$) (□) or PEG 300 alone (control)(●). Each point represents the mean, and vertical bars denote the s.e. mean (ethinyloestradiol, n=7; norethisterone acetate, n=6; PEG 300, n=6).

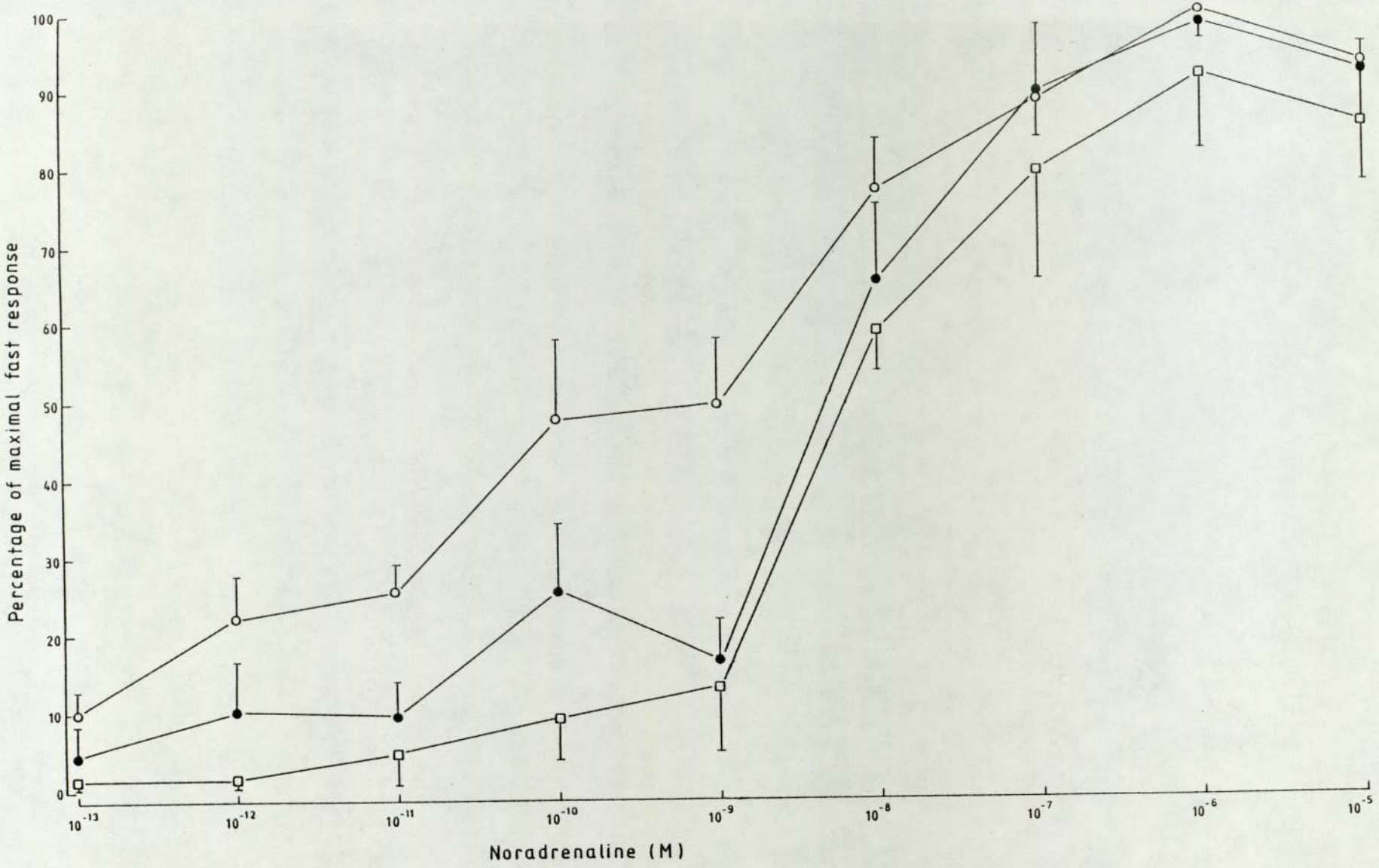

Percentage of maximal fast response
100
90
80
70
60
50
40
30
20
10
0
10^{-13}
10^{-12}
10^{-11}
10^{-10}
10^{-9}
10^{-8}
10^{-7}
10^{-6}
10^{-5}
Noradrenaline (M)

Figure 52

Sustained increase in force induced by noradrenaline administration in isolated aortic strips from female rats pre-treated with ethinyloestradiol in PEG 300 (822 $\mu g.kg^{-1}.day^{-1}$)(o), norethisterone acetate in PEG 300 (67 $mg.kg^{-1}.day^{-1}$)(□) or PEG 300 alone (control)(●). Each point represents the mean, and vertical bars denote the s.e. mean (ethinyloestradiol, n=7; norethisterone acetate, n=6; PEG 300, n=6).

* Indicates statistical significant difference compared with control responses.

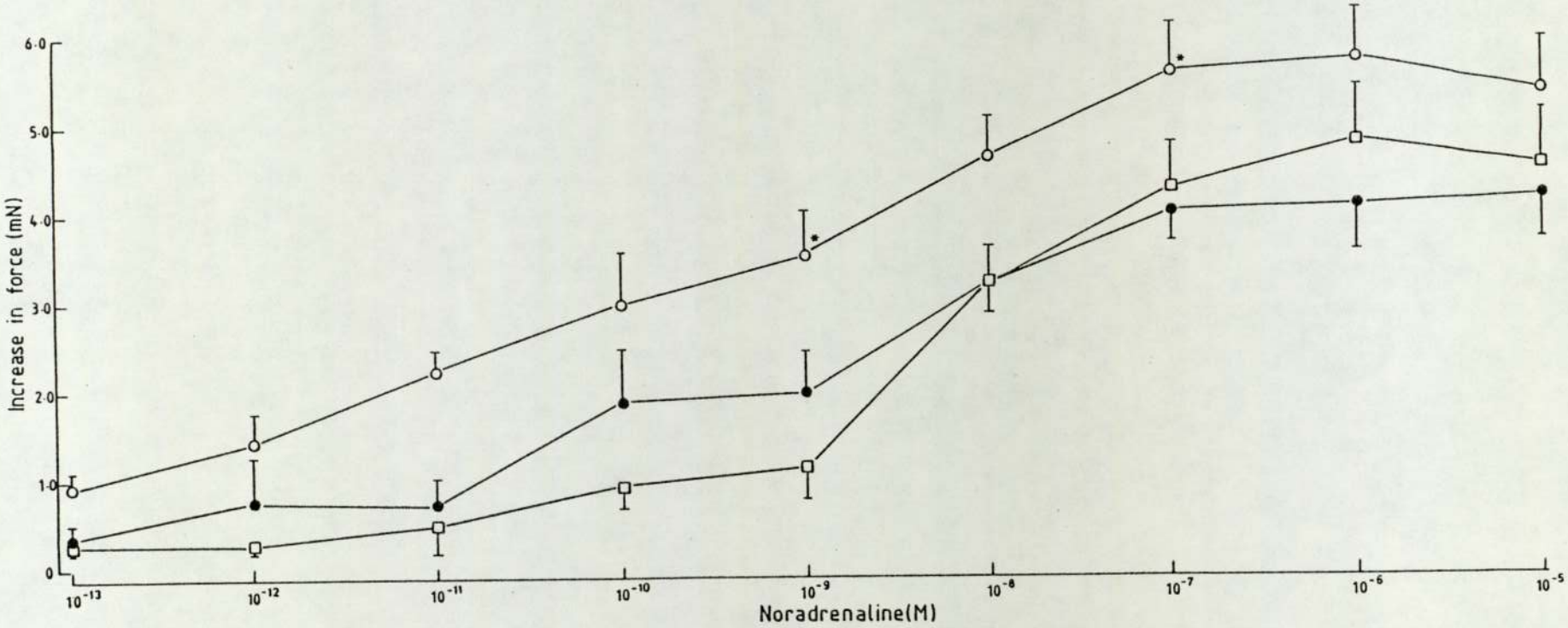
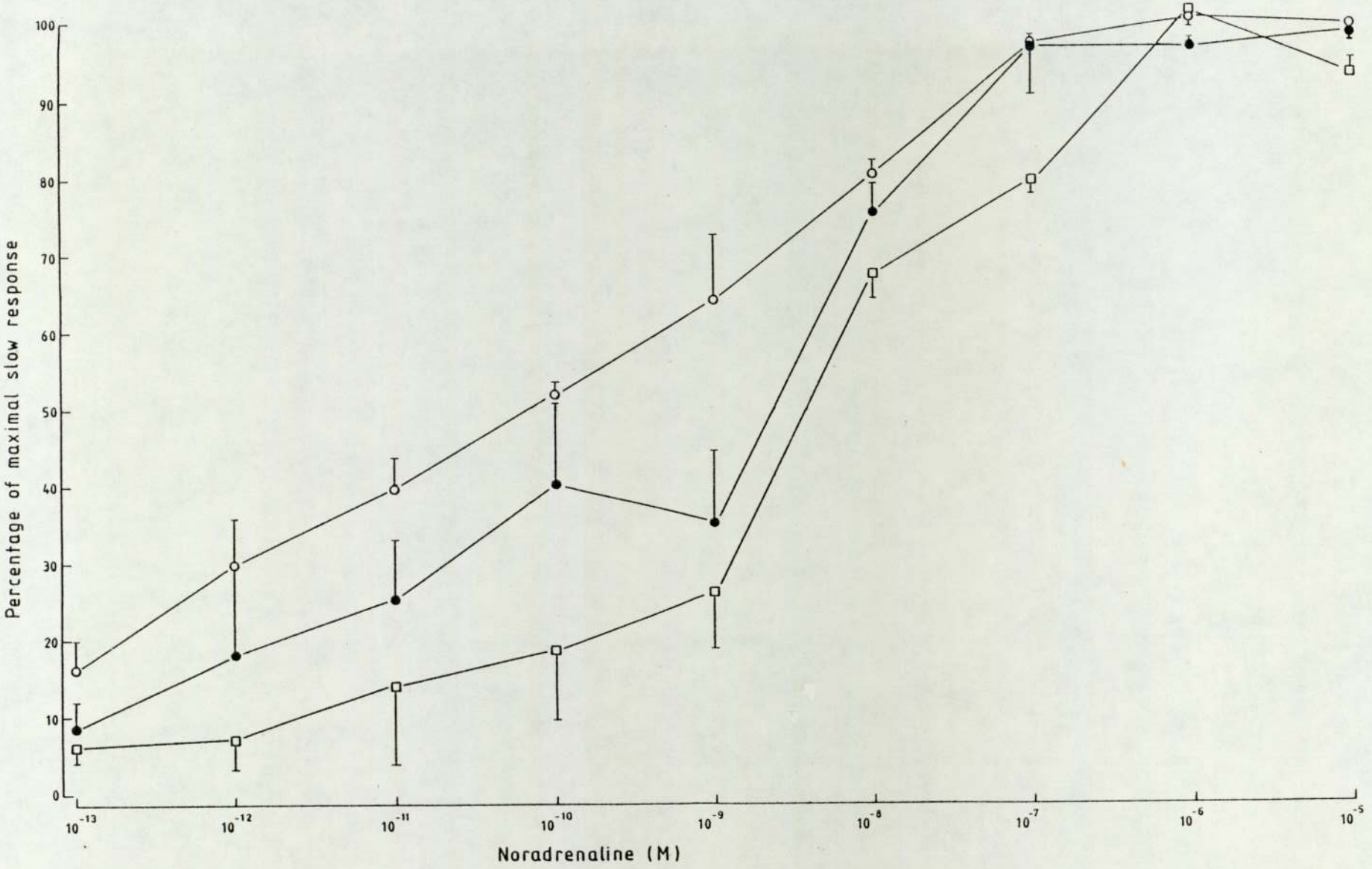

Increase in force (mN)
6·0
5·0
4·0
3·0
2·0
1·0
0
*
*
10^{-13}
10^{-12}
10^{-11}
10^{-10}
10^{-9}
10^{-8}
10^{-7}
10^{-6}
10^{-5}
Noradrenaline(M)

Figure 53

Percentage of the maximal sustained increase in force induced by noradrenaline administration in isolated aortic stips from female rats pre-treated with ethinyloestradiol in PEG 300 (822 $\mu g.kg^{-1}.day^{-1}$)(o), norethisterone acetate in PEG 300 (67 $mg.kg^{-1}.day^{-1}$) (□) or PEG 300 alone (control)(•). Each point represents the mean, and vertical bars denote the s.e. mean (ethinyloestradiol, n=7; norethisterone acetate, n=6; PEG 300, n=6).


Percentage of maximal slow response
100
90
80
70
60
50
40
30
20
10
0
10^{-13}
10^{-12}
10^{-11}
10^{-10}
10^{-9}
10^{-8}
10^{-7}
10^{-6}
10^{-5}
Noradrenaline (M)

b) Effect of hormone pre-treatment upon contractile responses of isolated aortic strips to angiotensin II

After sacrifice, the aorta of each rat was excised, cut into a helical strip and mounted in an organ bath under a resting force of 9.8 mN. After one hour, a non-cumulative concentration-response curve to angiotensin II was constructed (concentration range, 10^{-8}-10^{-5} M). Figure 54 (p. 185) shows a typical response of the aortic strip to angiotensin II. Responses to angiotensin II did not have an easily-identifiable fast phase. Responses were therefore measured as the sustained increase in force due to the slow type of contraction (see figure 54, p. 185).

Figure 55 (p. 186) shows the increase in force induced by angiotensin II (10^{-8}-10^{-5} M) in aortic strips from control rats and from rats pre-treated with ethinyloestradiol or with norethisterone acetate. At all concentrations of angiotensin II, there was no significant difference in the sustained increase in force of aortic strips from ethinyloestradiol pre-treated rats compared with aortic strips from control rats ($P>0.2$ throughout). At two of four concentrations of angiotensin II (10^{-6} and 10^{-5} M), the sustained increase in force was significantly greater in aortic strips from norethisterone acetate pre-treated rats compared with aortic strips from control rats ($P<0.05$).

The maximal contractile responses of aortic strips to angiotensin II were not determined in present experiments, since it was not possible to obtain a stock solution of angiotensin II which was of a sufficiently high concentration. An increase in force of 1.5 mN lay on the linear portion of each concentration-response curve

(see figure 55). Table 14 (p. 184) shows the concentration of angiotensin II required to elicit this standard contractile response in aortic strips from control rats and from rats pre-treated with ethinyloestradiol or with norethisterone acetate. Following ethinyloestradiol pre-treatment, the concentration of angiotensin II required to elicit this standard response was reduced ($P<0.02$). Similarly, the concentration required to elicit this standard response was significantly reduced following norethisterone acetate pre-treatment ($P<0.005$).

TABLE 14 - Concentration of angiotensin II required to elicit an increase in force of 1.5 mN in individual aortic strips taken from female rats pre-treated with hormonal agents and with PEG 300 (controls).

Agent		Concn ($\times 10^{-7}$ M)	P	Concn-ratio
EE	(7)	7.77 ± 1.13	0.01 -0.02*	2.57
NAc	(7)	2.56 ± 0.44	0.001-0.005*	7.81
PEG 300	(7)	20.00 ± 4.13		

Number of observations in parentheses.

Results are mean $\pm$ s.e. mean.

P values are with reference to aortic strips from PEG 300 pre-treated rats (controls).

* Indicates statistical significant difference.

Concn-ratio is the ratio of the mean concentrations in aortic strips from hormone pre-treated, compared with control, rats.

EE - Ethinyloestradiol.

NNAc - Norethisterone acetate.

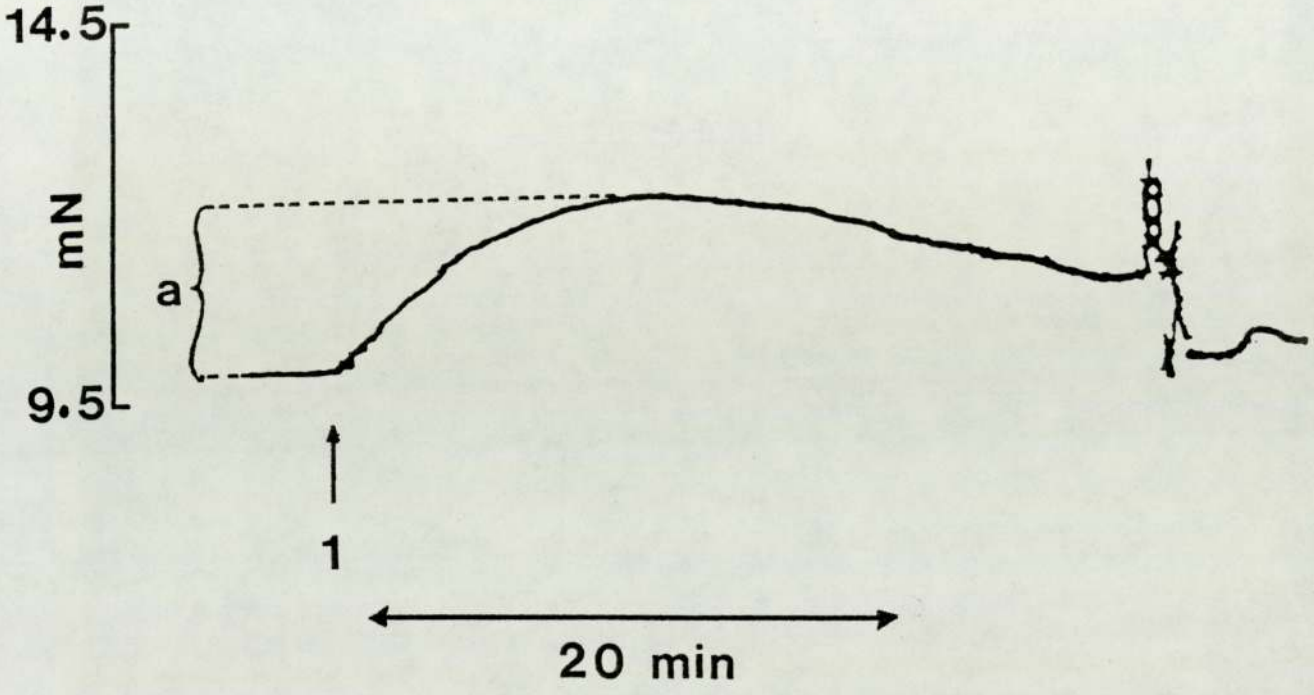


Figure 54

A typical trace of the response to angiotensin II of the isolated aortic strip of the rat.

1 = Point of administration of angiotensin II.

a = Increase in force.

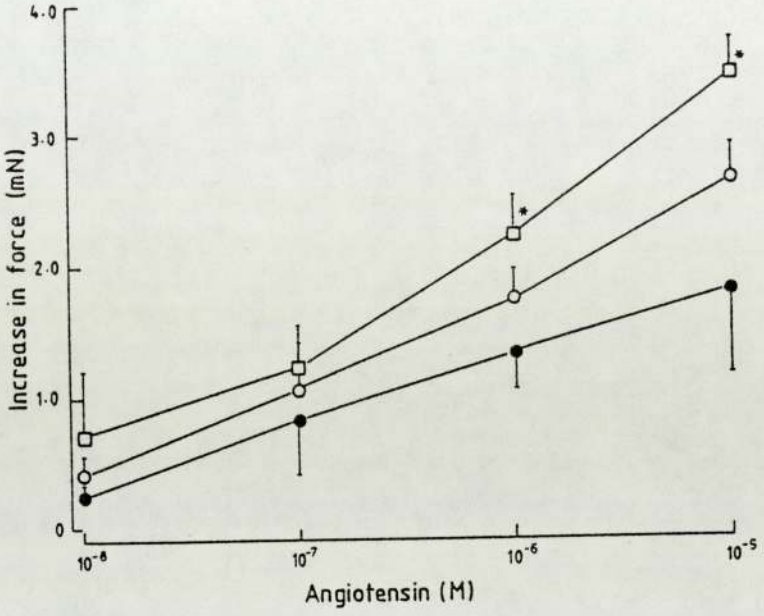


Figure 55

Sustained increase in force induced by angiotensin II administration in isolated aortic strips from female rats pre-treated with ethinyloestradiol in PEG 300 (822 ug.kg^{-1}.day^{-1})(o), norethisterone acetate in PEG 300 (67 mg.kg^{-1}.day^{-1})(□) or PEG 300 alone (control)(●). Each point represents the mean, and vertical bars denote the s.e. mean (n=7).

* Indicates statistical significant difference compared with control responses.

Summary

Following in vivo ethinyloestradiol pre-treatment, fast responses and maximal fast responses of the isolated aortic strip to noradrenaline were potentiated and the $E.C._{50}$ was significantly reduced. The maximal slow response to noradrenaline was potentiated following ethinyloestradiol pre-treatment and the $E.C._{50}$ was significantly reduced, although there was no change in other fast responses. Although responses were not altered at standard concentrations of angiotensin II following ethinyloestradiol pre-treatment, there was a significant decrease in the concentration of angiotensin II required to elicit a standard contractile response in isolated aortic strips.

Fast responses and maximal fast responses to noradrenaline and the $E.C._{50}$ were not significantly different in isolated aortic strips following norethisterone acetate pre-treatment. Slow responses and maximal slow responses to noradrenaline and the $E.C._{50}$ were not significantly different in isolated aortic strips following norethisterone acetate pre-treatment. Aortic strip responses to angiotensin II were significantly increased following in vivo norethisterone acetate pre-treatment. Also, the concentration required to elicit a standard contractile response in isolated aortic strips was significantly reduced following norethisterone acetate pre-treatment.

Experiment 2

Effect of hormone pre-treatment upon contractile responses of isolated portal veins

After sacrifice, the portal vein of each rat was excised and mounted in an organ bath under a resting force of 4.9 mN. After one hour, cumulative concentration-response curves were constructed to noradrenaline (10^{-8}-10^{-5} M), potassium chloride ($0.5x10^{-2}$-$3x10^{-2}$ M), electrical stimulation (100 V at 1, 5 and 10 ms and variable frequency) and angiotensin II (10^{-10}-10^{-6} M).

Figure 56 (p. 195) shows typical concentration-response curves to noradrenaline in the rat portal vein. The responses of portal veins to potassium chloride, electrical stimulation and angiotensin II were similar in appearance to those induced by noradrenaline. The portal vein exhibits spontaneous mechanical activity. The amplitude of spontaneous mechanical activity at rest was approximately 0.3 mN and no differences were observed in the amplitude of spontaneous mechanical activity of portal veins from control rats and from rats pre-treated with ethinyloestradiol or with norethisterone acetate.

At the highest concentrations of noradrenaline, potassium chloride and angiotensin II used, and at two electrical stimulation parameters (100 V, 5 s^{-1} at 5 and 10 ms), the amplitude of spontaneous mechanical activity was decreased in portal veins from control rats and from norethisterone acetate pre-treated rats, but not in portal veins from rats pre-treated with ethinyloestradiol. Reduction in the amplitude of spontaneous mechanical activity at 10^{-5} M noradrenaline can be seen in figure 56a (p. 195) which shows the response of an

isolated portal vein from a control rat. Figure 56b shows the response of an isolated portal vein, from an ethinyloestradiol pre-treated rat, in which the spontaneous mechanical activity is better maintained at the highest concentration of noradrenaline.

Force of contraction of portal veins was integrated automatically and all responses were measured as the total height of the integrator trace recorded for two minutes after stimulation.

Figure 57 (p. 196) shows contractile responses to noradrenaline (10^{-8}-10^{-5} M) of portal veins from control rats and from rats pre-treated with ethinyloestradiol or with norethisterone acetate. There were no significant differences in the responses of portal veins from ethinyloestradiol pre-treated rats compared with portal veins from control rats at any concentration of noradrenaline used ($P>0.2$ throughout). Similarly, there were no significant differences in the responses of portal veins from norethisterone acetate pre-treated rats compared with portal veins from control rats at any concentration of noradrenaline used ($P>0.2$ throughout).

The maximal contractile response to noradrenaline was not significantly different in isolated portal veins from ethinyloestradiol or norethisterone acetate pre-treated rats compared with portal veins from control rats ($P>0.2$ throughout). Figure 58 (p. 197) shows responses of portal veins to noradrenaline plotted as percentage of the maximal response. Table 15 (p. 192) shows the $E.C._{50}$ for contractile responses to noradrenaline in portal veins from control rats and from rats pre-treated with ethinyloestradiol or with norethisterone acetate. Following ethinyloestradiol pre-treatment, the $E.C._{50}$ for responses to noradrenaline was significantly reduced ($P<0.02$). The $E.C._{50}$ for responses to noradrenaline was not significantly different following norethisterone acetate pre-treatment ($P>0.2$).

Figure 59 (p. 198) shows contractile responses to potassium chloride ($0.5x10^{-2}$-$3x10^{-2}$ M) of portal veins from control rats and from rats pre-treated with ethinyloestradiol or with norethisterone acetate. There were no significant differences in the responses of portal veins from ethinyloestradiol pre-treated rats compared with control rats at any concentration of potassium chloride used ($P>0.2$ throughout). Similarly, there were no significant differences in the responses of portal veins from norethisterone acetate pre-treated rats compared with portal veins from control rats at any concentration of potassium chloride used ($P>0.2$ throughout).

The maximal contractile response to potassium chloride was not significantly different in isolated portal veins from ethinyloestradiol or norethisterone acetate pre-treated rats compared with portal veins from control rats ($P>0.3$ throughout). Figure 60 (p. 199) shows responses of portal veins to potassium chloride plotted as percentage of the maximal response. Table 16 (p. 193) shows the $E.C._{50}$ for contractile responses to potassium chloride in portal veins from control rats and from rats pre-treated with ethinyloestradiol or with norethisterone acetate. Following in vivo ethinyloestradiol or norethisterone acetate pre-treatment, there was no significant difference in the $E.C._{50}$ for responses to potassium chloride in isolated portal veins ($P>0.3$ throughout).

There were no significant differences in the contractile responses of portal veins from ethinyloestradiol pre-treated rats compared with portal veins from control rats to electrical stimulation ($P>0.2$ throughout). Similarly, there were no significant differences in the contractile responses of portal veins from norethisterone acetate pre-treated rats compared with portal veins from control rats to electrical stimulation ($P>0.1$ throughout).

Figure 61 (p. 200) shows contractile responses to angiotensin II (10^{-10}-10^{-6} M) of portal veins from control rats and from rats pre-treated with ethinyloestradiol or with norethisterone acetate. At all but one concentration of angiotensin II (10^{-7} M), there were no significant differences in the responses of portal veins from ethinyloestradiol pre-treated rats compared with portal veins from control rats ($P>0.1$ throughout). Similarly, there were no significant differences in the responses of portal veins from norethisterone acetate pre-treated rats compared with portal veins from control rats at any concentration of angiotensin II used ($P>0.5$ throughout).

The maximal contractile response to angiotensin II was not significantly different in isolated portal veins from ethinyloestradiol or norethisterone acetate pre-treated rats compared with portal veins from control rats ($P>0.2$ throughout). Figure 62 (p. 201) shows responses of portal veins to angiotensin II plotted as percentage of the maximal response. Table 17 (p. 194) shows the $E.C._{50}$ for responses to angiotensin II in portal veins from control rats and from rats pre-treated with ethinyloestradiol or with norethisterone acetate. Following ethinyloestradiol pre-treatment, the $E.C._{50}$ for responses to angiotensin II was significantly reduced ($P<0.05$). Similarly, the $E.C._{50}$ for responses to angiotensin II was significantly reduced following norethisterone acetate pre-treatment ($P<0.05$).

TABLE 15 - Concentration of noradrenaline required to elicit 50 % of maximal contractile responses in individual portal veins taken from female rats pre-treated with hormonal agents and with PEG 300 (controls).

Agent		$E.C._{50}$ ($x10^{-8}$ M)	P	Concn-ratio
EE	(6)	6.35 ± 5.00	0.01-0.02*	4.45
NAc	(6)	19.00 ± 5.44	0.2 -0.3	1.49
PEG 300	(6)	28.25 ± 5.72		

Number of observations in parentheses.

Results are mean $\pm$ s.e. mean.

P values are with reference to portal veins from PEG 300 pre-treated rats (controls).

* Indicates statistical significant difference.

Concn-ratio is the ratio of the mean concentrations in portal veins from hormone pre-treated, compared with control, rats.

EE - Ethinyloestradiol.

NAc - Norethisterone acetate.

TABLE 16 - Concentration of potassium chloride required to elicit 50 % of maximal contractile responses in individual portal veins taken from female rats pre-treated with hormonal agents and with PEG 300 (controls).

Agent		$E.C._{50}$ ($x10^{-3}$ M)	P	Concn-ratio
EE	(6)	6.55 ± 0.63	0.3-0.4	1.38
NAc	(6)	6.41 ± 0.34	0.3-0.4	1.41
PEG 300	(6)	9.02 ± 2.38		

Number of observations in parentheses.

Results are mean $\pm$ s.e. mean.

P values are with reference to portal veins from PEG 300 pre-treated rats (controls).

Concn-ratio is the ratio of the mean concentrations in portal veins from hormone pre-treated, compared with control, rats.

EE - Ethinyloestradiol.

NAc - Norethisterone acetate.

TABLE 17 - Concentration of angiotensin II required to elicit 50 % of maximal contractile responses in individual portal veins taken from female rats pre-treated with hormonal agents and with PEG 300 (controls).

Agent		$E.C._{50}$ ($\times 10^{-9}$ M)	P	Concn-ratio
EE	(6)	2.04 ± 0.94	0.025-0.05*	3.84
NAc	(6)	1.40 ± 0.65	0.025-0.05*	5.59
PEG 300	(6)	7.83 ± 2.38		

Number of observations in parentheses.

Results are mean $\pm$ s.e. mean.

P values are with reference to portal veins from PEG 300 pre-treated rats (controls).

* Indicates statistical significant difference.

Concn-ratio is the ratio of the mean concentrations in portal veins from hormone pre-treated, compared with control, rats.

EE - Ethinyloestradiol.

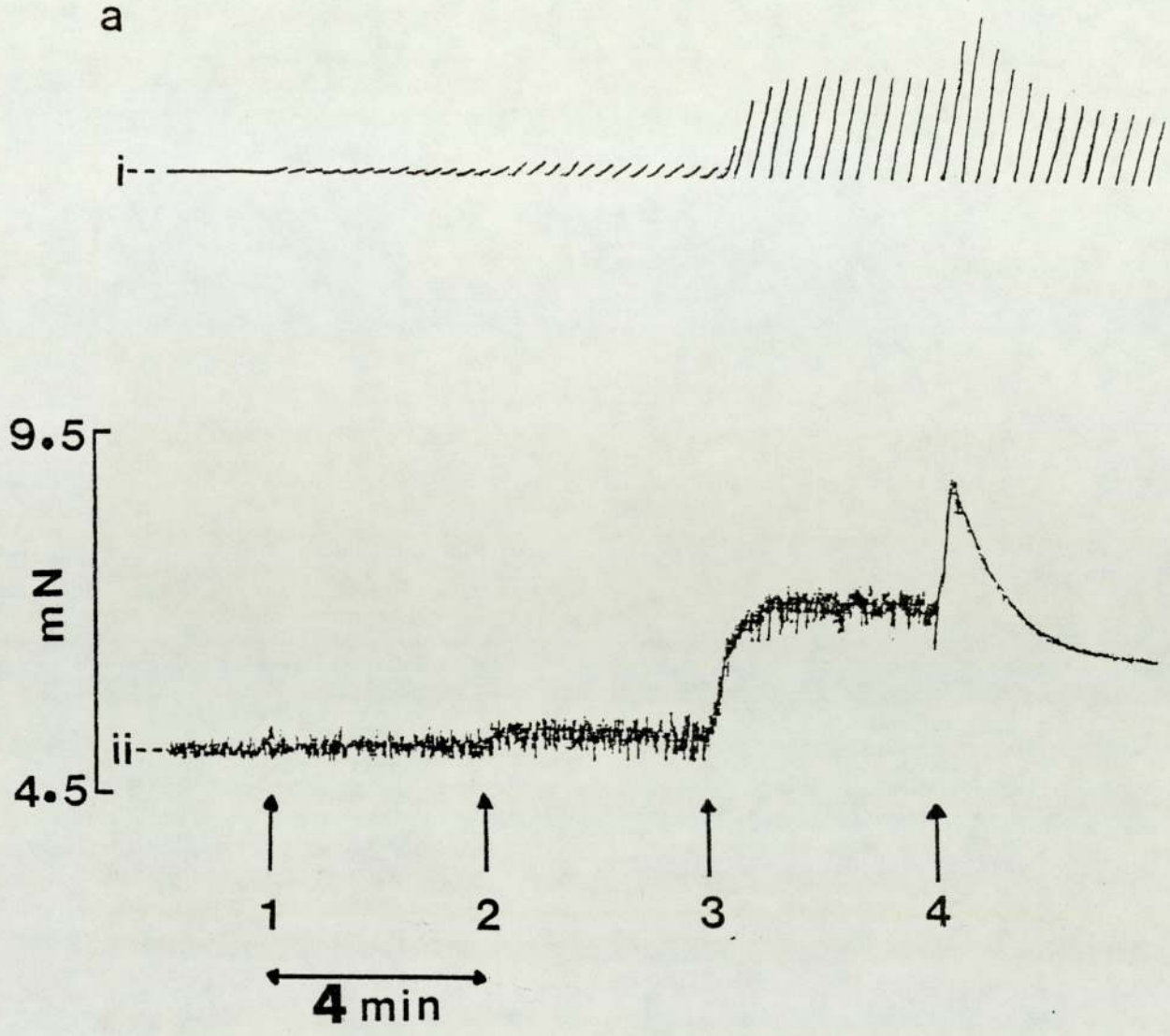
NAc - Norethisterone acetate.

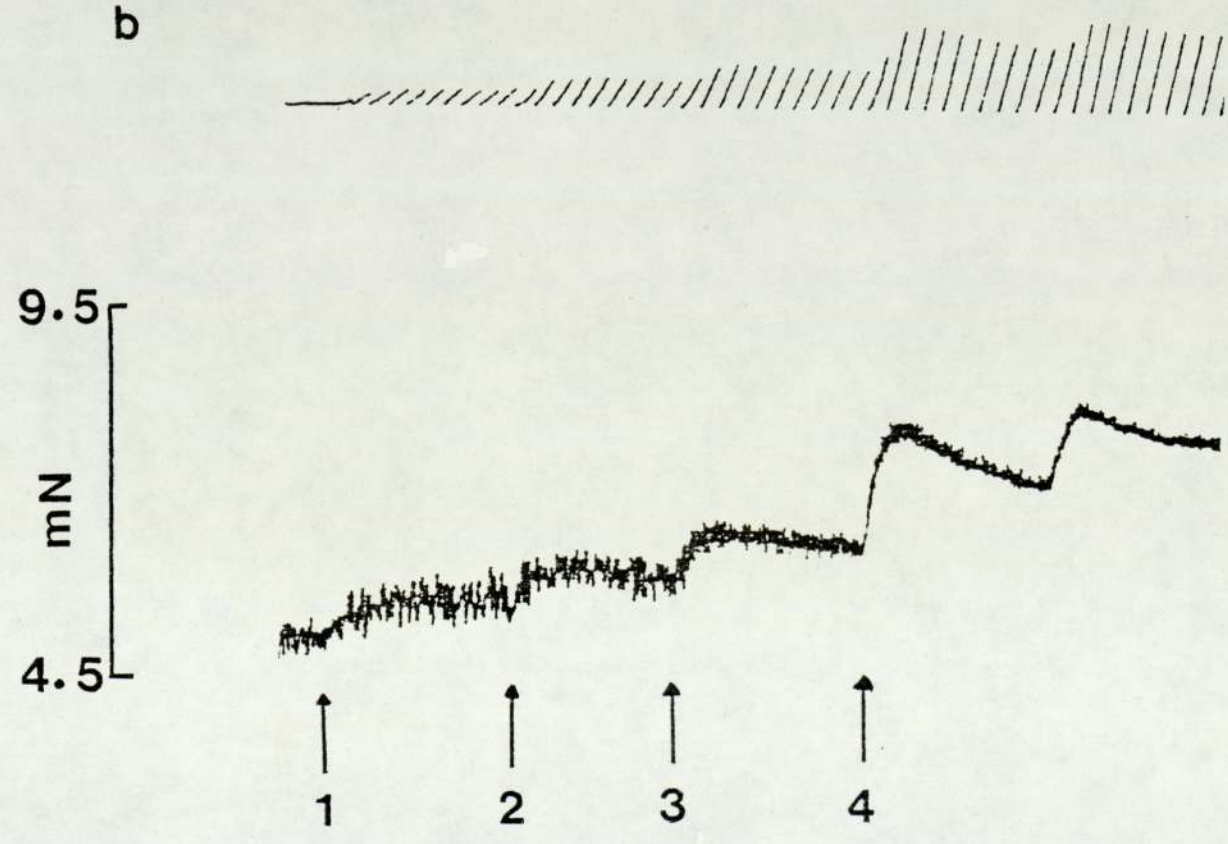
Figure 56

Typical traces of responses to noradrenaline of isolated portal veins from (a) control and (b) ethinyloestradiol pre-treated rats.

$1 = 10^{-8}$ M
$2 = 10^{-7}$ M
$3 = 10^{-6}$ M
$4 = 10^{-5}$ M

i = Integrated force of contraction.
ii = Spontaneous mechanical activity.


a
i
9.5
mN
ii
4.5
1
2
3
4
4 min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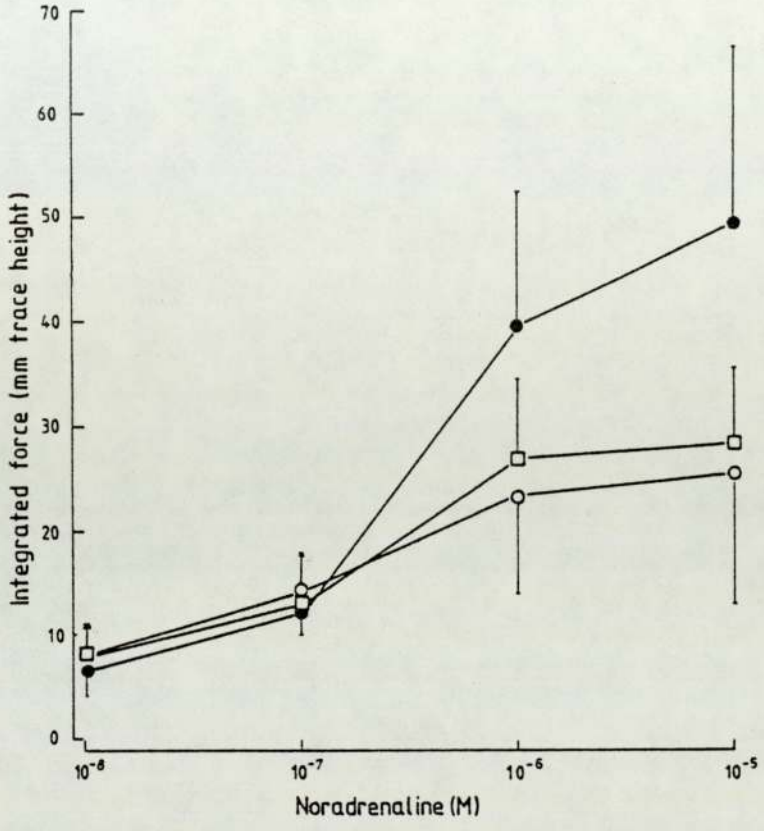


Figure 57

Force of contraction, integrated over two minutes, induced by noradrenaline administration in isolated portal veins from female rats pre-treated with ethinyloestradiol in PEG 300 (822 $\mu g.kg^{-1}.day^{-1}$) (o), norethisterone acetate in PEG 300 (67 $mg.kg^{-1}.day^{-1}$)(□) or PEG 300 alone (control)(•). Each point represents the mean, and vertical bars denote the s.e. mean (n=6).

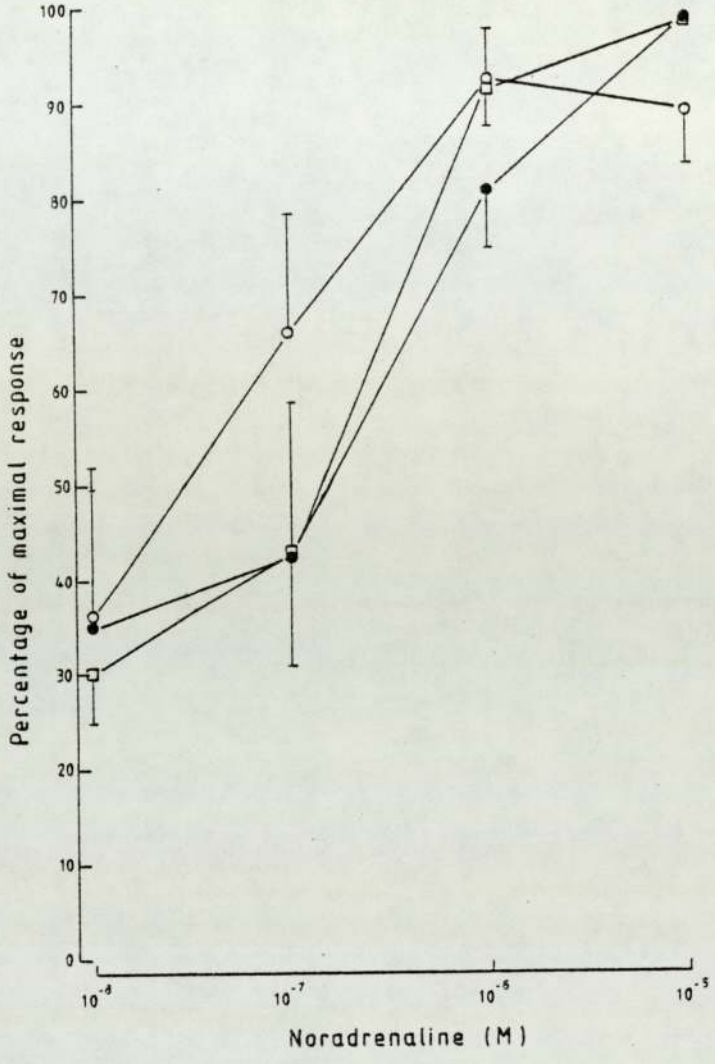


Figure 58

Percentage of the maximal contractile response induced by noradrenaline administration in isolated portal veins from female rats pre-treated with ethinyloestradiol in PEG 300 (822 $\mu g.kg^{-1}.day^{-1}$)(o), norethisterone acetate in PEG 300 (67 $mg.kg^{-1}.day^{-1}$) (□) or PEG 300 alone (control)(•). Each point represents the mean, and vertical bars denote the s.e. mean (n=6).

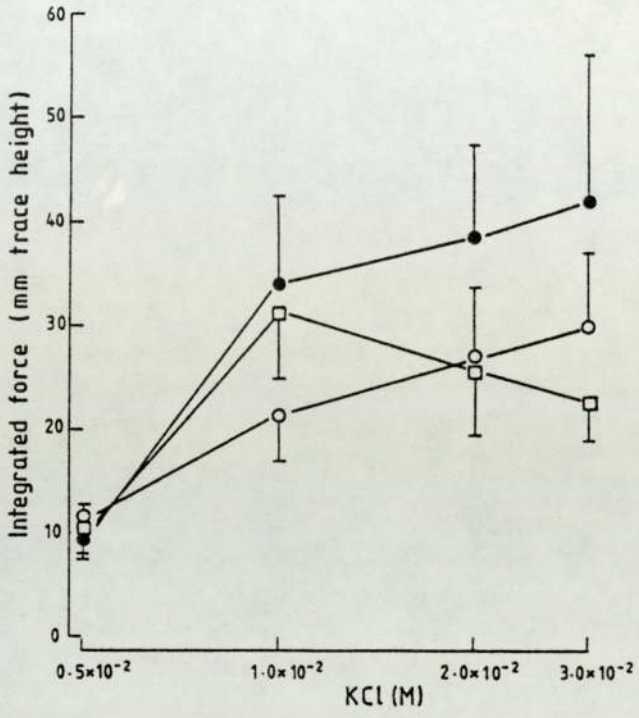


Figure 59

Force of contraction, integrated over two minutes, induced by potassium chloride administration in isolated portal veins from female rats pre-treated with ethinyloestradiol in PEG 300 (822 $\mu g.kg^{-1}.day^{-1}$)(o), norethisterone acetate in PEG 300 (67.$mg.kg^{-1}.day^{-1}$)(□) or PEG 300 alone (control)(•). Each point represents the mean, and vertical bars denote the s.e. mean (n=6).

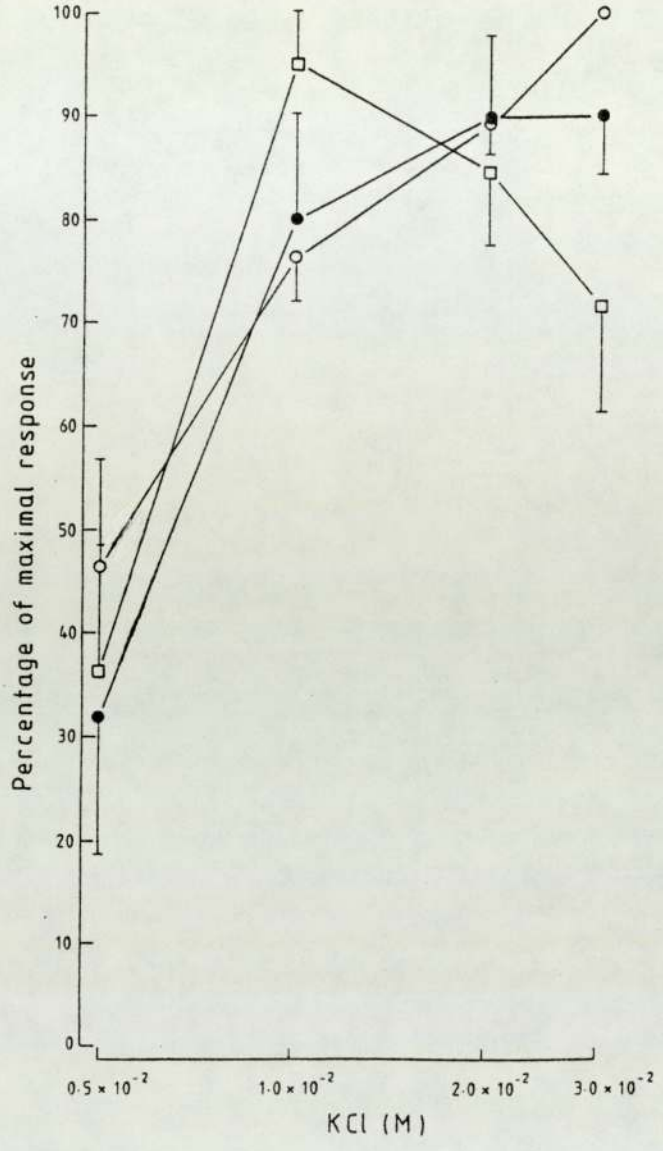


Figure 60

Percentage of the maximal contractile response induced by potassium chloride administration in isolated portal veins from female rats pre-treated with ethinyloestradiol in PEG 300 (822 µg. $kg^{-1}.day^{-1}$)(o), norethisterone acetate in PEG 300 (67 $mg.kg^{-1}.day^{-1}$)(□) or PEG 300 alone (control)(•). Each point represents the mean, and vertical bars denote the s.e. mean (n=6).

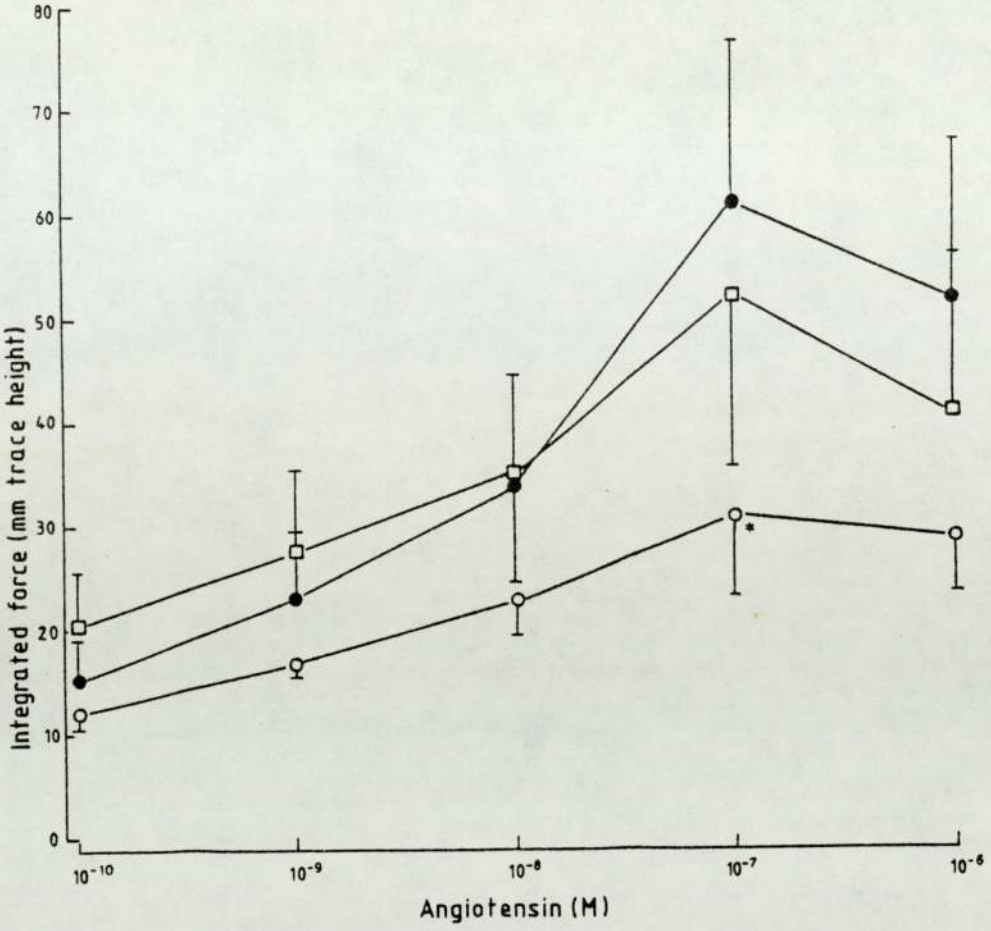


Figure 61

Force of contraction, integrated over two minutes, induced by angiotensin II administration in isolated portal veins from female rats pre-treated with ethinyloestradiol in PEG 300 (822 $\mu g.kg^{-1}.day^{-1}$) (o), norethisterone acetate in PEG 300 (67 $mg.kg^{-1}.day^{-1}$)(□) or PEG 300 alone (control)(•). Each point represents the mean, and vertical bars denote the s.e. mean (n=6).

* Indicates statistical significant difference compared with control responses.

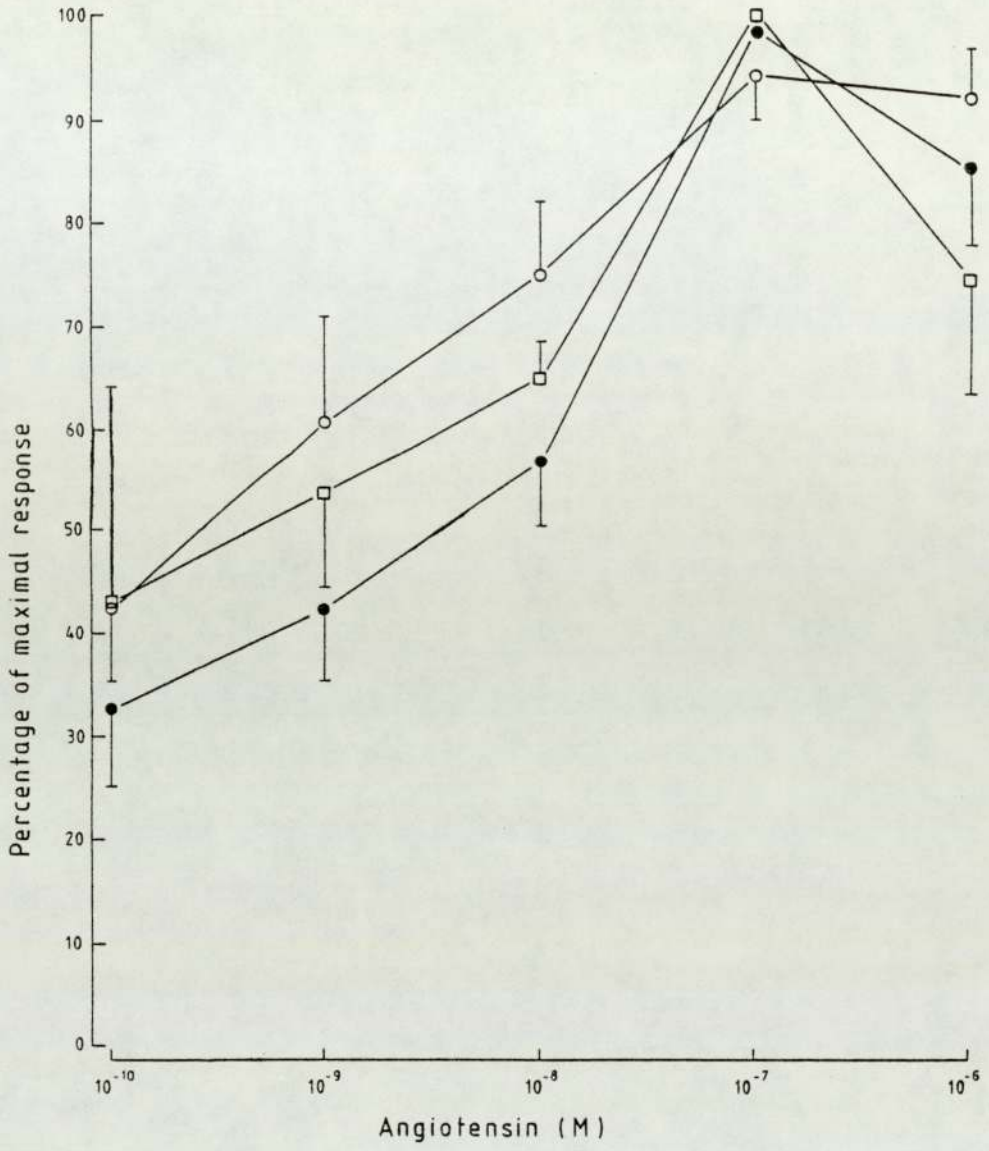


Figure 62

Percentage of the maximal contractile response induced by angiotensin II administration in isolated portal veins from female rats pre-treated with ethinyloestradiol in PEG 300 (822 $\mu g.kg^{-1}.day^{-1}$)(o), norethisterone acetate in PEG 300 (67 $mg.kg^{-1}.day^{-1}$) (□) or PEG 300 alone (control)(•). Each point represents the mean, and vertical bars denote the s.e. mean (n=6).

Summary

No differences were observed in the spontaneous mechanical activity of isolated portal veins following pre-treatment of rats with ethinyloestradiol or with norethisterone acetate compared with that of portal veins from control rats. Spontaneous mechanical activity during contractile responses to noradrenaline, angiotensin II, potassium chloride and electrical stimulation was better maintained in portal veins following ethinyloestradiol pre-treatment.

The concentrations required to elicit 50 % of maximal contractile responses to noradrenaline and to angiotensin II in individual isolated portal veins were reduced following pre-treatment with ethinyloestradiol. Similarly, the concentration required to elicit 50 % of maximal contractile response to angiotensin II in individual portal veins was reduced following norethisterone acetate pre-treatment. No change was observed in the maximal contractile responses of isolated portal veins to noradrenaline, angiotensin II and potassium chloride following pre-treatment with either ethinyloestradiol or norethisterone acetate.

Experiment 3

a) Effect of hormone pre-treatment upon responses of isolated paired atria to noradrenaline

After sacrifice, paired atria from each rat were excised and mounted in an organ bath under a resting force of 9.8 mN. The atria beat spontaneously and recordings of the average force developed during each spontaneous contraction (f) were made, together with measurements of the rate of change of force (df/dt) and the rate of beating (r_1)(see figure 63, p. 210). Table 18 (p. 207) shows f, df/dt and r_1 in atria from control rats and from rats pre-treated with ethinyloestradiol or with norethisterone acetate prior to administration of noradrenaline. No significant difference in f, df/dt or r_1 was recorded between atria from ethinyloestradiol pre-treated rats compared with atria from control rats ($P>0.1$ throughout). Similarly, f, df/dt and r_1 were not significantly different in atria from norethisterone acetate pre-treated rats compared with atria from control rats ($P>0.5$ throughout).

After 30-60 minutes, cumulative inotropic concentration-response curves to noradrenaline were constructed (concentration-range, 3.1×10^{-11}-3.1×10^{-5} M). Following administration of noradrenaline, the increase in force (c), the rate of change of force (df/dt) and the sustained rate of beating (r_2) were recorded. Figure 63 (p. 210) shows a typical concentration-response curve to noradrenaline and how c, df/dt and r_2 were measured.

Figure 64 (p. 211) shows the mean increase in force (c) following administration of noradrenaline (3.1×10^{-11}-3.1×10^{-5} M) to

paired atria from control rats and from rats pre-treated with ethinyloestradiol or with norethisterone acetate. At all concentrations of noradrenaline used, there were no significant differences in the increase in force (c), of atria from ethinyloestradiol pre-treated rats compared with atria from control rats ($P>0.2$ throughout). Similarly, at all but the lowest concentration of noradrenaline ($3.1x10^{-11}$ M), c was not significantly different in atria from norethisterone acetate pre-treated rats compared with atria from control rats ($P>0.1$ throughout). An increase in force of 0.75 mN lay on the linear portion of the concentration-response curve (see figure 64). Table 19 (p. 208) shows the concentration of noradrenaline required to elicit this standard response in atria from hormone pre-treated and control rats. There was no significant difference in the concentration of noradrenaline required to elicit this standard inotropic response in atria from ethinyloestradiol pre-treated and control rats ($P>0.7$). Similarly, there was no significant difference in the concentration of noradrenaline required to elicit this standard response in atria from norethisterone acetate pre-treated rats compared with atria from control rats ($P>0.3$).

Figure 65 (p. 211) shows the increase in force (c) induced by noradrenaline ($3.1x10^{-11}$-$3.1x10^{-5}$ M) in paired atria from individual control rats. Similarly, figure 66 (p. 212) shows the increase in force (c) induced by noradrenaline ($3.1x10^{-11}$-$3.1x10^{-5}$ M) in paired atria from individual ethinyloestradiol pre-treated rats. The responses of atria from rats pre-treated with ethinyloestradiol appeared to consist of two groups. Paired atria from five ethinyloestradiol pre-treated rats demonstrated concentration-response curves to noradrenaline which were similar to control curves. Paired

atria from two ethinyloestradiol pre-treated rats demonstrated concentration-response curves to noradrenaline which lay to the left of control curves. Ethinyloestradiol pre-treated rats from which the more responsive atria were isolated did not differ in body weight, post mortem findings or oestrous cycle length compared with other ethinyloestradiol pre-treated rats. Figure 67 (p. 214) shows the increase in force (c) induced by noradrenaline ($3.1x10^{-11}$-$3.1x10^{-5}$ M) in paired atria from individual rats pre-treated with norethisterone acetate. These atria demonstrated a wide spread of inotropic responses to noradrenaline. Comparison of figures 65, 66 and 67 indicate that inotropic responses of rat isolated atria to noradrenaline may not be normally distributed following hormone pre-treatment. For this reason, the Mann-Whitney test was applied (see page 59). However, application of this test indicated that there was no significant difference in the responses of atria from ethinyloestradiol pre-treated and norethisterone acetate pre-treated rats compared with the responses of control atria ($P>0.1$ throughout). Comparison of figures 65 and 67 (pp. 212 and 214) shows that the maximal force of atrial contraction may be elevated following pre-treatment with norethisterone acetate.

The rate of change of force (df/dt) at all concentrations of noradrenaline was significantly greater in atria from rats pre-treated with ethinyloestradiol compared with atria from control rats ($P<0.05$ throughout). For example, at $3.1x10^{-9}$ M noradrenaline, df/dt in atria from ethinyloestradiol pre-treated rats was 8.49 ± 1.20 $x10^{-2}$ $mN.s^{-1}$ (n=7), whereas df/dt in atria from control rats was 4.84 ± 0.90 $x10^{-2}$ $mN.s^{-1}$ (n=6)($P<0.05$). The rate of change of force (df/dt) was not significantly different in atria from norethisterone acetate pre-treated rats compared with atria from control rats at any concentration of noradrenaline used ($P>0.05$ throughout).

At all but one concentration of noradrenaline used ($3.1x10^{-8}$ M), the sustained rate of beating (r_2) was not significantly different in atria from ethinyloestradiol pre-treated rats compared with atria from control rats ($P>0.3$). Similarly, r_2 was not significantly different in atria from norethisterone acetate pre-treated rats compared with atria from control rats at any concentration of noradrenaline used ($P>0.3$ throughout).

Figure 68 (p. 215) shows the increase in rate (r_2-r_1) following administration of noradrenaline ($3.1x10^{-11}$-$3.1x10^{-5}$ M) to paired atria from control rats and from rats pre-treated with ethinyloestradiol or with norethisterone acetate. The increase in rate was not significantly different in atria from ethinyloestradiol and norethisterone acetate pre-treated rats compared with atria from control rats at any concentration of noradrenaline used ($P>0.2$ throughout). Table 20 (p. 209) shows that the concentration of noradrenaline required to elicit a standard increase in heart rate of 80 beats.min^{-1} was not significantly different in atria from either ethinyloestradiol or norethisterone acetate pre-treated rats compared with atria from control rats ($P>0.4$ throughout). There was no separation of positive chronotropic responses to noradrenaline following ethinyloestradiol or norethisterone acetate pre-treatment.

TABLE 18 - Force of spontaneous contraction, rate of change of force and rate of beating of isolated paired atria from hormone pre-treated rats prior to administration of noradrenaline.

	Pre-treatment				
Parameter	EE (7)	P	NAc (6)	P	PEG 300 (6)
Force (f)(mN)	1.10±0.13	0.8-0.9	1.03±0.02	0.5-0.6	1.13±0.18
Rate of change of force (df/dt) ($\times 10^{-2}$ mN.s^{-1})	6.62±1.01	0.1-0.2	4.22±0.41	0.8-0.9	4.42±0.75
Rate (r_1)(min^{-1})	238.6 ±31.1	0.5-0.6	205.8 ±15.5	0.5-0.6	217.5 ±8.0

Number of observations in parentheses.

Results are mean±s.e. mean.

P values are with reference to atria from PEG 300 pre-treated rats (controls).

EE - Ethinyloestradiol.

NAc - Norethisterone acetate.

TABLE 19 - Concentration of noradrenaline required to elicit an increase in force of 0.75 mN in individual paired atria taken from female rats pre-treated with hormonal agents and with PEG 300 (controls).

Agent		Concn ($\times 10^{-6}$ M)	P	Concn-ratio
EE	(7)	4.02 ± 1.46	0.7-0.8	2.59
NAc	(5)	8.81 ± 2.21	0.3-0.4	5.68
PEG 300	(5)	1.55 ± 6.93		

Number of observations in parentheses.

Results are mean $\pm$ s.e. mean.

P values are with reference to atria from PEG 300 pre-treated rats (controls).

Concn-ratio is the ratio of the mean concentrations in atria from hormone pre-treated, compared with control, rats.

EE - Ethinyloestradiol.

NAc - Norethisterone acetate.

TABLE 20 - Concentration of noradrenaline required to elicit an increase in rate of 80 beats.min^{-1} in individual paired atria taken from female rats pre-treated with hormonal agents and with PEG 300 (controls).

Agent		Concn ($\times 10^{-8}$ M)	P	Concn-ratio
EE	(7)	46.56 ± 51.96	0.8-0.9	1.47
NAc	(6)	11.80 ± 11.33	0.4-0.5	5.79
PEG 300	(6)	68.30 ± 67.90		

Number of observations in parentheses.

Results are mean $\pm$ s.e. mean.

P values are with reference to atria from PEG 300 pre-treated rats (controls).

Concn-ratio is the ratio of the mean concentrations in atria from hormone pre-treated, compared with control, rats.

EE - Ethinyloestradiol.

NAc - Norethisterone acetate.

Figure 63

A typical trace of responses to noradrenaline of isolated paired atria of the rat.

$1 = 3.1 \times 10^{-11}$ M
$2 = 3.1 \times 10^{-10}$ M
$3 = 3.1 \times 10^{-9}$ M
$4 = 3.1 \times 10^{-8}$ M
$5 = 3.1 \times 10^{-7}$ M
$6 = 3.1 \times 10^{-6}$ M
$7 = 3.1 \times 10^{-5}$ M

A = Force of contraction.
B = Rate of contraction.
C = Rate of change of force of contraction.

c = Increase in force after administration of noradrenaline.
f = Force developed during each spontaneous contraction.
r = Rate of beating.
df/dt = Rate of change of force.

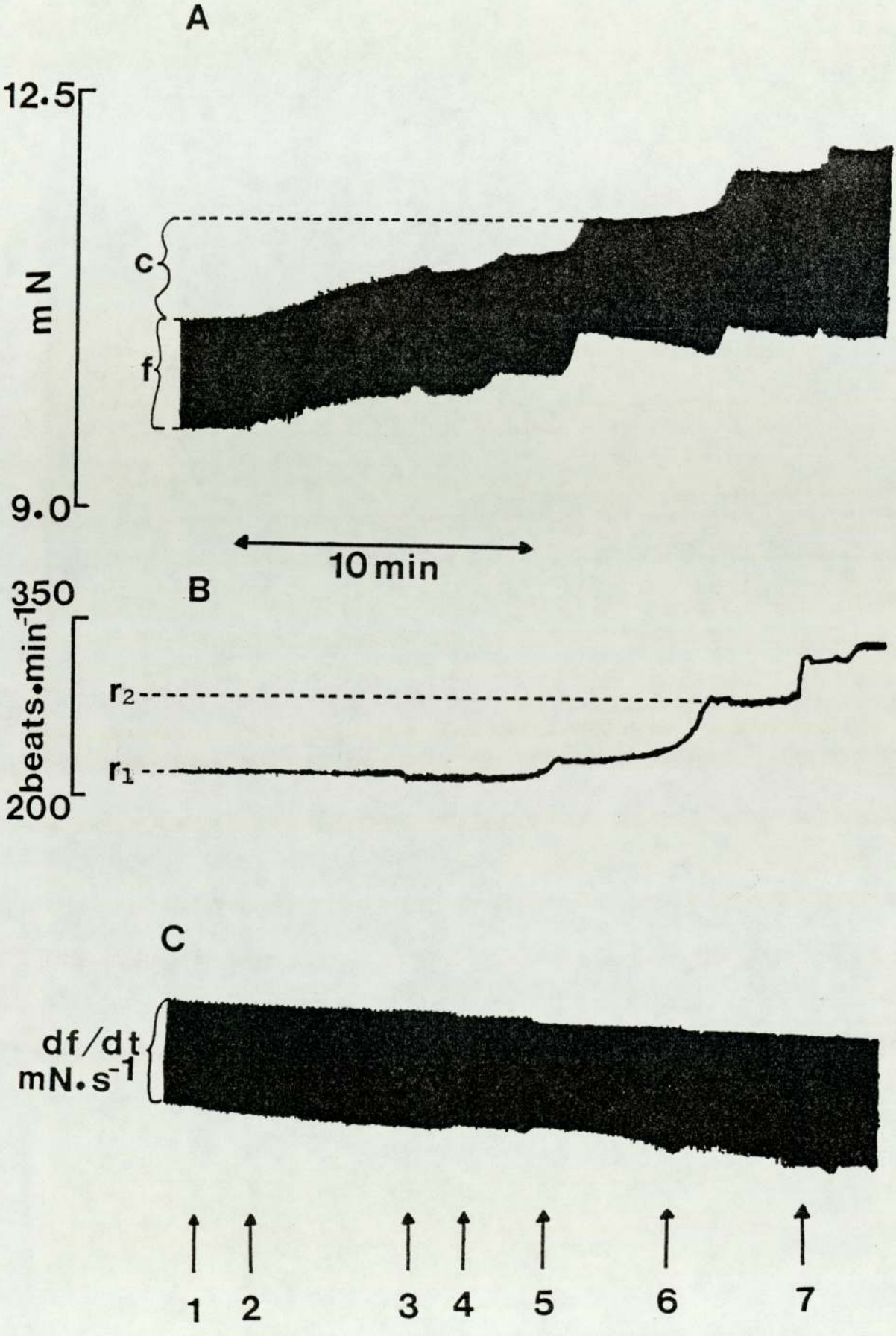

A
12.5
mN
9.0
c
f
10 min
B
350
beats·min⁻¹
200
r2
r1
C
df/dt
mN·s⁻¹
1
2
3
4
5
6
7

Figure 64

Increase in force (c) induced by noradrenaline administration in isolated paired atria from female rats pre-treated with ethinyloestradiol in PEG 300 (822 $\mu g.kg^{-1}.day^{-1}$) (o), norethisterone acetate in PEG 300 (67 $mg.kg^{-1}.day^{-1}$)(□) or PEG 300 alone (control) (•). Each point represents the mean, and vertical bars denote the s.e. mean (ethinyloestradiol, n=7; norethisterone acetate, n=6; PEG 300, n=6).

* Indicates statistical significant difference compared with control responses.

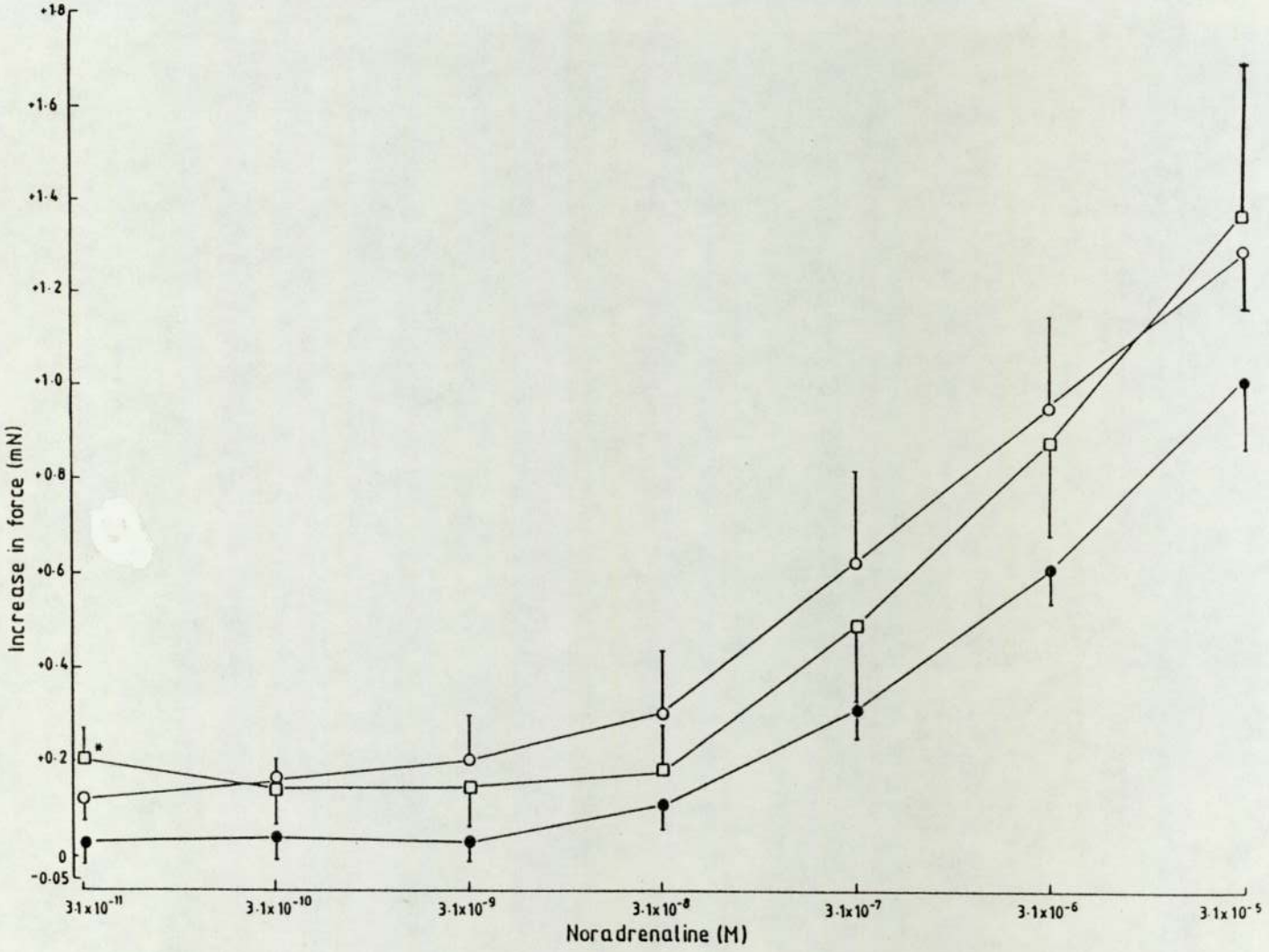

+1·8
+1·6
+1·4
+1·2
+1·0
+0·8
+0·6
+0·4
+0·2
0
-0·05
Increase in force (mN)
*
3·1x10⁻¹¹
3·1x10⁻¹⁰
3·1x10⁻⁹
3·1x10⁻⁸
3·1x10⁻⁷
3·1x10⁻⁶
3·1x10⁻⁵
Noradrenaline (M)

Figure 65

Increase in force (c) induced by noradrenaline administration in isolated paired atria from individual female rats pre-treated with PEG 300 alone (controls).

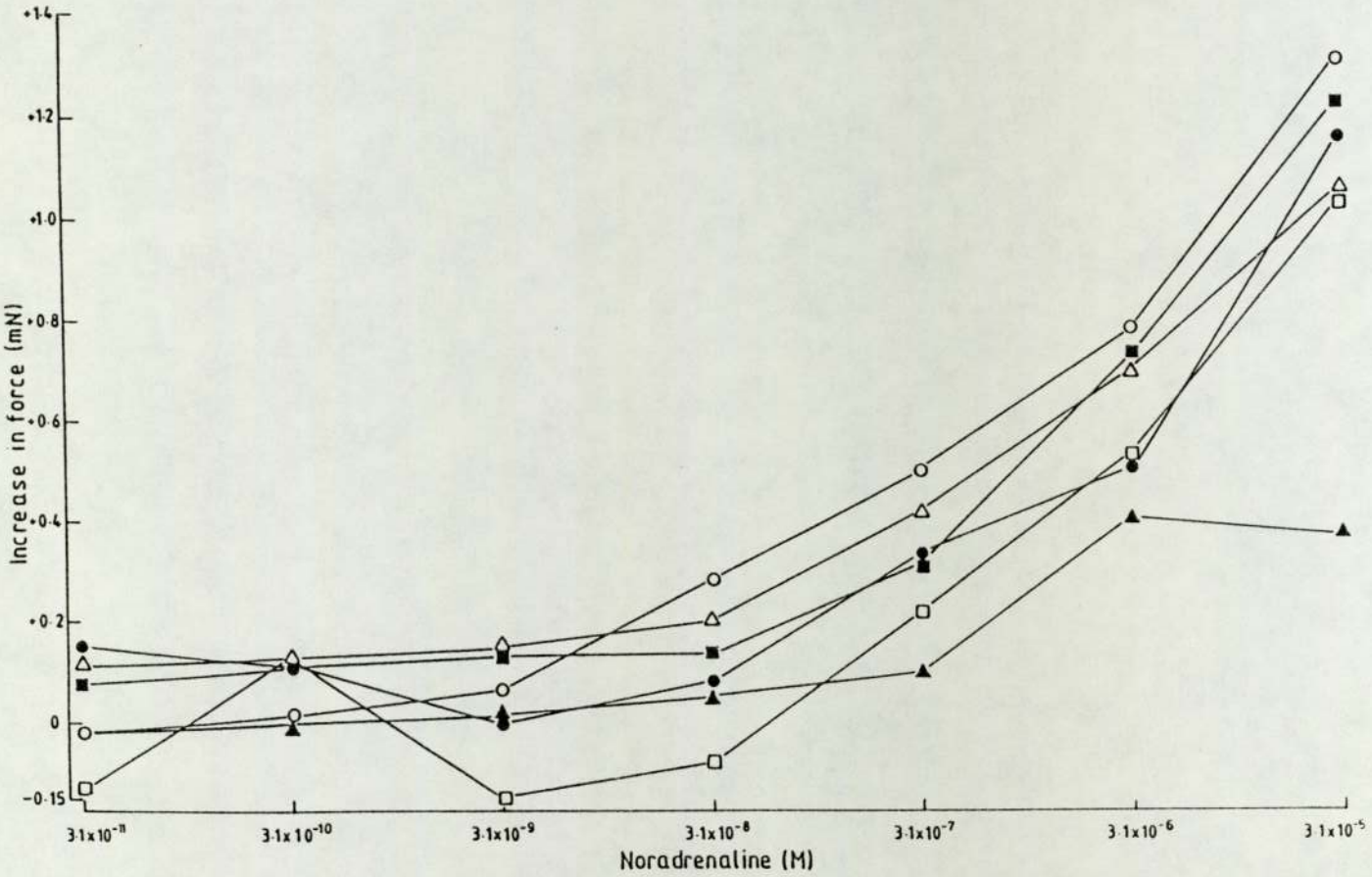
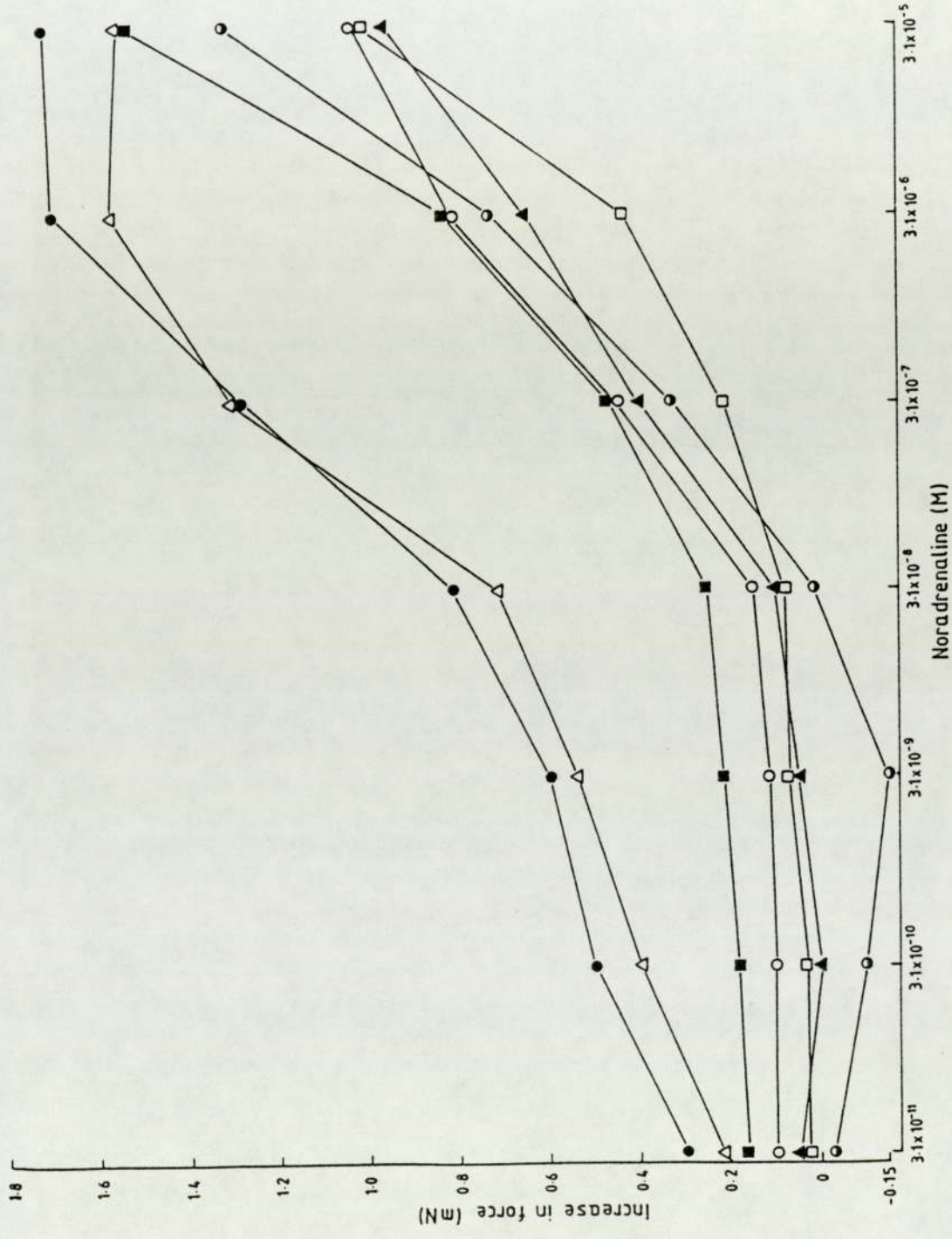

+1·4
+1·2
+1·0
+0·8
+0·6
+0·4
+0·2
0
-0·15
Increase in force (mN)
3·1x10⁻¹¹
3·1x10⁻¹⁰
3·1x10⁻⁹
3·1x10⁻⁸
3·1x10⁻⁷
3·1x10⁻⁶
3·1x10⁻⁵
Noradrenaline (M)

Figure 66

Increase in force (c) induced by noradrenaline administration in isolated paired atria from individual female rats pre-treated with ethinyloestradiol in PEG 300 (822 $\mu g.kg^{-1}.day^{-1}$).


Increase in force (mN)
1·8
1·6
1·4
1·2
1·0
0·8
0·6
0·4
0·2
0
-0·15
3·1x10⁻¹¹
3·1x10⁻¹⁰
3·1x10⁻⁹
3·1x10⁻⁸
3·1x10⁻⁷
3·1x10⁻⁶
3·1x10⁻⁵
Noradrenaline (M)

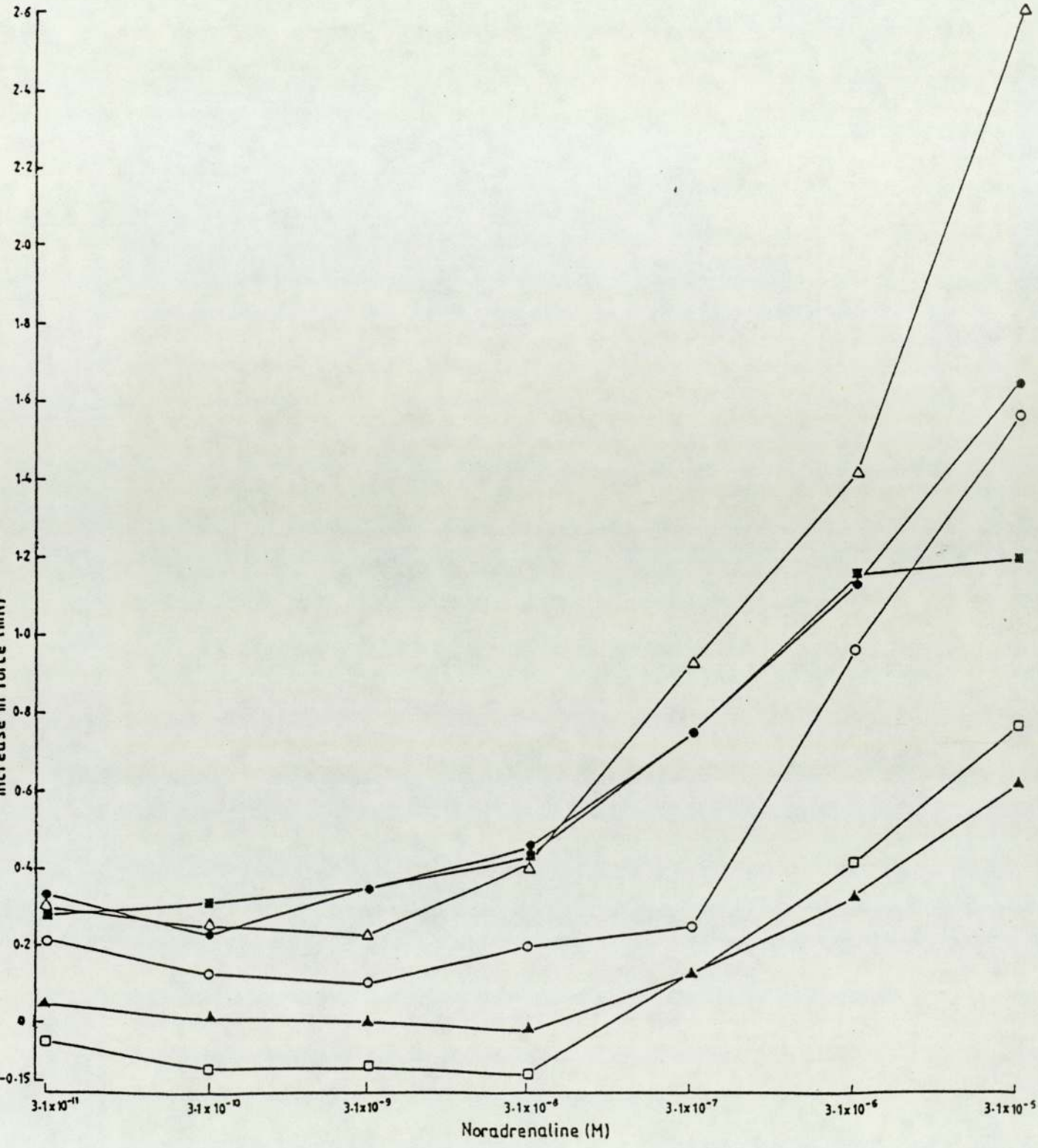
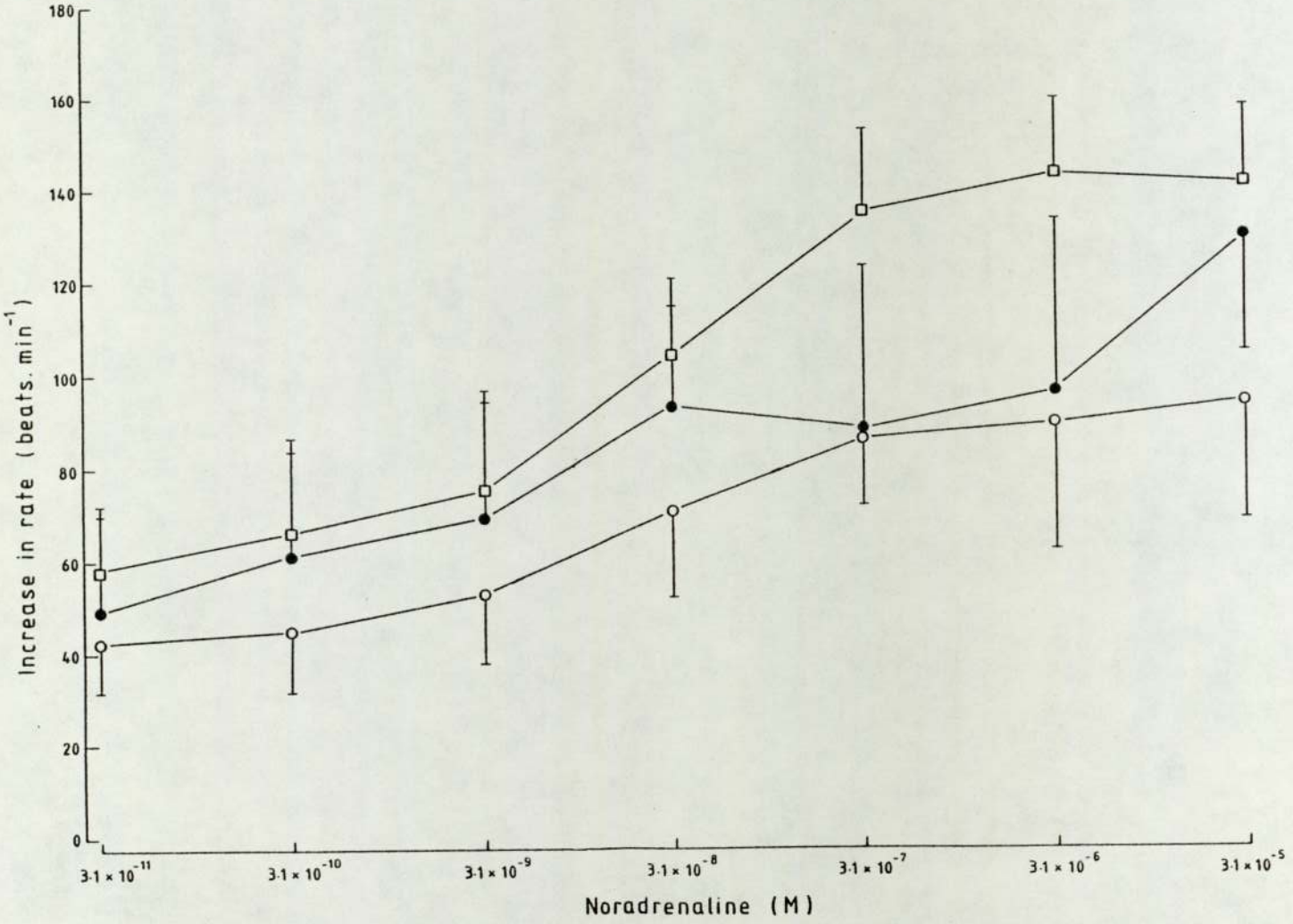


Figure 67

Increase in force (c) induced by noradrenaline administration in isolated paired atria from individual female rats pre-treated with norethisterone acetate in PEG 300 (67 $mg.kg^{-1}.day^{-1}$).

Figure 68

Increase in rate induced by noradrenaline administration in isolated paired atria from female rats pre-treated with ethinyloestradiol in PEG 300 (822 $\mu g.kg^{-1}.day^{-1}$) (o), norethisterone acetate in PEG 300 (67 $mg.kg^{-1}.day^{-1}$)(▫) or PEG 300 alone (control) (•). Each point represents the mean, and vertical bars denote the s.e. mean (ethinyloestradiol, n=7; norethisterone acetate, n=6; PEG 300, n=6).


Increase in rate (beats. min^{-1})
180
160
140
120
100
80
60
40
20
0
3·1 × 10^{-11}
3·1 × 10^{-10}
3·1 × 10^{-9}
3·1 × 10^{-8}
3·1 × 10^{-7}
3·1 × 10^{-6}
3·1 × 10^{-5}
Noradrenaline (M)

b) Effect of hormone pre-treatment upon responses of isolated paired atria to isoprenaline

After sacrifice, paired atria from each rat were excised and mounted in an organ bath under a resting force of 9.8 mN. The atria beat spontaneously and recordings of the average force developed during each spontaneous contraction (f) were made, together with measurements of the rate of change of force (df/dt) and the rate of beating (r_1)(see figure 63, p. 210). Table 21 (p. 219) shows f, df/dt and r_1 in atria from control rats and from rats pre-treated with ethinyloestradiol or with norethisterone acetate prior to administration of isoprenaline. No significant difference in f, df/dt or r_1 was recorded between atria from ethinyloestradiol pre-treated rats compared with atria from control rats ($P>0.2$ throughout). Similarly, f, df/dt and r_1 were not significantly different in atria from norethisterone acetate pre-treated rats compared with atria from control rats ($P>0.1$ throughout).

After 30-60 minutes, cumulative inotropic concentration-response curves to isoprenaline were constructed (concentration range, 2.8×10^{-16}-$2.8\text{-}10^{-10}$ M). Following administration of isoprenaline, the increase in force (c), the rate of change of force (df/dt) and the sustained rate of beating (r_2) were recorded. Figure 63 (p. 210) shows how c, df/dt and r_2 were measured.

Figure 69 (p. 221) shows the increase in force (c) following administration of isoprenaline (2.8×10^{-16}-2.8×10^{-10} M) to paired atria from control rats and from rats pre-treated with ethinyloestradiol or with norethisterone acetate. At all concentrations of isoprenaline used, there were no significant differences in the increase in force

(c) of atria from ethinyloestradiol pre-treated rats compared with atria from control rats ($P>0.4$ throughout). Similarly, at all but one concentration of isoprenaline (2.8×10^{-14} M), c was not significantly different in atria from norethisterone acetate pre-treated rats compared with atria from control rats ($P>0.1$ throughout). The increase in force induced by isoprenaline administration appeared to lie in the threshold region of the concentration-response curve. For this reason, no comparison was made of the concentrations of isoprenaline required to elicit the same response in hormone pre-treated and control rats.

At all but three concentrations of isoprenaline (2.8×10^{-16}, 2.8×10^{-15} and 2.8×10^{-12} M), the rate of change of force (df/dt) was significantly greater in atria from rats pre-treated with ethinyloestradiol compared with atria from control rats ($P<0.05$ throughout). For example, at 2.8×10^{-14} M isoprenaline, df/dt in atria from ethinyloestradiol pre-treated rats was $7.73\pm0.95\ \times10^{-2}$ mN.s^{-1} ($n=5$), whereas df/dt in atria from control rats was $4.75\pm0.83\ \times10^{-2}$ mN.s^{-1} ($n=6$)($P<0.05$). At all concentrations of isoprenaline used, there were no significant differences in the rate of change of force (df/dt) in atria from norethisterone acetate pre-treated rats compared with atria from control rats ($P>0.05$ throughout).

The sustained rate of beating (r_2-r_1) was not significantly different in atria from ethinyloestradiol pre-treated rats compared with atria from control rats at any concentration of isoprenaline used ($P>0.1$ throughout). Similar findings were obtained for norethisterone acetate pre-treated rats. Figure 70 (p. 222) shows the increase in rate (r_2-r_1) following administration of isoprenaline (2.8×10^{-16}-2.8×10^{-10} M) to paired atria from control rats and from rats

pre-treated with ethinyloestradiol or with norethisterone acetate. Table 22 (p. 220) shows that the concentration of isoprenaline required to elicit a standard positive increase in rate of 80 beats.min^{-1} was not significantly different in atria from ethinyloestradiol or norethisterone acetate pre-treated rats compared with atria from control rats ($P>0.5$ throughout).

TABLE 21 - Force of spontaneous contraction, rate of change of force and rate of beating of isolated paired atria from hormone pre-treated rats prior to administration of isoprenaline.

	Pre-treatment				
Parameter	EE (5)	P	NAc (6)	P	PEG 300 (6)
Force (f)(mN)	1.49±0.16	0.3-0.4	1.64±0.13	0.1-0.2	1.25±0.20
Rate of change of force (df/dt) ($x10^{-2} mN.s^{-1}$)	5.61±0.70	0.3-0.4	5.70±0.62	0.2-0.3	4.53±0.82
Rate (r_1)(min^{-1})	196.0 ±9.3	0.2-0.3	200.7 ±8.2	0.4-0.5	209.2 ±6.5

Number of observations in parentheses.

Results are mean±s.e. mean.

P values are with reference to atria from PEG 300 pre-treated rats (controls).

EE - Ethinyloestradiol.

Nac - Norethisterone acetate.

TABLE 22 - Concentration of isoprenaline required to elicit an increase in rate of 80 beats.min^{-1} in individual paired atria taken from female rats pre-treated with hormonal agents and with PEG 300 (controls).

Agent		Concn ($\times 10^{-14}$ M)	P	Concn-ratio
EE	(5)	2.38 ± 2.74	0.8-0.9	1.60
NAc	(6)	7.45 ± 4.34	0.5-0.6	1.96
PEG 300	(6)	3.81 ± 4.03		

Number of observations in parentheses.

Results are mean $\pm$ s.e. mean.

P values are with reference to atria from PEG 300 pre-treated, compared with control, rats.

Concn-ratio is the ratio of the mean concentrations in atria from hormone pre-treated, compared with control, rats.

EE -Ethinyloestradiol.

NAc - Norethisterone acetate.

Figure 69

Increase in force (c) induced by isoprenaline administration in isolated paired atria from female rats pre-treated with ethinyloestradiol in PEG 300 (822 $\mu g.kg^{-1}.day^{-1}$) (●), norethisterone acetate in PEG 300 (67 $mg.kg^{-1}.day^{-1}$)(□) or PEG 300 alone (control) (•). Each point represents the mean, and vertical bars denote the s.e. mean (ethinyloestradiol, n=5; norethisterone acetate, n=6; PEG 300, n=6).

* Indicates statistical significant difference compared with control responses.

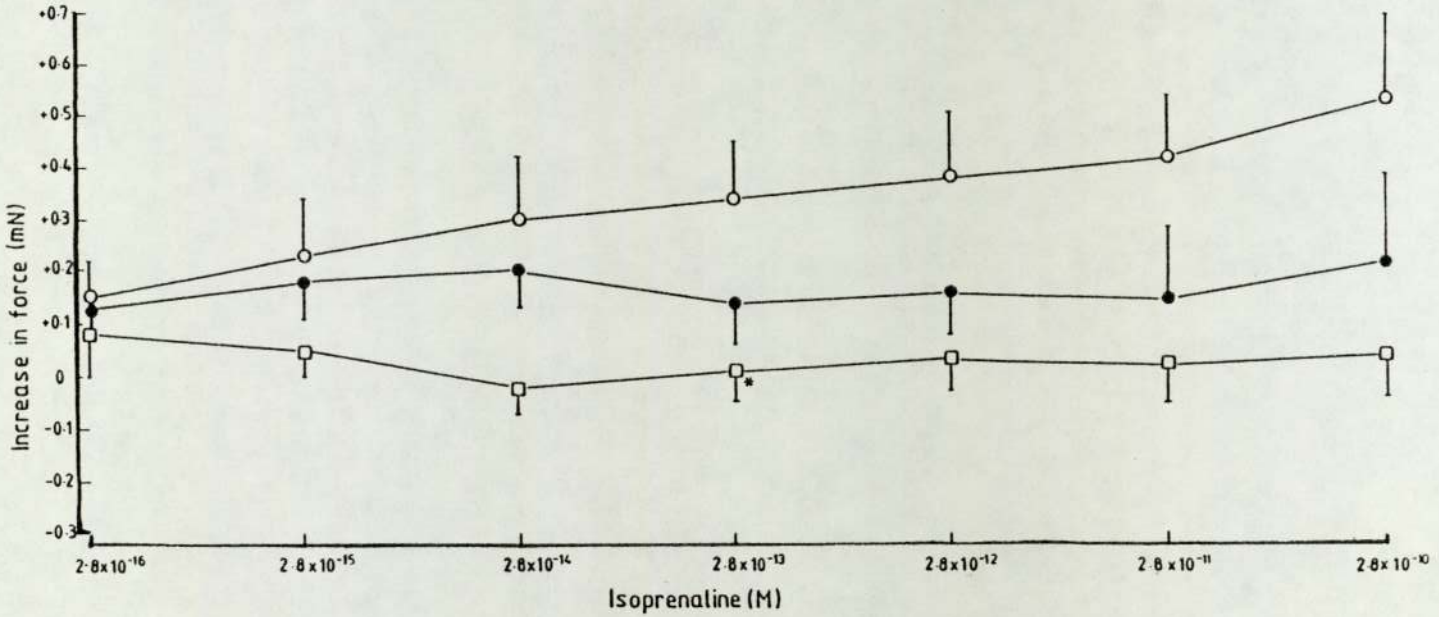
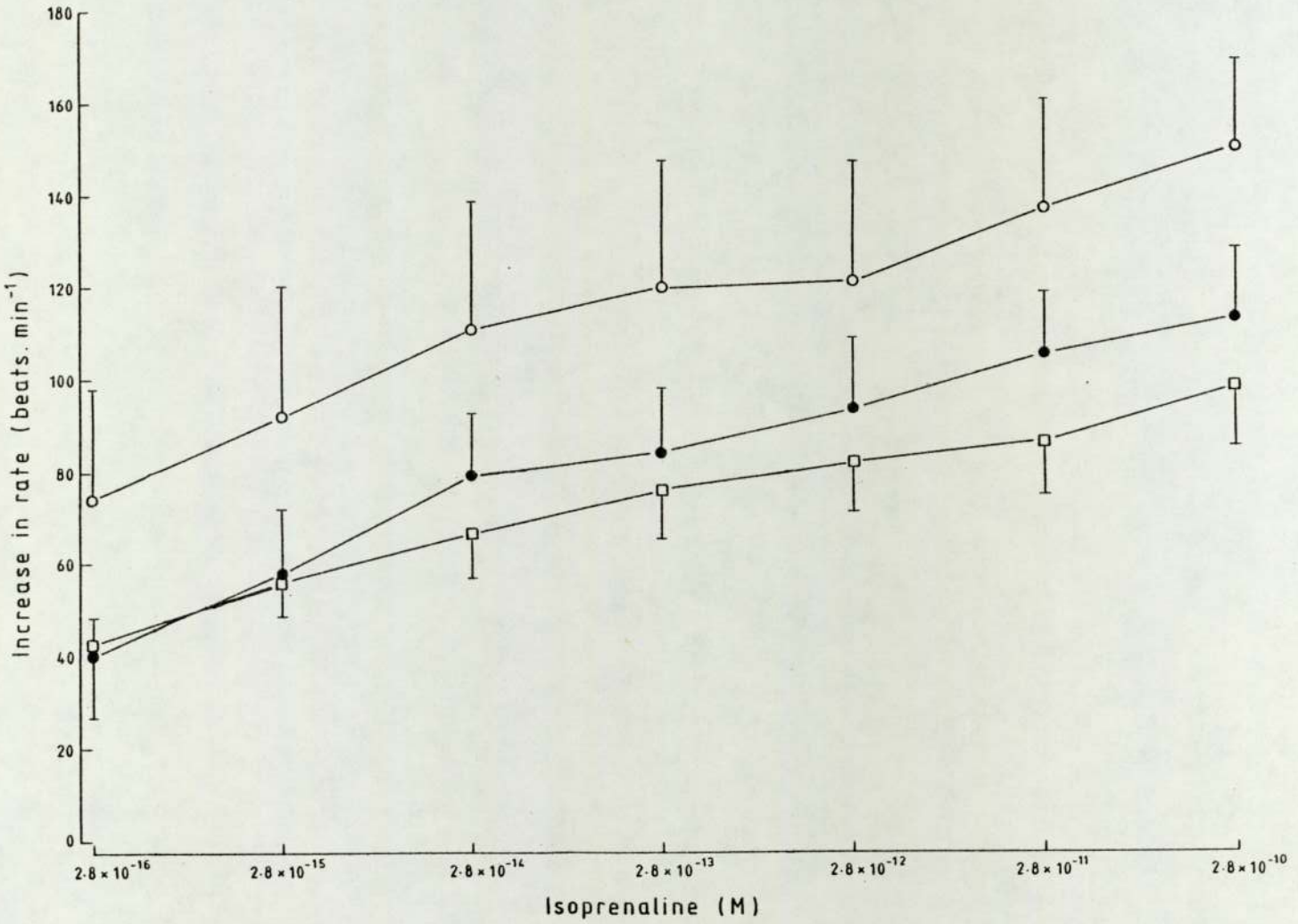

+0·7
+0·6
+0·5
+0·4
+0·3
+0·2
+0·1
0
-0·1
-0·2
-0·3
Increase in force (mN)
2·8x10⁻¹⁶
2·8x10⁻¹⁵
2·8x10⁻¹⁴
2·8x10⁻¹³
2·8x10⁻¹²
2·8x10⁻¹¹
2·8x10⁻¹⁰
Isoprenaline (M)
*

Figure 70

Increase in rate induced by isoprenaline administration in isolated paired atria from female rats pre-treated with ethinyloestradiol in PEG 300 (822 $\mu g.kg^{-1}.day^{-1}$) (o), norethisterone acetate in PEG 300 (67 $mg.kg^{-1}.day^{-1}$)(▫) or PEG 300 alone (control)(•). Each point represents the mean, and vertical bars denote the s.e. mean (ethinyloestradiol, n=5; norethisterone acetate, n=6; PEG 300, n=6).


Increase in rate (beats. min^{-1})
180
160
140
120
100
80
60
40
20
0
$2{\cdot}8 \times 10^{-16}$
$2{\cdot}8 \times 10^{-15}$
$2{\cdot}8 \times 10^{-14}$
$2{\cdot}8 \times 10^{-13}$
$2{\cdot}8 \times 10^{-12}$
$2{\cdot}8 \times 10^{-11}$
$2{\cdot}8 \times 10^{-10}$
Isoprenaline (M)

Summary

Pre-treatment of female rats with ethinyloestradiol did not alter the force developed during spontaneous contractions, the rate of change of force or the rate of beating of isolated atria. There appeared to be a separation of positive inotropic responses to noradrenaline following ethinyloestradiol pre-treatment. Also, the rate of change of force induced by noradrenaline and all but three concentrations of isoprenaline was increased in atria from ethinyloestradiol pre-treated rats compared with atria from control rats. Positive chronotropic responses to noradrenaline and to isoprenaline were not altered following ethinyloestradiol pre-treatment.

Pre-treatment of female rats with norethisterone acetate did not alter the force developed during spontaneous contractions, the rate of change of force or the rate of beating of isolated atria. Atria from norethisterone acetate pre-treated rats demonstrated a wide spread in inotropic responses to noradrenaline compared with those from control rats. The rate of change of force induced by noradrenaline or isoprenaline administration was not altered following norethisterone acetate pre-treatment. Positive chronotropic responses to noradrenaline and to isoprenaline were not altered following norethisterone acetate pre-treatment.

Discussion

Previous studies were concerned with an examination of the effect of alterations in the plasma concentration of ovarian hormones upon cardiovascular responses in the pithed rat, with a view to increasing the understanding of ovarian hormone-induced hypertension. These studies have been extended to an examination of the effect of pre-treatment of rats with oral contraceptive agents upon responses of isolated cardiac and vascular tissue.

Ethinyloestradiol is an oestrogenic agent and norethisterone acetate is a progestogenic agent, both of which are present in oral contraceptive preparations. Rats were administered either ethinyloestradiol or norethisterone acetate to determine the effect of the individual agents upon cardiovascular responses. Rats were given the equivalent of one thousand times the amount of the agents administered to women in oral contraceptives, that is 822 $\mu g.kg^{-1}.day^{-1}$ ethinyloestradiol or 67 $mg.kg^{-1}.day^{-1}$ norethisterone acetate. Hormonal agents were administered in the solvent PEG 300. Tissues from rats which were administered PEG 300 alone were used as controls.

It has been shown that, when hormonal agents are administered orally to women, either separately or in a combined oestrogen-progestogen oral contraceptive preparation, there is a marked increase in the plasma concentration of the agent until a maximal level is attained one to two hours after ingestion (Warren & Fotherby, 1973; Pasqualini, Castallet, Portois, Hill & Kincl, 1977; Nilsson & Nygren, 1978). This is followed by a rapid decrease in the plasma concentration of the agent such that levels twenty four hours after administration do not exceed plasma blank levels (Warren & Fotherby,

1973; Pasqualini et al., 1977; Nilsson & Nygren, 1978). Since Butcher and co-workers (1978) observed a similar pharmacokinetic profile when they administered a single subcutaneous injection of the oestrogen, 17β-oestradiol, to ovariectomised rats, oral contraceptive agents in present experiments were injected subcutaneously into rats at the same time each day. In this way it was hoped to induce a pharmacokinetic profile of an oral contraceptive agent in rats which was similar to that observed in human oral contraceptive users.

Since Cargille, Vaitukaitis, Bermudez & Ross (1973) have shown that the contraceptive effect of exogenous hormonal agents is dependent upon the stage of the menstrual cycle at which they are first administered, initial injections were given to rats at the same stage of the oestrous cycle, this being dioestrus.

There is mounting evidence (see reviews by Webb & Bohr, 1981; Weidmann, 1981) that potentiation of cardiac and vascular responses may lead to the development of a hypertensive condition. In 1976 Gammal observed that thirty daily intramuscular injections of ethinyloestradiol increased the smooth muscle content of renal arteries of rats. Since vascular medial hypertrophy may be the result of prolonged elevation of blood pressure (see review by Folkow, 1978), ethinyloestradiol may have induced the increase in smooth muscle content observed by Gammal (1976) by elevating the blood pressure. Since it was the purpose of this study to determine whether cardiac and vascular responses are potentiated prior to the establishment of a hypertensive condition, hormonal agents were administered for only ten days.

In order to examine the effect of oral contraceptive agents upon contractile responses of arterial and venous tissue, responses of

isolated rat aortic strips and portal veins were examined following pre-treatment of rats with ethinyloestradiol or with norethisterone acetate. The aortic strip and portal vein were selected because of the ease of isolatation and preparation and because of the extensive literature concerning these preparations (Funaki & Bohr, 1964; Fleisch, 1974; Hagemeijer, Rorive & Schoffeniels, 1965; Peiper, Klemt & Popov, 1979).

Bohr, Greenberg & Bonaccorsi (1978) and Johansson (1971) have reviewed the evidence which indicates that drugs may initiate vascular smooth muscle contractile responses either by electrical or non-electrical membrane-activation pathways. Johansson (1971) has cited evidence which indicates that, although spasmogens may initiate changes in rat aorta membrane potential, contraction is not coupled to this change in membrane potential. The rat portal vein, however, exhibits spike potentials and action potentials such that changes in membrane potential may initiate contractile responses (Funaki & Bohr, 1964; Bohr et al., 1978). The responses of isolated aortic strip and portal vein were therefore studied to determine whether hormone pre-treatment affects vascular contractile responses elicited by either an electrical or non-electrical membrane-activation mechanism.

Previous experimental findings, described in chapter 1, indicated that administration of 17β-oestradiol may reduce the speed of atrial conduction in vivo (see page 143). Also, Stumpf and co-workers (1977) have presented evidence which indicates that the atria of male and female rats concentrate the oestrogen, 17β-oestradiol, whereas ventricles do not. As an extension of these studies, responses of isolated paired atria were examined to determine whether administration of oral contraceptive agents affects atrial contractile responses.

Responses of isolated aortic strips to noradrenaline were examined, since noradrenaline is an endogenous catecholamine involved in the control of arterial pressure (Vanhoutte, 1978b). Noradrenaline may induce both α- and β-adrenoreceptor-mediated responses (Su, 1977), although at concentrations below 10^{-4}, α-adrenoreceptor-mediated responses dominate in the rat aortic strip (Wilson, 1982, personal communication) and concentrations below 10^{-4} M were used throughout present experiments. Responses of isolated aortic strips to angiotensin II were also examined, since the plasma concentration of angiotensin II, which is a potent pressor agent and constrictor of vascular smooth muscle (Devynck & Meyer, 1976), may be elevated in women taking oral contraceptives (Zakheim et al., 1976). Also, the plasma concentration of angiotensin II may alter vasoconstrictor responses to this spasmogen (Gross, Brunner & Ziegler, 1965).

The isolated portal vein of the rat shows spontaneous rhythmical contractions which are myogenic in nature (Johansson & Ljung, 1967). Funaki & Bohr (1964) have shown that the regular changes in muscle tension are preceded by rapid changes in membrane potential (spike potentials) such that there is a direct relationship between spike potential and individual contraction. Funaki & Bohr (1964) have also shown that administration of some spasmogens induces changes both in membrane potential and in the frequency and amplitude of contraction.

Noradrenaline has been shown to have a direct effect upon the portal vein contractile mechanism and also to increase the frequency of membrane electrical discharge (Axelsson, Wahlström, Johansson & Jonsson, 1967). Responses of isolated portal veins to angiotensin II were examined, since the plasma concentration of angiotensin II,

which is a potent constrictor of vascular smooth muscle (Devynck & Meyer, 1976), may be elevated in women taking oral contraceptives (Zakheim et al., 1976) and the concentration of plasma angiotensin II may alter vasoconstrictor responses to this spasmogen (Gross et al., 1965). In studies using the rabbit aortic strip, Paterson (1965) demonstrated that the mechanism of field stimulation is most likely to be neurogenic and sited at post-ganglionic adrenergic elements. Paterson (1965) believed that the transmitter at the site was probably noradrenaline. Since rat portal veins appear to be supplied by adrenergic nerves (Bengtsson, 1977), field stimulation of the portal vein may also induce contractile responses by activation of nervous innervation. Portal veins were therefore subjected to electrical field stimulation to determine whether oral contraceptive agents affect vascular responses to nervous stimulation. High concentrations of extracellular potassium have been shown to induce smooth muscle contraction by membrane depolarisation (see review by Altura & Altura, 1978). The effects of ethinyloestradiol and norethisterone acetate upon contractile responses of isolated portal veins to potassium chloride were therefore examined to determine whether oral contraceptive agents affect vascular smooth muscle membrane depolarisation.

Positive inotropic and chronotropic responses of isolated paired atria to noradrenaline were examined, since this catecholamine is endogenous (Vanhoutte, 1978b) and it may induce responses by stimulating α- and β-adrenoreceptors (Su, 1977). Findings of experiments described in chapter 1 indicate that positive chronotropic responses to the β-adrenergic agonist, isoprenaline (see page 142), are reduced following 17β-oestradiol pre-treatment. To determine whether

administration of all ovarian hormonal agents depress responses to isoprenaline, the effects were also examined of ethinyloestradiol and norethisterone acetate administration upon inotropic and positive chronotropic responses of isolated atria to isoprenaline.

Effects of ethinyloestradiol pre-treatment

The maximal fast and slow contractile responses to noradrenaline were potentiated following ethinyloestradiol pre-treatment. It is therefore possible that ethinyloestradiol pre-treatment induces an alteration in the contractile system or an increase in the energy supply for contraction. There is some support in the literature for the possibility that an alteration in the plasma concentration of an oestrogenic agent alters the contractility or energy supply in isolated smooth muscle. Csapo (1950) has shown that ovariectomy decreased, and subsequent in vivo administration of oestrogen increased, the actomyosin content of rat uterus smooth muscle. Also using the rat uterus, Wilson, Crocker & Willavoys (1974) have shown that energy reserves alter during the course of the oestrous cycle, when there is a variation in the plasma concentration of ovarian hormones (Yoshinaga et al., 1969).

Following in vivo ethinyloestradiol pre-treatment, there was also a left shift in the concentration-response curves of both fast and slow aortic strip responses to noradrenaline, as shown by a decrease in the $E.C._{50}$ values. This indicates an increase in the potency of noradrenaline. Unfortunately, the maximal contractile response to angiotensin II could not be achieved in present experiments. For this reason, it was not possible to record the effect of ethinyloestradiol pre-treatment upon the maximal contractile response or the $E.C._{50}$. However, the concentration of angiotensin II required to induce a standard contractile response was reduced following ethinyloestradiol pre-treatment.

Since ethinyloestradiol pre-treatment appears to increase the potency of both noradrenaline and angiotensin II, it seems likely that

ethinyloestradiol pre-treatment may have a generalised action upon the aortic strip. However, a number of possible mechanisms for the increase in potency of noradrenaline and angiotensin II must be considered. These include: a) an alteration in calcium mobilisation, b) an alteration in agonist/receptor interaction and c) an alteration in agonist metabolism or elimination.

a) The responses of rat aortic strips to noradrenaline in present experiments were found to be composed of two phases. Immediately after stimulation, there was a fast contraction. This was followed by a slower, more sustained, contraction. Contraction of smooth muscle is believed to be mediated by activation of the contractile elements by calcium (Ca^{2+}). This is believed to be made available from two sources; an extracellular pool which may be loosely bound to superficial sites on the cell membrane and an intracellular pool bound to a location which varies for different types of smooth muscle (Paiva et al., 1977). The current consensus is that mobilisation of intracellular calcium produces a fast contraction of the type observed immediately after administration of noradrenaline to aortic strips. Transfer of extracellular calcium into the cell is believed to cause the slower, more sustained type of noradrenaline-induced contraction (Paiva et al., 1977; Bohr et al., 1978). Only slow contractile responses to angiotensin II were observed in aortic strips taken from hormone pre-treated and control rats. In present experiments, therefore, angiotensin II appears to have induced contractile responses in aortic strips by initiating transfer of extracellular calcium into the smooth muscle cell (Paiva et al., 1977; Bohr et al., 1978). It is therefore possible that ethinyloestradiol pre-treatment facilitates the mobilisation of intracellular and extracellular calcium by independent mechanisms.

However, there is evidence in the literature for a unification of the fast and slow aortic strip α-adrenoreceptor responses. Bohr and co-workers (1978) have suggested that stimulation of α-adrenoreceptors leads to calcium release from the plasma membrane. This then triggers release of intracellular calcium from the sarcoplasmic reticulum, and both of these actions produce the fast phase of aortic strip contraction (Bohr et al., 1978). The slow phase of contraction is then induced by the influx of calcium, caused by an increase in membrane permeability which results from calcium being dislodged from the membrane during the fast phase of contraction (Bohr et al., 1978). Alteration in noradrenaline-induced responses due to apparent increases in intracellular and extracellular calcium mobilisation may therefore be due to a common action of ethinyloestradiol pre-treatment at the α-adrenoreceptor.

b) Webb & Bohr (1981) have suggested that an alteration in response to a vasoconstrictor agent may be due to an alteration in agonist/receptor interaction. The findings of Colucci, Gimbrone, McLaughlin, Halpern & Alexander (1982) indicate that in vivo pre-treatment with oestrogenic agents enhance α-adrenoreceptor affinity in isolated rat arteries. Colucci and co-workers (1982) observed a left shift in the concentration-response curves of the contractile responses of isolated rat mesenteric arteries to noradrenaline following changes in the plasma concentration of oestrogen. These studies were extended to an examination of α-selective binding in the arteries. Colucci and co-workers (1982) concluded that oestrogen increases catecholamine potency, and that this may be due to enhanced vascular α-adrenoreceptor affinity. It is possible that in vivo pre-treatment with any oestrogenic agent enhances vascular α-adrenoreceptor affinity

in isolated rat arteries. In this way, present increase in noradrenaline potency may be due to an increase in α-adrenoreceptor affinity as a result of ethinyloestradiol pre-treatment.

c) Another possible explanation for present findings is an alteration in the metabolism and/or elimination of noradrenaline or angiotensin II.

Several reported studies have been concerned with the effect of oestrogenic agents upon the amount of noradrenaline at adrenoreceptors and consequent responses of arterial vessels to noradrenaline. These studies have examined the possibility that oestrogenic agents may alter the metabolism or elimination of noradrenaline at adrenoreceptors. Nicol & Rae (1972) demonstrated that direct administration of several oestrogenic agents was associated with inhibition of extraneuronal uptake of noradrenaline in rabbit ear arteries. Rat aorta appears to be devoid of adrenergic innervation (Patil, Fudge & Jacobowitz, 1972) and Lightman & Iversen (1969) have provided evidence which indicates that, where smooth muscle cells are not in close proximity to adrenergic nerves, extraneuronal uptake is an important means of noradrenaline inactivation. It is therefore possible that, in present experiments, the potency of noradrenaline was increased as a consequence of reduction in extraneuronal uptake following ethinyloestradiol pre-treatment. However, Avakian & Gillespie (1976) have shown that, when noradrenaline is taken into arteries it may be re-released and so potentiate noradrenaline-induced responses. If this applies to isolated rat aortic strips, it is unlikely that an increase in noradrenaline potency is the result of reduction in extraneuronal uptake and it may be the result of an increase in extraneuronal uptake.

Using an oil-immersion technique, Kalsner (1969) examined the effect of direct application of the oestrogen 17β-oestradiol upon

noradrenaline-induced contractions of rabbit aortic strips. He observed that 17β-oestradiol administration potentiated responses. However, when known COMT inhibitors were administered prior to administration of noradrenaline, 17β-oestradiol did not potentiate contractile responses. Kalsner (1969) therefore proposed that 17β-oestradiol administration potentiated contractile responses to noradrenaline by inhibiting the enzymatic activity of COMT and, thereby, increasing the noradrenaline available to elicit contractions. Although this is an interesting possibility, the study of Kalsner (1969) was fundamentally different to the present study, for the following reasons; aortic strips were from different animal species, hormone application was direct by Kalsner (1969) and subcutaneous in the present study and the oestrogenic agents used were different. For this reason, the findings of Kalsner (1969) cannot be accurately compared with those of the present study. To determine whether ethinyloestradiol pre-treatment alters COMT activity in rat aortic strips, it would be necessary to examine noradrenaline-induced responses following addition of known COMT inhibitors such as pyrogallol or tropolone to the aortic strips (Kalsner, 1969).

Gross and co-workers (1965) and Devynck & Meyer (1976) have reviewed the evidence which suggests that vasoconstrictor responses to angiotensin II are inversely related to the plasma concentration of angiotensin II in several species. Devynck & Meyer (1976) have suggested that constrictor responses may be dependent upon the number of angiotensin II receptors available, which is itself inversely related to the plasma concentration of angiotensin II. It is therefore possible that increase in potency of angiotensin II observed in present experiments may be attributable to a decrease in plasma angiotensin II concentration as a consequence of ethinyloestradiol administration.

It is interesting to note that the gradient of the concentration-response curve to angiotensin II and not to noradrenaline appeared to be increased following pre-treatment with ethinyloestradiol. Folkow (1978) has demonstrated how an increase in the gradient of the concentration-response curve may be due to hypertrophic structural changes in the vascular wall as a result of a sustained increase in blood pressure. Since only responses to angiotensin II demonstrated this change, it is possible that aortic contractions are potentiated following ethinyloestradiol pre-treatment which leads to vascular hypertrophy and that present findings represent this period of vascular change. To clarify this point, it will be useful in future to determine the dry weight of aortae after ethinyloestradiol pre-treatment and to repeat the present studies after an administration period of less than ten days.

In present experiments, pre-treatment of female rats with ethinyloestradiol did not alter the amplitude of isolated portal vein spontaneous mechanical activity. Pre-treatment with ethinyloestradiol, therefore, appeared to alter neither membrane potential nor activity. However, McCalden (1975) observed that direct administration of another oestrogenic agent, 17β-oestradiol, had a biphasic effect upon rat portal veins in vitro. At low concentrations of 17β-oestradiol the amplitude of spontaneous mechanical activity was potentiated, whereas at high concentrations the amplitude was reduced. McCalden (1975) proposed that low concentrations of 17β-oestradiol moved the membrane potential of portal vein smooth muscle into a more excitable range, and that higher concentrations hyperpolarised the membrane out of the firing range.

It is possible that, in present experiments, the plasma concentration induced an effect mid-way between the effects observed by McCalden (1975), such that a lower plasma concentration would have potentiated, and a higher concentration would have reduced, the amplitude of spontaneous mechanical activity. However, it may not be reasonable to interpret present findings in the light of those of McCalden (1975), since the experiments were fundamentally different. Present experiments examined the effect of ethinyloestradiol, whereas McCalden (1975) examined the effect of 17β-oestradiol. Also, present experiments examined the influence of a circulating oestrogenic agent upon portal vein responses, whereas McCalden (1975) applied an oestrogenic agent directly to the portal vein in vitro.

Maximal contractile responses to noradrenaline, angiotensin II and potassium chloride were not altered following ethinyloestradiol pre-treatment. This indicates that the contractile mechanism or energy supply is unlikely to be altered by in vivo ethinyloestradiol pre-treatment.

A reduction in $E.C._{50}$ indicated that the potency of noradrenaline was increased following ethinyloestradiol pre-treatment. This effect may have been mediated by an increase in the amount of noradrenaline at the receptor site, or as a result of a decrease in its elimination and/or metabolism. Since sympathetic nerves appear to supply the portal vein (Bengtsson, 1977), elimination may be by neuronal or extraneuronal uptake (Lightman & Iversen, 1969). Reduction in either of these means of elimination may therefore be responsible for potentiation of noradrenaline-induced contractions.

Since increase in noradrenaline potency was also observed in isolated aortic strips following ethinyloestradiol pre-treatment,

ethinyloestradiol pre-treatment may induce similar actions on isolated portal vein and aortic strip. Isolated rat aortae have been shown to be devoid of adrenergic innervation (Patil et al., 1972) and Lightman & Iversen (1969) have shown that extraneuronal uptake is an important means of inactivation of noradrenaline where adrenergic innervation is absent. If ethinyloestradiol pre-treatment has a uniform effect upon isolated aortic strips and portal veins, it is possible that extraneuronal uptake is reduced in these isolated blood vessels. COMT is an important extraneuronal noradrenaline-metabolising enzyme (Vanhoutte, 1978a). It is therefore possible that inhibition of COMT is the means whereby the potency of noradrenaline is increased following ethinyloestradiol pre-treatment. However, the possibility that an increase in the potency of noradrenaline in isolated portal veins was due to decrease in neuronal uptake or to a decrease in the activity of MAO, the neuronal noradrenaline-metabolising enzyme (Vanhoutte, 1978a), cannot be eliminated. To determine whether the activity of noradrenaline-metabolising enzymes COMT or MAO was reduced following ethinyloestradiol pre-treatment, the present experimental protocol may be repeated, with the exception that COMT-inhibiting agents pyrogallol or tropolone (Kalsner, 1969) or the MAO-inhibiting agent iproniazid (Kalsner, 1969) may be administered directly to the isolated portal vein prior to addition of noradrenaline. If portal veins taken from ethinyloestradiol pre-treated rats no longer demonstrated potentiation of noradrenaline-induced responses compared with control tissues then the appropriate enzyme may be inhibited following ethinyloestradiol pre-treatment.

It is also possible that the potency of noradrenaline is

elevated by an effect of circulating ethinyloestradiol upon cell membrane function. Johansson, Jonsson, Axelsson & Wahlström (1967) have shown that noradrenaline exerts its contractile action in portal veins by changing the pattern of membrane electrical activity. Using extracellular recording techniques similar to those employed by Jetley & Weston (1980) it would be possible to determine whether changes in noradrenaline-induced contractions are related to any changes in membrane electrical activity.

Although pre-treatment with ethinyloestradiol was associated with an increase in the potency of noradrenaline on portal vein responses, there was no change in potassium chloride-induced contractions following ethinyloestradiol pre-treatment. Since Bengtsson (1977) has shown that, in the isolated rat portal vein, potassium chloride may release noradrenaline, this finding was unexpected. In vitro denervation by the method of Aprigliano & Hermsmeyer (1976) would make possible a determination of the influence of nervous release of noradrenaline upon potassium chloride-induced responses.

However, this apparent discrepancy between the influence of ethinyloestradiol pre-treatment upon potassium chloride- and noradrenaline-induced contractile responses may have been due to an inadequacy in experimental method. The entire concentration-response curve was produced by four concentrations of potassium chloride and by four concentrations of noradrenaline. If present experiments were repeated using several concentrations of potassium chloride and noradrenaline in the linear portion of the concentration-response curve, it would be possible to make a more accurate determination of any shift in the concentration-response curves. It may even be shown

that the $E.C._{50}$ for potassium chloride-induced contractions is reduced following ethinyloestradiol pre-treatment. However, if there was no alteration in pressor responses to potassium chloride following ethinyloestradiol pre-treatment, since Altura & Altura (1978) have shown that high concentrations of extracellular potassium may induce smooth muscle contraction by membrane depolarisation, it would appear that ethinyloestradiol administration does not alter portal vein membrane depolarisation.

Paterson (1965) has shown that electrical field stimulation is likely to exert its contractile action by releasing noradrenaline from post-ganglionic nerves. Responses to electrical field stimulation were not altered following ethinyloestradiol. Unfortunately, portal vein responses to electrical stimulation did not attain a maximum and it was not possible to record any change in potency following ethinyloestradiol pre-treatment. However, since ethinyloestradiol pre-treatment appeared to increase noradrenaline potency in the isolated rat portal vein, present experimental protocol could only demonstrate reduction in post-gangionic noradrenaline release.

The potency of angiotensin II on the isolated portal vein was increased following ethinyloestradiol pre-treatment. Similarly, there is evidence that ethinyloestradiol pre-treatment increased the potency of angiotensin II upon the isolated aortic strip (see page 230). It is therefore possible that all vasoconstrictor responses to angiotensin II are potentiated following in vivo ethinyloestradiol pre-treatment, possibly as a result of an increase in the number of vascular receptors as a consequence of reduced plasma concentration of angiotensin II (see page 234).

Several workers (Somylo & Somylo, 1971; Robertson & Khairallah, 1971; Volicer & Hynie, 1971; Devynck, Pernollet, Meyer, Fermandjian & Fromageot, 1973; Devynck, Pernollet, Meyer, Fermandjian, Fromageot & Bumpus, 1974) have provided evidence that angiotensin II exerts its contractile action both by changing the pattern of membrane electrical activity and by a direct influence upon the portal vein contractile system. Since the maximal contractile response was not altered by ethinyloestradiol pre-treatment, it seems unlikely that the contractile mechanism was affected by ethinyloestradiol pre-treatment. It is therefore more likely that ethinyloestradiol pre-treatment alters membrane electrical activity. This was also suggested as an explanation for potentiation of noradrenaline-induced portal vein responses. This gives a further reason why isolated portal vein membrane electrical activity should be measured in vitro after in vivo ethinyloestradiol pre-treatment (see page 238). Such a measurement would help in the understanding of ethinyloestradiol-induced changes in portal vein responses to noradrenaline and to angiotensin II.

Following ethinyloestradiol pre-treatment, inotropic responses, recorded as the increase in force, to noradrenaline appeared to separate into two groups. The concentration-response curve of atria from two ethinyloestradiol pre-treated rats appeared to lie to the left of control response curves, while concentration response curves from five ethinyloestradiol pre-treated rats appeared to be co-incident with control curves. Application of the Mann-Whitney test indicated that the apparent difference between the groups was not significant. However, there was no such apparent separation of positive chronotropic responses to noradrenaline following ethinyloestradiol pre-treatment.

Ethinyloestradiol pre-treatment may therefore have different effects upon atrial rate and force responses.

The rate of change of force recorded during noradrenaline and isoprenaline administration was significantly greater following ethinyloestradiol pre-treatment. Since no changes in positive chronotropic responses to noradrenaline or isoprenaline were observed, these findings are consistent with the view that ethinyloestradiol pre-treatment has a selective action upon inotropic responses. The observation of Broadley (1972) that an increase in the rate of the isolated heart induces a reduction in active force, gives further reason to believe that ethinyloestradiol pre-treatment selectively affects inotropic responses of isolated atria.

To determine whether force responses may be potentiated without any interference from rate responses, separation of left and right atria was attempted (see page 40). Unfortunately, for the reasons outlined on pages 40 and 41, it was not possible to determine whether rate and force responses are affected independently. In another attempt to determine whether force responses to noradrenaline are potentiated without interference by change in rate, isolated atria were partially depolarised with potassium chloride. This stopped spontaneous beating and a controlled heart rate was induced by electrical stimulation from punctate electrodes (see figure 2, p. 44). Noradrenaline was then administered to determine whether inotropic responses are altered following ethinyloestradiol pre-treatment, after the influence of the chronotropic effect has been removed. Unfortunately, basal force gradually decreased during these experiments, such that results were difficult to interpret.

It is possible that in vivo ethinyloestradiol pre-treatment alters the contractile mechanism or energy availability of isolated rat atria. Two studies demonstrate how these factors may be linked. In 1959 King and co-workers showed that ovariectomy reduced the actomyosin content and force developed to ATP in isolated rat ventricular columnae and that α-oestradiol administration to ovariectomised rats returned the force developed to ATP to the level observed in intact animals. Although King and co-workers (1959) examined the influence of a natural oestrogenic agent upon the rat ventricle, this study was concerned with an examination of in vivo changes in hormone levels upon a muscular component of isolated rat heart. For this reason, it is interesting to speculate whether an increase in the circulating level of any oestrogenic agent would increase the actomyosin content and force developed in all rat cardiac muscle. It is also interesting to note that in 1950 Csapo found that ovariectomy reduced the ATPase activity of rat uterus and that subsequent administration of oestrogen to rats returned the ATPase activity to pre-ovariectomy levels and increased the uterus actomyosin content. These findings indicate that circulating oestrogenic agents may increase the actomyosin content and force development of all muscle.

Subcutaneous implantation of ethinyloestradiol in silastic tubing was observed by Fregly & Thrasher (1977) to reduce the positive chronotropic responses induced by subcutaneous administration of isoprenaline in female rats. In present experiments, however, administration of ethinyloestradiol was not found to increase positive chronotropic responses to isoprenaline. Since Fregly & Thrasher (1977) administered approximately 36 $\mu g.kg^{-1}.day^{-1}$, and 822 $\mu g.kg^{-1}.day^{-1}$ ethinyloestradiol was administered in present experiments, it

appears unlikely that raised plasma concentration of ethinyloestradiol alone was responsible for the attenuation of isoprenaline-induced positive chronotropic responses observed by Fregly & Thrasher (1977). Since Fregly and co-workers (1978) found that subcutaneous implantation of steroids in silastic tubing gives reasonably constant drug release for long periods, it is possible that the plasma concentration of the oestrogenic agent must be stable in order to reduce isoprenaline-induced positive chronotropic responses. This is supported by present in vivo findings which indicate that constant administration of 17β-oestradiol may reduce isoprenaline-induced positive chronotropic responses (see page 142).

It seems unlikely that reduction in β-adrenoreceptor-mediated responses following ovarian hormone administration to female rats observed in present experiments and by Fregly & Thrasher (1977) is related to use of whole animal preparations, since Ćiric & Šusić (1980) observed in vivo potentiation of positive chronotropic responses to isoprenaline following pre-treatment with 17β-oestradiol.

An initial study was carried out to determine whether a pharmacokinetic profile characterised by variation in plasma ovarian hormone levels induces any changes in positive chronotropic responses in vivo. Unfortunately, daily subcutaneous administration of oestrogenic agents appeared to alter responses to general anaesthesia and several rats died following administration of an anaesthetic agent (see page 123).

It is interesting to note that the action of isoprenaline on isolated paired atria seemed to be more selective for increase in rate than for increase in force, since the concentration-response curve for

rate appeared to lie to the left of that for force. This is similar to the findings of Broadley & Lumley (1975) on separated guinea-pig atria.

Body weight gain

In present experiments, daily subcutaneous injections of ethinyloestradiol reduced body weight gain in female rats. Reduction in the body weight gain of rats may be a property common to all oestrogenic agents, since when Hoeg and co-workers (1977) administered mestranol to female rats, Saruta, Nakamura, Saito, Kondo & Matuki (1975) administered oestrogen and stilboestrol diproprionate to male rats and Douglas (1974) administered a combined mestranol-norethynodrel preparation to female rats, reduction in body weight gain was observed. Since Douglas (1974) observed reduction in body weight gain with no reduction in food intake, it is unlikely that oestrogenic agents reduce body weight gain by reducing appetite. Cardiovascular responses appeared to be unchanged or potentiated following pre-treatment with ethinyloestradiol in present experiments, which may lead to an increase in blood pressure (Webb & Bohr, 1981; Weidmann, 1981). Since there tends to be a positive correlation between blood pressure and body weight gain (see review by Cruz-Coke, 1981), it appears unlikely that reduction in body weight gain was responsible for alterations in cardiovascular responses observed following pre-treatment with ethinyloestradiol.

Oestrous cycle length and post-mortem examination findings

Daily subcutaneous injections of ethinyloestradiol induced in some rats the appearance of a continuous vaginal smear composed of a few leucocytes. Since the vaginal smear which is characteristic of the oestrous stage was absent in these rats, these findings indicate that ovulation may have been inhibited by administration of the oestrogenic agent to some rats. However, when Gammal (1976) administered ethinyloestradiol to rats by daily intramuscular injections, oestrous cycles were not affected. Gammal (1976) administered approximately one tenth of the amount of ethinyloestradiol (80 $\mu g.kg^{-1}.day^{-1}$) which was administered in present experiments and this may have been insufficient to inhibit ovulation. Alternatively, inhibition of ovulation may be dependent upon the pharmacokinetic profile produced by daily subcutaneous injections of ethinyloestradiol.

The uteri of some rats pre-treated with ethinyloestradiol in the present study were very vascular and fluid-filled compared with those of control rats. Douglas (1974) found an increase in the wet weight of uteri from rats pre-treated with a combined oestrogen-progestogen preparation. However, administration of the progestogenic agent, norethisterone acetate, to rats in present experiments did not alter the appearance of uteri and therefore the increase in uterus weight observed by Douglas (1974) may have been induced by the oestrogenic component of the preparation he administered.

Effects of norethisterone acetate

Following pre-treatment with norethisterone acetate, there was no change in the fast response, maximal fast response or $E.C._{50}$ of aortic strip responses to noradrenaline. Similarly, there was no change in the slow response, maximal slow response or $E.C._{50}$ of aortic strip responses to noradrenaline. In vivo norethisterone acetate pre-treatment therefore did not appear to alter the contractile mechanism or energy availabity of isolated aortic strips. However, responses to angiotensin II were increased following norethisterone acetate pre-treatment, and there appeared to be a left shift in the concentration-response curve, which indicated an increase in angiotensin II potency.

Since angiotensin II-induced responses were potentiated in aortic strips taken from norethisterone acetate pre-treated rats, whereas noradrenaline-induced responses were not, it is possible that norethisterone acetate pre-treatment affects angiotensin II/receptor interaction. Gross and co-workers (1965) and Devynck & Meyer (1976) have reviewed the evidence which suggests that vasoconstrictor responses to angiotensin II are inversely related to the plasma concentration of angiotensin II in several species. Devynck & Meyer (1976) have suggested that the constrictor responses may be dependent upon the number of angiotensin II receptors available, which is itself inversely related to the plasma concentration. It is therefore possible that responses to angiotensin II are potentiated as a consequence of reduced plasma concentrations of angiotensin II. However, studies carried out in vivo in women and in experimental animals have shown that progestogenic agents may increase the plasma concentration of angiotensin II and, as a consequence, reduce

pressor responses to angiotensin II. These studies include the findings of Zakheim and co-workers (1978) which indicated that norethynodrel administration increases the plasma concentration of angiotensin II in women, and those of Hettiaratchi & Pickford (1968) which showed that administration of progesterone to female rats reduced pressor responses to angiotensin II. Although this appears to indicate a discrepancy between present findings and those in the literature, it may not be reasonable to compare present findings with those in women or with those in whole animals.

Another possibility is that potentiation of the responses of isolated aortic strips to angiotensin II following pre-treatment with norethisterone acetate may have been due to a direct action of the progestogenic agent at the receptor. If norethisterone acetate potentiated contractile responses of the aorta to angiotensin II by reducing the plasma concentration of angiotensin II, administration of norethisterone acetate is unlikely to lead to an elevation in pressor responses or arterial blood pressure. However, if norethisterone acetate acts at the angiotensin II to facilitate angiotensin II/receptor interaction without a concomitant decrease in plasma angiotensin II, pressor responses may be potentiated. Since potentiated vaso-constrictor responses may lead to elevated arterial pressure (Webb & Bohr, 1981), administration of norethisterone acetate may result in raised blood pressure.

The slope of the concentration-response curve to angiotensin II appeared to be increased following pre-treatment with norethisterone acetate (see figure 55, p. 186). Folkow (1978) has shown that this type of change may be due to hypertrophy. Hypertrophy may therefore account for potentiation of contractile responses. To examine this, it

will be necessary in future to determine the dry weight of aortae after norethisterone acetate pre-treatment. If hypertrophy had taken place, present studies may be repeated with a shorter norethisterone acetate pre-treatment period. In this way, it would be possible to determine whether norethisterone acetate pre-treatment potentiates aortic strip responses prior to development of hypertrophy.

Pre-treatment of female rats with norethisterone acetate did not alter the amplitude of isolated portal vein spontaneous mechanical activity. Pre-treatment with norethisterone acetate, therefore, appeared to alter neither membrane potential nor activity. Similarly, pre-treatment of female rats with norethisterone acetate did not alter the amplitude of spontaneous mechanical activity or the magnitude of the contractile responses of isolated portal veins to noradrenaline, potassium chloride or electrical stimulation. Also, the maximal contractile responses of the portal vein to noradrenaline, potassium chloride, electrical stimulation and angiotensin II were not altered following pre-treatment with norethisterone acetate. The contractile system of the portal vein does not, therefore, appear to be affected by pre-treatment with norethisterone acetate. However, the $E.C._{50}$ for portal vein contractile responses to angiotensin II was reduced following pre-treatment with norethisterone acetate. This is similar to the action of norethisterone acetate pre-treatment upon aortic strip responses to angiotensin II (see page 247). Administration of norethisterone acetate may therefore alter all angiotensin II-induced vasoconstrictor responses, possibly as a result of an increase in the number of vascular angiotensin II receptors as a consequence of reduced plasma concentration of angiotensin II (see page 247).

Resting atrial contraction was recorded as the force, the rate of change of force and the rate of beating. None of these measures of atrial contraction was affected by pre-treatment of rats with norethisterone acetate.

Rate of change of force and positive chronotropic responses to noradrenaline or isoprenaline administration were not different following norethisterone acetate pre-treatment.

There was a wide spread in inotropic responses, recorded as the increase in force, to noradrenaline in atrial from norethisterone acetate pre-treated rats. Although application of the Mann-Whitney test did not show any significant difference between the responses of atria from norethisterone acetate pre-treated, compared with control, rats, it is possible that norethisterone acetate pre-treatment may have independent actions upon rate and force responses. It is unfortunate that an examination could not be made on separated left and right atria or upon driven paired atria to confirm whether norethisterone acetate pre-treatment has independent actions upon rate and force responses (see pages 40-41 and 241).

There was some indication that the maximal increase in force to noradrenaline may have been potentiated in some atria following norethisterone acetate pre-treatment. Since potentiated myocardial contractility may lead to an increase in blood pressure (Tarazi, 1977), those rats whose atria demonstrate an increase in maximal inotropic response following norethisterone acetate pre-treatment may develop hypertension. Since only part of the population of oral contraceptive users develops oral contraceptive-induced hypertension (Saruta et al., 1970; Mackay et al., 1971; Weir et al., 1971), these rats may be analogous to that part of the oral-contraceptive-taking population which develops hypertension.

Body weight gain

In present experiments, daily subcutaneous injections of norethisterone acetate did not alter body weight gain in female rats. Since Black, Fregly, Thrasher & Moreland (1976) also found no alteration in body weight gain when the progestogenic agent, norethynodrel, was implanted subcutaneously into male rats, all progestogenic agents may have no effect upon the body weight gain of rats.

Oestrous cycle length

Daily subcutaneous injections of norethisterone acetate did not alter the oestrous cycle length in female rats in present experiments. Similarly, Yoshinaga and c-workers (1969) observed no change in oestrous cycle length when progesterone was administered to female rats. Although Kulesár-Gergely & Kulesár (1979) observed lengthening of rat oestrous cycles following repeated subcutaneous injections with several different progestogenic agents, they observed no inhibition of ovulation during the fifteen and thirty day injection periods. Present findings and those of Yoshinaga and co-workers (1969) and of Kulesár-Gergely & Kulesár (1979) indicate that ovulation is generally not inhibited by administration of progestogenic agents to rats.

Effect upon blood pressure

Since daily subcutaneous injections of ovarian hormonal agents to female rats appear to induce a pharmacokinetic profile similar to that observed in human oral contraceptive users (see page 224), it is possible that similar changes in the cardiovascular system are induced by administration of oral contraceptive agents to women and to female rats. The possible effect of daily pre-treatment with either ethinyloestradiol or norethisterone acetate upon the blood pressure of female rats is therefore discussed below, together with the possible implications for human oral contraceptive users.

The potency of noradrenaline was enhanced in isolated aortic strips and portal veins following in vivo ethinyloestradiol pre-treatment. Since Webb & Bohr (1981) have shown that potentiation of vasoconstrictor responses may lead to the development of a hypertensive condition, administration of ethinyloestradiol may lead to elevated arterial blood pressure. Similarly, administration to women of ethinyloestradiol in oral contraceptive preparations may elevate resting blood pressure. Atrial inotropic responses to noradrenaline also appeared to be potentiated following ethinyloestradiol administration. Since potentiation of cardiac contractile responses may also lead to an increase in blood pressure (Tarazi, 1977; Weidmann, 1981), this is another means whereby pre-treatment with ethinyloestradiol by daily subcutaneous injections may increase blood pressure in female rats. The potency of noradrenaline appeared to be potentiated in a minority of atria from ethinyloestradiol pre-treated rats compared with atria from other ethinyloestradiol pre-treated rats. Rats from which these atria were taken may therefore be more likely to develop hypertension

following administration of ethinyloestradiol than other rats. To verify this hypothesis, it will be necessary, in future, to perform similar studies on a very large number of animals and to measure the blood pressure of female rats following prolonged treatment with ethinyloestradiol. Since only part of the population of oral contraceptive users develops oral contraceptive-induced hypertension (Saruta et al., 1970; Mackay et al., 1971; Weir et al., 1971), a group of rats sensitive to the stimulating action of ethinyloestradiol upon atrial inotropic responses to noradrenaline may be analogous to that part of the population of oral contraceptive users which develops hypertension.

An increase in the plasma concentration of ethinyloestradiol compared with other ethinyloestradiol pre-treated rats may account for the increase in noradrenaline potency in a minority of isolated atria. Ahluwalia, Curry, Crocker & Verma (1977) have provided evidence which indicates that women who become hypertensive while taking oral contraceptives have higher plasma concentrations of ethinyloestradiol than women who remain normotensive on oral contraceptives. An alteration in metabolism or excretion of this oestrogenic agent in some women may therefore predispose them toward developing hypertension. Similarly, an alteration in metabolism or excretion of ethinyloestradiol may also predispose rats toward potentiation of cardiac contractility and toward development of hypertension.

In conclusion, any elevation of blood pressure due to daily administration of ethinyloestradiol may be the result of potentiated vasoconstrictor and cardiac inotropic responses to noradrenaline.

Administration of norethisterone acetate was associated with potentiation of aortic strip and portal vein responses to angiotensin II. If this potentiation occurs without a concomitant decrease in plasma angiotensin II, administration of norethisterone acetate may elevate blood pressure, since Webb & Bohr (1981) have shown that potentiation of vasoconstrictor responses may lead to development of a hypertensive condition. However, some workers (Gross et al., 1965; Devynck & Meyer, 1976) have shown that plasma angiotensin II and vascular contractile responses to angiotensin II are inversely related. Administration of norethisterone acetate may therefore not lead to an elevation of resting blood pressure. Inotropic responses to noradrenaline were wide spread in individual norethisterone acetate pre-treated rats. It is therefore possible that norethisterone acetate pre-treatment may reduce inotropic responses in some rats, and potentiate inotropic responses in other rats. Since potentiation of cardiac contractile responses may lead to an increase in blood pressure (Tarazi, 1977; Weidmann, 1981), pre-treatment with norethisterone acetate by daily subcutaneous injections may increase blood pressure in a minority of female rats. In a majority of rats, however, pre-treatment with norethisterone acetate may not elevate resting blood pressure by potentiating cardiovascular responses.

Cardiac contractility may therefore be increased in ethinyloestradiol pre-treated rats and in a minority of norethisterone acetate pre-treated rats. Several workers (Walters & Lim, 1970; Clezy et al., 1972; Weir et al., 1974; Fischer & Swain, 1980) have shown that systolic blood pressure elevations are greater than elevations in diastolic pressure in oral contraceptive users. Walters & Lim (1969)

and Koch-Weser (1974) have suggested that this type of change in blood pressure profile may indicate an increase in myocardial contractility. Present findings therefore provide further evidence that daily administration of oral contraceptive agents induces similar changes in the cardiovascular system of rats and of women.

The present findings also suggest that administration of ethinyloestadiol is more likely to elevate the blood pressure of female rats than administration of norethisterone acetate. Thus if daily administration of oral contraceptive agents to rats and to women induces similar changes in the cardiovascular system, present findings provide further evidence to support the widely-held belief (see pages 12-14) that it is the oestrogenic component of oral contraceptives which elevates blood pressure in some women.

GENERAL DISCUSSION

General Discussion

Human pregnancy and oral contraceptive therapy have been associated with an increase in the blood pressure of some women (Fregly & Fregly, 1977; Welt & Crenshaw, 1978). Since potentiation of cardiovascular responses may lead to a permanent elevation in blood pressure (Webb & Bohr, 1981; Weidmann, 1981), present experiments were performed to determine whether potentiation of cardiovascular responsiveness may be involved in the aetiology of ovarian hormonal agent-induced hypertension.

It is difficult to determine the effect of ovarian hormonal agents upon the responsiveness of the human cardiovascular system. For this reason, studies have been carried out using experimental animals. Several other workers have examined the influence of ovarian hormonal agents upon cardiovascular responsiveness in experimental animals (King et al., 1959; Kalsner, 1969; Nicol & Rae, 1972; DeFelice & Joiner, 1973; McCalden, 1975; Fregly & Thrasher, 1977; Fregly et al., 1978). It is interesting, however, that none of these workers appear to have continued this type of investigation. Also, it is very difficult from the above studies to determine the mode of action of ovarian hormonal agents, since these studies were concerned with different hormones, different routes of administration and different tissue preparations from different animal species.

Lloyd and co-workers (Lloyd, 1959a, b; Lloyd & Pickford, 1961; Hettiaratchi & Pickford, 1968) performed a series of related studies to determine the action of alteration in circulating ovarian hormone levels upon pressor responses in female rats. However, experiments performed by Lloyd and co-workers were executed using anaesthetised

rats. Since rats under general anaesthesia are maintained with a nervous system capable of modifying cardiovascular responses (Price, 1960), these studies did not involve examination of the direct effect of changes in ovarian hormone levels upon the heart and blood vessels.

To determine the influence of ovarian hormonal agents upon cardiovascular responsiveness in present experiments, a consistent regimen was devised for a series of experiments in which as many variables as possible were controlled.

Since the studies of Lew (1975) and Fischer & Swain (1980) have shown that the blood pressure of female rats may be elevated following administration of ovarian hormonal agents, and since the purpose of the present study was to examine the development of ovarian hormonal agent-induced hypertension, present experiments were all performed using female rats. Established hypertension may lead to hypertrophy of blood vessels (Folkow, 1978) and to potentiation of vasoconstrictor responses (Webb & Bohr, 1981). In order, therefore, to determine whether ovarian hormonal agents induce hypertension by potentiating cardiovascular responsiveness, the effect upon the cardiovascular system of short-term changes in the plasma concentration of ovarian hormonal agents was examined in present experiments. To determine the effect of changes in the concentration of plasma ovarian hormones upon responses of the cardiovascular system of intact animals, blood pressure and heart rate responses of pithed female rats were examined, since pithing has been shown by Gillespie & Muir (1967a) to remove nervous innervation of the cardiovascular system. Also, responses were examined in isolated cardiac and vascular tissue after pre-treatment of rats with oral contraceptive agents.

The relevance and limitations of individual experiments have been discussed in chapters 1 and 2. It is the purpose of the present discussion to examine the implications of all the present findings in an integrated manner.

Several studies have shown that discontinuous administration of oestrogenic agents is associated with no change or an increase in the blood pressure of female rats (Douglas, 1974; Lew, 1975; Hoeg et al., 1977; Fischer & Swain, 1980). However, in present experiments, constant administration of 17β-oestradiol was found to reduce systolic blood pressure in female rats. It has been suggested that the difference between present findings and those in the literature may be due to the difference in the pharmacokinetic profile of the oestrogenic agents, which is dependent upon the mode of administration (see page 164). Present studies show that the responsiveness of components of the cardiovascular system was unchanged or potentiated following discontinuous ethinyloestradiol pre-treatment. Since potentiation of cardiovascular responses may lead to an elevation of blood pressure (Webb & Bohr, 1981; Weidmann, 1981), findings from in vitro studies appear to be consistent with the published work, which suggests that the effect of an administered oestrogenic agent upon the cardiovascular system of the rat is dependent upon the mode of its administration.

Administration of both 17β-oestradiol from osmotic minipumps and ethinyloestradiol by daily subcutaneous injection was associated with reduction in body weight gain in female rats. Other workers have observed similar effects following administration of a variety of oestrogenic agents: mestranol to female rats (Hoeg et al., 1977), oestrogen and stilboestrol to male rats (Saruta et al., 1975) and a combined mestranol-norethynodrel preparation to female rats (Douglas,

1974). However, daily subcutaneous injections of norethisterone acetate did not alter body weight gain in female rats in present experiments. Similarly, Black and co-workers (1976) found no alteration in body weight gain when the progestogenic agent, norethynodrel, was implanted subcutaneously into male rats. It is therefore suggested that administration of oestrogenic agents to rats by any route reduces body weight gain, whereas administration of progestogenic agents does not. In present experiments, constant administration of 17β-oestradiol was associated with reduction in systolic blood pressure whereas discontinuous administration of ethinyloestradiol induced changes in the cardiovascular system consistent with an increase in blood pressure. Changes in the cardiovascular system therefore do not appear to be related to body weight gain changes induced by administration of the oestrogenic agents.

The pharmacokinetic profile is only one of the variables involved in any study of the effect of ovarian hormonal agents upon the cardiovascular system and it is one which does not appear to have been considered in previous studies.

Another variable in such a study is the absolute plasma concentration of ovarian hormone or ovarian hormonal agent under study. However, it is difficult to examine the effect of a change in the plasma concentration of only one ovarian hormone or hormonal agent, since such a change may induce changes in the concentration of other endogenous ovarian hormones or gonadotrophins (Rabii & Ganong, 1976; Miller & Riegle, 1980; Sipinen, Lähteenmäki & Luukkainen, 1980).

In present experiments, the oestrus stage of the cycle was lengthened following 17β-oestradiol pre-treatment in some rats. In 1980 this type of phenomenon was shown by Miller & Riegle to be

associated with reduction in the plasma concentration of progesterone. It is therefore possible that observations presently ascribed to the effect of a constant and high plasma concentration of 17β-oestradiol may have been due to reduction in plasma progesterone, or to an increase in the plasma concentration of 17β-oestradiol in conjunction with reduction in plasma progesterone. Similarly, although the prime purpose in bilaterally ovariectomising rats was to reduce the plasma concentration of 17β-oestradiol, ovariectomy is also associated with reduction in the plasma concentration of progesterone (Feder, Resko & Goy, 1968). It is also possible, therefore, that the effects presently ascribed to reduction in plasma 17β-oestradiol may have been due to a decrease in the plasma concentrations of both 17β-oestradiol and progesterone.

It is interesting to note that few workers have extended initial studies on the effect of ovarian hormonal agents upon cardiovascular responses. It is possible that these workers observed discrepancies in experimental responses and so did not publish their findings. However, some apparent discrepancies may be due to this difficulty in controlling the levels of circulating hormones which leads to difficulties in the interpretation of findings.

Although present findings showed that continuous pre-treatment with 17β-oestradiol was associated with reduction in cardiovascular responsiveness and that discontinuous pre-treatment with ethinyloestradiol or norethisterone acetate was associated with no change in potentiation in cardiovascular responsiveness, there was evidence of a marked variability in response. Thus, in a majority of rats pressor responses to electrical stimulation of the sympathetic spinal outflow were reduced, but in one rat pressor responses were increased (see page 157). Similarly, although discontinuous

administration of ethinyloestradiol generally led to potentiation of atrial inotropic responses to noradrenaline, the responses of a few paired atria appeared to be greater than those in a majority. Also, a minority of isolated paired atria appeared to demonstrate potentiation of inotropic responses to noradrenaline following discontinuous in vivo norethisterone acetate pre-treatment.

Present findings therefore suggest the possibility that a minority of rats respond to administration of ovarian hormonal agents with cardiovascular responses which are potentiated compared with the majority. Potentiation of cardiovascular responses may lead to hypertension (Webb & Bohr, 1981; Weidmann, 1981). It is therefore possible that this minority of rats may develop ovarian hormonal agent-induced hypertension.

It has been suggested that constant administration of 17β-oestradiol to female rats induced changes in the cardiovascular system similar to those observed during human pregnancy (see page 166). It has also been proposed that similar changes are induced by administration of oral contraceptive agents to women and to female rats (see pages 224-225). Since only a minority of pregnant women and oral contraceptive users develop hypertension (Welt & Crenshaw, 1978; Laragh, 1972; Fregly & Fregly, 1977), present findings give further reason to believe that administration of ovarian hormonal agents to female rats is a suitable animal model for the action of ovarian hormonal agents upon the cardiovascular system of women. The minority of rats with enhanced cardiovascular responsiveness may be similar to those women who develop ovarian hormonal agent-induced hypertension.

Since the effect of ovarian hormonal agent administration upon the cardiovascular system appears to be dependent upon the pharmacokinetic profile of the administered agent, it is possible that a minority of rats may have a different pharmacokinetic profile compared with the majority. It is also possible that a minority of women respond to pregnancy or to ovarian hormonal agent administration with a different pharmacokinetic profile compared with that of the majority and it is this minority of women which develops ovarian hormonal agent-induced hypertension.

Other studies have not shown any lack of consistency in cardiovascular responses to ovarian hormonal agent administration (King et al., 1959; Kalsner, 1969; Nicol & Rae, 1972; DeFelice & Joiner, 1973; McCalden, 1975; Fregly & Thrasher, 1977; Fregly et al., 1978).

Since only a minority of women develop pregnancy-induced hypertension (Welt & Crenshaw, 1978) or oral contraceptive-induced hypertension (Laragh, 1972; Fregly & Fregly, 1977), it is difficult to determine the reasons for elevation of blood pressure during oral contraceptive therapy. Unfortunately, present findings indicate that similar problems exist in animal models of human pregnancy and oral contraceptive use. It appears that potentiation of cardiovascular responses may be involved in ovarian hormonal agent-induced hypertension. However, to determine the reasons for this potentiation would involve examination of a perhaps prohibitively large number of animals.

An increase in blood pressure may lead to hypertrophy of blood vessels (Folkow, 1978) and to potentiation of vasoconstrictor responses (Webb & Bohr, 1981). In order to determine whether ovarian hormonal agents induce hypertension by potentiating cardiovascular

responsiveness, it was necessary to examine the effects upon the cardiovascular system of short-term changes in the plasma concentration of ovarian hormonal agents. To this end, therefore, hormonal agents were administered for ten days. However, present findings indicate that isolated aortic strips may have undergone hypertrophy following ten days' administration of ethinyloestradiol or norethisterone acetate (see pages 235 and 248). It is therefore possible that a period of ten days' pre-treatment may be too long to determine whether oral contraceptive agents induce hypertension by potentiating cardiovascular responses.

It has been suggested that reduction in pressor responses to exogenous noradrenaline in vivo following 17β-oestradiol pre-treatment is due to an increase in the activity of COMT (see page 139). It has also been proposed that potentiation of in vitro vasoconstrictor responses to exogenous noradrenaline following ethinyloestradiol pre-treatment is the result of a decrease in the activity of COMT (see page 237). If alteration in COMT activity is the means whereby cardiovascular responses to exogenous noradrenaline are altered, the change in COMT activity may be dependent upon a) the use of whole animal or isolated animal tissue, b) the particular oestrogenic agent administered or c) the pharmacokinetic profile induced by the mode of ovarian hormonal agent administration.

In conclusion, present findings show an interesting similarity between the effect of constant administration of 17β-oestradiol to rats and human pregnancy upon the cardiovascular system. Also, daily subcutaneous injections of oral contraceptive agents appeared to induce changes in the cardiovascular system of rats which may have led to an increase in blood pressure. However, many of the findings were

difficult to interpret and this may have been due to interaction of plasma ovarian hormone levels or to the apparent variability in response of cardiovascular systems to alteration in the plasma concentration of ovarian hormonal agents. One suggested explanation for the present findings is that cardiovascular responses are dependent upon the mode of administration of an ovarian hormonal agent. Neither interaction of ovarian hormone levels, nor variability in responses nor the effect of the mode of administration appear to have been considered as possible explanations for the effect of ovarian hormonal agents upon the cardiovascular system in published studies.

Present findings therefore contribute to the understanding of the influence of ovarian hormonal agents upon the cardiovascular system and provide a basis for further investigation.

Summary

Present findings were performed in an attempt to increase the understanding of oral contraceptive-induced hypertension. However, constant administration of 17β-oestradiol was associated with reduction in systolic pressure and an apparent reduction in stroke volume which therefore appeared to model the effect of pregnancy upon the human cardiovascular system, rather than that of oral contraceptive therapy. Daily injections of oral contraceptive agents to female rats appeared to induce changes in isolated components of the cardiovascular system which may elevate blood pressure. It therefore appears from present experiments that the effect of administered ovarian hormonal agents upon cardiovascular responses is dependent upon the pharmacokinetic profile of the administered agent.

However, present findings do provide support for the belief that the cardiovascular system of a minority of rats may react to administration of oestrogenic agents in a way dissimilar to that of the majority. It was suggested that this minority may be analogous to the minority of women who develop ovarian hormonal agent-induced hypertension.

Future work

Present observations were difficult to interpret, and this appeared to be due, in part, to difficulty in controlling the plasma concentration of ovarian hormones (see page 261). For this reason, present experiments may be extended to determine the effects of constant administration of progesterone or Danazol from osmotic

minipumps, since Danazol has been shown by Fraser (1979) to inhibit pituitary release of gonadotrophins. An examination of the plasma concentration of ovarian hormonal agents would extend the understanding of the influence of the absolute level and pharmacokinetic profile of ovarian hormonal agents upon cardiovascular responsiveness.

The results of in vivo and in vitro experiments indicate that changes in the plasma concentration of oestrogenic agents may affect the activity of COMT and/or extraneuronal uptake. To examine this possibility, present experiments may be repeated in the presence of a COMT inhibitor. These experiments may be extended to determine whether any changes in COMT activity are due to a) the use of whole animal or isolated animal tissue, b) the particular oestrogenic agent administered or c) the pharmacokinetic profile induced by the mode of ovarian hormonal agent administration.

There is some indication that ten days' ethinyloestradiol pre-treatment may have induced aortic hypertrophy. This may be assessed by determining the dry weight of aortic strips in untreated and treated rats. If hypertrophy does take place after ten days' treatment, the treatment period may be reduced to determine whether cardiovascular responses are potentiated following pre-treatment with ovarian hormonal agents prior to any change in vascular wall structure.

It will be necessary in future to determine whether changes in the responses of isolated cardiovascular tissue following pre-treatment with ethinyloestradiol or with norethisterone acetate lead to an elevation in the blood pressure of female rats. If an elevation of blood pressure is observed, daily subcutaneous injections of oral contraceptive agents will be confirmed as a suitable animal model for oral contraceptive-induced hypertension in women.

It has been proposed that reduction in systolic pressure observed in present experiments was due to constant administration of 17β-oestradiol which induced constant high plasma concentrations of this oestrogenic agent and that constant high plasma concentrations of 17β-oestradiol may also reduce systolic pressure in pregnant women. If the results of these, and future, studies indicate that constant administration of 17β-oestradiol induces similar effects on the cardiovascular system of rats and women, it may be advisable in future to administer hormones for contraception at a constant rate. Since constant administration of 17β-oestradiol in present experiments appeared to inhibit ovulation, constant administration of ovarian hormonal agents may be as effective in reducing fertility as those preparations used at present as oral contraceptives, which may have a different pharmacokinetic profile and which elevate blood pressure.

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