INVESTIGATION INTO THE VASOMOTOR SIGNS AND SYMPTOMS OF THE MENOPAUSE

by

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The mechanism of the menopausal hot flush was investigated. Physiological changes, as well as changes in plasma and whole blood pharmacological activity were examined in post-menopausal women, at rest and during a hot flush, and in a group of pre-menopausal controls, at rest and during heat induced vasodilatation. The hot flush was found to be associated with an altered cardiovascular activity and an increase in skin surface temperatures. There was a difference in the contractile activity of plasma from post-menopausal women, at rest and during a hot flush, upon the methysergide blocked rat fundic strip. This suggested the involvement of a humoral component in the mechanism of the hot flush. This humoral component did not appear to be simply due to vasodilatation since there was no difference in the contractile activity of plasma from pre-menopausal women at rest and during heat induced vasodilatation. 17β-oestradiol appeared to affect the responsiveness of both vascular and non-vascular sympathetically innervated smooth muscle. 17β-oestradiol depressed the responses of the rat isolated vas deferens to transmural stimulation to noradrenaline and to potassium chloride. This suggested a direct action of 17β-oestradiol upon the smooth muscle. Furthermore, 17β-oestradiol, given in a dose which is considered physiological, increased the pressor responses of the female pithed rat to stimulation of the spinal sympathetic outflow but left responses to noradrenaline and angiotensin more or less unaffected. In addition 17β-oestradiol given at a higher amount decreased the pressor responses of the female pithed rat to stimulation of the spinal sympathetic outflow. The pressor responses to noradrenaline and angiotensin were again left more or less unaffected. These results suggested a biphasic action of 17β-oestradiol on the presynaptic site, at the neuroeffector junction of the vascular smooth muscle. Although the mechanism of the menopausal hot flush has not been completely elucidated, it appears to involve both a humoral and a nervous component. Furthermore, the hot flush may be related to the hormonal changes occurring at the menopause, since 17β-oestradiol was found to affect the vascular smooth muscle.

menopause, vasomotor, 17β-oestradiol, smooth muscle

CONTENTS

GENERAL INTRODUCTION

The term menopause describes the time in a woman's life when ovarian function ceases thus marking the end of her reproductive years (Finn 1976). The menopause is accompanied by various disorders, most of which are directly or indirectly associated with the hormonal changes occurring at this time. One of these disturbances is the hot flush, a vasomotor disorder which affects a great proportion of post-menopausal women. Its exact mechanism has not received much investigation and only a few studies are available in the literature (see Literature Review, page 17). In the present study, an attempt has been made to elucidate the nature of this mechanism.

Evidence presented by previous workers suggests that several physiological changes occur in post-menopausal women while experiencing a hot flush. These changes mainly include an alteration in cardiovascular activity and a rise in the skin temperature of certain areas (Hannan 1927; Molnar 1975). Most of these observations were derived from studies involving very few subjects. The most recent one performed by Molnar (1975) which provided most of the information listed above involved a single patient. It therefore appeared of interest to examine any physiological changes possibly occurring in post-menopausal women during a hot flush and to establish their occurrence in a number of patients suffering from severe and frequent hot flushes.

The study was carried out in collaboration with Dr. D.W. Sturdee, the Director of the Birmingham Menopause Clinic and depended entirely on volunteers who were patients at this clinic. An equal number of pre-menopausal women have been studied at rest and during heat-induced vasodilatation for purposes of comparison.

The study on human subjects had to finish 15 months after its commencement because of lack of volunteers and therefore many questions concerning the mechanism of the hot flush which required the participation of human subjects remained unsolved.

The physiological parameters which appeared of interest to measure were:

Heart rate
Cutaneous blood flow
Blood pressure
Skin resistance
Skin temperature

The electrocardiogram (ECG) of these women was also studied, to confirm the results of a previous worker (Molnar 1975) that fluctuations of the ECG base line occur during a hot flush.

In addition to the physiological study of the hot flush, studies were carried out on the contractile activity of the plasma on smooth muscle and on prostaglandin E-like and kinin-like activity in the blood of post-menopausal women at rest and during a hot flush. The contractile activity of plasma from pre-menopausal women at rest and during heat-induced vasodilatation was also examined. It was thus hoped to establish any changes in the biological activity of plasma or whole blood of post-menopausal women during a hot flush which might be indicative of the involvement of a circulating substance in the mechanism of the flush.

Several substances have been implicated in the flushes of various pathological conditions. These substances include 5-hydroxytryptamine, histamine, catecholamines, prostaglandins and kinins (see Literature Review, page 25). The possible involvement of these substances and of acetylcholine was examined. All these

compounds with the exception of catecholamines, are vasodilator in most vascular beds. Therefore the possibility of their involvement was examined in the vasodilatation observed during a hot flush.

The possibility that catecholamines were responsible for the triggering mechanism of the hot flush was examined. This 'trigger hypothesis' evolved from the following observations and evidence:

1. Catecholamines are capable of releasing vasodilator substances such as kinins and prostaglandins (see pages 26, 28).
2. Catecholamines have been shown to cause typical flushes in patients suffering from the carcinoid syndrome (see page 26).
3. The results of the physiological study in the present project as well as the results obtained by other workers indicated a sympathetic activation at the onset of the hot flush (see pp 17,81).

It might be that an initial increase in the sympathetic nerve activity occurs, possibly of vasoconstrictor nature, and as a consequence, the homeostatic mechanisms for the regulation of the local blood flow become activated in order to re-establish normal flow.

A possible reason for this sudden increase in the sympathetic activity might be the changes in the hormonal status of post-menopausal women. Oestrogen levels are known to fluctuate during the peri-menopausal years. In the case of 17β-oestradiol these fluctuations may reach a peak value of approximately 10 times the normal levels seen in women before the menopause (Campbell 1976). It may be that this sudden increase in oestrogen levels interferes with the activity of the sympathetic nerve fibres innervating the blood

vessels and initiates the instability in the peripheral circulation leading to the hot flush. Alternatively it may be that this fluctuation in oestrogen levels renders the blood vessels more sensitive to any given stimuli. Thus the same stimulus might evoke a greater response in post-menopausal women than in pre-menopausal women.

Finally, the possibility exists that oestrogen or lack of oestrogen might affect the activity of the 'vasomotor centre'. It has been shown that castration in rats causes hyperactivity of certain hypothalamic nuclei of the anterior hypothalamus as well as increasing the noradrenaline synthesis in the same area (Donoso and Stefano 1967; Donoso, De Gutirrez Moyano *et al* 1969). Although the 'vasomotor centre' is not directly implicated in the above mentioned study, its location is believed to be extended to the anterior hypothalamus and the possibility cannot be excluded that it might also be affected. If this was true then a fall in oestrogen levels during the fluctuations of the peri-menopause might result in a hyperactivity of the vasomotor centre which may lead to the sequence of events seen during a hot flush.

The following possibilities should be therefore considered as far as the interaction between oestrogen, vascular smooth muscle, and the sympathetic nervous system is concerned.

1. Oestrogen affects the vascular smooth muscle directly.
2. Oestrogen interferes with the sympathetic innervation at (a) post-ganglionic level, (b) pre-ganglionic level, (c) central level. In the second part of the present study, possibilities 1 and 2(a) were considered and tested on two experimental systems:

(i) On the pithed rat preparation as a model for the study of the effect of oestrogen on the vascular smooth muscle and its innervation.

(ii) On the *vas deferens* preparation. This preparation was used as a model for the study of the effect of oestrogen on sympathetically innervated non-vascular smooth muscle tissues.

LITERATURE REVIEW

The literature review aims to cover the following subjects which are considered relevant to the present study:

1: The menopause.

2: The vasomotor disorders of the menopause.

3: Studies of flushes associated with pathological conditions.

4: Regulation of the peripheral circulation.

5: Oestrogens, the cardiovascular system and its innervation.

1: THE MENOPAUSE

The term menopause actually indicates the time that the last bleeding occurs from the uterus of the human female and consequently it also indicates the end of her reproductive years. Women seem to be unique in the animal kingdom in having a menopause in which ovulation ceases due to exhaustion of oocytes in the ovary (Finn 1976). The situation in other primates is not very clear owing to scarcity of data. In a study performed by Hodgen, Goodman, O'Connor and Johnson (1977), it was shown that rhesus monkeys exhibited patterns similar to the human menopause during the third decade of their life. In fact vaginal bleeding declined progressively and eventually ceased and serum hormonal profiles were consistent with those described in peri- and post-menopausal women (Sherman, West and Konerman 1976; Treloar, Boynton and Ben 1967).

The basic feature of the menopause is that the primordial follicle and its derivatives, the granulosa cells and the surrounding theca cells, degenerate or fail to react to endogenous gonadotrophins. The granulosa cells in the pre-menopausal ovary are capable of producing oestrogens from acetate, the most rudimentary steroid

precursor, but tend to accumulate progesterone and 17α-hydroxy-progesterone (Figure 1). The theca cells tend to produce the androgens dehydroepiandrosterone and particularly testosterone and androstenedione from progesterone and may continue the biosynthetic pathway to oestradiol and oestrone. Granulosa and theca cells function synergistically to achieve maximum steroid production. The stromal cells produce the androgens, androstenedione, testosterone and dehydroepiandrosterone, in the normal pre-menopausal ovary and this process continues in the post-menopausal ovary (Cooke 1976). The change in the function of the ovaries of the ageing woman results in the precipitation of a number of disorders of varying severity and frequence of occurrence. In the following section these disorders are discussed together with the age of the women when the menopause occurs.

1. The age of the menopause

The age of menopause in Caucasian populations has been found to be around 50 years (Benjamin 1960; McKinlay, Jefferys and Thompson 1972; Treloar 1974; Benedek Jaszman 1976; Gray 1976). It appeared to be independent of environmental factors, education or physical type. No relationship could be demonstrated either with the age of menarche, the number of pregnancies or the time of last pregnancy. However the age of the onset of the menopause did appear to be related to whether or not the women had been married.

The age of the menopause in non-Caucasian women tends to be lower than that in Caucasians and ranges from 49.7 years among the Bantu in South Africa (Frere 1971) to 43.6 years among poorly nourished women in New Guinea (Scragg 1973). These results suggest

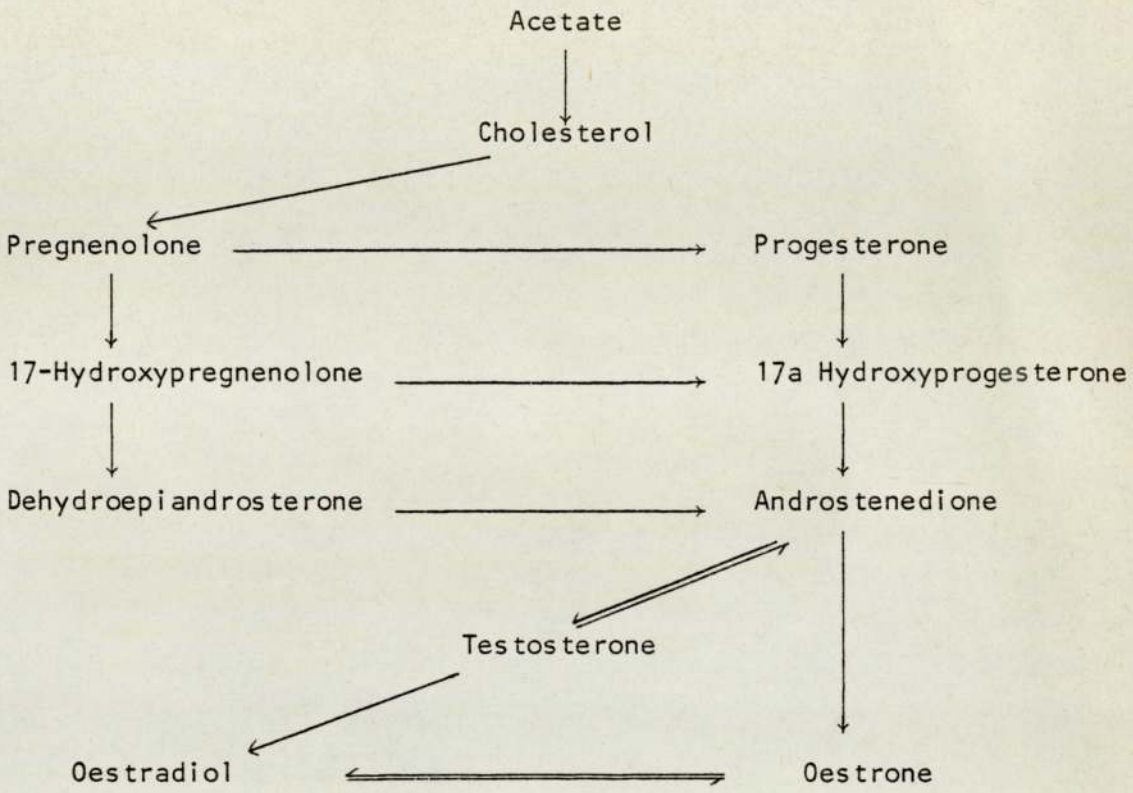


FIGURE 1: Pathway for oestrogen synthesis from acetate (from Smith, O.W. and Ryan, K.J. 1962. Am. J. Obstet. Gynecol. 84,141)

that poor nutrition might be associated with premature menopause though the influence of racial factors cannot be excluded.

2. The disorders of the menopause

The disorders of the menopause can be divided into two classes:

2.1: Genital disorders.

2.2: Other disorders due to autonomic, hormonal and psychosomatic causes.

2.1: Genital disorders

The genital disorders involve the transition from normal menses via a changed pattern of menstruation, accompanied by infertility, to cessation of uterine bleeding and post-menopausal genital atrophy. The cause of these changes is entirely hormonal and can be seen in every woman. The hormonal changes that occur at the time of the menopause are discussed below.

(a) Hormonal changes of the menopause

As mentioned before, the basic feature of the menopause is that the primordial follicle and its derivatives, the granulosa and theca cells, degenerate or fail to react to endogenous gonadotrophins. As pituitary gonadotrophins are primarily controlled by feedback of ovarian steroids to the hypothalamus, failing ovarian steroid production has been shown to result in excessive hypothalamo-pituitary responses (Adamopoulos, Loraine, Dove 1971). In addition, low urinary excretion of oestrogens has been shown and particularly lower oestradiol excretion. The main circulating oestrogen after the menopause appears to be oestrone.

Peripheral levels of progesterone 17-hydroxyprogesterone, testosterone, dehydroepiandrosterone and androstenedione also fall during the post-menopausal years (Maroulis and Abraham 1976).

Table 1 shows the levels of the main circulating steroids found in the plasma of pre- and post-menopausal women as measured by Maroulis *et al* (1976).

TABLE 1: Peripheral plasma levels of steroids in pre- and post-menopausal women

Steroid	Pre-menopausal mean ± se ng/ml	Post-menopausal mean ± se ng/ml
Progesterone	0.52 ± 0.12	0.28 ± 0.03
17-Hydroxyprogesterone	0.58 ± 0.12	0.15 ± 0.02
Testosterone	0.27 ± 0.06	0.19 ± 0.03
Dehydroepiandrosterone	4.56 ± 1.00	1.46 ± 0.14
Androstenedione	1.25 ± 0.11	0.27 ± 0.02
Oestradiol	0.09 ± 0.003	<0.02
Oestrone	0.05 ± 0.003	0.036 ± 0.003

Data taken from Maroulis and Abrahams. Obstetrics and gynaecology Vol. 48 No. 2 1976.

Apart from the fall in circulating steroid levels, another very important difference between the pre- and post-menopausal women is the contribution of the post-menopausal ovary to ovarian steroids in the circulation. Thus in the post-menopausal woman about 40 µg/day of oestrone is excreted but virtually none of it is secreted by the ovary and almost all of it is derived from androstenedione (Cooke 1976). The adrostenedione is secreted largely by the adrenal gland and conversion occurs peripherally, that is outside the ovary. The site of conversion is likely to be the adipose tissue (Grodin, Siiteri and MacDonald 1973).

During the normal menstrual cycle, both follicle-stimulating hormone (FSH) and luteinising hormone (LH) fluctuate signficantly. These fluctuations include the early follicular phase rise (particularly FSH) and the mid-cycle surge (particularly LH) of both gonadotrophins (Speroff and Vande Wiele 1971). As women approach the menopause FSH levels appear to rise out of the normal range while LH levels remain normal (Sherman and Korenman 1975). These elevated FSH levels can occur throughout a normal cycle, despite the attainment of oestrogen levels that would be expected to suppress FSH secretion sufficiently to maintain normal concentrations (Judd 1976). This observation has raised the question as to whether ovarian factors other than oestrogen and progesterone may play a role in FSH-feedback control. In post-menopausal women both LH and FSH levels are significantly elevated over the concentrations seen in pre-menopausal women with FSH usually being higher than LH. The higher FSH levels are thought to be due to the slower clearance of this gonadotrophin (Yen, Llerena, Pearson and Littell 1968, 1970).

2.2: Other disorders due to autonomic, hormonal and psychosomatic causes

This class of disorders do not necessarily occur in all women. They differ in intensity and frequency from one woman to another and are often regarded as unpleasant and referred to as 'complaints' (Benedek Jaszman 1976).

These are:

(a) Joint and muscle pains

These pains probably reflect a reduction in muscle strength, with a possible reduced ability to disperse the build up in muscle of lactic acid after exercise.

(b) Vasomotor disorders

The precise mechanism of these disorders is not known. It has been shown however that women who have marked premenstrual changes commonly suffer from hot flushes (Cope 1976). It was suggested by the same worker that in this group of women there exists a very delicately balanced relationship between ovarian feedback and the activity of the hypothalamus and the pituitary. It was also proposed that an element of their autonomic system might be particularly labile. Finally in the natural menopause many women show a marked fluctuation of oestrogen production and it might be that this fluctuation is important for the precipitation of vasomotor symptoms. These vasomotor symptoms will be discussed in more detail later in the introduction.

(c) Atrophic vaginitis and the urethral syndrome

These conditions are being considered together, as the vulva, the lower part of the vagina, the urethra and the trigone are embryologically similar and have been shown to respond in a similar manner both to oestrogen deprivation and to its replacement (Cope 1976). Atrophic vaginitis consists of symptoms such as discomfort and disability for sexual intercourse, whereas the urethral symptom is manifested by 'urge incontinence'. This latter group of women has been shown to be largely prone to infections such as cystitis,

urethritis, kolpitis and bacterial and fungal infections (Workshop Report 1976).

(d) Cardiovascular system

The precise relationship between cardiovascular disease and the menopause is not yet clearly established. However, there is some evidence of a relationship, in that the incidence of hypertension in women increases with age (National Centre for Health Statistics 1964; Weiss 1972). It has been shown that hypertension is more common in men than women until after the menopause when this difference is reversed (Weiss 1972). Furthermore, in non-smoking women before the menopause, coronary heart disease is extremely rare, whereas after the menopause the incidence approaches that of men (Cope 1976). Parrish and co-workers (Parrish, Carr, Hall and King 1967) showed that there is an association between plasma cholesterol concentration and oestrogen secretion. Plasma cholesterol was lowest during mid-cycle when oestrogen secretion was maximal whereas following bilateral oophorectomy or in the post-menopause the levels of cholesterol, lipoproteins and triglycerides rose. The same workers have demonstrated that in women castrated before the age of 40 and in women who have had a premature menopause, coronary heart disease incidence increased, though serious atherosclercosis did not appear for about 14 years.

(e) Skin and connective tissues

The menopause is not only the time of decreasing ovarian function, it is also a period of progressive involution of body functions. Therefore it has been considered inappropriate to assume that all the skin problems in menopausal women are due to oestrogen deficiency.

However, the skin is an important bearer of secondary sex characters and a major target for oestrogenic action (Shahrad and Marks 1976). Frost and co-workers (Frost, Weinstein and Hsia 1966) have shown that skin actively metabolises oestrogens, in that infant foreskin had the ability to interconvert oestradiol to oestrone. It has also been shown that hair follicle epithelium actively takes up oestrogens, and interconverts them to a degree which is dependent upon the stage of the hair cycle (Rampini, Davis, Moretti and Hsia 1971). At the menopause oestrogen deprivation has been shown to cause thinning of the epidermis (Cope 1976). It has also been shown to cause a negative nitrogen balance with diminution of muscle which is replaced by fibrous tissue. Finally it causes subcutaneous fat to atrophy and lose its elasticity.

(f) *Osteoporosis*

In 1941, Albright and co-workers (Albright, Smith and Richardson 1941) showed that there was a close relationship between the menopause and osteoporosis. In 1957 Henneman and Wallach reported that height loss could be prevented if women at the beginning of their menopause were given oestrogen. More recently, Aitken, Hart and Lindsay (1973) and Gallagher and Nordin (1973) have demonstrated a relationship between bone resorption and oestrogen deficiency in castrated women. Gallagher *et al* (1973) finally suggested that oestrogen reduced the sensitivity of bone to parathyroid hormone which is known to promote loss of calcium from the bone.

(g) Psychosomatic symptoms

Psychosomatic symptoms include fatigue, headache, irritability, dizziness, palpitations and depression. These symptoms usually occur during the menopause and early post-menopausal years. In the case of endogenous depression, evidence has been suggested that abnormally low levels of free plasma tryptophan are associated with oestrogen deficiency (Aylward 1976). Furthermore, oestrogen administration which seems to alleviate depression in post-menopausal women, was shown to increase the levels of free plasma tryptophan.

2: THE VASOMOTOR DISORDERS OF THE MENOPAUSE

1. Manifestation

The manifestation of the vasomotor disorders occurring at the menopause is termed the 'Hot Flush'. It is one of the major and more frequently occurring disorders of the menopause and it consists of a sudden feeling of warmth and accompanied by flushing of the face and sometimes sweating (Molnar 1975). The face and neck are the parts usually affected and where the hot flush becomes obvious.

Hannan (1927) described the hot flush as a 'vasomotor crisis' and suggested that it may be divided into three clinical stages: (a) a premonitory stage associated with symptoms such as headache and palpitations and which were relieved with the onset of the flushing; (b) a stage of flushing which was associated with a feeling of warmth; and (c) a stage of reaction which was associated with symptoms such as perspiration and faintness.

However, Molnar (1975) in his single patient study was unable to find evidence for a warning stage prior to the full development of the hot flush.

2. Incidence, severity and frequency

Few studies are available concerning the incidence, severity and frequency of the hot flushes. McKinlay and Jefferys (1974) found that 74 per cent of the women who had last menstruated between 3 and 12 months previously, suffered from hot flushes. This is consistent with the finding by Thompson, Hart and Darno (1973) who reported that 74 per cent of the post-menopausal women in a general practice in north-east Scotland suffered from hot flushes. Jaszman (1976) also found that hot flushes were most

frequently reported by women in the early post-menopause along with perspiration and formication. However, McKinlay's study (McKinlay *et al* 1974) indicated that the hot flushes persist much longer than it is generally believed.

The severity of the hot flush is varied and symptoms can be graded from 'embarrassment' to 'acute physical discomfort'. The acute discomfort associated with hot flushes has been reported to be worse when they occur at night (McKinlay *et al* 1974).

Finally the frequency of hot flushes per day varies. Hannan (1927) reported that some patients complained of experiencing as many as 12 hot flushes a day where as others complained of one or two a week. The factors which determine the frequency of hot flushes per day are not yet established.

3. Studies on the mechanism of the hot flush

Although the hot flush is the most common disorder of the menopause, it has received very little scientific investigation and its aetiology still remains obscure. One major reason for this is that the hot flush can only be studied in human beings who are otherwise healthy and that therefore any study is dependent upon volunteers.

The first detailed study on 'The Flushings of the Menopause' was performed by Hannan (1927). His study aimed at an elucidation of their mechanism in addition to recommending methods of treating the hot flushes and their associated symptoms.

The blood pressure and heart rate observations performed in this study showed a rise of the systolic blood pressure and the pulse rate just prior to the onset of the hot flush. After the hot

flush had ceased there was a considerable fall in blood pressure which was restored after 30 minutes. The increased pulse rate was maintained during the hot flush but returned to its normal value after 30 minutes. These findings led Hannan (1927) to suggest that there was a strong resemblance between the changes in blood pressure and pulse rate associated with a flushing attack and those produced by an intravenous injection of adrenaline in normal individuals.

Consequently, he investigated the effect of adrenaline injection in women suffering from hot flushes and the following phenomena were observed:

(a) Blood pressure and pulse rate changes which were similar to those associated with
   - the injection of adrenaline in normal individuals,
   - the 'vasomotor crisis' (hot flush) of the menopause.

(b) A premonitory stage associated with symptoms such as headache and palpitations.

(c) A stage of flushing.

(d) A stage of reaction associated with perspiration and faintness.

Failure of other drugs to produce the same symptoms led Hannan to the suggestion that 'the flushings' are produced by a reflex discharge of adrenaline into the blood stream, and that the flushes are produced by a peculiar sensitivity of the vasomotor system at the time of the menopause due to a general disturbance of the endocrine system, consequent upon a deficiency of the internal secretion of the ovaries.

Other workers since then have attempted to investigate the mechanism of the hot flush by measuring the changes in physiological parameters such as body temperature, or basal metabolic rate.

Klaften (1944) measured uterine and other internal body temperatures daily in 24 menopausal women but was unable to obtain measurements during a flush. Collett (1949) found that during hot flushes the Basal Metabolic Rate rose 5 to 15 per cent which was attributed to a "faster heart beat and stiffening of the muscles". In the same study it was found that cheek temperature increased during a hot flush from 0.5°C to 2.0°C.

Reynolds (1941) reported a finger volume change and face temperature rise during a flush in one post-menopausal woman.

In a more recent study Molnar (1975) studied one post-menopausal patient suffering from hot flushes. He measured internal and external body temperatures, sweating, cardiac activity by means of electrocardiography and blood glucose concentration during hot flushes. The results of this study were:

(a) The flushes were well defined by marked undulations of the ECG base line, and tachycardia.

(b) The mean duration of the flushes was 3.8 minutes.

(c) There was profuse sweating on the forehead and nose, moderate sweating on the sternum and adjacent areas and little or none on the cheek or leg.

(d) There was a marked rise in finger, toe and cheek temperature.

(e) There was a fall in forehead temperature immediately after the onset of the flush due to evaporation of sweat.

(f) The internal temperatures showed a downward trend. The fall was always very slow and lasted 15 - 30 minutes.

(g) No significant difference was found between the blood glucose concentration immediately after the hot flushes compared with normal values.

Finally, Molnar concluded that "a hot flush is apparently an explosive activation of certain areas of the brain resulting in subjective heat distress, in the inhibition of ongoing discharges of vasoconstrictive impulses to the extremities and in the excitation of stimulating impulses to the heart, to sweat glands and to vasodilators of the face. This activation of brain centers, lasts a few minutes. The interval of excitation-inhibition is followed by a subliminal state of a few minutes during which a passing stimulus, eg a finger prick, can evoke another flush of short duration."

In addition, the "possibility of cellular accumulation and sudden discharge of a neurohumor" is considered. "This substance," according to Molnar, "converts the status quo of the affected brain centers - a condition of comfort associated with a particular modulation of hypothalamic neuronal discharges by afferent impulses from peripheral temperature receptors - into one of intense discomfort and disequilibrium with the environment. This substance or its formation can usually be counteracted in post-menopausal years by oestrogens."

Hot flushes are successfully treated by hormone replacement therapy. However, this can only provide an explanation for their possible mechanism if correlation can be made between the occurrence of vasomotor symptoms and peripheral oestrogen levels in post-menopausal women.

Campbell (1976) has examined the possible correlation between hormonal changes and the occurrence of hot flushes in a systematic study of a 24-hour intensive steroid and protein profile in post-menopausal women with and without vasomotor symptoms.

Seven post-menopausal women and four age matched controls who had no vasomotor symptoms were studied. The results of this study would be summarised as follows.

Two non-flushing women had high sustained levels of oestrone and oestradiol. All the flushing women and two non-flushing had low levels of oestrone and oestradiol but sharp peaks were observed especially in flushing women which in the case of oestradiol were up to 10 times the basal values.

Both plasma androstenedione and testosterone showed a well marked diurnal variation suggesting that these hormones are mainly derived from the adrenal. Plasma androstenedione levels were significantly higher in non-flushing than in flushing women and the latter group showed a somewhat different diurnal rhythm.

Plasma testosterone levels were similar in both groups. Serum follicle stimulating hormone (FSH) values were lowest in those patients with the highest plasma oestrogen levels which suggested a negative feedback control of FSH release, but luteinizing hormone (LH) levels were a poor guide to oestrogen status.

There was no difference in plasma prolactin levels between flushing and non-flushing patients, but the surges of growth hormone appear to be higher in flushing patients.

In individual cases the best correlation between hormone levels and flush was demonstrated by oestrogen and androstenedione but the relationship was not sufficiently close to suggest that this was causal.

In an attempt to elucidate the mechanism of the hot flushes several workers have attempted to treat the hot flushes with more specific agents.

It was hoped that the use of these agents might clarify the possible involvement of either endogenous substances or the nervous system in the hot flush.

Ferriman and Purdie (1965) considered the increase in circulating gonadotrophin concentration as a causative factor of hot flushes, and they investigated this possibility by treating their patients with dithiocarbamoylhydrasine (ICI 33828), a compound which has been shown to have antigonadotrophin activity both in animals (Paget, Walpole and Richardson 1961; Brown 1963) and in humans (Bell, Brown, Fotherby, Loraine and Robson 1962). A double blind trial was carried out in 33 patients for 15 days. It was found that ICI 33828 significantly reduced the number of hot flushes in all cases but did not abolish them completely.

However, this compound has been found to affect thyroid function (Tulloch, Crook and Brown 1963) and lactation (Zagni and Benson 1964) as well as gonadotrophin levels. Therefore there are some reservations as to whether the hypothalamus is a specific site of action for this drug and therefore its antigonadotrophin action needs to be established. In contrast Clorinda, Bohler and Greenblatt (1976) examined the possible factors involved in the pathophysiology of the hot flush and reached the conclusion that increased gonadotrophins were not the causative factor. They supported this with the following observations:

(a) Prepubertal castrates or people suffering from primary gonadal failure and consequently elevated gonadotrophins did not experience hot flushes.

(b) Small amounts of oestrogen without producing significant lowering of gonadotrophin levels are frequently effective in arresting the hot flush.

(c) When individuals with hypopituitarism or primary gland failure were treated for many years with oestrogens, they experienced hot flushes when medication was discontinued.

(d) Gonadotrophin therapy did not produce hot flushes.

Clayden, Bell and Pollard (1974) carried out a double-blind trial of a non-hormonal medication using Clonidine (Dixarit). Clonidine has been shown to diminish vascular reactivity when given chronically to cats (Zaimis and Hannington 1969). It has also been shown to be effective in the prophylaxis of migraine (Wilkinson 1969; Shafar, Tallett and Knowlson 1972). During the double-blind trial a pronounced placebo response was observed. This was attributed to both a continuous improvement in condition with time and to the benefit of the increase in attention received during the trial. This is in contrast with the results on the effectiveness of a non-steroidal oestrogen analogue (Utian 1973) where no placebo response was found. In spite of the placebo effect, Clayden and co-workers (1974) concluded that clonidine was effective in relieving hot flushes and they suggested this might be due to reduced responsiveness of the small blood vessels to various stimuli. They also suggested that the vasculature of post-menopausal women is more labile and consequently more sensitive to stimuli, which may account for the

occurrence of hot flushes in these women. Coope and Williams (1978) examined the possible involvement of an adrenergic component in the mechanism of the hot flush by treating patients suffering from hot flushes with propranolol. Propranolol causes both central and peripheral blockade and has been shown to be effective in relieving both palpitations and bradycardia.

The results of the above study which involved a prospective double-blind randomised comparison of propranolol and matching placebo showed propranolol to be no more effective than placebos in controlling hot flushes. However, a very close correlation was established between the daily atmospheric temperature and the number of flushes occurring in their patients.

3: STUDIES ON FLUSHES ASSOCIATED WITH PATHOLOGICAL CONDITIONS

Although the mechanism of the menopausal hot flush has received little scientific investigation and therefore remains obscure the mechanisms of the flushes associated with several pathological conditions have been extensively investigated. This has resulted in the implication of several vasoactive substances in these flushes.

The menopausal hot flushes are manifestations of a natural process (ie physiological) whereas the flushes of other syndromes are manifestations of disease (ie pathological). However, it is relevant to review the current knowledge of their mechanism since this may lead to a better understanding of the menopausal hot flushes.

Four pathological conditions which exhibit hot flushes will be considered and these are:

1. The carcinoid syndrome
2. Phaechromocytoma
3. Diencephalic autonomic epilepsy
4. The dumping syndrome

1. The carcinoid syndrome

The carcinoid syndrome consists of cutaneous flushing, asthma, intermittent diarrhoea, a skin rash resembling pellagra and collagen deposits on the endocardium including valve surfaces of the heart. It is a manifestation of carcinoid tumours and particularly of metastatic carcinoma of the liver (Sjoerdsma and Melmon 1964).

Several substances have been implicated in the mechanism of carcinoid syndrome and in particular with the carcinoid flush.

In a review of the carcinoid spectrum, Dollinger and Gardener (1966) examined the involvement of serotonin, catecholamines, kinin peptides, and histamine in the mechanism of the syndrome.

Serotonin has been isolated from carcinoid tumours (Lambeck 1952) and it was subsequently postulated that most of the symptoms of the syndrome could be ascribed to excess serotinin produced by the tumour (Thorson, Bjoru, Bjorkman and Waldenström 1954). However, normal blood serotonin levels have been observed in a patient during four days of continuous and severe flushing. Elevated levels of blood serotonin are present in most patients suffering from this syndrome but they may not always correlate with flushing episodes (Robertson, Peart and Andrews 1962). Furthermore, intravenous infusion of large doses of serotonin produces an atypical flush of less intensity than that usually in the carcinoid syndrome (Robertson *et al* 1962; Levine and Sjoerdsma 1963). In addition serotonin antagonists frequently failed to prevent or modify the flushing episodes (Sanders and Axtell 1964).

Catecholamines have also been implicated in the mechanism of the flushes of the carcinoid syndrome. Intravenous administration of catecholamines (Peart, Andrews and Robertson 1961), adrenaline or noradrenaline, to patients with spontaneous flushes reproduced the typical flushing attack.

Elevated levels of 4-hydroxy 3-methoxymandelic acid, an adrenaline and noradrenaline metabolite, have been observed in some patients with carcinoid tumours (Sandler and Ruthven 1960). Furthermore, CA are able to release a kinin peptide from the sub-maxillary gland of the cat (Hilton and Lewis 1956) and it has been

suggested by Robertson *et al* (1962) that some of the manifestations of the carcinoid syndrome, particularly the cutaneous flush, might be related to increased levels of kinin peptides. Furthermore, a kininogen-consuming kinin generating system which is activated by either adrenergic or cholinergic agonists has been demonstrated in rat blood (Rothschild, Gomes and Castania 1976).

Finally, it appears that catecholamines are involved in the mechanism of these flushes but they do not seem to be the sole endogenous substance related to flush production since spontaneous flushes may occur despite administration of phenoxybenzamine, an antagonist of administered noradrenaline (Dollinger *et al* 1966).

As it has been mentioned previously kinin peptides can be released by the action of catecholamines (Hilton *et al* 1956), and it has been suggested that they may be the causative factor of the hot flush (Robertson *et al* 1962). Kinin peptides are potent vasodilators and infusion of bradykinin into five patients with carcinoid syndrome was shown to produce flushes similar to those occurring spontaneously and those induced by noradrenaline administration (Oates, Melmon, Sjoerdsma, Gillespie and Mason 1964). High concentrations of kinins have been found in hepatic venous blood of several carcinoid patients during catecholamine-induced flushes compared with lower levels in those patients before noradrenaline and with negligible amounts after noradrenaline in patients without carcinoid disease (Oates *et al* 1964). All the above evidence strongly implicates the kallikrein-kinin systems in the production of the carcinoid flush.

On the other hand, excess histamine has been demonstrated in some patients with carcinoid syndrome (Pernow and Wandelström 1957) but it does not seem to be the direct cause of the flush (Robertson *et al* 1962). However, it might be that serotonin or histamine cause local release of catecholamines which then cause release of kinin peptides causing the flush (Dollinger *et al* 1966).

In more recent studies prostaglandins have been implicated in the carcinoid syndrome. The fact that administration of prostaglandin E_1 or E_2 in man causes intense flushing associated with diarrhoea (Bergström, Duner, v. Euler, Pernow and Sjoväll 1959; Bergström, Carlson, Ekelund and Öro 1965; Horton, Main, Thompson and Wright 1968) and the finding that prostaglandins E_2 and F_{2a} were present in high concentrations in patients with bronchial carcinoma as well as in patients with a-cell tumours of the pancreatic islets (Sandler, Karim and Williams 1968) lead Smith and Greaves (1974) to investigate involvement of prostaglandins in the carcinoid flush. It was shown that in one patient suffering from carcinoid syndrome, the concentrations of total blood prostaglandins increased almost six-fold during a noradrenaline provoked flush as compared with concentrations before the flush. The concentrations of total blood prostaglandins before and during the flush were considered by the authors to fall within the normal range. However, of the total prostaglandin activity recovered before the flush, approximately two-thirds was due to prostaglandins of the E-series and the remainder due to those of the F-series, whereas during the flush the recovered prostaglandin activity was almost entirely due to prostaglandins of the E-series. The authors concluded that the rise in

blood prostaglandin activity could be due to an expulsion of a pool of prostaglandin-rich blood from the hepatic vasculature into the systemic circulation in response to the vasoconstrictive action of noradrenaline.

2. Phaeochromocytoma

According to Anderson (1948) phaeochromocytoma is due to a catecholamine secreting tumour, usually benign, composed of a differentiated mature type of cell resembling the adrenal medulla. This condition is manifested by attacks of palpitations, sweating, flushing or blanching, vertigo, headache, dyspnea, chest pain or tremor and hypertension (Hutchison, Evans and Davidson 1958). In phaeochromocytoma flushes occur in association with fluctuating levels of catecholamines (Goldfien, Zilleli, Goodman and Thorn 1961). Exceptionally high levels of catecholamines have been demonstrated ranging from 5-4, 200 ng/ml of plasma (Robinson 1963; Goldfien *et al* 1961; Priestley, Kvale and Gifford 1963) during paroxysmal attacks.

3. Diencephalic autonomic epilepsy

This is a rare syndrome of paroxysmal autonomic discharge. It was first described by Penfield (1929) in a patient with autonomic paroxysms characterised by hypertension, tachycardia, salivation and lacrimation and generalised seizures. In addition, flushing is one of the symptoms characterising the autonomic epilepsy. Although adrenergic signs have been attributed to paroxysmal catecholamine release, excretion of catecholamines and their metabolites have been reported normal in some cases (Cameron and Doig 1970). However, in a recent study performed by Metz and

associates (Metz, Halter, Ponte and Robinson 1978) it was found that plasma catecholamine levels in one patient with transient paroxysms of hypertension, tachycardia and flushing, rose from normal basal level to increased (twofold) levels at the peak of the spells. Prostaglandin E levels were found to be normal at basal state but rose to almost threefold values shortly after a spell.

4. Dumping syndrome

The dumping syndrome appears in some patients as a sequel to gastric surgery and may be provoked by a meal or, experimentally, by ingestion of a hypertonic glucose solution (Machella 1949). The symptoms may vary widely in different individuals but generally they include borborygmi, nausea, vomiting and diarrhoea as the intestinal component, and dizziness and pallor followed by flushing, tachycardia, sweating and weakness (Zeitlin and Smith 1966). Therefore it consists of both intestinal and vasomotor symptoms similarly to the carcinoid syndrome.

Serotonin and kinins have both been implicated in the mechanism of the dumping syndrome. The study of Zeitlin and Smith (1966) showed no changes in blood serotonin during 'dumping' attacks. However, their 24-hour urinary excretion 5-hydroxyindoloacetic acid, a serotonin metabolite was more than twice normal. Furthermore, the vasomotor symptoms of dumping were found to be clearly related to the appearance of raised levels of free kinin in the circulation.

5. Summary

The humoral factors that have been implicated as contributing to the mechanism of the flushes of the syndromes mentioned above are:

serotonin, catecholamines, kinin peptides and prostaglandins. Since all these substances are implicated in the regulation of the local blood flow, it seems probable that all these syndromes arise from a disorder of local circulatory control.

4: REGULATION OF THE PERIPHERAL CIRCULATION

In the present section the general principles of the control of the peripheral circulation are discussed. In addition, special attention is given to the control of the cutaneous circulation as this is most relevant to the study of the mechanism of the hot flush.

1. Basal vascular tone and its control

Inherent myogenic activity, particularly pronounced in 'single unit' smooth muscles (according to Bozler's classification of smooth muscle 1948) in the precapillary resistance and sphincters, is responsible for a basal vascular tone which keeps the vessels in a state of partial constriction (Folköw and Neil 1971). The venous capacitance vessels manifest little basal myogenic activity and their tone is determined mainly by the influence of sympathetic vasoconstrictor activity. The vasculature of these vessels conforms to 'multi-unit' type of smooth muscle (Bozler 1948).

Myogenic activity, though a primary consequence of an intrinsic membrane instability in 'single unit' muscles may be facilitated or dominated by extrinsic neurogenic or humoral factors. Changes in the chemical and physical environment are important modulators of the vascular tone. Factors such as electrolyte concentration, pH, oxygen tension, osmolarity, temperature etc, affect vascular smooth muscle activity because they are more or less directly involved in the basic machinery of the muscle cell itself (Mellander and Johansson 1968). Other vascular control systems, particularly those which mediate central or other remote influences on peripheral vessels, operate by means of transmitters or circulating substances. Differences in the distribution of

receptors to such substances between vessels may contribute to differentiation of the circulatory control.

The neurogenic and humoral mechanisms by which the vascular tone is influenced are discussed below in more detail.

2. Nervous control

Although the level of basal vascular tone in a local circuit is determined by the interplay of local mechanical and chemical factors, neurogenic mechanisms exert a 'remote control' of the overall situation which permits the optimal distribution of the circulating blood in emergency states and which serves to adjust the circulation to the requirements of the body as a whole. The most important influence in this respect is furnished by the activity of sympathetic vasoconstrictor fibres. In addition there exist vasodilator fibre systems of sympathetic, parasympathetic or dorsal origin which also are involved in the vascular control. These systems of vasoconstrictor and vasodilator fibres are described separately.

2.1: Vasoconstrictor fibres

The adrenergic vasoconstrictor fibres act by releasing noradrenaline in direct contact with the outer sheath only of the vascular media (Folköw, Öberg and Rubinstein 1964). Noradrenaline couples with a-receptors in the membrane of the vascular effector cell to cause constriction in all vascular beds with the possible exception of the coronary vascular bed (Gregg and Fisher 1963). In addition to the post-synaptic a-receptors there exist a-receptors in the terminal varicosities of adrenergic neurons which are

stimulated by the released transmitter and this stimulation results in a decrease in the further release of transmitter (Farnebo and Hamburger 1971; Kirpekar and Puig 1971; Langer, Adler, Energo and Stefano 1971). These receptors presumably provide a mechanism for the negative feedback control of noradrenergic transmission (Westfall 1977).

Modulation of the discharge of the vasoconstrictor nerve fibres achieves central and reflex control of the vascular bed in most instances.

Adjustment of regional flow by sympathetic constrictor nerves depends on several factors such as density of regional fibre distribution, differences in effector cell sensitivity and variations in vasoconstrictor discharge frequency (Folköw and Neil 1971).

There exist quantitative differences in the density of adrenergic fibres distribution both between regions and between consecutive sections of a particular vascular bed.

Adrenergic nerve endings have been identified in all the consecutive sections of a vascular bed with the exception of the true capillaries (Ehinger, Falck and Sporrong 1966). Precapillaries show in general a rich innervation. Arterial vessels are characterised by a plexus of nerve fibres located on the outside of the media and only few branches penetrate into this layer and then only for short distances. On the post-capillary side, the venular segments seem to have a sparser nerve supply than larger veins which in turn have fewer constrictor fibres than the precapillary vessels (Falck 1962).

Three major effects are produced by variations of vasomotor nerve supply (Folköw and Neil 1971). First there is the adjustment of total regional flow resistance. Second there is the adjustment of the pre/post-capillary resistance ratio which secures a mobilisation of fluid when required. Third there is the adjustment of venous capacitance so as to secure appropriate priming of the cardiac pump by the venous return.

Variations in vasoconstrictor discharge frequency may be achieved either by reactions involving the higher centres such as the hypothalamus (Folköw 1960) or by reflex influences. It is worth mentioning that the range of discharge frequency in tonically active vasoconstrictor fibres varies from less of 1 impulse to 8 impulses/ sec (Folköw 1955).

2.2: Vasodilator fibre systems

(a) The sympathetic cholinergic fibre system

The existence of a vasodilator fibre system in the sympathetic nerves to the hind limbs of the cat and dog was demonstrated by Bülbring and Brown (1935) and Folköw and Ünvas (1948). Blockade of the dilator response by atropine gave evidence for the cholinergic nature of the system (Folköw, Hager and Ünvas 1948). The existing evidence suggests that these fibres are distributed only to the vessels of the skeletal muscle (Mellander *et al* 1968; Ross 1971). A central representation of the sympathetic cholinergic vasodilator fibre system was demonstrated in the hypothalamus (Eliasson *et al* 1951) and the sites of the brain structures from which active vasodilatation in skeletal muscle can be elicited have later been mapped out in greater detail (Abrahams, Hilton and Zbrozyna 1960; Hilton and Zbrozyna 1963; Lindgren 1955).

As to the functional significance of the sympathetic cholinergic vasodilator system it is generally agreed that these fibres are not involved in cardiovascular homeostatic reflexes of chemoreceptor or baroreceptor origin (Ünvas 1960). Existing evidence suggests that activation of the cholinergic dilator fibres in animals is part of the autonomic adjustments associated with the alerting response and the defense reaction (Abrahams *et al* 1960, 1964; Unvas 1966). A large increase in skeletal muscle blood flow has been demonstrated in people subjected to emotional stress (Barcroft, Brod, Hejl, Hirsjarvi and Kitchin 1960) but conclusive evidence as to the role or even the existence of sympathetic cholinergic vasodilator fibres in man is not yet available (Ross 1971).

(b) The parasympathetic cholinergic fibre system

Activation of autonomic fibres distributed by way of the cranial nerves produces vasodilatation in areas such as the genital area, the tongue and the salivary glands (Folköw 1955; Ünvas 1960). Hilton and Lewis (1956) suggested that such vasodilatation may be caused through the release of a vasodilator substance, namely bradykinin.

(c) Other vasodilator fibre systems

The neurogenic vasodilatation elicited during body heating is discussed in detail in the section concerned with the control of cutaneous circulation (page 45).

Finally, antidromic stimulation of afferent C-fibres causes a pronounced and sustained vasodilator response preferentially in the cutaneous vascular bed (Celander and Folköw 1953). It is now

agreed that dorsal root vasodilator fibres are not reflexly activated from the central nervous system but that the afferent C-fibres may participate in local vascular effects (Mellander *et al* 1968).

3. Humoral control

In addition to the nervous control, humoral mechanisms are also important in the vascular control. These perform slower adjustments and are therefore concerned with long term regulation (Guyton 1977). This control involves:

(a) Metabolic and non-metabolic autoregulation of local blood flow.
(b) The renin-angiotensin system.
(c) The antidiuretic hormone.
(d) Other substances involved in the humoral regulation of blood flow such as prostaglandins, kinins and histamine.

In the following section the involvement of these substances (prostaglandins, kinins and histamine) is discussed in detail as more relevant to the present study.

3.1: Prostaglandins

Prostaglandins (PGs) have been recently shown to modulate neurotransmitter release at sympathetic postgaglionic nerve endings (Hedqvist 1977). This is also one of the ways of regulating vascular reactivity since all the blood vessels of the systemic circulation are autonomically and mainly sympathetically innervated. In addition prostaglandins may exert direct effects upon the blood vessels themselves. The pharmacological effects of prostaglandins on several vascular beds, are well recognised and they appear to be

dependent upon species, dose, and the individual vascular bed studied (Messina, Weiner and Kaley 1976).

Most of the blood vessels respond to the prostaglandins of the E-series with vasodilator responses (Stovall and Jackson 1967) and the basis for their vasodepressor activity appears to be a widespread vasodilatation of the principal circulatory beds. Analysis of the cardiovascular actions of prostaglandins of the F-series has been complicated by qualitatively different effects of these substances in different species. It has been concluded however by several workers that the pressor activity of prostaglandin $F_{2\alpha}$ is due to cardiac stimulation and increased total peripheral resistance (Nakano and Cole 1969; Emerson, Jelks, Daugherty and Hodgman 1971).

Aside from their cardiac and peripheral vascular effects, prostaglandins of the F-series also exert central effects that may be an integral part of their overall cardiovascular action (Horton and Main 1967).

Prostaglandins also exert their cardiovascular effects by interacting with other vasoactive substances such as catecholamines, angiotensin and vasopressin (Bergström *et al* 1965; Holmes, Horton and Main 1963), and bradykinin (McGiff, Terragno and Lonigro 1972; Messina, Weiner and Kaley 1975; Ferreira, Moncada and Vane 1973; Needleman, Marshall and Sobel 1975).

Until recently it was thought the only members of the prostaglandin series with substantial biological activity were PGE_2 and $PGF_{2\alpha}$. However, since 1973 there have been important discoveries on the nature of the products of arachidonic acid

(Moncada and Vane 1978b). Such products include thromboxane A_2 and prostacyclin, substances with potent biological activities. Thromboxane A_2 is a very potent vasoconstrictor and plays a major role in platelet agreggation, whereas prostacylin is a potent vasodilator and inhibits platelet agreggation (Moncada and Vane 1978a). These two substances are believed to play an important role in thrombosis and haemostosis. In addition, prostacyclin has been suggested to be a circulating hormone possibly involved in vascular autogregulation (Moncada and Vane 1978b).

In conclusion prostaglandins, because of their ability to affect vascular smooth muscle directly, their capacity to modulate vasopressor responsiveness and their participation in the mediation of diverse vasodilator reactions, may be considered as playing a fundamental role in the regulation of blood flow.

3.2: Kinins

Kinins are released from all mammalian tissues (Erdös 1970). The kinins belong to the class of 'tissue or local hormones', a group of active materials which are locally released in the body not having specialised glands of secretion and being rapdily inactivated at the site of release (Rocha e Silva 1970). They include linear polypeptides such as bradykinin, the first kinin to be isolated in pure form and synthesised. The pharmacological effects of bradykinin are well established. When bradykinin is injected intra-arterially in humans, it produces marked vasodilatation in the arms (Fox, Goldsmith, Kidd and Lewis 1960, 1961). Intravenous infusion (Fox *et al* 1960) produced symptoms of flushing in the face, a short-lived choking sensation, an awareness of cardiac

action, mild sensations of hot and cold over most of the body surface, pulsation of the lips and a metallic taste in the mouth.

As for the haemodynamic effects of bradykinin, it is assumed that an increased cardiac output combined with a large fall in total systemic resistance as consequence of peripheral vasodilation results in increase in flow in most visceral segments with fall in systemic blood pressure, increased volume of the capillary bed and a possible constriction of smaller veins (Rocha e Silva 1970). The possible involvement of kinins in pathological conditions as well as their interactions with prostaglandins have already been discussed in earlier sections of the introduction. The fact that kinins can be formed anywhere in the circulatory system together with the fact that they exert powerful effects on the circulation might suggest a role in regulation of circulatory function. However this role is not yet elucidated.

3.3: Histamine

Histamine has been implicated in reflex vasodilatation, vasodilatation after excercise, the dilator phase of spontaneous vasomotion and the gradual opening of capillary beds observed in slowly developing inflammation and in stress states (Schayer 1965). Support for its possible involvement is provided by the fact that it is synthesised continuously within cells of the blood vessels, it acts primarily on intrinsic vasodilator receptors and its rate of synthesis can be adjusted to suit the environment through an adaptive mechanism (Schayer 1965).

Histamine receptors both H_1 and H_2 are found in the cardiovascular system (Johnston and Owen 1977) and their involvement in the cardiovascular response is now well documented.

The direct effect of histamine on the small vessels is a vasodilatation which is followed by an increase in the vascular permeability (Rocha e Silva 1966). The action of histamine upon the isolated mammalian heart is also well documented and may involve stimulation or inhibition, depending upon the species examined (Reite 1972). Since in some species these effects are strikingly similar to those of sympathomimetic amines there is a possibility that the response of the heart to histamine is either wholly or in part, secondary to a release of adrenaline or noradrenaline (v. Euler 1966).

Although the pharmacological actions of histamine are well established its physiological role is less clear. Histamine has been shown to be released into the circulation during the sudden withdrawal of sympathetic tone (Heitz and Brody 1975) and following direct stimulation of the sympathetic nerves and spinal ventral roots (Tuttle and McCleary 1970; Lioy and White 1973). These observations as well as the observations of McGrath and Shepherd (1976) suggest the possibility that histamine has a regulatory role in adrenergic neurotransmission and hence in that part of the circulatory apparatus which is innervated adrenergically. McGrath *et al* (1976) have suggested that this effect of histamine might be due to an inhibition of calcium influx which is necessary for the nerve depolarisation to occur, or to a change in membrane resting potential. Furthermore the possibility has been considered that the sympathetic nerves supply histaminergic post-ganglionic fibres to the blood vessels but the reports on the subject have been highly controversial (Campbell 1970).

The release of histamine during prolonged vasodilatation has been suggested by Morganroth, Young and Sparks (1977) but further data are needed to confirm or refute the hypothesis.

Experiments performed by Johnston and Owen (1977) using histamine H_1 and H_2 antagonists have led to the suggestion that histamine is not directly involved in the control of resting peripheral circulation. However, they did not exclude an involvement of histamine in circulatory regulation when released in abnormally large amounts as occurs in several pathological states such as endotoxaemia anaphylaxis and poisoning by animal venoms (McGrath *et al* 1976).

4. The cutaneous circulation

In the present section the cutaneous vascular patterns and the control and function of the cutaneous circulation are described.

4.1: Vascular patterns

There is a general pattern and structure of the vessels of the skin which is found in most skin areas. Such a pattern is described as follows (Ryan 1973):

An artery about 100μ in diameter enters the lower dermis. It has a lining of flat endothelium cells which lie on a relatively thick elastic membrane - the internal elastic lamina. The latter is composed of longitudinal and spiral fibres, which are more closely packed on the internal surface, than in veins. Some of the fibres penetrate into the media and some smooth muscle cells are anchored to them, others merely separated by them.

The smooth muscle fibres are arranged in a fine collagen or reticular network which is continuous with an outer coat composed

of an external elastic lamina and collagen fibres but this is not well developed in the small arteries of the lower dermis. The arteries divide once or twice as they pass through the lower dermis and their branches run vertically or obliquely before reaching the mid dermis where they divide again. In the mid dermis they are about 50μ in diameter and have only a single layer of smooth muscle cells. The smooth muscle cell layer becomes discontinuous and spiral and the vessels are now arterioles. The internal elastic lamina is no longer a feature, and few elastic fibres are detectable in the network supplying the muscle layers. The arterioles divide further and when they reach the upper dermis their calibre is reduced to approximately 15μ when they finally become capillaries.

Further branching of the vessels gives rise to a network of horizontal capillaries lying parallel to the surface. The terminal capillaries are vertical loops that drain into the horizontal venous plesus.

The venules in the upper and middle dermis are more numerous than the arterioles. The diameter of the venules ranges from 40 to 60μ in the upper and mid dermis to 100 - 400μ in the deeper tissues. Veins are thinner walled than arteries and their layers are less well defined. The endothelial lining is richer in cells, many of which show more metabolic activity. There is a gradual increase in elastic fibres and medium sized veins may have an ill-defined elastic lamina.

As a rule, blood flows from arteries and arterioles, through capillaries to venules and veins. The skin, however, has certain mechanisms that enable blood to pass directly from arteries to veins

through structures called the arteriovenous anastomioses (AVA) or shunts. AVAs originate as branches of arteries or arterioles and connect directly with the accompanying vein or venule. At the point of their origin, AVAs have a typical arterial wall and at the efferent end they resemble veins. AVAs are rich in contractile tissue and in nerves of mainly sympathetic origin although nerve fibres of parasympathetic origin have also been found to innervate these structures (Montagna and Parakkal 1974). Acerylcholine and histamine have been found to dilate AVAs whereas noradrenaline and adrenaline to constrict them. AVAs main function is to serve thermoregulation. In addition, however, they are suggested to act as pressure regulators (Burnton 1961) thus attributing an additional function to the skin which is not usually mentioned, that of 'pressure regulatory' system.

4.2: The control and function of the cutaneous circulation

The circulation through the skin is characterised by its enormous variability and the extent to which this is under control by the central nervous system. The cutaneous circulation is adapted to the roles of nutrition and heat regulation by special structures in addition to the usual nutritive arteries and veins. There exists large subcutaneous venous plexus that can cause a considerable amount of blood to flow within the dermis at a short distance from the ambient atmosphere (see page 43). In addition there are arteriovenous anastomoses that open up as quite large vascular connections between the arteries and the venous plexus. When dilated, they flood the venous plexuses, allowing a rapid flow of warm blood into this heat exchanger. Arteriovenous anastomoses are

found in the hands, feet, lips, nose and ears. Finally, the skin contains a large number of eccrine sweat glands in addition to the above heat-dissipation technique.

The nerves innervating the eccrine sweat glands are postgonglionic sympathetic cholinergic fibres (Dale and Feldberg 1934). Stimulation of the sweat glands not only leads to the production of fluid at the surface, but it also initiates a sequence of events leading to further vasodilatation of the skin blood vessels (Mellander *et al* 1968). The most important control of the skin circulation is through the thermosensitive cells in the preoptic regions of the hypothalamus which are delicately adjusted to respond to the temperature of the blood (Ström 1960). Information coming from the receptors in the skin is integrated with that of central neurons. The output of these neurons stimulates the sympathetic vasoconstrictor fibres running to the vessels of the skin.

In addition to the sympathetic vasoconstrictor fibre system the blood vessels of the skin are believed to be supplied by a sympathetic vasodilator fibre system (Mellander *et al* 1968; Burnstock 1977). The neurogenic vasodilatation elicited in the human arm during body heating roughly corresponds to that obtained by regional nerve blockade, which indicated that the thermoregulatory response in this vascular bed accounted for by inhibition of sympathetic constrictor fibres (Roddie, Shepherd and Whelan 1957a). However, in other skin areas such as the forearm and calf, the blood flow increase produced by body heating greatly exceeded that occurring after nerve blockade (Roddie, Shepherd and Whelan 1957). Grant and Holling (1938) observed that local anaesthesia of

cutaneous nerves prevented the flushing and rise in temperature in the forearm which normally follows body heating. These observations appeared to offer good evidence that cutaneous nerves contain fibres which when stimulated by impulses from a heat sensitive centre cause dilatation of vessels in the forearm. This vasodilatation would not be due to a mere inhibition of the normal vasoconstrictor impulses, because these should be blocked by the anaesthesia, so that vasodilatation would then occur passively. Edholm, Fox *et al* (1956) using adrenaline iontophoresis prevented the normal increase in forearm flow as measured by venous plethysmography. Thus the response appears to be due to dilatation of the blood vessels of the skin rather than to vasodilatation of deeper vessels. This was confirmed by Barcroft, Bock *et al* (1956) who used a thermocouple located in the muscle. In addition, Roddie *et al* (1956) measured the oxygen saturation of venous blood in veins draining the skin and compared it with that from veins draining muscle in subjects whose bodies had been heated. These workers found that only the skin vessels had an increased oxygen content which supported the hypothesis that the observed vasodilatation was confined to the cutaneous vasodilatation.

The pronounced vasodilatation in the areas of forearm and calf was found to be closely related in time to the activation of the sweat glands (Edholm, Fox *et al* 1957) although the two events can be dissociated by atropinisation which abolished the sweating response but merely delayed the increase in blood flow.

Several substances have been suggested as mediators of this vasodilatation including histamine (Brody 1966; Ryan and Brody 1972), bradykinin (Fox and Hilton 1958), prostaglandins (Bergström, Carlson,

Ekelund and Öro 1965), dopamine (Bell and Lang 1976) and angiotensin (Godfraind 1970). Adenine nucleotides and nucleosides have also been shown to be potent vasodilators (Burnstock 1972), so the possibility that purinergic nerves might be involved cannot be excluded. However, no conclusive evidence is yet available concerning the exact nature of the mediator of this vasodilatation of sympathetic origin.

5: OESTROGENS, THE CARDIOVASCULAR SYSTEM AND THE AUTONOMIC NERVOUS SYSTEM

In this section the interactions between oestrogens and the cardiovascular system and its innervation are discussed.

As early as 1940, Reynolds (Reynolds 1941) in an attempt to elucidate the mechanism by which oestrogen relieves the hot flushes of the climacteric examined the peripheral vascular effect of oestrogen in the rabbit, in the human male, and in the human menopausal female. It was observed that in the rabbit ear the effect of oestrogen was limited to the smallest vessels lying beyond the arterioles, namely the capillaries and venules and possibly the arteriovenous anastomoses. It was characterised by dilatation of the vessels within the first 3 to 20 minutes. In the human male, local oestrogen injection caused an increase in finger volume which was demonstrated by plethysmography. This increase persisted for approximately two hours but both nail bed temperature and the temperature of the plethysmograph remained unaltered. From this observation it was concluded that the volume change is effected without a significant alteration in the rate of blood flow, and that vasodilatation occurred beyond the smallest arterioles (Reynolds 1941). In menopausal women two types of dermovascular responses were observed. One, similar to that seen in the male, consisted of a slow rise in the blood flow to a sustained level. The second type of response to oestrogen, which appeared to occur less frequently was characterised by a slow increase in finger blood volume to a maximum, followed by partial subsidence to a plateau which was maintained throughout the two-hour period of observation. This type of response was shown to be

similar to the response occurring during a true spontaneous hot flush and it was observed either when the subject was in a highly nervous state or when some exciting or disturbing factors were present. When it occurred the patient experienced sensations similar to those experienced during a hot flush. Finally, Reynolds (1941) concluded that although both the menopausal hot flush and the 'dermovascular' responses involve dilatation, in the menopausal hot flush this effect is partly due to arteriolardilation where as in the oestrogen-response it is the smallest vessels beyond the arterioles that mainly are concerned. The former involves 'intermediation' of nervous activity (inhibition of arteriolar tone), the latter appears to be a direct effect of the hormone on tissues in or about the blood vessels.

More recently, Tacchi (1960) observed that both local and intravenous administration of oestrone or synthetic oestrogens induced vasodilatation of the conjuctival vascular bed. Schiff and Brown (1961) suggested a mechanism whereby oestrogen could increase capillary strength. Clemetson and colleagues (Clemetson, Blair and Reed 1962) investigated this aspect further in a group of post-menopausal females and showed that capillary strength was increased when ethinyl-oestradiol or conjugated equine oestrogens were given orally or intravenously. Lloyd and Pickford in the late fifties, early sixties, studied extensively the effect of oestrogens on the vascular responsiveness to various stimuli. Lloyd (1959 a,b) showed that the vascular responses of the rat to oxytocin and vasopressin varied with the concentration of ovarian hormones in the body and also during pregnancy. In fact oxytocin was dilator

in the dioestrous rat, though without effect on the blood pressure, while in the oestrous animals, during late pregnancy or after ovarian hormone administration, oxytocin was pressor and constrictor. Lloyd and Pickford (1961) investigated this further by looking at the effect of other dilator substances on the responses to oxytocin and vasopressin in the rat and they also attempted to determine if any part was played by the central and peripheral nervous system. It was shown that none of the vasodilator substances tested altered the responses to oxytocin or vasopressin. Pithing or decerebration as well as ganglionic or peripheral sympathetic blockade, but not atropine, altered the responses to oxytocin and caused an increased sensitivity to the pressor action of vasopressin, in a similar manner to oestrogen administration. The possibility was therefore considered that oestrogens might depress sympathetic nervous activity or block the hypothetical central dilator action of oxytocin thus unmasking a peripheral constrictor effect. Similar results were obtained in the dog in observations on the hind limb blood flow which confirmed the hypothesis that oestrogens might affect sympathetic impulses either by reducing their outflow or their reception (Lloyd and Pickford 1962). The same workers (Lloyd and Pickford 1963) showed, using dogs and rats with abnormal sexual development, that gonadal hormones are important for at least some vascular reactions including those to oxytocin and vasopressin. In an attempt to elucidate mechanism of the identical change in vascular reactivity by procedures as diverse as oestrogen administration and interference with the sympathetic nervous system Haigh and co-workers (Haigh, Lloyd and Pickford 1965) suggested

that adrenaline is involved in the mode of action of oxytocin and oestrogen on vascular smooth muscle. It was proposed that oestrogens interfere with the manufacture of adrenaline in, or its release from the sympathetic nerves, in addition to increasing the acetylcholine-like content of organs, as previously shown by Reynolds (1939) and Reynolds and Foster (1940). Adrenaline derived from the adrenals did not appear to be involved in the mode of action of oxytocin and oestrogens on the smooth muscle. Only that produced locally in the periphery and presumably in the nerve ending was involved and it was suggested that sympathetic nerves may be responsible for some activity in addition to impulse transmission (Lloyd and Pickford 1967).

More recently, Rosenfeld and co-workers (Rosenfeld, Morris, Battaglia, Makowski and Meschia 1976) investigated the effect of oestrogen on regional blood flow in non-anaesthetised unstressed animals and on the contribution of changes in cardiac output. It was shown that only the skin and myocardium demonstrated a significant change in blood flow following 17β-oestradiol administration. Blood flow to the kidneys, adrenals, spleen, brain, pancreas and skeletal muscle did not appear to be affected. In the same study, infusion of 17β-oestradiol caused a significant increase in the cardial output of the animals. On the other hand, Altura (1972) demonstrated that intact mesenteric arterioles of female rats are much more sensitive to the constrictor actions of catecholamines than are arterioles of male rats. This sex difference appeared to be specific for catecholamine molecules exhibiting not only a catechol nucleus but a hydroxyl group on the β-carbon on the side

chain. Furthermore, it was demonstrated that by treatment of male rats with a single administration of an oestrogen their vascular responsiveness became very much similar to that of the female rats (Altura 1975). It was concluded that circulating levels of oestrogen might play an important role in potentiating responses to other autonomic stimuli. Furthermore, it seems probable that oestrogens may inhibit extra neuronal uptake of noradrenaline in the vascular smooth muscle and this might account for their potentiation of the actions of exogenous noradrenaline in such tissues (Kalsner 1969).

Oestrogens have been reported to affect the cardiac muscle as well as the vascular smooth muscle. Iversen and Salt (1970) showed that steroid hormones including 17β-oestradiol inhibited the extraneuronal uptake mechanism with potencies comparable with those of the most active inhibitors of extraneuronal uptake. Furthermore, oestrogens have been shown to have a glycosidic effect in that they may alter the actinomyosin-ATP mechanism of the myocardium, which might result in increased efficiency of myocardioel contractility (Walter and Lim 1969).

METHODS

1: STUDY OF THE MENOPAUSAL HOT FLUSH

1. Subjects

A total of 11 post-menopausal women and six pre-menopausal women were studied at the laboratories at the University of Aston in Birmingham. Both groups were volunteers. The post-menopausal women were patients who had been referred to the Birmingham Menopause Clinic. They were between 45 - 50 years of age and suffered from severe and frequent incidents of flushing.

2. Experimental protocol

All the women, post- and pre-menopausal were studied intensively during approximately two hours. They were seated in a room with air temperature maintained at approximately 21°C by a thermostatic heater. Each woman was allowed to rest for 10 minutes and so to become familiar with environment and the people involved in the study. Then the antecubital vein was cannulated with an indwelling cannula (butterfly type). They were then allowed to relax (for 30 minutes) and get used to the cannula. Subsequent to that the study began.

The study involved taking blood samples at rest and during flushing in addition to recording several physiological variables.

During the study all women remained quiet and any unnecessary movement was avoided since it appeared to interfere with the recordings. Care was taken that the women could not see the recordings. Each procedure was carried out by the same person throughout the study so that the women might feel at ease.

Each subject was asked to indicate the onset of a flush and its termination and this subjective sensation was recorded.

Since it was not always possible to obtain sufficient spontaneous flushes for the study, a standard method was devised to induce a hot flush. This involved the administration of a hot drink together with slight warming-through application of blankets. In most of the post-menopausal subjects this procedure resulted in a hot flush.

The pre-menopausal women were between 20 - 25 years of age and had not been taking any oestrogen treatment for at least three months prior to the study.

The study of these women involved taking blood samples at rest and during heat induced vasodilatation, in addition to recording the same physiological variables as in the post-menopausal women.

The pre-menopausal women were subjected to the same procedures as the post-menopausal ones, only the degree of warming was greater since the desired effect was a vasodilatation comparable with a hot flush. In order to induce this vasodilatation the room was heated to 26°C after the initial 15 minutes of basal recordings. In addition, a hot drink, blankets and a hot water bottle were used to provoke the desired degree of vasodilatation.

All recordings were made simultaneously so that time relationships between them might be studied. Continuous recordings were made with the exception of blood pressure measurements which were intermittent. The frequency of blood pressure measurements is discussed in the appropriate section (page 56).

3. Physiological measurements

All women were subjected to the following procedures:

Electrocardiography

Recordings of
- heart rate
- skin temperature
- blood pressure
- digital blood volume pulse
- galvanic skin resistance

3.1: Electrocardiography

A single lead (AVR) electrocardiogram (ECG) was recorded on a DEVICES M4 recorder with an isolated input ECG channel. The plates were placed on the inner surface of the wrists.

(a) Heart rate

Heart rate was monitored with a DEVICES instantaneous rate meter (type 2751) which was triggered by the signal from the photoplethysmograph (see page 57) and recorded on a DEVICES M4 recorder. The recorder was calibrated to cover a range from 0 - 250 beats/minute. Although there was some individual variations in the resting heart rate of the subjects, this variation was limited to 7.7% ($n = 13$) which indicated that the results recorded with the present technique were fairly reliable.

(b) Skin temperature

The skin temperature of the palm of the hand, forearm and forehead were recorded throughout the experiment by bead thermistors placed in the respective areas. The temperatures were displayed on a galvanometer made by Light Laboratories. There was a variation

in the resting skin temperatures of the subjects (see Appendix) but this variation was not due to inaccuracy of the recording apparatus but due to genuine individual variation as this was confirmed by concomittant recordings of resting blood flow (see page 58).

(c) Blood pressure

Blood pressure was recorded by alternative mercury sphygmonanometry at the brachial artery at 30-minute intervals and also during a hot flush. However, because the blood pressure recording was taken after the blood samples were taken there was always a time-lag between this measurement and the onset of the hot flush.

(d) Digital blood volume pulse

Changes in the blood volume of the index finger were measured by plethysmography. Plethysmography is a means of recording changes in volume per unit time (Barcroft and Swan 1953).

In the present study, photoplethysmography was used. This type of plethysmography also depends on the volume of blood entering the tissues under observation and is based on the principle that light is attenuated when it is shone through the skin (Ryan 1973). This attenuation is due to reflection, scattering, or absorption of light. Red light is predominantly absorbed by oxygenated blood. Body tissues are transparent to red light and red light is transmitted through the full thickness of the skin, but there is a big difference between the transparency of tissue and blood. Blood absorbs almost ten times more red light than the tissues and the

difference between the amount of light transmitted through skin in which the vessels are dilated and skin in which the vessels are constricted depends on the greater absorption due to the greater volume of blood in the vasodilated skin. This method was discovered by Hentzmann (1939) and has been extensively modified during the past thirty years (Ramsay 1972). It has the advantage that it can be applied at any site unlike occlusion plethysmography which is confined to limb circulation studies.

In an area such as the finger, records may be made by measuring light passing through the skin or it may be measured as it is reflected back to the surface. In the present study the latter technique was applied. Special care was taken to avoid pressure on the skin as this would interfere with the blood flow and might give misleading results.

Photoplethysmography measures the expansion of the skin vessels which is a summation effect of the arterial pulse and the opposing elastic properties of the vessel wall. The results obtained are records of changes in blood volume, their relative size compared with a previous reading. Therefore they do not provide information concerning absolute amounts of blood flowing in the particular skin area where measurements are made (Ryan 1973).

The photoplethysmographic transducer used in the present study converts blood volume changes into electrical signals and consists of a light source which is an infra-red radiator. The transducer was applied on the index finger and the signal output was recorded by a DEVICES M4 recorder.

The recorder was calibrated for each individual experiment until a signal from the plethysmograph could be picked up by the recorder. Changes in the blood volume were assessed as percentage increases in the excursion of the recorder pen, the excursion of the recorder pen at rest taken as 100 per cent.

The only way of assessing the accuracy of the photoplethysmograph was by relating it to the concomitant measurements of skin temperature, and the reciprocal was true as previously mentioned (page 56). For instance, in a subject with low resting skin temperature a small excursion of the recorder pen connected to the plethysmograph was observed, or higher sensitivity of the recorder was needed to display a plethysmographic signal in the first place.

Nature of the measurements

The blood volume pulse consists of a series of pulsatile changes produced by the passage of the pulse wave through the monitored area (Brown 1972). The tracing of the blood volume pulse resembles that of blood pressure waves obtained by cannulation and exhibits systolic and diastolic peaks, dicrotic notching etc. It cannot be interpreted in the same way, however, since the data obtained by this technique is only relative.

(e) Galvanic skin resistance

If two electrodes are placed on the skin surface and a small current is driven through them, the skin behaves as a resistor. A voltage develops across the electrodes and by application of Ohm's law one can calculate the apparent resistance which is known as the skin resistance level (SRL) (Edelberg 1972). Stimuli such as a

sudden noise or a question asked of the subject may, to varying degrees, be followed by a rapid fall in skin resistance. This response is known as galvanic skin resistance response (SRR) or electrodermal reflex.

Several models have been proposed in order to elucidate the way by which the changes in the skin resistance are related to physiological processes. In most of these studies the electrodermal activity has been correlated with either the secretory and pre-secretory activity of the sweat glands exclusively (Adams 1966; Martin and Venables 1967) or with the activity of both sweat glands and that of other skin structures such as the epidermis and corneum (Darrow and Gullickson 1970).

Edelberg (1972), after careful consideration of the models proposed by the previous workers, suggested the following model as giving an adequate explanation for the mechanism of the skin resistance response in man:

The sweat gland may be considered as a relatively steady source of sweat production and its lumen as having a substantial negative potential with respect to the surrounding tissue. In response to external stimulation, this tonic level may increase somewhat or phasic neural discharges above the tonic level may occur. For the purposes of that model it was assumed that these secretory events were accompanied by any resistance changes. When the glands are inactive, sweat is presumed to normally fill the ducts up to the level of the Malpighian layer. When endomotor activity commences the sweat rises up the coiled portion of the duct, reducing the resistance between the surface and the negative

electrical generator. However, in an actively sweating subject the ducts would be full most of the time and no resistance changes would be produced by this mechanism. Nevertheless an actively sweating person does produce responses and these responses must be attributed to either epidermal activity or activity of the wall at the level of the Malpighian layer. Which of the two levels is responsible is not clear yet. However, there is additional evidence for the involvement of an epidermal structure in SRR afforded by the demonstration that SRR can be elicited by the centre of the nail bed where no sweat glands occur.

Both the sympathetic and parasympathetic divisions of the autonomic nervous system have been implicated as mediators of the skin reflex. Now, however, it is generally accepted that the control is in fact sympathetic but with many parasympathetic characteristics, especially the involvement of acetylcholine at the neuroeffector junction.

Nature of the measurements

In the present study the skin resistance level (SRL) and skin resistance response (SRR) of the hand were measured in both post-menopausal and pre-menopausal subjects. The palmar SRL of normal subjects ranges from a few $K\Omega$ to several hundred $K\Omega$ (Venables and Martin 1967). This observation was confirmed in the present study (see Appendix). SRRs occur as a sudden drop in resistance and they range again from a few $K\Omega$ to several hundred $K\Omega$. The hand SRL and SRR were measured by placing electrodes on the palmar and dorsal area of the hand. A small current of the order of 6 μA was passed through the electrodes and the resistance signal was displayed on a galvanometer.

The apparatus for the measurement of SRL and SRR was designed and built in the Department of Pharmacy at the University of Aston in Birmingham. It consisted of a constant current source (6 μA) that provided the current needed for the skin resistance to be elicited. The electrodes were linked to a galvanometer where skin resistance changes were displayed as resistance units (K^{Ω}). The measurements were recorded every five minutes at rest and every minute during a hot flush.

3.2: Thermography

The thermographic study was carried out at the Birmingham Menopause clinic by Dr. D.W. Sturdee. Four post-menopausal women who had had sufficiently frequent hot flushes took part in the study. Each woman was seated in a chair and was naked to the waist. Room temperature was maintained between 18 - 20°C and 15 minutes were allowed for the body surface temperature to stabilise and reach equilibrium with the room temperature before commencing the study. Thermogram pictures were taken with an AGA Thermovision I Model 680 Medical Camera System, which had a colour isotherm camera attachment taking Polaroid film. Temperature of the individual isotherms were standardised by comparison with a black body reference source set at a constant temperature. Thermograms were taken at five-minute intervals to provide a film base line in anticipation of a hot flush and more frequently when a hot flush was experienced.

4. Pharmacological study

4.1: Blood collection

The blood was withdrawn, using ice-cold plastic syringes, from the antecubital vein by means of an indwelling cannula and was squirted into ice-cold tubes containing anticoagulant. When kinin-like activity was tested the kinase inhibitor SQ 20881 (Erdos 1976) was also added at a concentration of 10^{-7}M. Samples were then centrifuged at 2000 g and 4°C for 20 minutes and the plasma collected and stored at -18°C. The plasma samples were thawed immediately prior to use.

When whole blood activity was investigated a separate collection procedure was followed (see pages 63,64).

The pharmacological investigations will be considered in two sections:

(a) Plasma screening

The following preparations were used to investigate the activity of plasma on smooth muscle.

- The rat isolated fundic strip to investigate any possible 5-hydroxytryptaminergic, cholinergic or histaminergic activity.
- The rat isolated ascending colon to investigate any catecholaminergic activity.
- The guinea pig isolated ileum to investigate histaminergic activity.
- The pithed rat preparation to investigate any pressor activity.

(b) Blood assays

Whole blood was assayed for:

- Prostaglandin-like activity.
- Kinin-like activity.

Prostaglandin-like activity assay

The extraction procedure of Greaves and McDonald Gibson (1972) was used and the protocol may be summarised as follows:

1. 10 ml of whole blood was drawn into ice-cold syringes with no anticoagulant.
2. The blood was haemolysed by addition of 10 ml distilled water and was maintained on ice.
3. Absolute ethanol was added to produce a final concentration of 85 per cent.
4. The resulting solution was allowed to stand overnight at $4^{o}C$ and then centrifuged at 3000 g for 15 minutes. The precipitate was washed with absolute ethanol and the supernatants were combined and evaporated to dryness *in vacuo* using a rotary evaporator.
5. The dry residue was redissolved in ethanol-water (2 : 1 v/v) and washed three times with equal volumes of petroleum ether.
6. The ethanol was removed from the aqueous phase *in vacuo* using a rotary evaporator and the latter was acidified to pH 3 with 1N hydrochloric acid.
7. The aqueous phase was extracted three times with equal volumes of diethyl ether.
8. The non-aqueous phases were combined and evaporated to dryness.

The dry extracts were subsequently stored at -18°C and redissolved into warm Tyrode solution immediately prior to assaying on the rat isolated fundic strip, against prostaglandin E_1 in randomised 2 x 2 assay.

Recovery was determined by addition of 50 ng prostaglandin E_1 to 5 ml of blood and the extraction was carried out as above. The calculated recovery was 75%.

The prostaglandin-like activity was checked by blocking the preparation with SC-19220, a prostaglandin receptor blocker (Bennet and Posner 1971).

Kinin-like activity assay

The procedure followed was that used by Mashford and Roberts (1972) for whole blood samples prior to bradykinin radioimmunoassay. The protocol may be summarised as follows:

1. 5 ml of whole blood was drawn into iced cold syringes and squirted into 20 ml of absolute ethanol.
2. The resulting solution was centrifuged at 2500 g at 4°C for 15 minutes and the supernatant collected into round bottomed flasks. The precipitate was resuspended in 20 ml of 75 per cent ethanol, recentrifuged and the supernatants combined.
3. The supernatant was reduced to 2 ml by evaporation at 40°C *in vacuo* using a rotary evaporator.
4. The residual solution was acidified with 5 ml of 0.01 HCl and partitioned twice with 20 ml of diethyl ether.
5. The remaining aqueous phase was evaporated to dryness at 40°C *in vacuo*.

The dry extracts were stored at -18°C and redissolved in warm Tyrode solution immediately prior to a randomised 2 x 2 bio assay on the oestrous rat uterus against standard bradykinin, by the method of de Jalon (1945). Tyrode solution was used instead of de Jalon's solution. To test recovery, 50 ng of bradykinin were added into 5 ml of blood and extraction was carried out as previously described. The calculated recovery was 69%.

To ensure that the activity measured was due to kinins the reconstituted extracts were incubated at 37°C for 30 minutes with chymotrypsin, a proteolytic enzyme, and any remaining activity was detected by bio assay.

2: DETAILS OF PHARMACOLOGICAL METHODS

1. Isolated *in vitro* preparation

In all cases male wistar rats weighing 200 - 250 gm or male albino guinea pigs weighing 300 - 400 gm were killed by cervical dislocation. The appropriate organ was dissected out and unless otherwise stated, suspended in a 30 ml organ bath containing either Tyrode or Krebs' solution at the appropriate temperature, gassed with either carbogen, oxygen or air.

Prior to use all animals were kept in cages containing four animals per cage and were supplied with tap water and a standard 41B pellet diet *ad libitum*.

1.1: Recording methods

In all the preparations with the exception of the pithed rat, the contractions were recorded istonically with DEVICES 2LD01 transducers under the appropriate tension for each preparation and on an M2 DEVICES recorder.

All tissues were equilibrated for an hour before the beginning of the experiment. 10 mm of pen excursion on the chart corresponded to 2mm muscle shortening. Results are expressed as responses in mm of pen excursion and not in mm of actual muscle shortening.

(a) The rat isolated fundic strip

The rat isolated fundic strip was prepared according to the method described by Vane (1957) for the bioassay of 5-hydroxytryptamine.

The fundus was cut along its lesser margin and opened to produce a flap of muscular tissue which was washed in physiological solution to remove any loose filaments and stomach contents. Cuts were made on alternate sides of the flap of fundal tissue and the

tissue was pulled from its upper and lower edges in order to produce a strip of muscular tissue. The fundic strip was cleaned with a pair of scissors and suspended under a 2 g load in an organ bath containing Krebs' solution at 37°C and gassed with carbogen. A dose cycle of 10 minutes was used with 60 - 90 seconds contact time.

(b) The rat isolated ascending colon

The rat isolated ascending colon was prepared as described by Gaddum, Peart and Vogt (1949) for the bioassay of adrenaline and noradrenaline in blood.

The first 3 cm of a rat's ascending colon, which is easily recognised by its diagonal striations on its surface were dissected out, flushed through, and suspended under a 1 g load in an organ bath containing Tyrode solution bubbled with air and maintained at 30°C. The dose cycle was 5 minutes and the contact time 60 seconds.

(c) The guinea pig isolated ileum

The guinea pig isolated ileum was prepared as described by Smith (1961).

After the ileum had been dissected out and flushed through with physiological solution, a 2 - 3 cm segment was suspended under a 1 g load in an organ bath containing Tyrode solution at 32°C and bubbled with air. The dose cycle was 10 minutes with 60 seconds contact time.

(d) The rat isolated vas deferens preparation

The isolated rat *vas deferens* preparation was prepared as described by Birmingham and Wilson (1963).

The abdomen was opened in the midline and the vasa deferentia were dissected out, without the hypogastric nerve. Each vas deferens was subsequently suspended under a 1 g load in an organ bath both containing Krebs' solution maintained at 37°C, in a holder suitable for transmural stimulation. The tissues were gassed with carbogen.

Stimulation was for periods of 15 seconds at five minute intervals and was provided by a square wave stimulator (Scientific Research Instruments Ltd). A frequency of 25 shocks/sec was used at supramaximal voltage (90 - 120 V) and a pulse width varying between 0.1 and 0.5 msec.

2. The pithed rat preparation

2.1: The intact pithed rat preparation

Male wistar rats weighing 200 - 250 gm or female wistar rats weighing 180 - 220 gm were anaesthetised with pentobarbitone (60 mg/Kg IP). The trachea was cannulated and the animal pithed via the right orbit by the method of Shipley and Tilden (1947). A steel pithing rod (1.5 mm in diameter) was used, suitable for the stimulation of the spinal sympathetic outflow (Gillespie and Muir 1967). Immediately after pithing, positive pressure artificial respiration was commenced using a Palmer 'Miniature-ideal' pump set to deliver 4 ml of air at a rate of 60 inflations/min.

The right jugular vein and the carotid artery were cannulated as described by Shipley and Tilden (1947) and the blood pressure measured by means of a pressure transducer (Bell and Howell) connected to a DEVICES M2 recorder.

The heart rate was measured by means of a DEVICES instantaneous rate meter (type 2751) triggered from the blood pressure signal.

The animal was maintained at 37°C by a thermostatically controlled animal operating table (Palmer).

Electrical stimulation of the spinal sympathetic outflow was performed as described by Gillespie *et al* (1967). The indifferent electrode, a hypodermic needle, was inserted subcutaneously into the right hind limb and connected to one pole of a square wave stimulator (Scientific and Research Instruments Ltd). The other pole was connected to the shaft of the pithing rod.

The preparation was injected intravenously with atropine sulphate (1 mg/kg) and (+) - tubocurarine hydrochloride (3 mg/kg), 30 minutes prior to the beginning of the experiment.

Intravenous injection volumes were 1 ml/kg followed by a flush of 0.4 ml/kg of 0.9 per cent W/V saline.

2.2: The ovariectomised and sham ovariectomised pithed rat preparation

Cycling female wistar rats weighing 180 - 220 gm were ovariectomised bilaterally and equal numbers of cycling female rats were sham-ovariectomised bilaterally. These animals were then used for the pithed rat preparation as described previously. The vaginal smear method which was used to determine the various stages of the oestrous cycle, as well as the surgical procedures followed during the ovariectomies and sham-ovariectomies are described below:

(a) The vaginal smear method

The various stages of the oestrous cycle were determined by examination of vaginal smears. The stages of the oestrous cycle are designated as follows:

Dioestrus: The smear mainly consisted of leucocytes.

Proestrus: The smear consisted mainly of epithelial cells.

Oestrus: Large number of squamous epithelial cells were present in the smear.

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

(b) Surgical procedures

All ovariectomies and sham-ovariectomies were carried out under sterile conditions. The animals were anaesthetised using halothane with an oxygen-nitrous oxide gas mixture. All instruments were sterilised by boiling at 100°C for 15 minutes and were then stored in a mixture of 5 per cent Hibitane (ICI Ltd) and 70 per cent methyl alcohol, 2 : 5 V/V. Prior to use the instruments were rinsed in distilled water.

The mid-lateral area was shaved and washed with 0.5 per cent aqueous hibitane and the abdomen opened on each side by two small incisions. The fallopian tubes and associated blood vessels were ligated and the ovary removed. The muscle wall was sutured using silk thread and a curved triangular needle. The skin was then clipped together using surgical clips. The area was subsequently washed with 0.5 per cent aqueous Hibitane and sprayed with 'aeroplast' dressing (Parke-Davis).

The animals were housed individually in clean cages for 72 hours following surgery and thereafter were housed four animals per cage until they were used.

Successful removal of the ovaries was verified by examining the vaginal smears from seven days after the operation until they were used. At least 15 days elapsed between the operation and the day the animals were used.

An equal number of animals were subjected to sham-ovariectomy using the same procedure with the exception that the ovaries were not removed but returned intact to the abdomen.

The sham-operated animals were checked after seven days to determine whether they were cycling, and vaginal smears were examined daily until the animals were used. Only cycling animals were used for the pithed rat preparation.

3: DRUGS AND CHEMICALS

BDH

Acetylcholine chloride
Adrenaline hydrogen tartrate
Atropine sulphate
Histamine acid phosphate
Hydrochloric acid (concentrated)
5-Hydroxytryptamine creatinine sulphate
Hyoscine hydrobromide
Potassium chloride (analar grade)
Diethyl ether (analar grade)
Petroleum spirit 40° - 60°C (analar grade)

SIGMA

L-arterenol bitartrate
Bradykinin triacetate
Chymotrypsin
17β-oestradiol

ICI

[1]Propranolol

CIBA

[1]Phentolamine mesylate

SANDOZ PRODUCTS LTD

[1]Methysergide

MAY & BAKER LTD DAGENHAM

[1]Mepyramine maleate

MERCK SHARP & DOHME

[1]Cyproheptadine hydrochloride

---

[1]compounds donated.

SEARLE

[1]SC-19220

SQIBB

[1]SQ-20881

UPJOHN COMPANY

[1]Prostaglandin E_1

CALMIC MEDICAL DIVISION, THE WELLCOME FOUNDATION LTD

(+) - tubocurarine

JAMES BURROUGH LTD

Absolute ethanol

Composition of physiological solutions

	Tyrode mMol l⁻¹	Krebs mMol l⁻¹
NaCl	137	118
KCl	2.7	4.7
$MgSO_4.7H_2O$	1	1.18
$NaH_2PO_4.2H_2O$	0.42	
KH_2PO_4		1.17
$NaHCO_3$	11.9	0.25
$CaCl_2.2H_2O$	1.8	0.25
Glucose	5.6	11.0

[1]compounds donated.

4: STATISTICAL EVALUATION OF RESULTS

The mean and standard errors for each set of results were calculated from the following formulae:

$$\bar{x} = \frac{\Sigma x}{n}$$

Where $\bar{x}$ is the mean, x is the sample parameter and n the number of observations per sample.

$$s = \sqrt{\frac{\Sigma x^2}{n-1} - \frac{(\Sigma x)^2}{n(n-1)}}$$

Where s is the standard deviation.

$$SE = \frac{s}{\sqrt{n}}$$

Where SE is the standard error.

The means of individual samples were compared by application of Student's 2-tailed t-test for sample means, viz:

$$t = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{(SE_1^2 + SE_2^2)}}$$

The coefficient of variation Vx was calculated from the following formula:

$$Vx = \frac{s}{\bar{x}} \times 100$$

Where s is the standard deviation and $\bar{x}$ is the mean.

The values of probability, p, were obtained from the appropriate tables.

The results were calculated using a Commodore calculator.

RESULTS

A

A STUDY OF THE POST MENOPAUSAL HOT FLUSH ON HUMAN SUBJECTS

1: AN EPIDEMIOLOGICAL SURVEY CARRIED OUT IN 25 POST-MENOPAUSAL WOMEN WHO WERE PATIENTS AT THE MENOPAUSE CLINIC AT THE WOMEN'S HOSPITAL IN BIRMINGHAM

Investigations were made on 25 post-menopausal women who were complaining of severe and frequent hot flushes and who had been referred to the menopause clinic by their General Practitioner. They were given a questionnaire to fill in at the day of their first visit. The questionnaire was composed by Dr. D.W. Sturdee and a copy of this is shown on the next page.

The purpose of this survey was to define any common features among post-menopausal women experiencing hot flushes. It also helped to assess whether there were any similarities between the subjective sensations experienced by these women and the records of the physiological changes which were observed during a hot flush, and are reported in the next section.

1. Results of the survey

1.1: Age and marital status

The average age of the post-menopausal women participating in the survey was 51.5 ± 0.8 years ($n = 23$). Two women gave no information concerning their age. Twenty-two women were married, 1 was single, 1 divorced and 1 did not provide information.

1.2: Type of menopause

Of the 25 women, 16 had experienced a spontaneous menopause, 6 had a menopause after oophorectomy and 2 had been having irregular periods for 8 and 12 months respectively.

questionnaire

NAME: _____ AGE: _____ HOSPITAL NO: _____

MARITAL STATUS _____ PARITY: _____

1. TYPE OF MENOPAUSE: Spontaneous ☐ Hysterectomy ☐ Hyst.+ BSO ☐
   XRT ☐ Reg.menses ☐ Irreg. in last 3 mnths ☐
   Date of last period _____ Time since menopause _____

TO BE COMPLETED BY PATIENT:-

2. Time since hot flushes began _____ Are they now:- Static ☐
   Average number per 24 hours _____ increasing ☐
   Average duration of flush _____ decreasing ☐
   Are the flushes worse during:- day ☐ night ☐ no difference ☐

3. Where does sensation of flush usually begin?
   (e.g. neck, tummy, face etc.) _____
   Where does sensation of flush spread to _____

4. Is sweating associated with flush? Yes ☐ Always ☐ Occasionally ☐ Never ☐
   Does sweating occur without a flush? Never ☐ Occasionally ☐ Frequently ☐
   Where does sweating occur? _____

5. Are there any situations or factors which provoke a flush? (e.g. alcohol, excitement, embarrassment, sex.) _____

6. Do you have any warning of impending flush/sweat? Yes ☐ No ☐
   Describe briefly _____

7. Do you have any symptoms accompanying the flush/sweat? Yes ☐ No ☐
   (e.g. nausea, palpitations, headache etc.)
   Describe briefly _____

8. Do you consider your sensitivity to cold is:-
   Normal ☐
   More than other people ☐
   Less than other people ☐

   Do you consider your sensitivity to heat is:-
   Normal ☐
   More than other people ☐
   Less than other people ☐

   If not normal, has this changed since the menopause? Yes ☐ No ☐

9. Did your mother have similar flushes? Yes ☐ No ☐ Do not know ☐
   Did your sister have similar flushes? Yes ☐ No ☐ Do not know/ not applicable ☐

10. Apart from hormones is there anything else which reduces the frequency or severity of the flushes/sweats? Yes ☐ No ☐
    If so describe briefly _____

11. Do you recognize a blush as different from a flush? Yes ☐ No ☐

12. Any other relevant comments:- _____

1.3: Time since the hot flushes began

In 10 women out of the 25, the hot flushes began before their last period. Only 1 of these 10 women had undergone oophorectomy. Of the 9 women, 8 had experienced a spontaneous menopause and 1 was still having irregular periods. In 7 women the hot flushes began simultaneously with or just after the menopause. Of these women 3 had been oophorectomised and 4 had experienced spontaneous menopause. Seven women gave insufficient information concerning either the time of the menopause or the time when the flushes began.

1.4: Number of hot flushes/day

The average number of hot flushes per 24 hours experienced by these women was 9.7 ± 1.2 ($n = 23$). Two gave no information. An attempt was made to correlate the number of hot flushes with either the time of the menopause or the time since the hot flushes began. Unfortunately these are insufficient observations to make any definite statements. However, it seems that in 5 of the 6 oophorectomised women the number of flushes per day regresses with the time since oophorectomy, as shown in Table 2.

TABLE 2: Time since the hot flushes began and number of hot flushes in 24 h in 5 oophorectomised women

Time since hot flushes began in years	Number of hot flushes/24 h
1.0	16
2.5	14
4.0	10
5.0	5
9.0	3

1.5: Time of the day when the hot flushes were reported to be worse in frequency and intensity

The time of the day when the hot flushes were worse in regard to frequency and intensity fell into three categories.

(a) Eight women reported that their hot flushes were worse during the day time.

(b) Thirteen women reported that their hot flushes were worse during the night.

(c) Two women reported that they found no diurnal variation

Finally, 2 women gave no information.

1.6: Progression of the hot flushes

The hot flushes were reported to be stable in 13 women, increasing in frequency and intensity in 7 women and decreasing in 2 women. Three women gave no information. The time since the hot flushes began did not seem to have any effect on the progression of the hot flushes. In 13 women where the hot flushes were stable, the mean time since they had began was 2.7 ± 0.6 years ($n = 13$). In 7 women where the hot flushes were increasing in frequency and intensity, the mean time since they had began was 2.9 ± 1.1 years. ($n = 7$). Finally, in the 2 women where the hot flushes were decreasing, the time elapsed since they began was 12 years and 1 year respectively.

1.7: Part of the body where the hot flushes began

In most women the sensation of the hot flush began in the face and neck. A few reported that the sensation began in the trunk or 'all over'. This sensation was reported to spread 'all over', or in the arms and the trunk.

1.8: Association of hot flushes with sweating

In 15 women the hot flush was always associated with sweating, in 8 women occasionally and in 2 women never. Ten women reported that sweating was experienced mainly in association with a hot flush. Nine women reported that they occasionally sweated and this sweating was not due to a hot flush and 5 women frequently experienced sweating which was not associated with a hot flush.

1.9 : Part of the body where sweating occurred

Sweating was reported to occur in the face, neck, trunk and arms.

1.10: Duration of the hot flushes

The average reported duration of the hot flush was found to be 3.0 ± 0.5 min ($n = 22$). Three women gave no information.

1.11: Possible provocation of a hot flush

Ten women out of 25 reported that the hot flushes could be provoked by embarrassment, by alcohol, by anxiety, by excitement or by a hot drink. Fifteen reported that there was nothing that could provoke hot flushes.

1.12: Warning symptoms of an impeding hot flush

Ten women reported that palpitations, intense feeling of heat, depression, and a feeling of being tense, warned them that a hot flush was being precipitated. Thirteen women reported that they had no warning and 2 gave no information.

1.13: Symptoms accompanying a hot flush

Nineteen women reported that hot flushes were accompanied by palpitations, tachycardia, breathlessness, dizziness, nausea, exhaustion and headache. Six reported no accompanying symptoms.

1.14: Sensitivity to heat or to cold

Ten women reported an increased sensitivity to cold, 10 normal sensitivity, 3 a decreased sensitivity to cold and 2 gave no information. Fifteen women reported increased sensitivity to heat, 9 normal sensitivities and 1 decreased sensitivity to heat. The change in sensitivity to cold did not necessarily accompany a change in sensitivity to heat and the converse was also true. Nine women reported that the change in sensitivity to cold or heat occurred after the menopause, 1 reported that it was unrelated to the onset of the menopause and 7 women with altered sensitivity to cold or heat gave no information.

1.15: Possible avoidance of the hot flushes

Three women out of the 25 reported that being in a cool environment and avoiding emotional stress reduced the incidence of hot flushes. Twenty-two women reported that nothing reduced their hot flushes.

All of the 25 women reported that a hot flush is a distinctly different sensation from a blush.

1.16: Summary

To summarise, the results of the survey gave an indication of the subjective sensations reported by 25 women experiencing hot flushes, at a mean frequency of 9.7 ± 1.2 per 24 h. According to

these subjective reports, the hot flush is a severe and unpleasant symptom of sudden onset and of a mean duration of 3.0 ± 0.5 min. The sensation usually starts in the face and neck and spreads in the arms and all over the body. It is normally accompanied by sweating of the face, neck, arms and trunk. It can be provoked by embarrassment, excitement, alcohol and a hot drink. In many cases palpitations, a feeling of heat, a feeling of depression and a general tense feeling precede the hot flush. It is usually accompanied by palpitations, breathlessness, dizziness, nausea, exhaustion and headaches. Finally, it appears to be a totally different sensation from a blush.

2: PHYSIOLOGICAL CHANGES RECORDED DURING HOT FLUSHES

Some of the physiological changes that occur during a hot flush and can be recorded have received in the past very little investigation. The most complete study of the physiological changes occurring during a hot flush was undertaken by Molnar (1975) in one patient only.

This section is concerned with a study of the physiological changes occurring during a hot flush in 11 post-menopausal women, and a comparison with physiological changes occurring during heat-induced vasodilatation, in 6 pre-menopausal women.

The reports of the symptoms preceding and accompanying a hot flush suggested that the following physiological variables ought to be recorded:

1. Heart rate
2. Blood pressure
3. Digital blood volume pulse
4. Galvanic skin resistance
5. Skin temperature

1. Heart rate

Heart rate was recorded from the signal provided by the photoplethysmograph (see page 56). Heart rate can also be recorded from the R wave of the electrocardiogram (ECG). However, as the ECG is affected by factors such as skin resistance, in addition to any possible changes in the pattern of the PQRST complex, it does not provide a very clear signal for the heart rate recording.

In 7 post-menopausal women the onset of the hot flush was associated by a transient increase of the heart rate. The heart rate increased from 71.0 ± 3.0 ($n = 7$) to 86.4 ± 4.0 ($n = 7$) beats per minute, which was a statistically significant increase ($p < 0.01$). The duration of the increase was variable. The time elapsing between the onset of the increase in the heart rate and its return to the resting level was 1.1 ± 0.04 minutes ($n = 7$). Figure 2a shows the heart rate recording of a post-menopausal woman before and during a hot flush. It can be seen that the increase in heart rate coincided with the initiation of the fluctuations in the base line of the ECG (these fluctuations are discussed in page 89). In this particular woman studied, the increase in heart rate was of considerably greater duration than the mean reported above. However, it was reported by this woman that this hot flush was "a particularly severe one".

Figure 3a shows the heart rate recording of the same woman 20 minutes after the onset of the hot flush. It can be seen that the heart rate has returned to the resting value recorded prior to the hot flush.

In pre-menopausal women the heart rate remained unchanged before, during and after the heat-induced vasodilatation. Figure 4a shows the record of the heart rate of a pre-menopausal woman during heat-induced vasodilatation and Figure 5a shows the heart rate recording of the same woman 20 minutes later when skin temperature (see page 97) had returned to normal. No difference could be observed between the heart rate recorded during and after the heat induced vasodilatation compared with the rate after recovery from

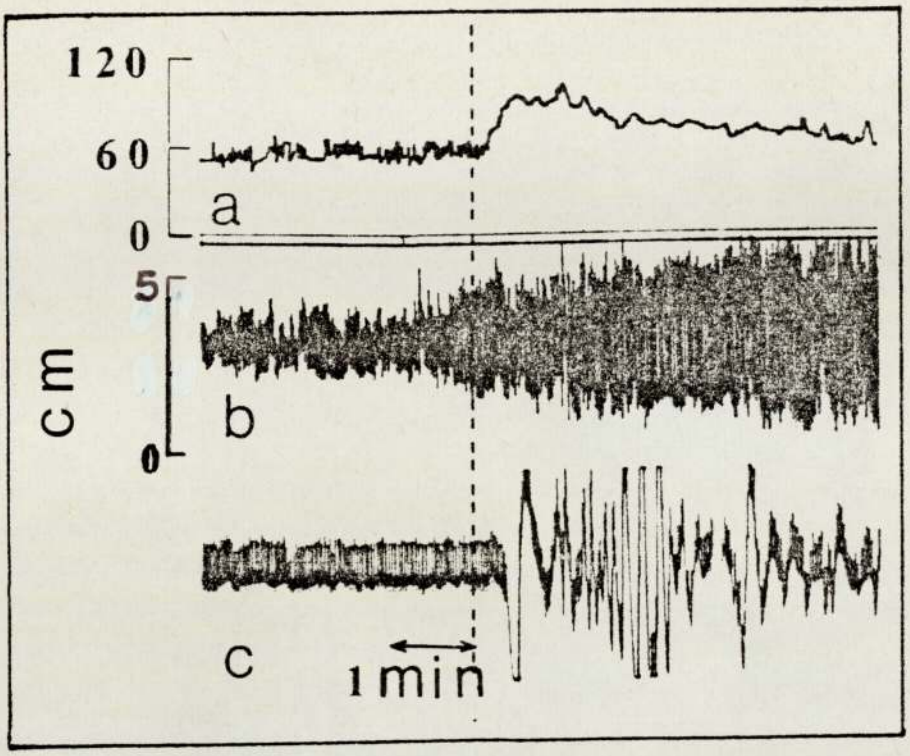


FIGURE 2: Physiological changes occurring in a post-menopausal woman during a hot flush. a: heart rate in beats/min; b: digital blood volume pulse; c: electrocardiogram. Broken line indicates the onset of the hot flush.

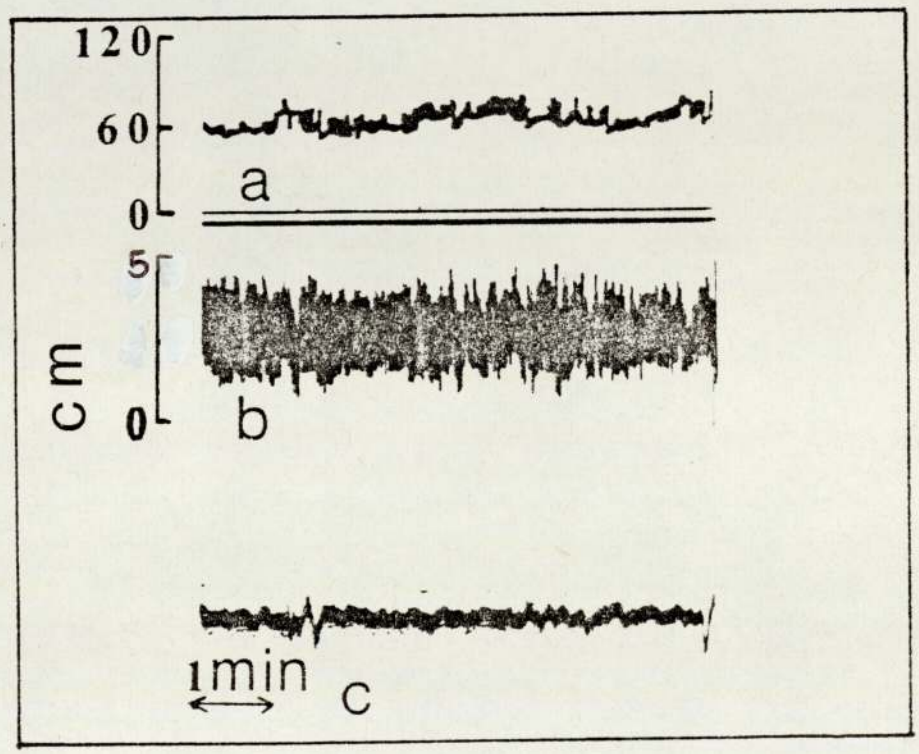


FIGURE 3: Recovery from a hot flush in the same post-menopausal woman as above. Twenty minutes have elapsed between the onset of the hot flush and the above recording. a: heart rate in beats/min; b: digital blood volume pulse; c: electrocardiogram.

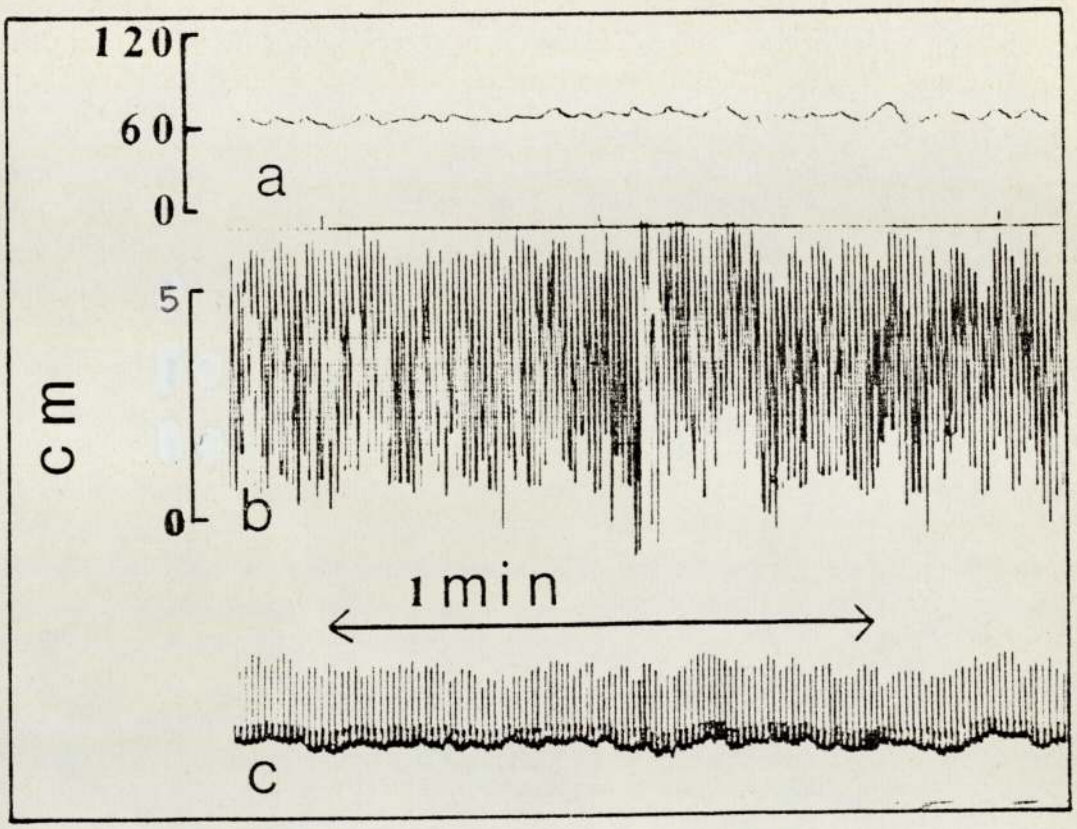


FIGURE 4: Physiological changes occurring in a pre-menopausal woman during heat induced vasodilatation. a: heart rate in beats/min; b: digital blood volume pulse; c: electrocardiogram.

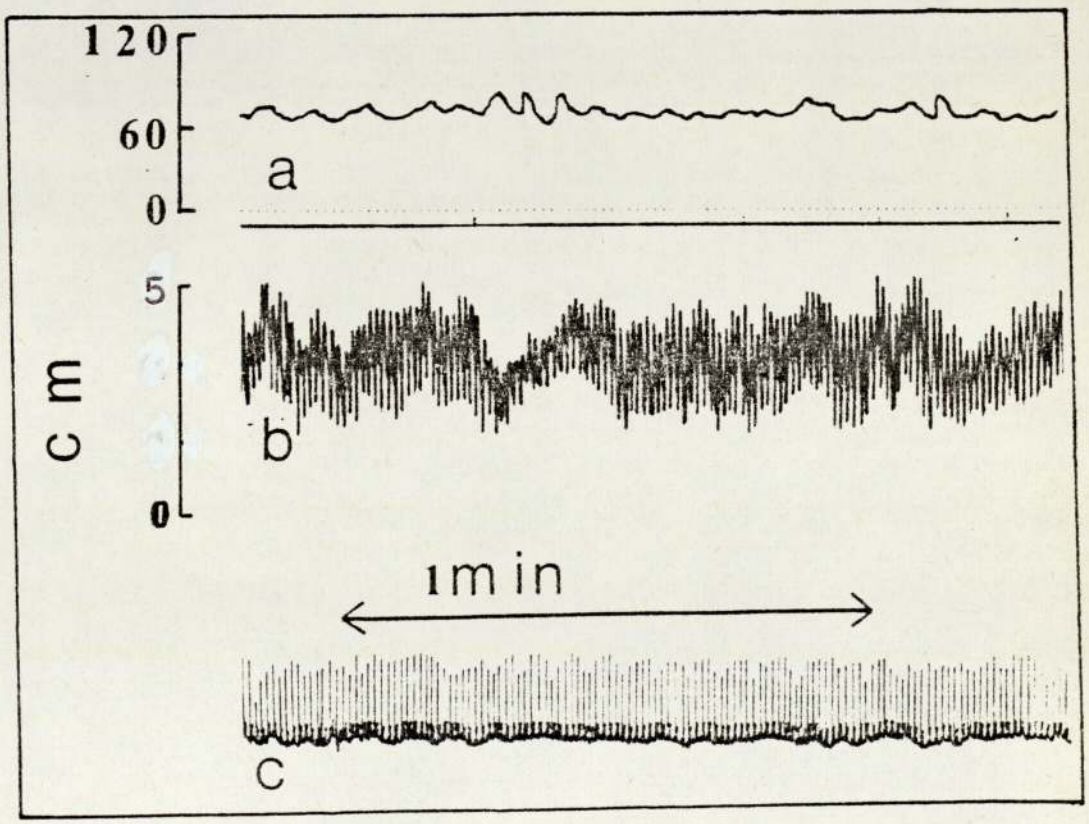


FIGURE 5: Recovery from heat induced vasodilatation in the same pre-menopausal woman as in Figure 4. Twenty minutes have elapsed between the records shown in Figure 4 and those in the above Figure. a: heart rate in beats/min; b: digital blood volume pulse; c: electrocardiogram.

vasodilatation. This suggested that the transient increase in heart rate observed in the post-menopausal women could not be simply due to vasodilatation.

2. Blood pressure

Blood pressure measurements were made, at time intervals stated in the methods (see page 56) in the post-menopausal women at rest and during hot flushes and from pre-menopausal women at rest and during heat induced vasodilatation.

There was no difference in the systolic or diastolic pressure of post-menopausal women at rest (121 ± 3.6, $n = 7$, over 85.4 ± 3.1, $n = 7$, mmHg) and during a hot flush (123 ± 4.4, $n = 7$, over 84.3 ± 3.2, $n = 7$, mmHg). Similarly no difference was found in the systolic or diastolic pressure of pre-menopausal women at rest (120 ± 4.1, $n = 6$, over 83 ± 2.8, $n = 6$, mmHg) and during heat induced vasodilatation (121 ± 3.5, $n = 6$, over 81 ± 3.3, $n = 6$, mmHg). However, a study with continuous recording of the blood pressure would be desirable in order to confirm the above findings.

3. Digital blood volume pulse

In post-menopausal women the onset of the hot flush was associated with a marked and persistent increase in the amplitude of the digital blood volume pulse by $157.0 \pm 16.0\%$ ($n = 7$) of its resting value. This increase preceded the increase in heart rate and the initiation of fluctuations in the ECG base line (see page 89) by a short time of no more than 30 seconds. The digital blood volume pulse was the last of the measurements to recover after the hot flush. The time of recovery was 21.0 ± 3.0 minutes ($n = 7$). Figure shows the recording of the digital blood volume pulse of

a post-menopausal woman before and during a hot flush. It can be seen that the increase was sudden and reached its maximal value 2.5 minutes after the initiation of the increase. Figure 3b shows the recording of the digital blood volume pulse in the same patient 20 minutes after the onset of the hot flush. It can be seen that the blood volume pulse has returned approximately to its resting value.

The observed increase in the digital blood volume pulse was indicative of a vasodilatation of the cutaneous blood vessels of the hand as digital photoplethysmography only provides evidence for the cutaneous blood flow in the hand (Ryan 1973). This finding was also confirmed by an increase in the skin temperature of the hand (see page 94) which also suggested an increase in the cutaneous blood flow of this area.

In pre-menopausal women, warming caused a large increase in the amplitude of the digital blood volume pulse by a maximum of $183.0 \pm 28.9\%$ ($n = 6$) of its resting value. The onset of the vasodilatation observed in these women was much more gradual and it took considerably longer to reach a maximum, usually about 20 minutes after the warming had started. Recovery started as soon as the means of inducing warming were removed. The time for recovery was 23.0 ± 5.0 minutes ($n = 6$). This time for recovery was similar to that seen in post-menopausal women (see page 85) recovering from a hot flush.

Figure 4b shows the recording of the digital blood volume pulse in a pre-menopausal woman made when this parameter was maximally increased. Figure 5b shows the recording of the same

variable in the same woman after she was allowed to recover. In this particular woman the time for recovery was 20 minutes. The slow onset of the vasodilatation cannot be seen in Figure 4b because it preceded the maximal increase by 18 minutes.

The maximal increase in the amplitude of the digital blood volume pulse observed in the pre-menopausal women was comparable to that seen in post-menopausal women (see page 85). However, the mechanism underlying this increase may not be the same in the two groups of women because of the observed difference in its onset and development in the pre- and post-menopausal women respectively. Nevertheless, this comparable increase in the digital blood volume provides some evidence that the thermally induced vasodilatation in pre-menopausal women as a model for the control of the physiological events occurring during a hot flush was a reasonable one, ie it did not produce too large or too small increases in the cutaneous blood flow.

4. Skin resistance

The symptoms preceding the hot flush reported by the post-menopausal women who participated in the epidemiological survey (see page 78) included palpitations and a feeling of being tense. Such symptoms may be classified in behavioural terms as symptoms indicating 'arousal'. Electrodermal activity may serve as an arousal indicator (Edelberg 1972) and it has been used in lie detection (Gustafson and Orne 1965) and measuring the tranquilising or stimulating effects of pharmacologic agents (Schneider and Costiloe 1957). The electrodermal reflex is believed to be mediated by the sympathetic nervous system (see page 60). The increase in

heart rate and in the amplitude of the digital blood volume pulse seen in post-menopausal women during a hot flush (both variables are mainly under sympathetic control) suggested the involvement of the sympathetic nervous system in the mechanism mediating a hot flush. In addition, Molnar (1975) observed a fluctuation in the base line of the ECG recording in a post-menopausal woman during a hot flush which he attributed to an altered conductivity of the skin due to sweat gland activity. The sweat gland activity (also under sympathetic control, see page 45) appears to be responsible for at least part of the electrodermal reflex, as already discussed in the Methods section, page 59. It therefore appeared of interest to examine the skin resistance of post-menopausal women at rest and during a hot flush and to compare to that of pre-menopausal women at rest and during heat induced vasodilatation. For this purpose both the base line of the ECG recording and the skin resistance level and response were examined in the two groups of women.

5. Electrocardiography

In the present study, electrocardiography was performed in order to confirm the observation of a previous worker (Molnar 1975) in a number of patients, as his study involved only one patient. In addition, it served as supportive evidence for the changes in skin resistance discussed below. The pattern of the ECG was not examined in the present study but D.W. Sturdee (personal communication) reported no changes in the PQRST complex, in post-menopausal women during a hot flush.

In post-menopausal women the onset of the hot flush was accompanied by marked fluctuations of the ECG base line. These fluctuations usually diminished as the hot flush subsided and the base line came back to normal. It usually lasted 2 - 3 minutes depending on the severity of the hot flush. Figure 2c shows the ECG trace from a patient just before and during a hot flush. It can be seen that the onset of the fluctuations of the ECG base line is accompanied by changes in the records already discussed. These fluctuations usually coincided with the patient's sensation of the hot flush. Figure 3c shows the ECG trace from the same patient 20 minutes after the onset of the hot flush. It can be seen that the ECG base line has ceased fluctuating and is similar to that seen before the onset of the hot flush.

When pre-menopausal women were warmed there were no marked changes in the ECG base line. Figure 4c shows the ECG record from a pre-menopausal woman during warming. Figure 5c shows the ECG recording from the same woman after she was allowed to cool down.

The heart rate and digital blood volume pulse records also seen in Figures 2,3,4,5, have already been discussed.

The fact that no changes in the ECG base line were observed in pre-menopausal women suggests that thermal vasodilatation alone cannot account for the changes seen in post-menopausal women.

6. Galvanic skin resistance (GSR)

The resting skin resistance level varied in both post-menopausal and pre-menopausal women which was in agreement with reports in the literature concerning individual variation (see page 60). Individual values of resting skin resistance level are shown in the Appendix.

In post-menopausal women the onset of the hot flush was marked by an acute fall in the skin resistance. This fall was the first event observed during a hot flush and it preceded the subjective feeling of an impending hot flush by a matter of a few seconds. The fall in GSR reached a minimum of $44.0 \pm 6.0\%$ ($n = 7$) of its resting value acutely and it recovered slowly over a period of approximately 20 minutes to its resting level. The time for this recovery was similar to that necessary for the fluctuations in the ECG base line to cease (see page 89).

In pre-menopausal women there was a very small gradual fall in the skin resistance during warming by $8.1 \pm 4.4\%$ ($n = 6$) of its resting value. This fall was not significantly greater than zero.

The absence of any significant changes in the skin resistance of pre-menopausal women suggested that the activation of the sweat glands (which is believed to be partly responsible for the skin resistance response) was not sufficient to provoke a skin resistance response, during thermal vasodilatation.

At this point it would be appropriate to mention the case of a post-menopausal who was normally experiencing hot flushes but in whom the usual slight heating stimulus failed to produce a hot flush. In this subject the effect of warming was observed and compared with both the hot flushes of post-menopausal women and the thermal vasodilatation of pre-menopausal women.

Figures 5 , 7 shows the records of the heart rate, digital blood volume pulse and ECG in this woman, during warming and the records of the same variables after she was allowed to

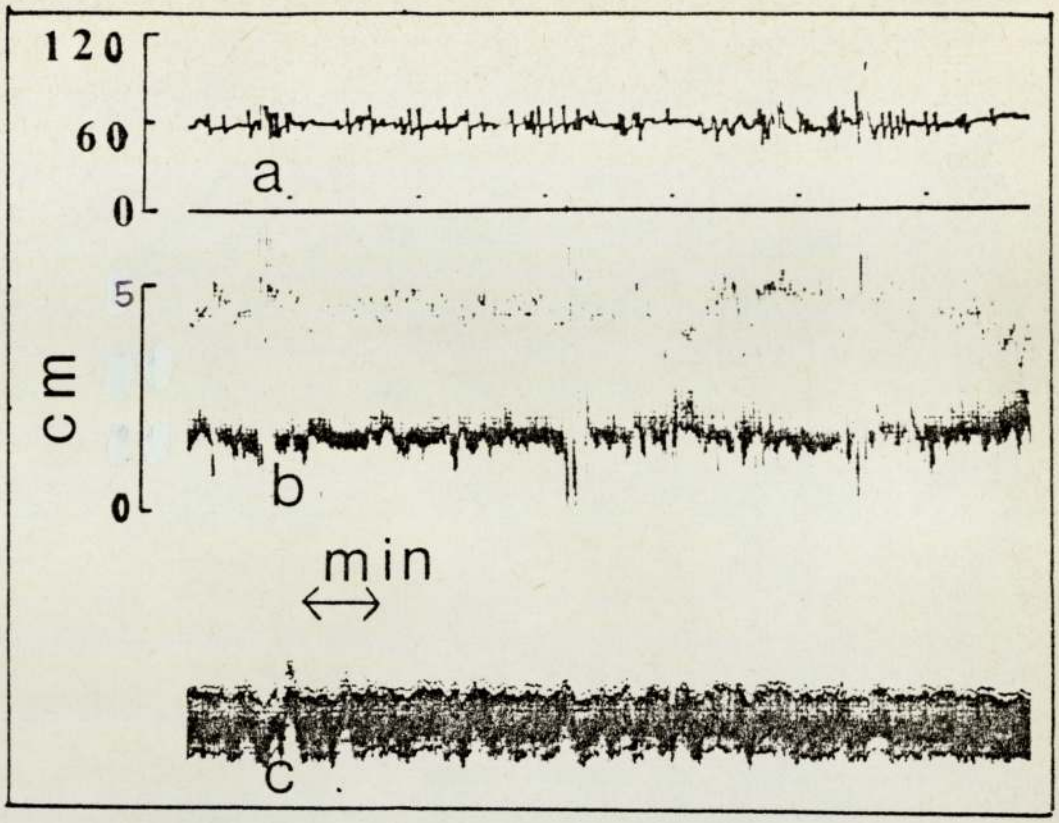


FIGURE 6: Physiological changes occurring in a post-menopausal woman in whom a slight heat stimulus failed to cause a hot flush.
a: heart rate in beats/min; b: digital blood volume pulse;
c: electrocardiogram.

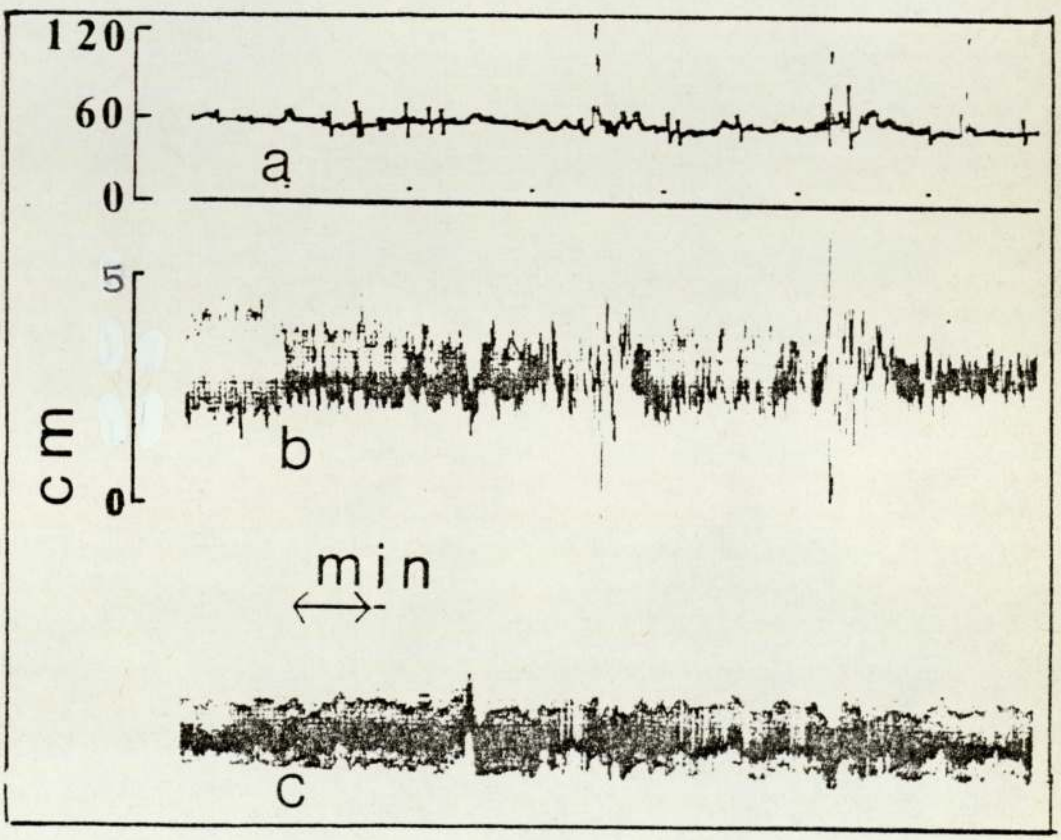


FIGURE 7: Recovery from heat induced vasodilatation in the same post-menopausal woman as in Figure 6. Twenty minutes have elapsed between the records shown in Figure 6 and those in the above Figure.
a: heart rate in beats/min; b: digital blood volume pulse;
c: electrocardiogram.

cool down. As in the case of pre-menopausal women (page 84), Figure 6b only shows the maximal increase in the amplitude of the digital blood volume pulse as the time between the beginning of the warming and that when the amplitude of the digital blood volume pulse was maximal,was approximately 20 minutes. The records shown in Figure 7 were similar to those before the warming started.

It can be seen that the only difference between the two figures consists of the greater amplitude of the digital blood volume pulse. The heart rate and the ECG base line remained unaltered. The observed pattern is similar to that seen in pre-menopausal women in Figures 4, 5 (page 84) during thermal vasodilatation and recovery, and this provided additional evidence that the hot flush is distinguishable from thermal vasodilatation in post-menopausal women.

7. Skin temperature

In post-menopausal women there was a rapid rise in skin temperature of the hand, arm and forehead. The hand temperature increased by $1.1 \pm 0.2^{\circ}C$ ($n = 7$), the arm temperature by $0.8 \pm 0.2^{\circ}C$ ($n = 7$) and the forehead temperature by $0.7 \pm 0.1^{\circ}C$ ($n = 7$). These temperature increases in the hand, arm and forehead corresponded to increases of $2.7 \pm 0.6\%$ ($n = 7$), $2.0 \pm 0.5\%$ ($n = 7$) and $2.0 \pm 0.3\%$ ($n = 7$) of their resting values.

The recovery was very slow and very often it was not complete before another hot flush occurred.

Figure 8 shows the changes in the skin temperatures plotted against time in a post-menopausal woman who had three hot flushes during the experiments. The broken lines indicate the onset of a

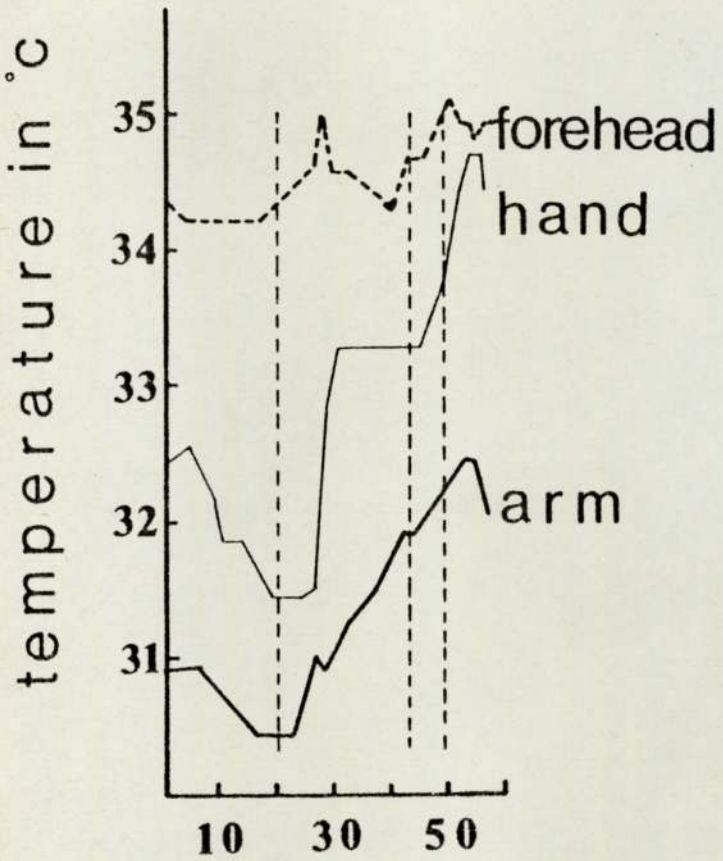


FIGURE 8.

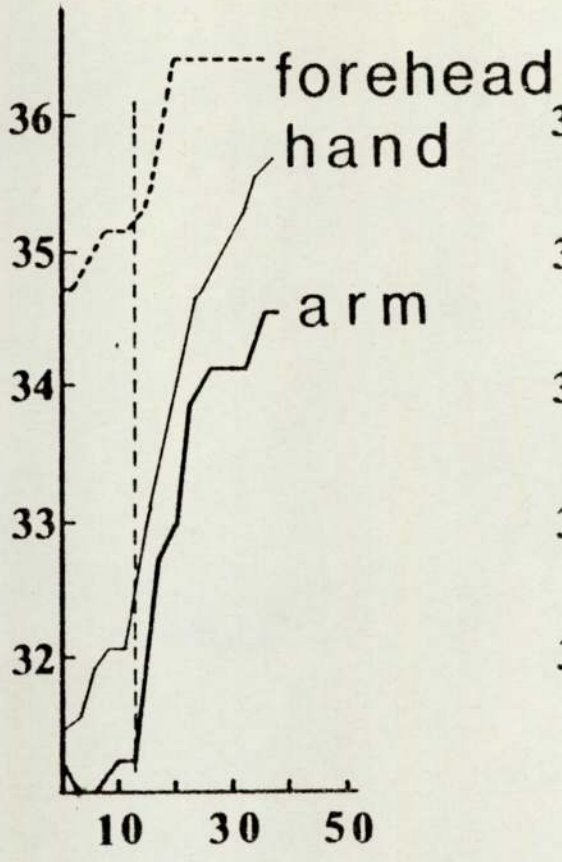


FIGURE 9

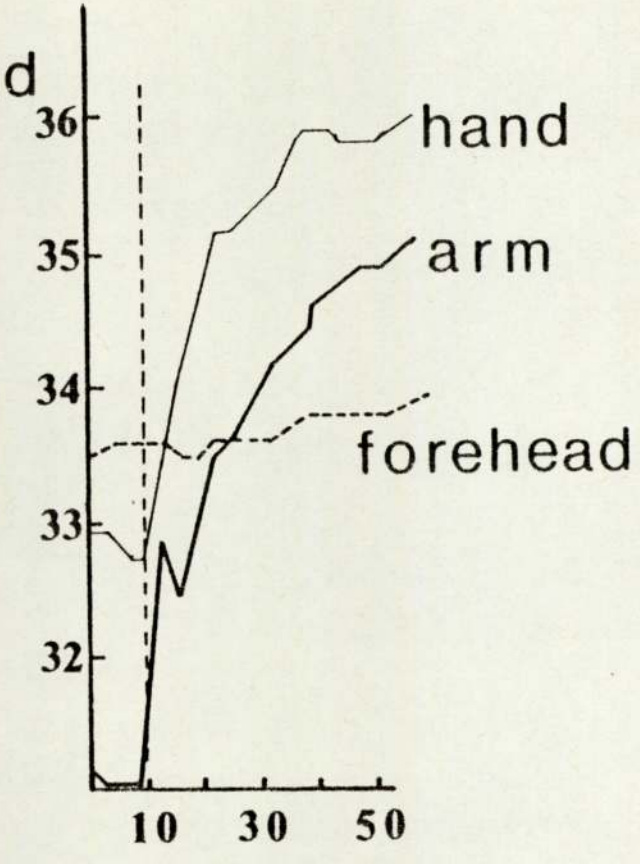


FIGURE 10

hot flush. It can be seen that the forehead temperature started to increase just before, and the arm temperature simultaneously with the onset of the hot flush, whereas the hand temperature started to increase a few minutes after the onset of the hot flush (3 minutes after the first flush and 2 minutes after the second; at the onset of the third flush all parameters were still rising). In pre-menopausal women warming caused much greater increases in temperature and these increases were dependent upon the length and intensity of warming. Increases in temperature were progressive in contrast to the acute changes associated with the hot flush in the post-menopausal women. The hand temperature rose by a maximum of $2.5 \pm 0.7^{\circ}C$ ($n = 6$), the arm temperature by a maximum of $3.3 \pm 0.8^{\circ}C$ ($n = 6$) and the forehead temperature by a maximum of $1.5 \pm 0.6^{\circ}C$ ($n = 6$). These temperature increases were equivalent to $8.4 \pm 2.5\%$ ($n = 6$), $8.9 \pm 1.0\%$ ($n = 6$) and $4.3 \pm 0.9\%$ ($n = 6$) increases of the resting hand, arm and forehead temperatures respectively. Figure 9 shows the temperature changes in the hand, arm and forehead in a pre-menopausal woman during warming. The broken line marks the initiation of warming.

Finally, Figure 10 shows the changes in hand, arm and forehead temperatures plotted against time in the post-menopausal woman in whom warming failed to cause a hot flush. It can be seen that the pattern of the changes in this post-menopausal woman differs greatly from that seen in post-menopausal women during a hot flush, but that it is similar to that seen in pre-menopausal women during warming.

8. Thermography

The two thermograms presented in this section are used for the purpose of illustration of the results obtained by D.W. Sturdee and to support the findings on skin temperature measurements by thermoprobes performed in the present study. Hence no quantification of these results could be obtained.

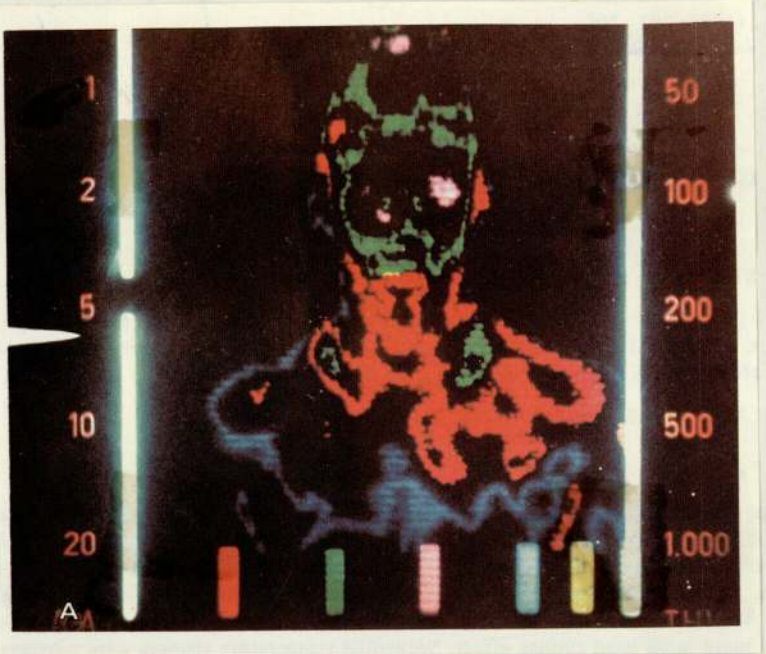
The thermograms of one subject before and during a hot flush are shown in Figures 11a and 11b. The resting thermogram in Figure indicates the skin temperature variation of the upper chest, shoulders, neck and face of the subject before she experienced a hot flush. The areas of black between the isotherms indicate that the temperature was between that of the two bordering isotherms. The warmest parts were the pink areas just below the left eye and right nostril, and on the trunk the supraclavicular fossae were typically warmer than the surrounding skin and are shown as 'islands' of green among the red isotherms.

The thermogram in Figure 11b was taken just a few seconds after the subjective sensation of the flushing. It can be seen that this thermogram was very different from that taken at rest and it demonstrated an overall increase of skin temperature of about 1° during the hot flush, which occurred within 10 - 15 seconds of the initial subjective sensation of warmth, which confirmed the findings on the temperature measurements in the present study, and which were $0.7 \pm 0.1^{\circ}C$ for forehead temperature.

9. Summary

To summarise, the results of the study of the physiological measurements in post-menopausal women at rest and during flushing

(a)



(b)

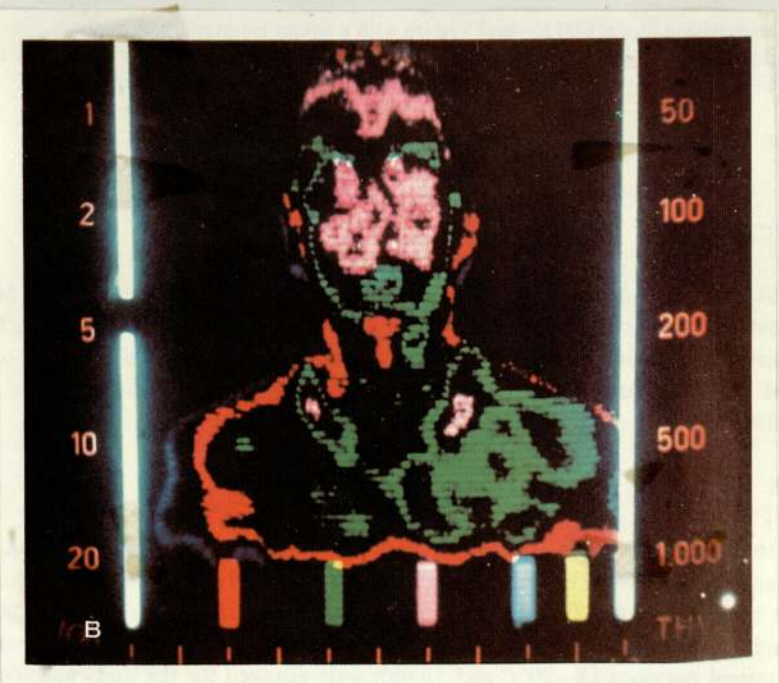


FIGURE 11: Thermograms of a post-menopausal woman before (a) and during a hot flush (b). The temperature scale of the isotherms is indicated beneath the thermograms increasing from left to right by about $1^{\circ}C$ for each colour. The dark blue isotherm is just off the scale to the left. The green isotherm was equivalent to $32^{\circ}C$.

and in pre-menopausal women at rest and during heat induced vasodilatation, suggest that the following changes take place.

9.1: Post-menopausal women

The onset of the hot flush is associated by a sudden initiation of fluctuations in the ECG base line, an increase in heart rate of short duration, a marked increase in the amplitude of the digital blood volume pulse and an acute fall in the skin resistance. These changes were accompanied by increases in the forehead, hand and arm temperature initiated in the stated order.

The increase in the skin temperatures suggested a peripheral vasodilatation. This was supported by the increase in amplitude of the digital blood volume pulse. As this variable started increasing simultaneously or shortly before the onset of the hot flush (the time stated by the patients), and the hand temperature started increasing a few minutes afterwards it would be reasonable to suggest that, at least in the hand, the increase in temperature was due to the vasodilatation observed in this area. It would be interesting to monitor the blood flow in areas such as the arm and forehead in order to examine whether such a correlation could be also made in these areas. However, in the present study such data are not available.

All the physiological variables studied, recovered completely after approximately 20 minutes unless another hot flush occurred in the meantime.

The stated duration of the hot flush as calculated from the reports of the post-menopausal women who participated in the survey

and which was 3.0 ± 0.5 minutes ($n = 22$) seems to correlate best to the time between the onset of the hot flush and the time needed for the temperatures to start falling.

9.2: Pre-menopausal women

Warming pre-menopausal women caused no change in the heart rate, ECG base line and skin resistance, but caused a large and progressive increase in the amplitude of the digital blood volume pulse, and of the forehead, hand and arm temperatures. Both changes were indicative of an increased cutaneous blood flow due to the thermoregulatory adjustment in response to heat load.

The changes in the heart rate, digital blood volume pulse, skin resistance and forehead, hand and arm temperatures both in post- and pre-menopausal women are summarised in Tables in the Appendix.

3: EXPERIMENTS TO INVESTIGATE A POSSIBLE RELEASE OF VASOACTIVE SUBSTANCES RESPONSIBLE FOR THE MECHANISM OF THE HOT FLUSH

The results of the previous section showed that the hot flush in post-menopausal women is associated with changes in their heart rate digital blood volume pulse, skin resistance and skin temperatures. These changes are of a different nature to those occurring during thermal vasodilatation in pre-menopausal women.

In the present section, it was examined whether any vasoactive substances possibly responsible for some of the physiological changes mentioned above were released into the circulation either before or during the hot flush.

Several vasoactive substances have been implicated in the flushes which accompany the following pathological conditions: carcinoid syndrome, dumping syndrome, phaeochromocytona and autonomic epilepsy. An extensive review on these conditions appears in the Literature Review (page 25). These substances include 5-hydroxy-tryptamine, histamine, catecholamines, prostaglandins and kinins. The present section is concerned with an examination of their release into the circulation of post-menopausal women before or during a hot flush, and their involvement in mediating the events of the hot flush. Furthermore, the possible involvement of acetylcholine is also examined.

1. Pharmacological screening of the plasma of post-menopausal women at rest and during a hot flush and of pre-menopausal women at rest and during heat-induced vasodilatation

The rat fundic strip preparation was used for an initial screening of the plasma of pre- and post-menopausal women.

This tissue was chosen because it contracts to a variety of physiological substances with high sensitivity. It is the tissue of choice for assaying very small amounts of 5-hydroxytryptamine (5HT) (10^{-11}M) (Vane 1957), prostaglandins (PGs) (5×10^{-12}M) (Horton 1972) and acetylcholine (Ach) (10^{-10}M) (Vane 1957). Histamine also contracts the rat fundic strip but in greater amounts than the already mentioned substances (10^{-5}M) (Vane 1957).

1.1: The effect of plasma on the isolated rat fundic strip when added directly to the preparation

The plasma from post menopausal women at rest and during a hot flush and from pre-menopausal women at rest and during heat induced vasodilatation was found to contract the isolated rat fundic strip.

To investigate whether there was any difference in the activity of the plasma, four volumes of plasma from post-menopausal women at rest (0.1, 0.2, 0.4 and 0.8ml) were compared with equal volumes of plasma during a hot flush. Similarly, 0.1, 0.2, 0.4 and 0.8 ml of plasma from pre-menopausal women were compared with equal volumes of plasma during heat-induced vasodilatation. The plasma of both pre- and post-menopausal women was also compared with 0.1, 0.2, 0.4 and 0.8 ml of a solution containing 10^{-9}M 5-hydroxytryptamine for control purposes. 5-hydroxytryptamine was used as a control because this substance is known to be present in abundance in plasma as a result of the breakdown of the platelets (Humphrey and Jacques 1954).

(a) Comparison of the activity of the plasma from post-menopausal women at rest and during a hot flush

Figure 12 shows the mean ± SE (n = 5) of the responses in mm of the four volumes of plasma at rest and during a hot flush. It can be seen that there were no significant differences in the responses of the two plasmas at any of the four volumes used.

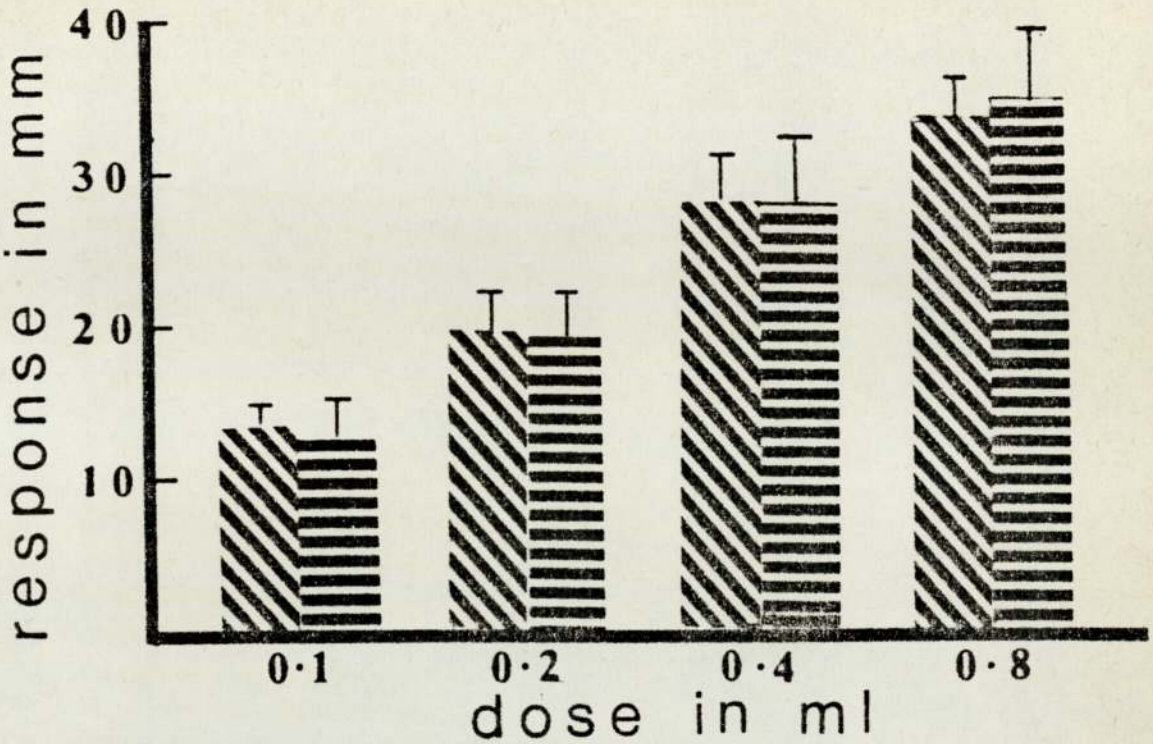
(b) Comparison of the activity of the plasma from pre-menopausal women at rest and during heat induced vasodilatation

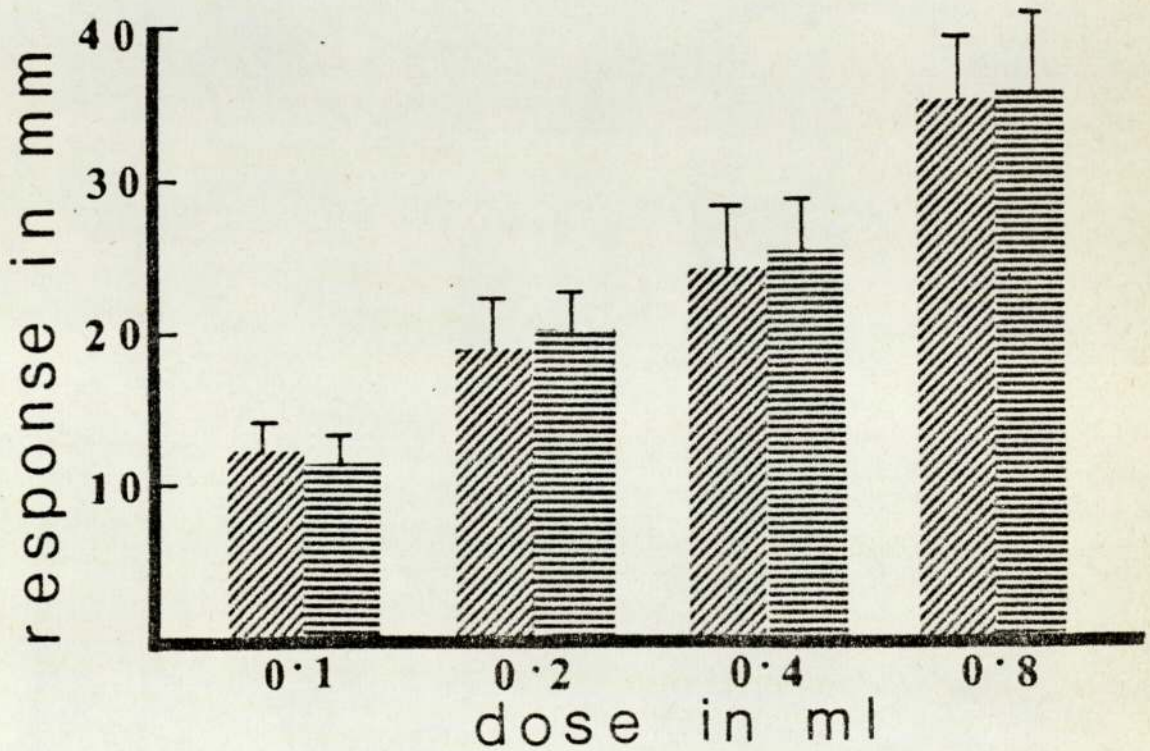
Figure 13 shows the mean ± SE (n = 5) of the responses in mm of the four volumes of plasma at rest and during heat induced vasodilatation. Again, no significant difference can be seen in the responses of the two plasmas at any of the four volumes used.

(c) Comparison of the activity of the plasma from post-menopausal women at rest and during a hot flush, with that of plasma from pre-menopausal women at rest and during heat induced vasodilatation and with a solution of 5-hydroxytryptamine of known concentration (10^{-9}M)

Figure 14 shows the mean ± SE (n = 5) of the responses in mm, of plasma from post-menopausal women at rest and during a hot flush, from pre-menopausal women at rest and during heat induced vasodilatation and of 5-hydroxytryptamine (10^{-9}M). It can be seen that there is no significant difference in these responses at any of the four volumes used.

The fact that the responses of the plasma were not significantly different from those to 5-hydroxytryptamine, suggested that a considerable portion of the response of the rat fundic strip to plasma from both post- and pre-menopausal women might be due to the presence of 5-hydroxytryptamine.


40
30
20
10
response in mm
0·1
0·2
0·4
0·8
dose in ml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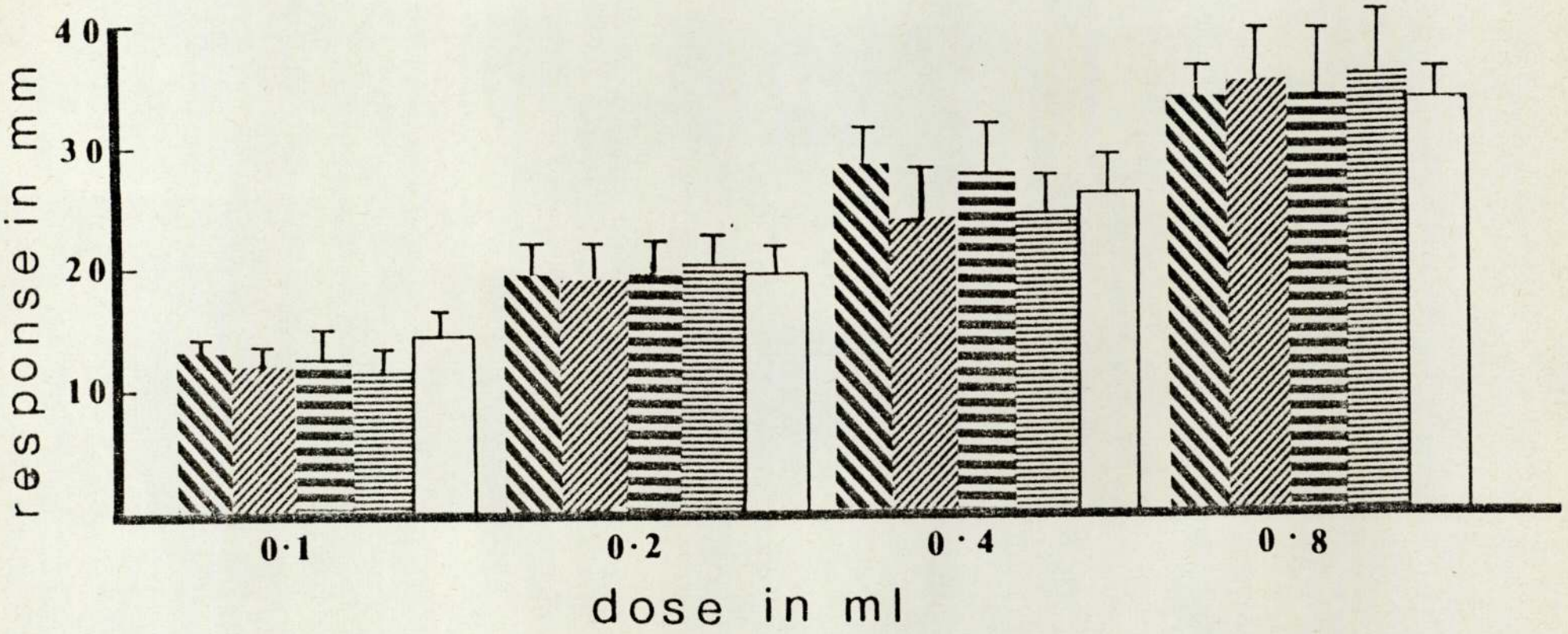


FIGURE 14: The dose response relationship for plasma from post-menopausal women at rest and during a hot flush and from pre-menopausal women at rest and during heat induced vasodilatation, and for 5-hydroxytryptamine (from a stock solution of 10^{-9} M) on the isolated rat fundic strip. The doses (abcissa) are expressed as volume in ml. The vertical bars represent SE of the means (n = 5). Ordinate scale: mm of pen excursion.

To investigate whether 5-hydroxytryptamine was masking any possible activity due to other vasoactive substances, the activity due to 5-hydroxytryptamine was blocked with methysergide (10^{-9}M), a specific 5-hydroxytryptamine receptor blocker.

1.2: The effect of methysergide (10^{-9}M) upon the contractile responses of rat fundic strip to plasma of post-menopausal women at rest and during a hot flush

Figure 16 shows the effect of methysergide (10^{-9}M) upon the control responses of the rat fundic strip to 5-hydroxytryptamine and to post-menopausal women at rest and during a hot flush. The control responses were approximately 50 per cent of the responses to the highest volume of the plasma used, and were matched with a response of similar height of 5-hydroxytryptamine. The final concentration of 5-hydroxytryptamine necessary to produce such a response was between 6×10^{-12}M - 1.2×10^{-11}M which was equivalent to volumes between 0.2 - 0.4 ml from a solution of 10^{-9}M (the volume of the organ bath was 30 ml). The responses to such concentrations of 5-hydroxytryptamine were on the linear portion of the dose response curve as it can be seen in Figure 15.

It was found that when methysergide was applied to the preparation at a concentration sufficient to reduce responses to 5-hydroxytryptamine to $14.9 \pm 4.5\%$ ($n = 16$) of the control, the responses to the plasma taken at rest were reduced to a significantly greater degree than those to plasma taken during a hot flush ($p < 0.01$). The response remaining after the application of the antagonist was $26.7 \pm 4.1\%$ ($n = 16$) of the control response to the plasma at rest and $56.7 \pm 9\%$ ($n = 16$) of the control response to the plasma taken during a hot flush. Thus, it appeared that the contractile activity of plasma may not be entirely due to 5-hydroxytryptamine.

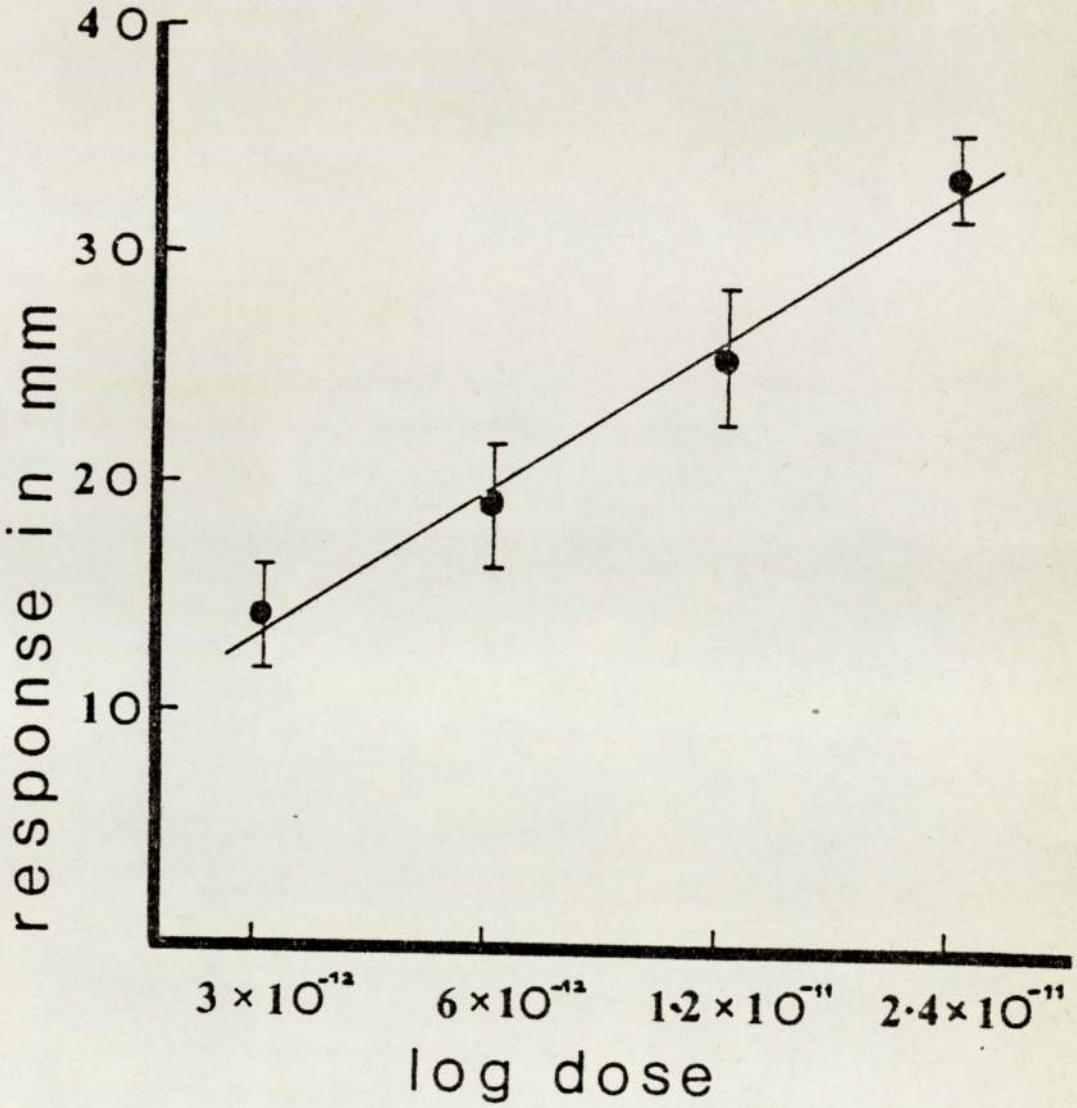
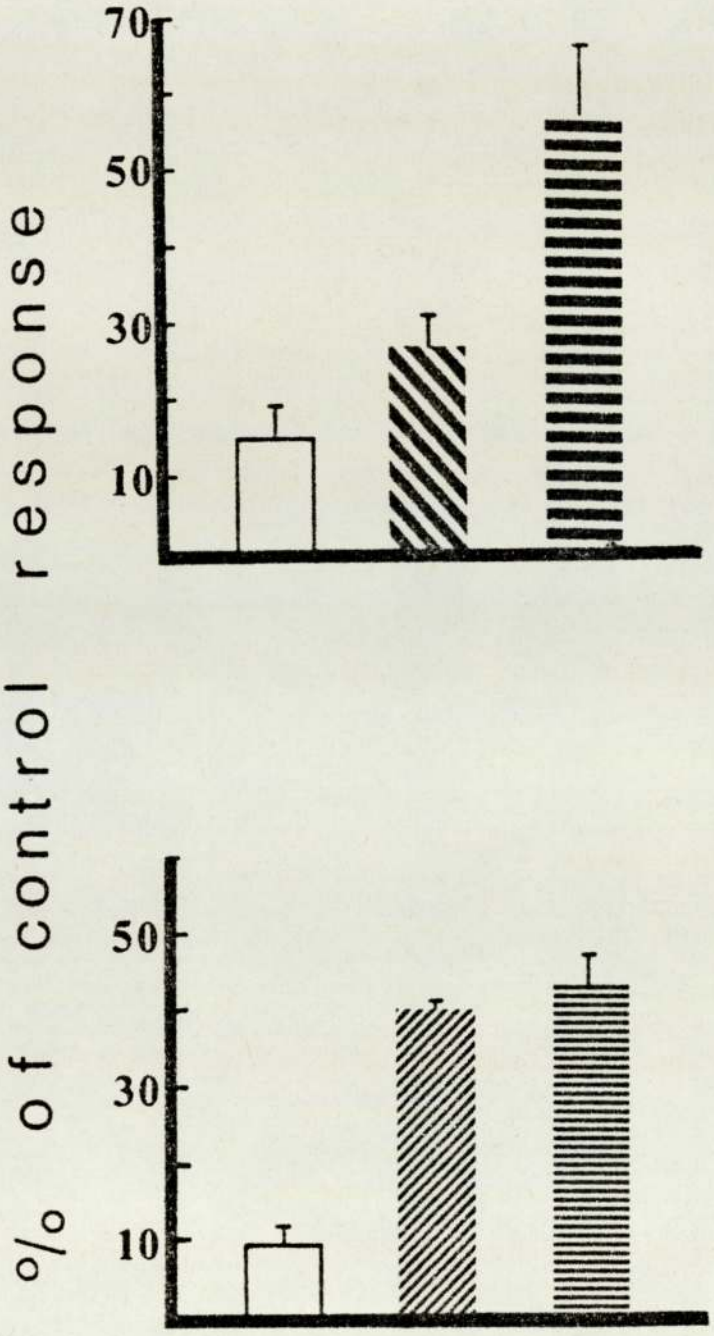


FIGURE 15: The regression line to the linear portion of the log-dose/response curve for 5-hydroxytryptamine on the rat fundic strip. The doses (abcissa) are expressed in molar concentrations. Ordinate scale: mm of pen excursion. The vertical bars represent the SE of the means (n = 5).


% of control response
70
50
30
10
50
30
10

1.3: The effect of methysergide (10^{-9}M) upon contractions of the rat fundic strip to plasma of pre-menopausal women at rest and during heat induced vasodilatation

Figure 17 shows the effect of methysergide (10^{-9}M) upon contractions of the rat fundic strip to 5-hydroxytryptamine and to plasma from pre-menopausal women at rest and during heat induced vasodilatation. Control responses to plasma and to 5-hydroxytryptamine were selected as in the case of post-menopausal women (page 106).

It was found that methysergide at a concentration sufficient to reduce responses to 5-hydroxytryptamine to $9.1 \pm 2.3\%$ ($n = 6$) of control caused a similar reduction of the responses to plasma taken at rest or during heat induced vasodilatation. The responses remaining after the application of the antagonist were $40.0 \pm 1.2\%$ ($n = 16$) and $42.3 \pm 4.1\%$ ($n = 16$) of the control responses to the plasma taken at rest and during heat induced vasodilatation respectively.

It therefore appeared that not all the contractile activity of the plasma of pre-menopausal women or post-menopausal women is due entirely to 5-hydroxytryptamine. Furthermore, the remaining activity of the plasma of post-menopausal women at rest, on the methysergide blocked rat fundic strip, was significantly smaller than that of the plasma during a hot flush. Such a difference was not observed in the case of the plasma of pre-menopausal women at rest or during heat induced vasodilatation.

However, the responses to plasma from post-menopausal women at rest that remained after the application of methysergide were significantly smaller ($p < 0.05$) than those to plasma from pre-menopausal women at rest. There was no statistically significant

difference between the responses to plasma from post-menopausal women during a hot flush compared to those of pre-menopausal women during heat induced vasodilatation, on the methysergide-blocked rat fundic strip. This may suggest that there is a significant difference in the activity of the plasma of post-menopausal women at rest compared to that of pre-menopausal women at rest. This difference might be due to the difference in the hormonal status of the two groups. However, no direct connection can be made between the differences in activity of the plasma and the difference in circulating levels of oestrogen from the present results.

As a difference was established between the activity of the plasma of post-menopausal women at rest compared to that during a hot flush, an attempt was made to determine the reason for such a difference. Therefore, the possible release of several vasoactive substances into the circulation of these women was tested. The substances that were examined were acetylcholine, histamine, catecholamines, prostaglandins and kinins. All these substances are normally found in varying amounts in the circulation. It was hoped, however, to detect any differences between the amounts of these substances released into the circulation at rest and those released during a hot flush.

1.4: Investigation of cholinergic activity of the plasma of post-menopausal women at rest and during flushing

To examine the presence of any cholinergic activity in the plasma of post-menopausal women at rest and during flushing, the effect of hyoscine, an acetylcholine muscarinic antagonist, was investigated upon contractions of the rat fundic strip to both plasmas.

Figure 18 shows the effect of hyoscine (10^{-7}M) upon control contractions of the rat fundic strip to acetylcholine and to plasma of post-menopausal women taken at rest and during flushing. Control responses were selected as previously mentioned (page 104). It was found that hyoscine at a concentration sufficient to reduce acetylcholine responses to 13.6 ± 3.8% (n = 5) of control had very little effect on the responses to both plasmas. The responses after the application of the antagonist were 92.3 ± 14.0% (n = 5) and 85.8 ± 13.1% (n = 5) of the control responses of plasma taken at rest and during flushing respectively.

It therefore appeared that a muscarine-like action was not responsible for the difference previously observed.

1.5: Investigation of histaminic activity of the plasma of post-menopausal women taken at rest and during flushing

To examine the presence of any histaminic activity in the plasma of post-menopausal women the effect of mepyramine, a histamine antagonist, was investigated upon contractions of rat fundic strip to histamine and to both plasmas. Control responses were selected as previously mentioned (page 104).

Figure 19 shows the effect of mepyramine (5×10^{-5}M) upon contractions of the rat fundic strip to plasma taken at rest and during flushing. It was found that mepyramine at a concentration sufficient to reduce histamine responses to 24.0 ± 7.2% (n = 5) of control left the responses to both plasmas almost unaffected. The responses after the antagonist were 94.1 ± 14.2% (n = 5) and 94.0 ± 13.8% (n = 5) of the control responses of plasma taken at rest and during flushing respectively.

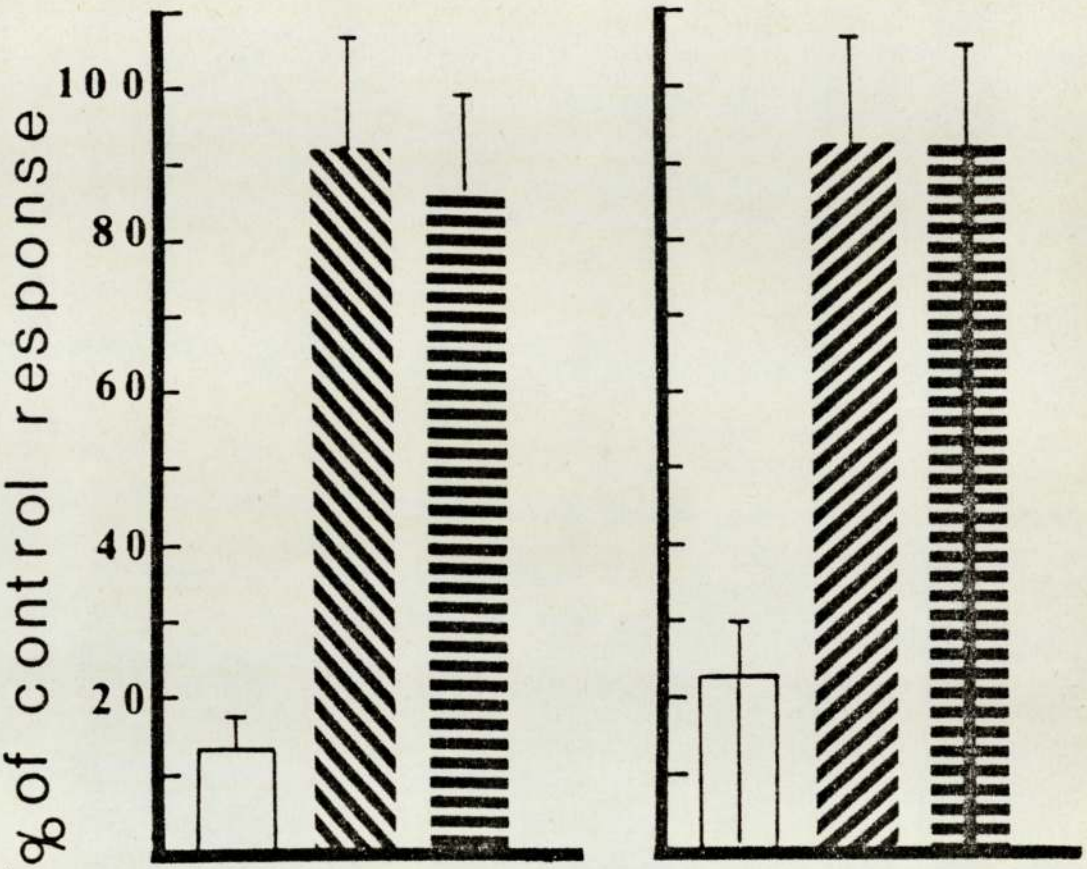


FIGURE 18

FIGURE 19

FIGURE 18: The effect of hyoscine (10^{-7}M) upon the control responses of the rat fundic strip to acetylcholine (6×10^{-10} - 1.2×10^{-9}M) ☐ and to plasma from post-menopausal women at rest ▨ and during a hot flush ▤. Ordinate scale: percentage of control response that remained after the application of the antagonist. Vertical bars are the SE of the means (n = 5).

FIGURE 19: The effect of mepyramine (5×10^{-5}M) upon the control responses of the rat fundic strip to histamine (3.3×10^{-6} - 6.6×10^{-6}M) ▥ and to plasma from post-menopausal women at rest ▨ and during a hot flush ▦. Ordinate scale: percentage of control response that remained after the application of the antagonist. Vertical bars are the SE of the means (n = 5).

It therefore appeared that histamine could not explain the difference previously observed. The possibility of the involvement of histamine in the hot flush was further investigated by examining the effect of plasma on the isolated guinea pig ileum, a preparation which is much more sensitive to histamine than the rat fundic strip (Smith 1961).

It was found that both plasmas failed to contract this preparation and this is consistent with the hypothesis that histamine does not account for the observed difference in activity of plasma taken at rest and that taken during flushing.

1.6: Investigation of catecholamine-like activity of the plasma of post-menopausal women at rest and during flushing

a) The effect of plasma taken at rest and during flushing upon contractions of the rat ascending colon to potassium chloride

Catecholamines have been shown to reduce contractions of the rat ascending colon to spasmogens (Gaddum, Peart and Voght 1949). This effect has been used to test for the presence of catecholamines in biological fluids. To investigate whether the presence of catecholamines might account for the difference in activity of plasmas observed previously, the effect of plasma of post-menopausal women taken at rest and during a hot flush was studied upon contractions of the rat ascending colon to potassium chloride (KCl).

In this series of experiments, it was hoped that if catecholamines were present in sufficient amounts (>5 ng of noradrenaline or >15 ng of adrenaline) in the resting plasma, then the contractions to KCl in the presence of this plasma should be smaller than those to KCl in the absence of any plasma. The presence of catecholamines in such amounts was expected to be found in the plasma at rest as this plasma appeared to contract the

methysergide-blocked rat fundic strip less than that during a hot flush, and catecholamines relax the smooth muscle of the digestive tract. The amounts of plasma (both at rest and during a hot flush) used did not contract or relax the rat ascending colon when added directly to it in the absence of KCl.

Figure 20 shows the effect of 0.2 ml of plasma taken at rest and during a hot flush, on the contractions in mm of the rat ascending colon to 10^{-2}M, 2 x 10^{-2}M and 4 x 10^{-2}M of KCl. The contractions to KCl in the presence of plasma were 12.4 ± 1.2 mm (n = 8), 23.8 ± 1.4 mm (n = 8) and 33.8 ± 4.2 mm (n = 8) for the 10^{-2}M, 2 x 10^{-2}M and 4 x 10^{-2}M concentrations respectively. The contractions to the same concentrations of KCl in the presence of 0.2 ml of plasma at rest were 12.3 ± 1.8 mm (n = 8), 22.0 ± 1.7 mm (n = 8) and 29.3 ± 2.3 mm (n = 8) and those in the presence of 0.2 ml plasma during a hot flush 12.5 ± 1.6 mm (n = 8), 27.5 ± 1.9 mm (n = 8) and 35.9 ± 1.5 mm (n = 8) respectively. There was no significant difference between the responses to KCl in the absence of any plasma and those in the presence of 0.2 ml plasma at rest or during a hot flush. However, there was a significant difference ($P < 0.05$) between the responses to KCl (2 x 10^{-2}M and 4 x 10^{-2}M) in the presence of plasma taken at rest compared to the responses to the same concentrations of KCl in the presence of plasma during a hot flush.

Thus although catecholamines did not appear to be present in the plasma in sufficient amounts to reduce the responses to KCl as it was originally hoped, the results of these experiments provided additional evidence that there exists a difference in the activity of the plasma at rest compared to that taken during a hot flush.

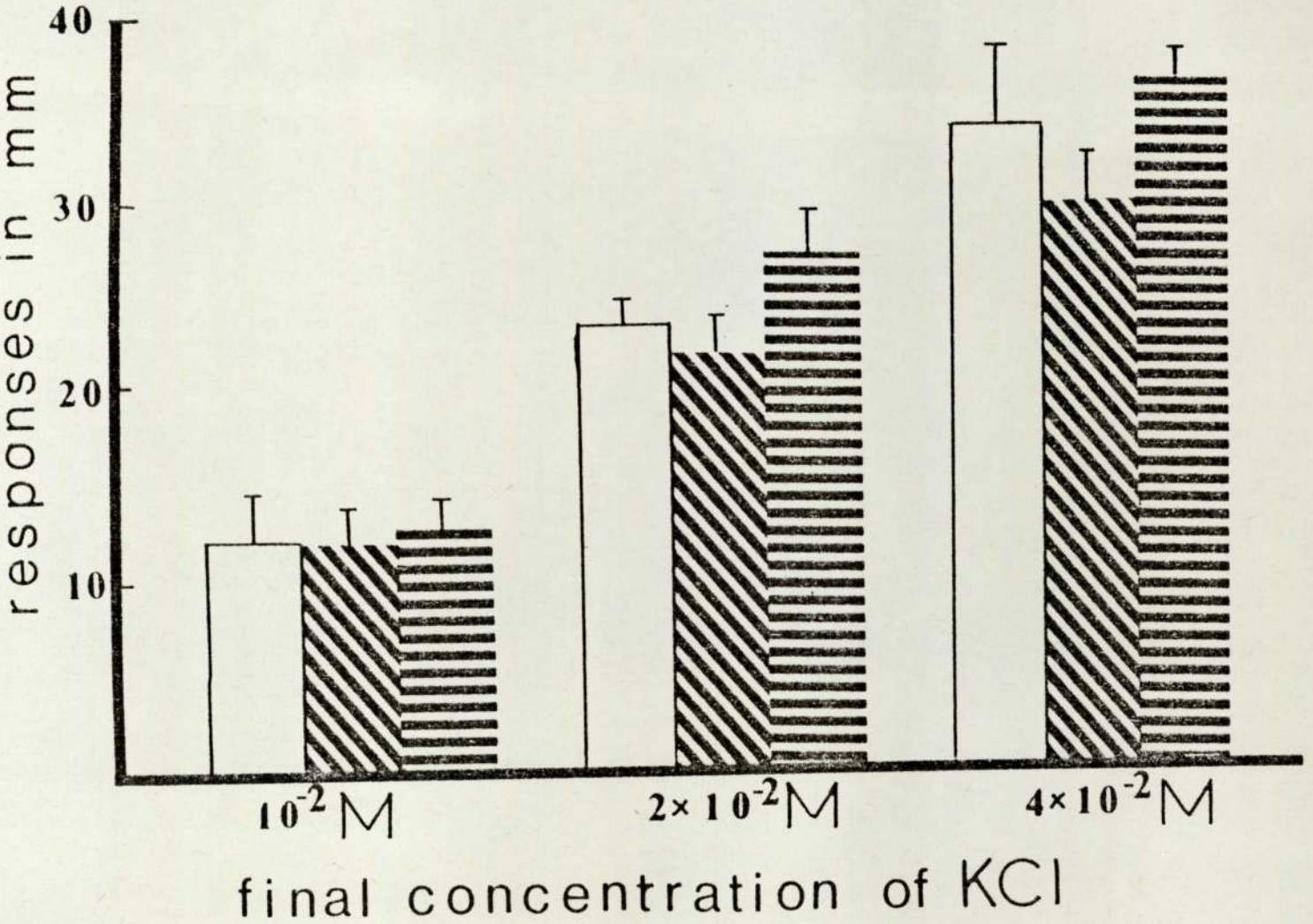


FIGURE 20: The effect of 0.2 ml plasma from post-menopausal women at rest and during a hot flush upon the responses of the rat ascending colon to 3 concentrations of potassium chloride (KCl): responses to KCl (control), responses to KCl in the presence of 0.2 ml of plasma at rest, responses to KCl in the presence of 0.2 ml of plasma during a hot flush. Ordinate scale: responses in mm of pen excursion. Vertical bars are the SE of the means (n = 8).

The absence of catecholamine-like activity in the plasma was further confirmed by a separate study on the pithed rat preparation.

b) *The pressor responses of the pithed rat preparation to plasma taken at rest or during a hot flush*

The pithed rat preparation is a suitable preparation to assess pressor activity. In this preparation both plasma taken at rest and during a hot flush had a very small effect on the blood pressure as it can be seen in Figure 21 which shows the trace of a typical experiment of this series. The preparation in this experiment responded to 5 ng of adrenaline, 2.5 ng noradrenaline and stimulation of the spinal sympathetic outflow at a frequency of 0.2 H_2 at supramaximal voltage and at 1 msec pulse with 18 mm Hg, 16 mm Hg and 11 mm Hg increases in systolic pressure respectively.

Subsequent addition of propranolol (0.2 mg/kg) and phentolamine (0.4 mg/kg) 5 minutes before the addition of the pressor stimuli, markedly reduced the responses to adrenaline, noradrenaline and stimulation of the spinal sympathetic outflow, as it can be seen in Figure 22 , but it left the responses to plasma unaffected. This suggested that the pressor responses to plasma at rest or during a hot flush were not likely to be due to catecholamines. It is possible that these responses were due to a volume effect as the same volume (0.2 ml) of saline produced responses of similar amplitude.

2. Investigation of whole blood bradykinin and prostaglandin-like activity

Prostaglandins and kinins have been largely implicated in flushes exhibited by patients suffering from certain pathological

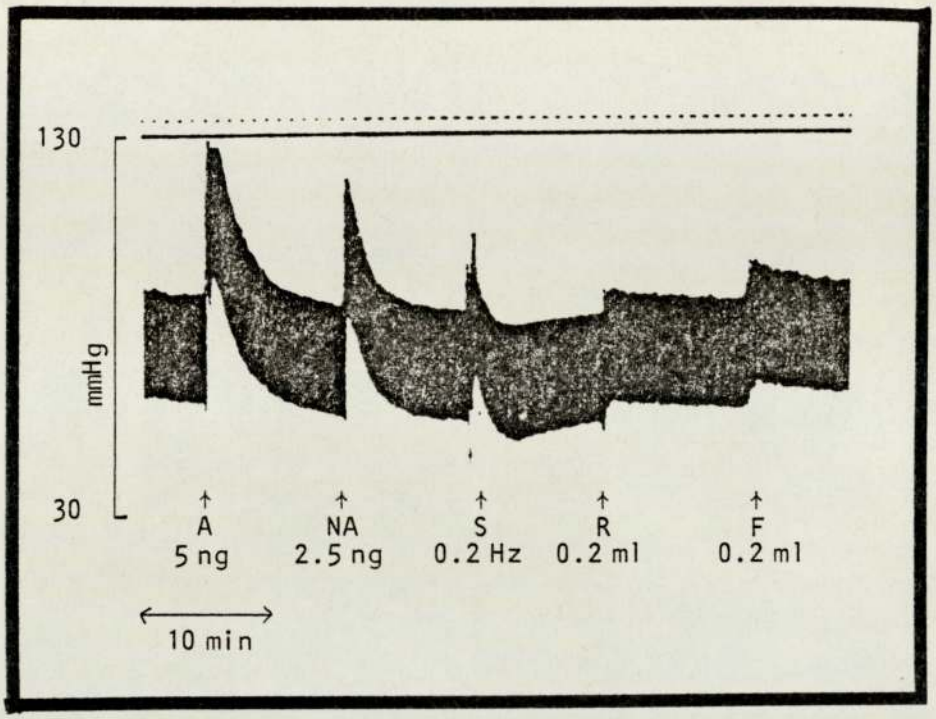


FIGURE 21: The pressor responses in mm Hg of the pithed rat preparation to 5 ng/kg adrenaline (A), to 2.5 ng/kg noradrenaline (NA), to electrical stimulation(s) (0.2 Hz) and to 0.2 ml of plasma from a post-menopausal woman at rest (R) and during a hot flush (F)

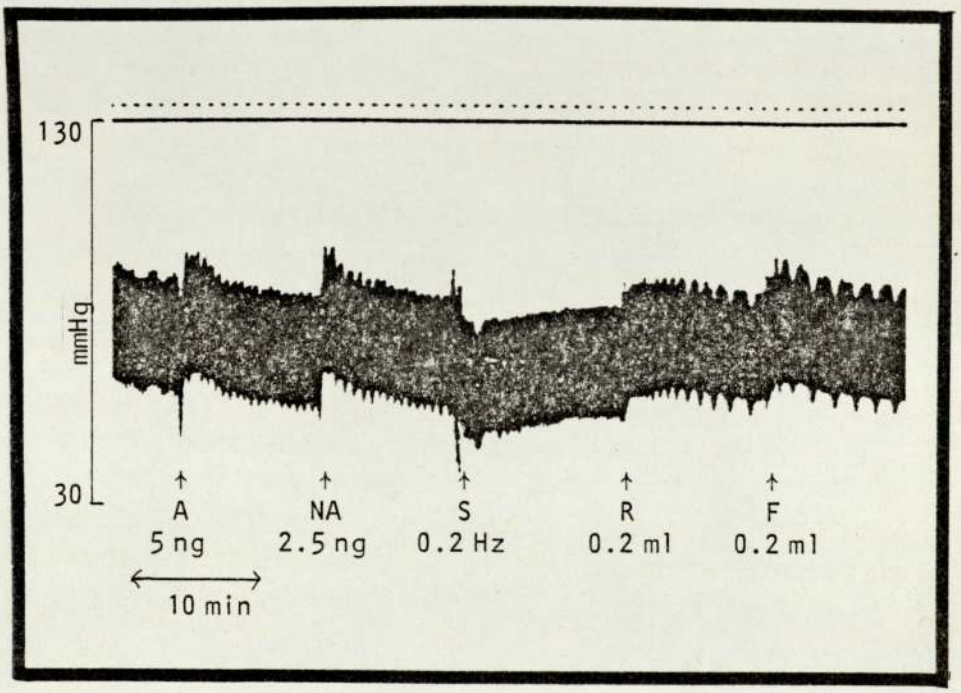


FIGURE 22: The effect of propranolol (0.2 mg/kg) and phentolamine (0.4 mg/kg) on the pressor responses in mm Hg of the pithed rat to 5 ng/kg adrenaline (A), to 2.5 ng/kg noradrenaline (NA), to electrical stimulation(s) (0.2 Hz) and to 0.2 ml of plasma from the same post-menopausal woman as in Figure 21, at rest (R) and during a hot flush (F).

conditions (page 25). These substances are produced locally, at their site of action, in very small amounts and are very short lived. To examine their possible involvement in the mechanism of the menopausal hot flush, bradykinin and prostaglandin-like activity was measured at rest and during a hot flush, using extractions followed by bioassay of the extracts on isolated smooth muscle preparations.

It was hoped that the use of extraction methods immediately after the collection of blood samples would prevent the destruction of the active substances prior to assay. The calculated recoveries (see Methods, pages 64, 65) provided evidence that these extraction procedures gave a good yield of the substances under examination.

2.1: Determination of whole blood prostaglandin E-like activity in blood samples of post-menopausal women taken at rest and during flushing

Total prostaglandin E-like activity expressed as prostaglandin E_1 activity was determined in whole blood after extraction by a randomised 2 x 2 assay on the isolated rat fundic strip. The prostaglandin E-like (PGE-like) nature of the reconstituted extracts was confirmed by blocking both responses to extracts and to prostaglandin E_1 with SC-19220 (Bennet *et al* 1971), a prostaglandin receptor antagonist at a concentration of 5×10^{-5}M.

Figure 23 shows the PGE-like content of blood samples taken at rest and during flushing, expressed as ngPGE_1/ml of blood. It was found that the PGE-like activity did not differ significantly between the blood samples taken at rest and those taken during flushing. The concentrations were 10.5 ± 3.0 ng/ml of blood ($n = 5$) and 12.2 ± 1.0 ng/ml of blood ($n = 5$) for the blood samples taken at

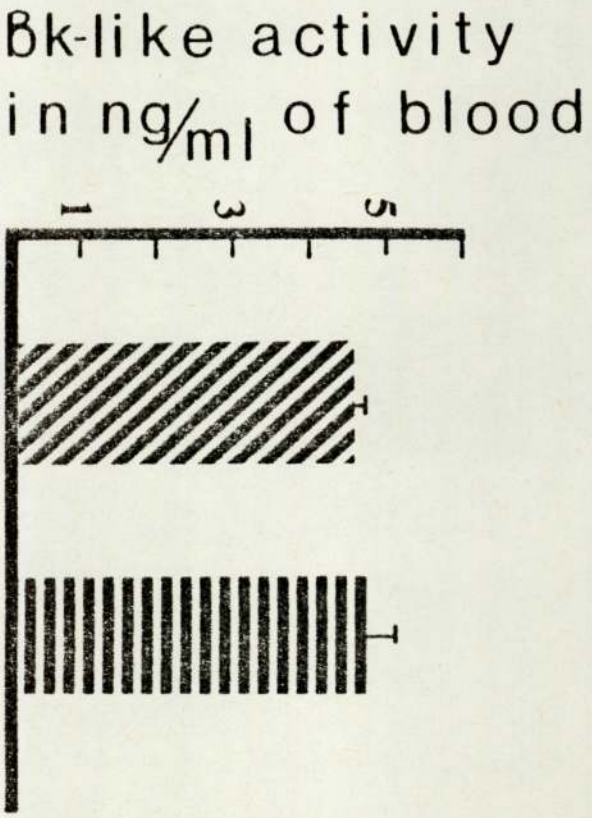


FIGURE 24: Whole blood bradykinin (Bk)-like activity in ng/ml of blood taken from post-menopausal women at rest and during a hot flush. Vertical bars are the SE of the means (n = 5).

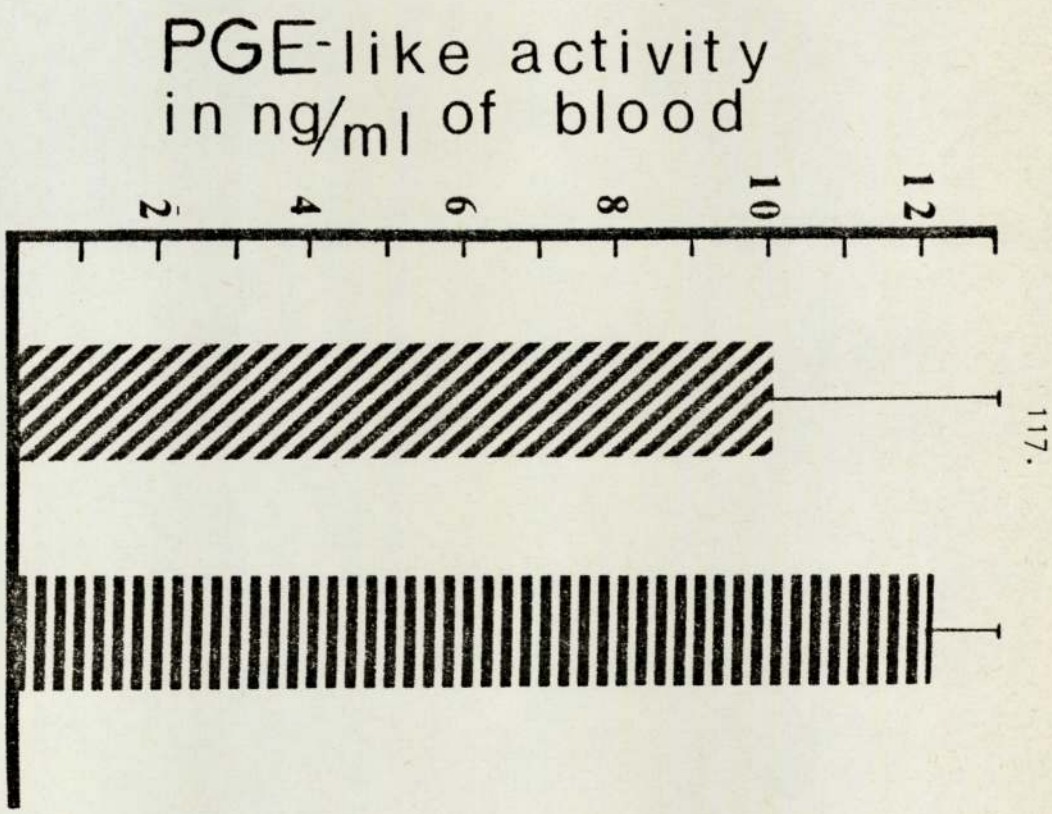


FIGURE 23: Whole blood prostaglandin E-like activity in ng/ml of blood taken from post-menopausal women at rest and during a hot flush. Vertical bars are the SE of the means (n - 5).

rest and during flushing respectively. These concentrations were within the physiological range established by the bioassay method used (Greaves and McDonald 1972).

2.2: Determination of whole blood kinin-like activity in blood samples of post-menopausal women taken at rest and during flushing

Total kinin-like activity, expressed as bradykinin (BK)-like activity, was determined in whole blood by an extraction procedure followed by a randomised 2 x 2 assay on the isolated rat oestrous uterus. The kinin-like nature of the reconstituted extracts was confirmed by prior incubation 37°C for 30 minutes with chymotrypsin (1.2×10^{-5}M) which abolished responses to both standard BK and to the extracts.

Figure 24 shows the kinin-like content of blood samples taken at rest and during flushing expressed as ng/ml of blood. No significant difference was found in the kinin-like activity of blood samples taken at rest and during flushing. The concentrations were 4.5 ± 0.2 ng/ml ($n = 5$) of blood and 4.6 ± 0.5 ng/ml ($n = 5$) of blood for the blood samples taken at rest and during flushing respectively. These concentrations were within the physiological range established by the bioassay method used (Zeitlin and Brocklehurst 1967).

3. Summary

The results of the investigation of the biological activity of plasma in pre-menopausal women and whole blood and plasma in post-menopausal women, provided evidence for the following:

a) The contractile activity of the plasma, from post-menopausal women and during a hot flush and of pre-menopausal women at rest and during thermal vasodilatation, was similar when tested directly on the rat fundic strip.

b) A major portion of this activity, but not all of it, is due to 5-hydroxytryptamine as the experiments on the methysergide-blocked rat fundic strip showed.

c) The remaining activity not due to 5-hydroxytryptamine is significantly smaller in the plasma of post-menopausal women at rest compared to that during a hot flush. In the pre-menopausal women the remaining activity is similar at rest and during thermal vasodilatation. This latter result suggested that the mechanism accounting for thermal vasodilatation (inhibition of vaso-constrictor tone, or active vasodilatation, see page 46) did not affect the biological activity of plasma on the rat fundic strip. Therefore an alternative mechanism would perhaps be responsible for the difference in biological activity of plasma in post-menopausal women at rest compared to that during a hot flush.

d) Acetylcholine, histamine and catecholamines did not seem to account for the observed difference.

e) The whole blood levels of prostaglandin E- and bradykinin-like activity were similar at rest and during a hot flush and they are within the physiological range established for the methods of determination used.

Possibilities of a mechanism for the observed difference in biological activity of the plasma of post-menopausal women at rest and during a hot flush are discussed in the General Discussion.

Unfortunately, the present study had to finish 15 months after its commencement because of the lack of patients and as mentioned in the introduction to the project many questions that required the use of human subjects remained unresolved.

We proceeded, however, to examine the possible mechanism underlying the hot flush in relation to the hormonal status of the post-menopausal woman. The following two sections describe the results of this investigation.

B

THE EFFECT OF 17β-OESTRADIOL ON THE SYMPATHETICALLY INNERVATED SMOOTH MUSCLE

In the present section the effect of 17β-oestradiol on the sympathetically innervated vascular and non-vascular smooth muscle was examined in order to test the 'trigger hypothesis' already discussed in the Introduction in detail. The results presented here concern experiments carried out on:

(a) The rat isolated vas deferens preparation

(b) The female pithed rat preparation

1: THE EFFECT OF 17β-OESTRADIOL ON THE RESPONSES OF THE ISOLATED RAT VAS DEFERENS TO TRANSMURAL STIMULATION, TO NORADRENALINE AND TO POTASSIUM CHLORIDE

In this series of experiments the effect of 17β-oestradiol was investigated upon the contractions of the isolated vas deferens, evoked by the release of the endogenous transmitter, by exogenous noradrenaline and by a direct depolarising agent, potassium chloride. The effect of 17β-oestradiol on each of the three stimuli, was examined separately in three different sets of experiments.

Control responses to transmural stimulation, to noradrenaline and to potassium chloride were obtained at the beginning of each experiment for each set of experiment. After these responses became reproducible, 17β-oestradiol (15 μg/ml) was added to the bathing fluid and the contractions were elicited in its presence. When the effect of 17β-oestradiol on the responses was established it was then removed from the bathing fluid and the responses were again elicited. Five minutes elapsed between any consecutive additions of stimulation, noradrenaline or potassium chloride. The responses to the three stimuli were reproducible for two hours (with no significant increase or decrease).

The responses were expressed as a percentage of the appropriate control responses obtained prior to the addition of 17β-oestradiol.

1. The effect of 17β-oestradiol upon the responses of the isolated rat vas deferens to transmural stimualtion

The isolated rat vas deferens preparation was stimulated at supramaximal voltage (90 - 120 V) and at a fixed frequency of 25 shocks/second using 0.1 - 0.5 Msec pulse width. These parameters

have been shown to stimulate directly the nerves at the post-ganglionic site but not the smooth muscle itself (Birmingham and Wilson 1963).

Figures 25 a,b show the effect of 17β-oestradiol on the control responses produced by transmural stimulation and how these responses recovered after 17β-oestradiol was removed from the bathing fluid, in a typical experiment of this series.

It can be seen that 17β-oestradiol caused an acute decline of the control responses which became maximal 23 minutes after the addition of 17β-oestradiol (Figure 25a). When 17β-oestradiol was removed responses started recovering rapidly and the recovery was complete 40 minutes after the removal of 17β-oestradiol.

Figure 26 shows the maximal decline obtained in the presence of 17β-oestradiol and the maximal recovery after its removal, in a total of five experiments. It can be seen that the responses declined to $23.7 \pm 5.7\%$ ($n = 5$) during exposure to 17β-oestradiol and that they subsequently recovered to $104.7 \pm 6.5\%$ ($n = 5$) of the control responses.

2. <u>The effect of 17β-oestradiol upon the responses of the isolated rat vas deferens to addition of noradrenaline 2×10^{-4}M</u>

Figure 27 shows the effect of 17β-oestradiol upon the control responses to exogenous noradrenaline (2×10^{-4}M) and how they recovered after 17β-oestradiol was removed from the bathing fluid in a typical experiment of this series. It can be seen that 17β-oestradiol caused an acute decline in the control responses which was maximal immediately after the addition of 17β-oestradiol. When 17β-oestradiol was removed the responses started

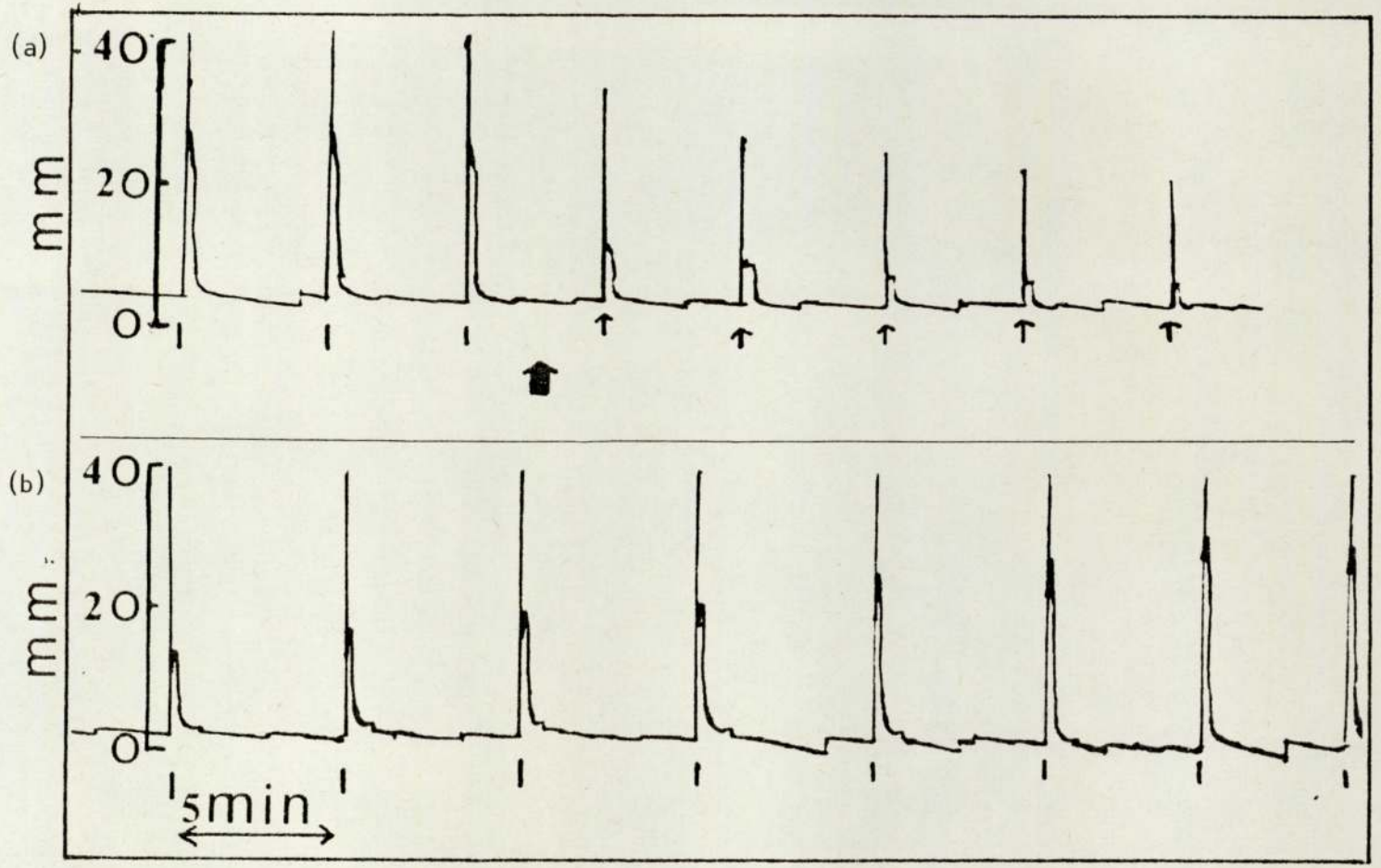


FIGURE 25: The trace of a typical experiment of this series showing the effect of 17β-oestradiol (15 μg/ml) upon control responses to transmural stimulation (25 shocks/sec, 0.2 msec, 90 V) of the rat vas deferens (top panel) and recovery of these responses after removal of 17β-oestradiol from the bathing fluid (lower panel). I: transmural stimulation in the absence of 17β-oestradiol; ↑: transmural stimulation in the presence of 17β-oestradiol; ⬆ : introduction of 17β-oestradiol to the bathing fluid.

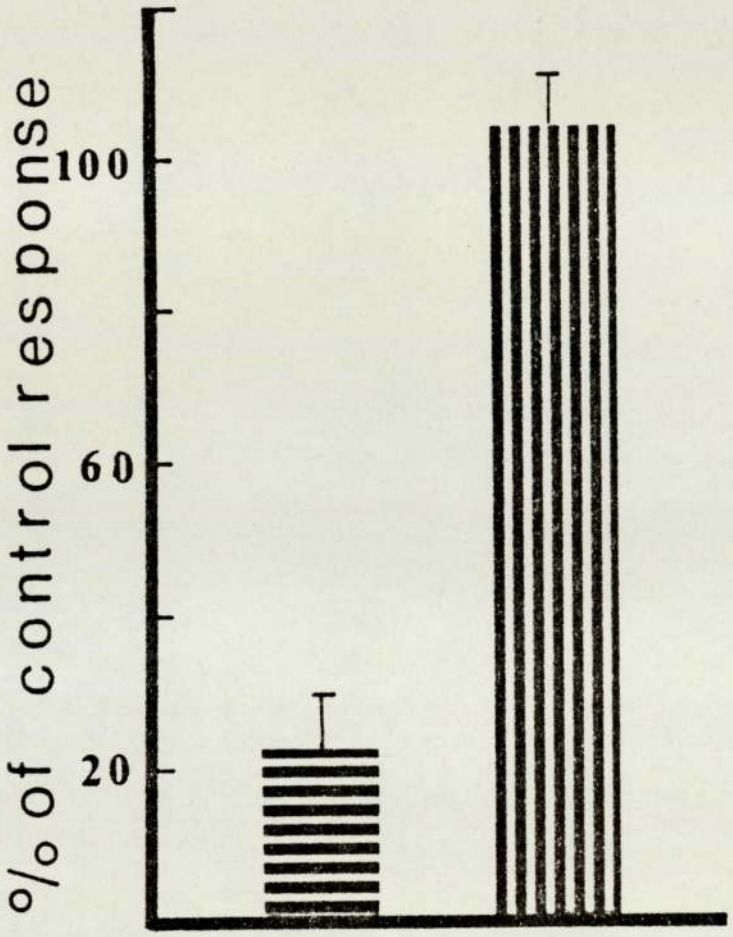


FIGURE 26: The effect of 17β-oestradiol (15 µg/ml) upon control responses to transmural stimulation (25 shocks/sec, 0.1 - 0.5 msec, 90 - 120 V) of the rat vas deferens. ▤ : Maximal decline of the control response in the presence of 17β-oestradiol. ▥: Maximal recovery after the removal of 17β-oestradiol. Ordinate scale: percentage of the control response that remained after addition of 17β-oestradiol and during recovery. Vertical bars are the SE of the means (n = 5).

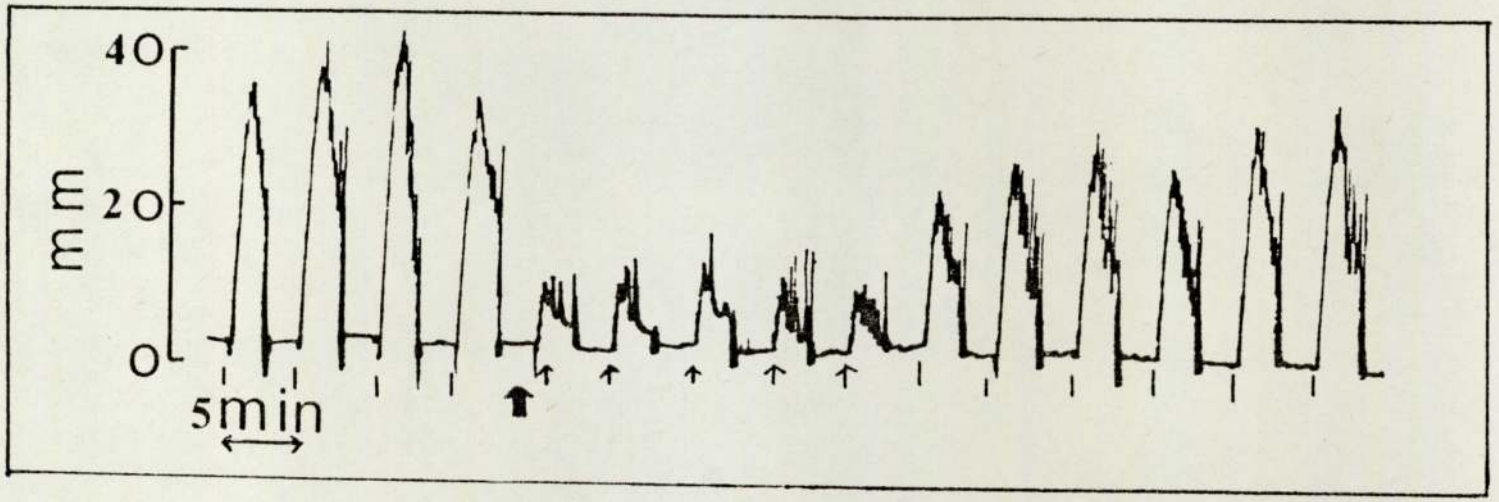


FIGURE 27: The trace of a typical experiment of this series showing the effect of 17β-oestradiol (15 μg/ml) upon the control responses of the rat vas deferens to noradrenaline (NA) (2×10^{-4}M) (top panel) and recovery of these responses after the removal of 17β-oestradiol from the bathing fluid (lower panel). I: NA responses in the absence of 17β-oestradiol; ↑: NA responses in the presence of 17β-oestradiol; ⬆ : introduction of 17β-oestradiol to the bathing fluid

to recover and the recovery reached a stable level 60 minutes after the removal of 17β-oestradiol from the bathing fluid.

Figure 28 shows the maximal decline in the presence of 17β-oestradiol and the maximal recovery in a total of five experiments. It can be seen that the responses declined to 30.2 ± 9.4% (n = 5) and recovered to 95.9 ± 6.7% (n = 5) of the control responses.

3. The effect of 17β-oestradiol upon the responses of the isolated rat vas deferens to potassium chloride 8×10^{-2}M

Figures 29 a,b show the effect of 17β-oestradiol upon the control responses to potassium chloride (8×10^{-2}M) and how they recovered after the removal of 17β-oestradiol during a representative experiment of this series.

It can be seen that 17β-oestradiol completely abolished the responses to potassium chloride 12 minutes after its addition. When 17β-oestradiol was removed from the bathing fluid the responses began to recover but the recovery was still incomplete 60 minutes after the removal of 17β-oestradiol from the bathing fluid.

Figure 30 shows the maximal decline in the presence of 17β-oestradiol and the maximal recovery in a total of five experiments. It can be seen that the responses were completely abolished and that they only recovered to 67.7 ± 9% (n = 5) of the control responses, prior to addition of 17β-oestradiol.

This maximal decline in responses to potassium chloride was significantly greater than the maximal decline in the responses to transmural stimulation and to exogenous noradrenaline in the presence of 17β-oestradiol ($p < 0.05$). Similarly, the maximal recovery in responses to potassium chloride was significantly smaller than the recovery in the responses to transmural stimulation and to exogenous noradrenaline at the 5% level of significance.

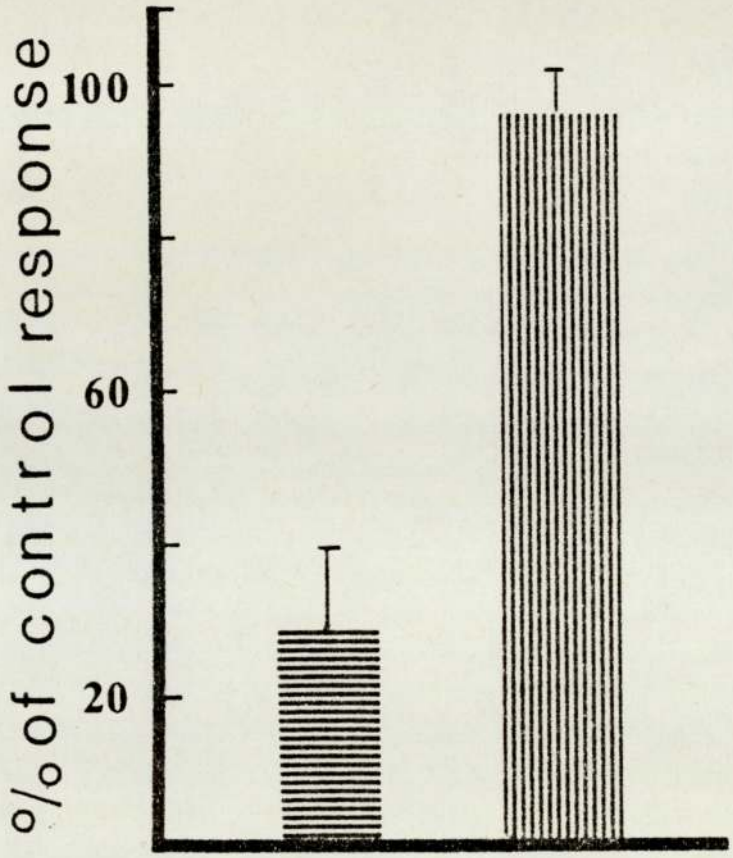


FIGURE 28: The effect of 17β-oestradiol (15 µg/ml) upon control responses of the rat vas deferens to noradrenaline (2×10^{-4}M). ▤ : Maximal decline of the responses to NA in the presence of 17β-oestradiol. ▥ : Maximal recovery after removal of 17β-oestradiol. Ordinate scale: percentage of the control response that remained after addition of 17β-oestradiol and during recovery. Vertical bars are the SE of the means (n = 5).

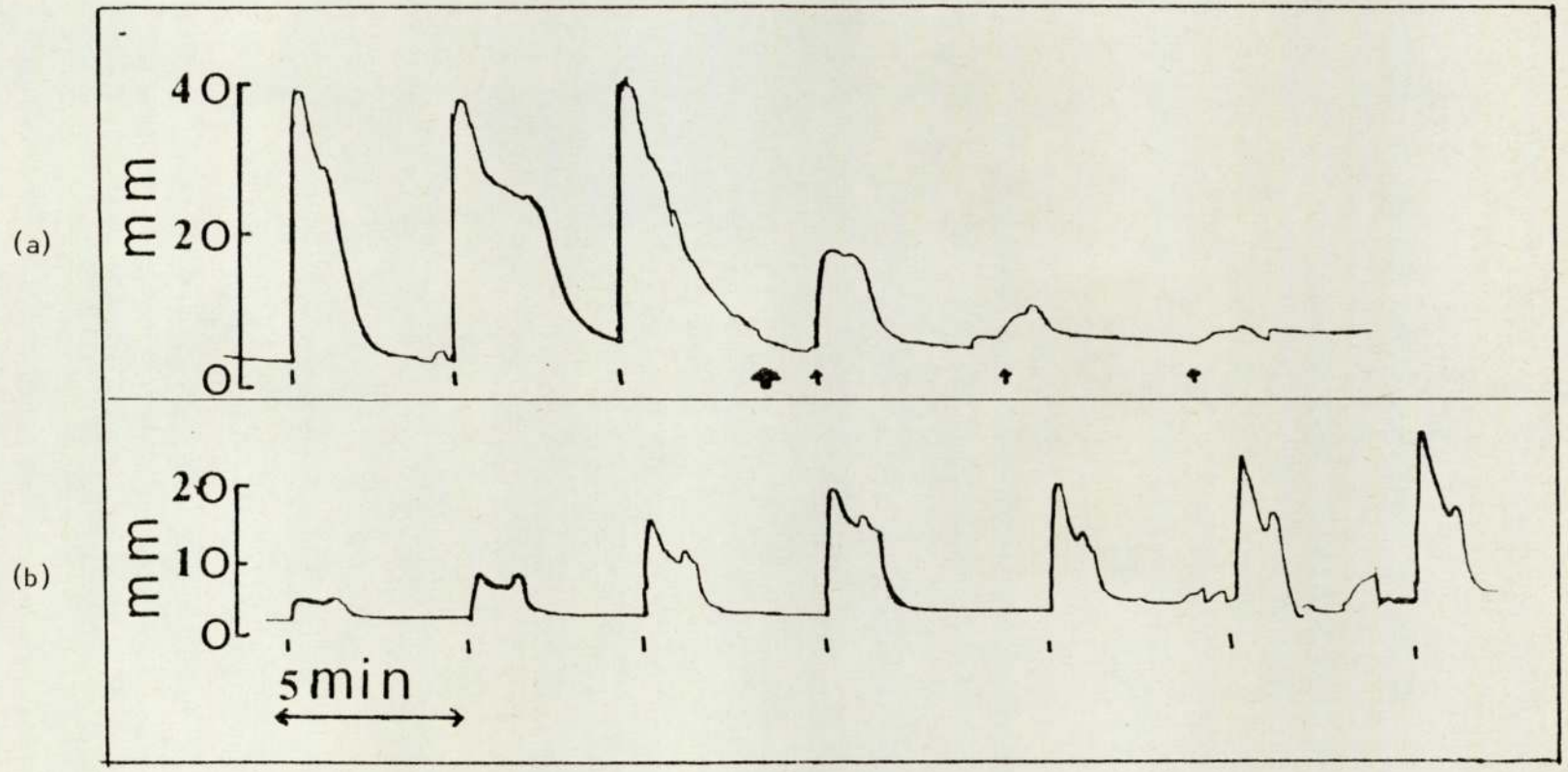


FIGURE 29: The trace of a typical experiment of this series showing the effect of 17β-oestradiol (15 μg/ml) upon the control responses of the rat vas deferens to potassium chloride (KCl) (8×10^{-2}M) (top panel) and recovery of these responses after the removal of 17β-oestradiol from the bathing fluid (lower panel). ↓: KCl responses in the absence of 17β-oestradiol; ↑ : KCl responses in the presence of 17β-oestradiol; ⬆ : introduction of 17β-oestradiol to the bathing fluid.

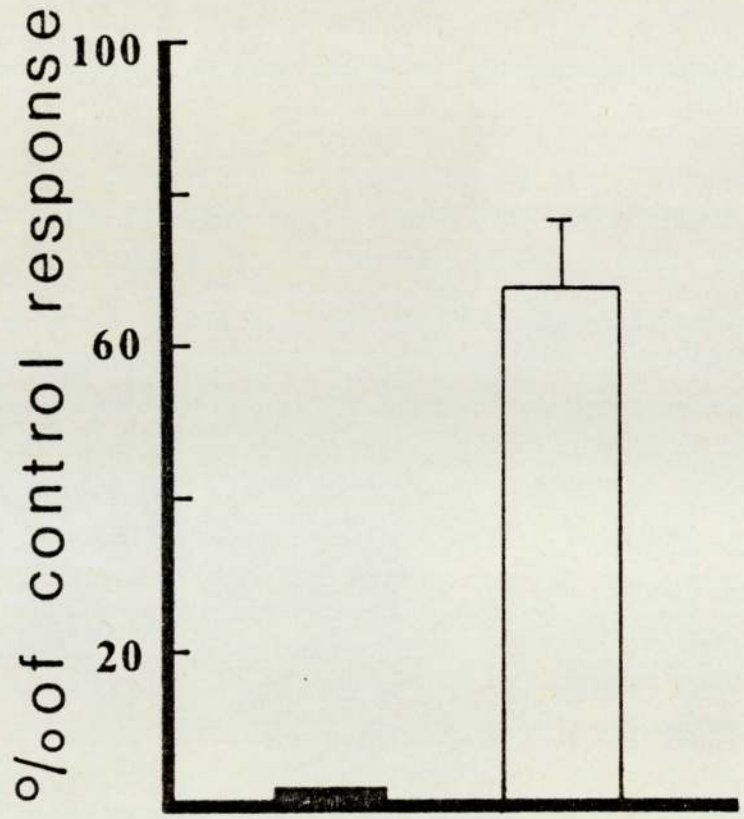


FIGURE 30: The effect of 17β-oestradiol (15 µg/ml) upon control responses of the rat vas deferens to potassium chloride (KCl) (8×10^{-2}M). ▬ : Maximal decline of the responses to KCl in the presence of 17β-oestradiol. □ : Maximal recovery after removal of 17β-oestradiol. Ordinate scale: percentage of the control response that remained after addition of 17β-oestradiol and during recovery. Vertical bars are the SE of the means (n = 5).

4. Summary

In summary, 17β-oestradiol (15 µg/ml) caused a great reduction in the responses of isolated rat vas deferens to transmural stimulation, to noradrenaline, and to potassium chloride. The responses to potassium chloride were reduced to a significantly greater degree than the responses to the other two stimuli. Responses to all three stimuli started recovering after the removal of 17β-oestradiol from the bathing fluid. However, although the responses to transmural stimulation and to noradrenaline showed complete recovery, the recovery of the responses to potassium chloride was incomplete and significantly lower than those of the responses to the other two stimuli.

It therefore appeared that 17β-oestradiol does affect the response of non-vascular adrenergically innervated smooth muscle, to various stimuli. The possible mechanism responsible for this effect is discussed in the General Discussion (page 176).

2: THE PRESSOR RESPONSES OF THE FEMALE PITHED RAT TO STIMULATION OF THE SPINAL SYMPATHETIC OUTFLOW, TO NORADRENALINE AND TO ANGIOTENSIN AT VARIOUS STAGES OF THE OESTRUS CYCLE, AFTER BILATERAL OVARIECTOMY AND SHAM-OVARIECTOMY AND AFTER INJECTION OF 17β-OESTRADIOL

In this series of experiments it was hoped to create an animal model to examine whether variations in circulating ovarian hormones (as they are known to occur during the perimenopause, see page 21) affect the responsiveness of the blood vessels to various stimuli. The aim of these experiments was to test whether the hot flush may be 'triggered' by such fluctuations (see page 21) in ovarian hormones, through an alteration in the vasomotor balance.

The animal model was the female pithed rat where pressor responses to stimulation of the spinal sympathetic outflow, to noradrenaline and to angiotensin were studied. These responses were studied at various stages (oestrus, dioestrus and ovariectomy) when different amounts of ovarian hormones were circulating in the blood and following intravenous administration of 17β-oestradiol.

Effects on pressor responses to stimulation of the spinal sympathetic outflow would provide information on possible interference with release of noradrenaline from the nerve endings and/or reception at the post-synaptic side.

Those on responses to noradrenaline would provide information on interference with the post-synaptic site exclusively.

Finally, effects on responses to angiotensin, which is a non-adrenergic stimulus would provide information on possible interference with the ability of the vascular smooth muscle to contract.

1. The basal systolic pressure and heart rate of intact and sham-ovariectomised at oestrus and dioestrus and of ovariectomised female pithed rats

Approximately 30 minutes after the pithed rat preparation had been set up and before the beginning of the experiment, the systolic blood pressure was noted. This blood pressure is referred to as basal systolic blood pressure.

Figure 31 shows the basal systolic blood pressure in mm of Hg in intact oestrous and dioestrous, ovariectomised, and sham-ovariectomised, oestrous and dioestrous, female pithed rats. The basal systolic blood pressures were expressed as mean ± SE of 10 observations.

It can be seen that all blood pressures were similar and these were:

a) intact rats at oestrus: 67.6 ± 4.4 (n = 10) mm Hg

b) intact rats at dioestrus: 69.2 ± 3.3 (n = 10) "

c) ovariectomised rats: 62.8 ± 3.4 (n = 10) "

d) sham-ovariectomised rats at oestrus: 65.1 ± 3.8 (n = 10) "

e) sham-ovariectomised rats at dioestrus: 66.4 ± 3.4 (n = 10) "

Similarly, the basal diastolic blood pressures of the same groups of animals, recorded as described previously, were not significantly different and they exhibited a pattern similar to that seen in basal systolic blood pressure of these animals. Thus, it appeared that the concentrations of circulating oestrogen had no significant effect upon the basal systolic and diastolic blood pressure of these animals. (The values for the basal systolic pressures were considered appropriate to be presented as all the pressor responses are expressed as % resting systolic pressure.)

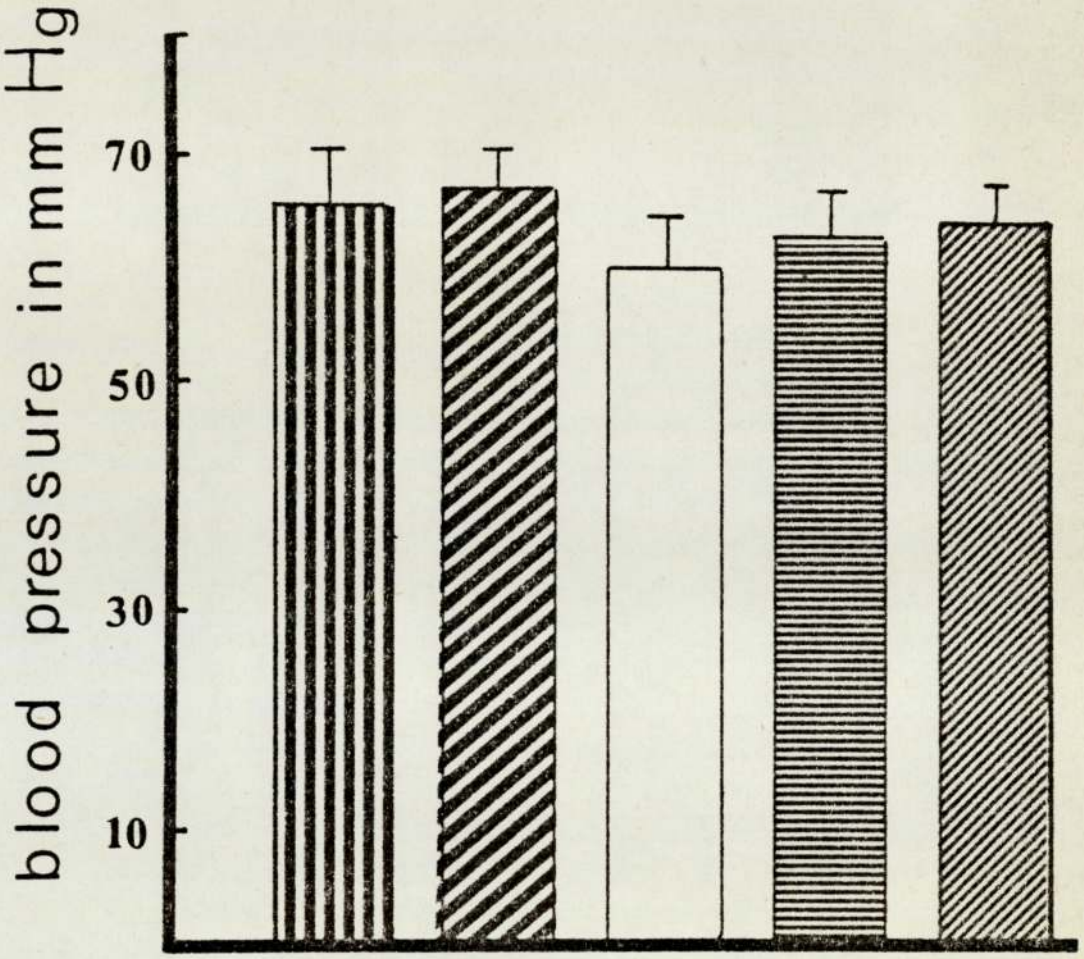


FIGURE 31: The basal systolic pressure (in mmHg) in the intact oestrous ▥, and dioestrous ▨ in the ovariectomised □ and in the sham-ovariectomised oestrous ▤ and dioestrous pithed rat ▨ Vertical bars are the SE of the means (n = 10).

Heart rate was also recorded at the same time as the basal blood pressure as described previously. Figure 32 shows the heart rate in beats per minute in intact, ovariectomised and sham-ovariectomised female pithed rats. Since the heart rate did not appear to vary with the stage of the oestrous cycle, the intact oestrous and dioestrous rats were grouped together and so were the sham-ovariectomised oestrous and dioestrous rats. The heart rates were expressed as the mean ± SE of 10 observations.

It can be seen that although in intact and sham-ovariectomised rats the heart rates were similar, and these were 376.6 ± 10.6 ($n = 10$) beats/minute and 360 ± 14.1 ($n = 10$) beats/minute, the heart rate in ovariectomised rats (325 ± 12 {$n = 10$}) was significantly lower when compared with that in intact ($p < 0.01$) and sham-ovariectomised ($p < 0.05$) rats. The lower heart rate in the ovariectomised rats suggested that complete lack of oestrogen might affect cardiac function.

2. A comparison of the pressor responses of the intact, ovariectomised and sham-ovariectomised pithed rat, to varying amounts of noradrenaline and angiotensin (25 - 200 ng/kg) and to varying frequencies of stimulation of the spinal sympathetic outflow (0.1 - 0.8 Hz)

The stimulus/pressor response relationship to nerve stimulation, noradrenaline and angiotensin was investigated in intact (oestrous and dioestrous), ovariectomised and sham-ovariectomised pithed rats. These relationships are not presented in the form of dose/response curves as the amounts or frequencies of stimuli used only cover mean percentage increases of the resting systolic pressure up to 90% and do not cover the whole range of responses necessary for a complete dose/response curve. Pressor responses in excess of this (90%) shortened the life of the pithed rat preparation.

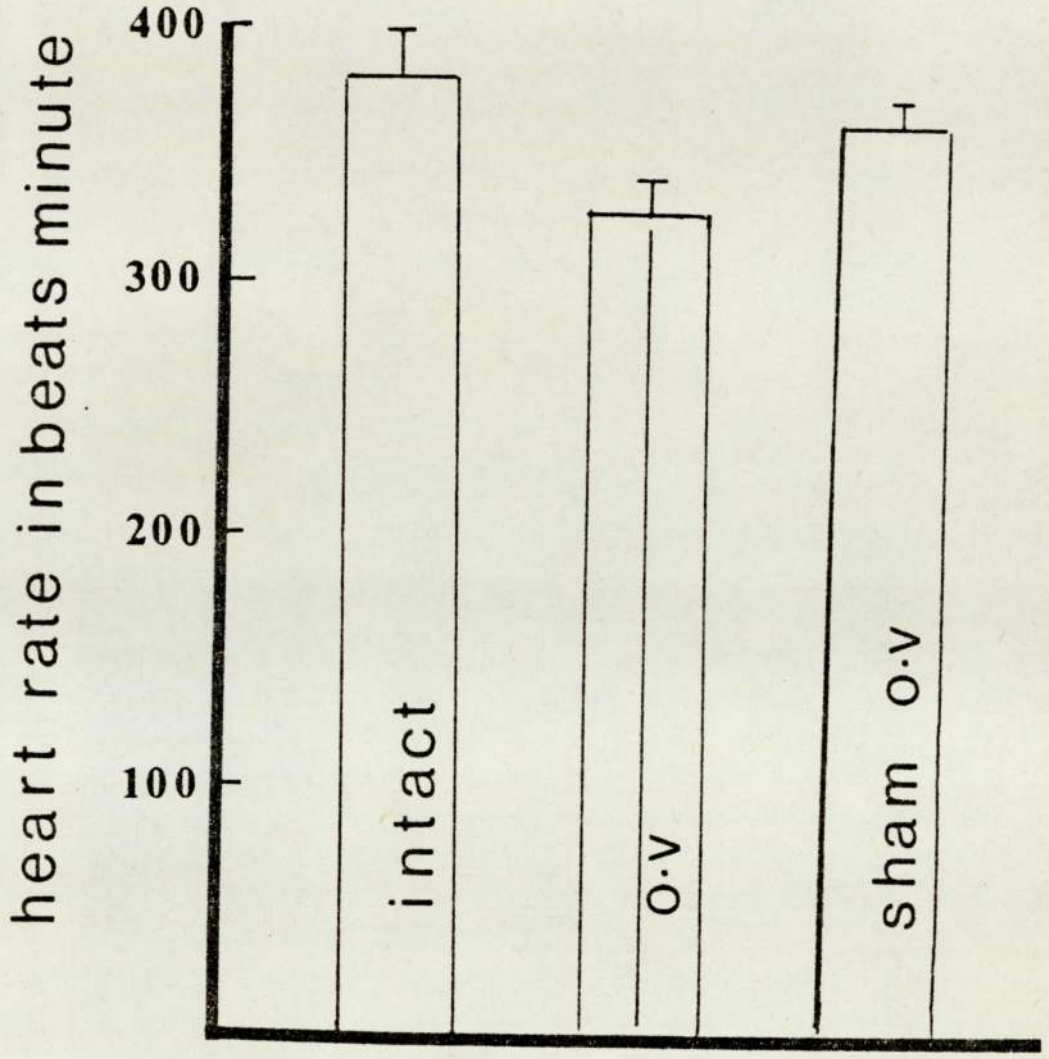


FIGURE 32: The heart rate (in beats/min) in the intact (oestrous + dioestrous) , ovariectomised (O.V) and sham-ovariectomised (oestrous + dioestrous) pithed rat.

Figure 33 shows the pressor responses to varying frequencies of stimulation of the spinal sympathetic outflow at fixed supra-maximal voltage and 1 msec pulse width. These pressor responses are expressed as mean ± SE % increase of the resting systolic pressure (n = 5). No significant differences can be seen in the pressor responses of intact pithed rats (oestrous and dioestrous) compared with those of ovariectomised or sham-ovariectomised (oestrous and dioestrous), at any frequency used.

Figure 34 shows the pressor responses (expressed as mean ± SE % increase of resting systolic pressure, n = 5) to 25, 50, 100 and 200 ng/kg of noradrenaline in the same groups of animals. It can be seen that there were no significant differences in the pressor responses to noradrenaline in intact (oestrous and dioestrous) compared with ovariectomised or sham-ovariectomised (oestrous and dioestrous) pithed rats.

Finally, Figure 35 shows the pressor responses (expressed as mean ± SE % increase of resting systolic pressure, n = 5) to 25, 50, 100 and 200 ng/kg of angiotensin in the same groups of animals. Again, all the responses were similar at all concentrations used in all the groups of animals.

Thus it appeared that the stimulus/pressor response did not vary significantly with the stage of the oestrous cycle or ovariectomy. These stimulus/pressor response relationships were constructed at the beginning of each experiment after the initial 30-minute period that the preparation was allowed to settle down after pithing.

The effect of time and 17β-oestradiol on the pressor responses of the intact (oestrous and dioestrous) ovariectomised

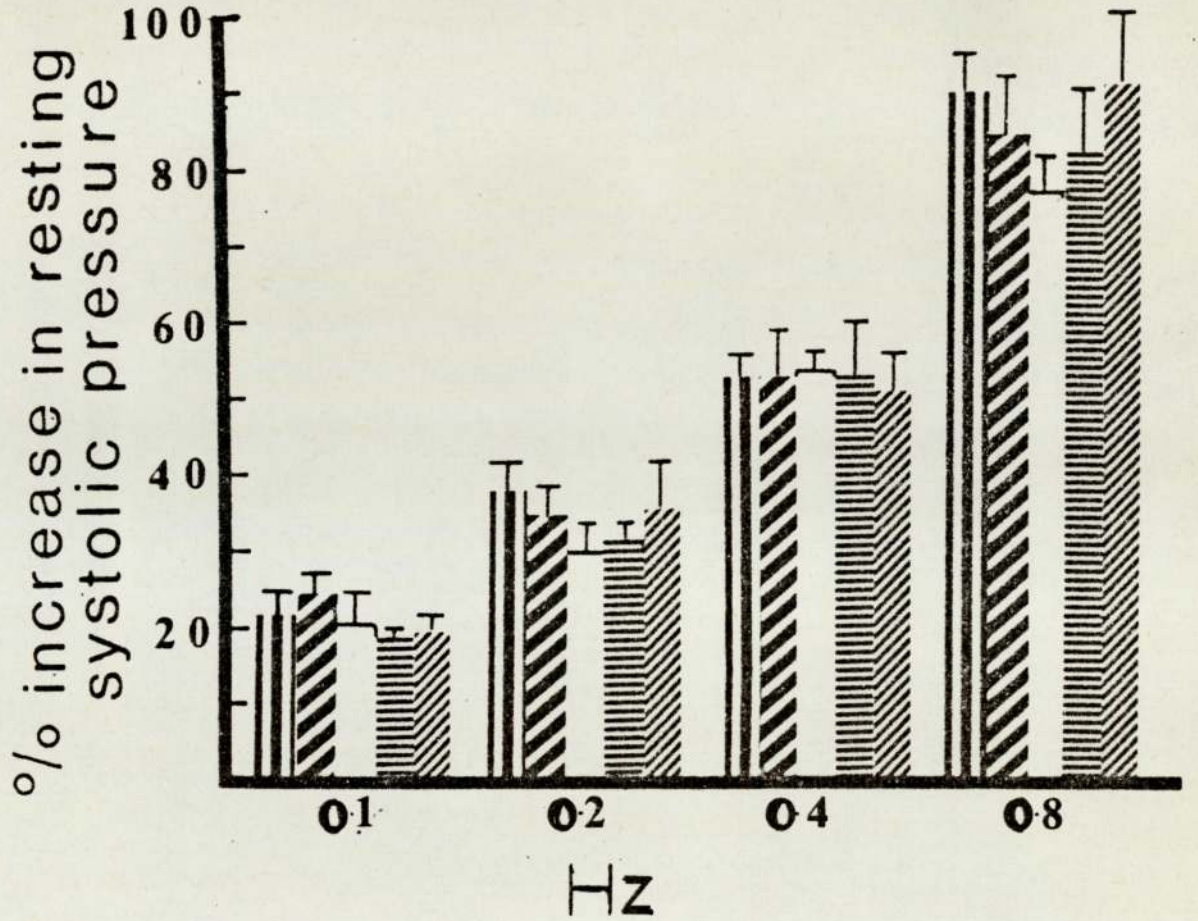


FIGURE 33: The stimulus/pressor response relationship to varying frequencies (0.1, 0.2, 0.4, 0.8 Hz) of stimulation of the spinal sympathetic outflow at fixed supramaximal voltage and at 1 msec pulse width, in the intact oestrous and dioestrous, ovariectomised and sham-ovariectomised oestrous and dioestrous pithed rat. Responses are expressed as percentage increase in resting systolic pressure ± SE of the mean (n = 5).

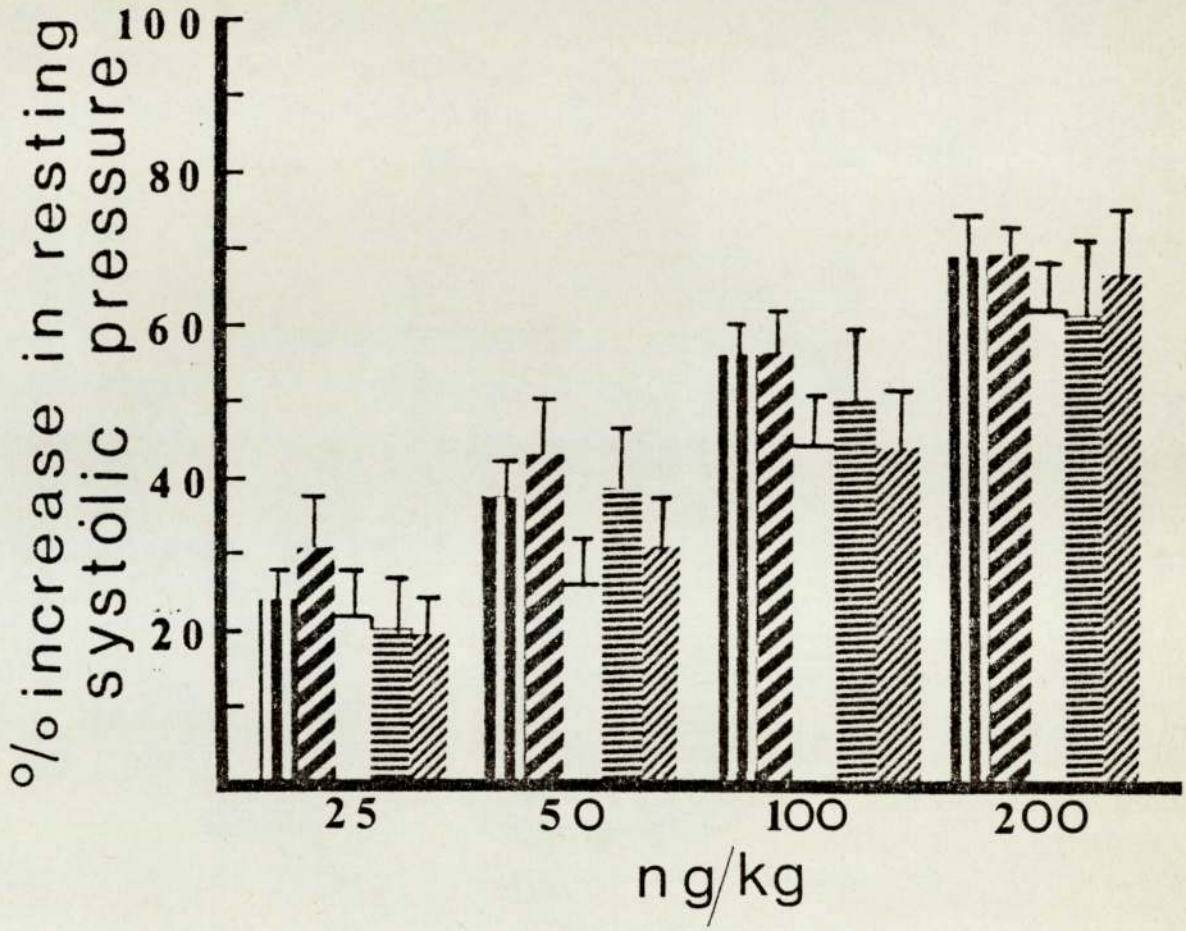


FIGURE 34: The dose/pressor response relationship to noradrenaline (NA) (25, 50, 100, 200 ng/kg) in the intact oestrous ▥ and dioestrous ▨, ovariectomised □, and sham-ovariectomised oestrous ▤ and dioestrous ▨ pithed rat. Responses are expressed as % increase in resting systolic pressure ± SE of the mean (n = 5).

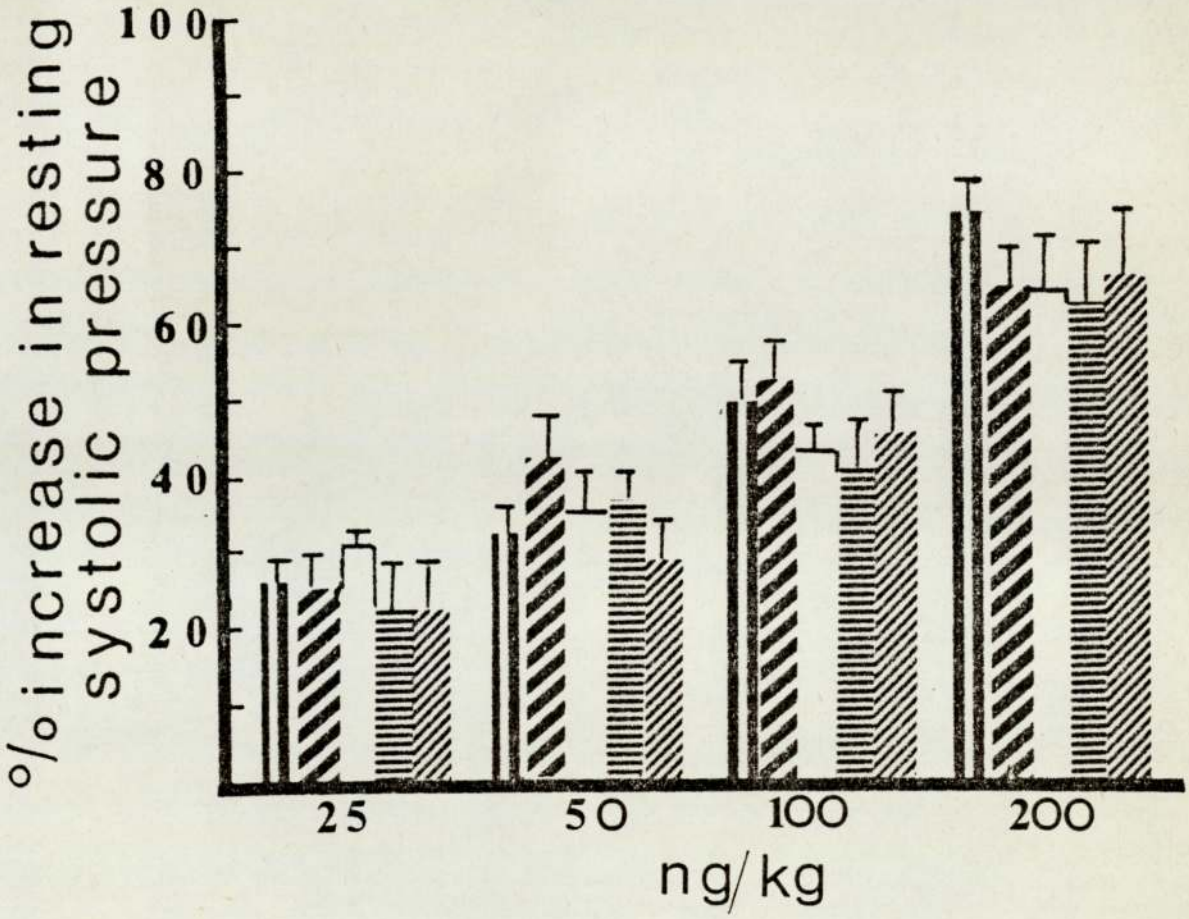


FIGURE 35: The dose/pressor response relationship to angiotensin (25, 50, 100, 200 ng/kg) in the intact oestrous and dioestrous, ovariectomised and sham-ovariectomised oestrous and dioestrous pithed rat. Responses are expressed as % increase in resting systolic pressure ± SE of the mean (n = 5).

and sham-ovariectomised (oestrous and dioestrous) pithed rat to stimulation of the spinal sympathetic outflow, to noradrenaline and to angiotensin were subsequently investigated.

3. The effect of time and of 17β-oestradiol on the pressor responses of the intact, ovariectomised and sham-ovariectomised pithed rat to stimulation of the spinal sympathetic outflow, to noradrenaline and to angiotensin

3.1: General experimental protocol

At the beginning of each experiment (in all experiments of this series) stimulus/pressor response relationships to stimulation of the spinal sympathetic outflow, to noradrenaline and to angiotensin were constructed. A suitable amount of stimulus (for nerve stimulation, noradrenaline and angiotensin) producing approximately 50% increase of the resting systolic pressure was chosen and repeated six times throughout the experiment. The order of the application of the stimuli was:

stimulation of the spinal sympathetic outflow,
noradrenaline ,
angiotensin.

A single application of all three stimuli in a consecutive manner formed a cycle. Therefore, each experiment consisted of six cycles. Five minutes elapsed between consecutive stimuli.

3.2: The effect of time

Six cycles of pressor responses were studied in the intact, (oestrous and dioestrous), ovariectomised and sham-ovariectomised pithed rat, in order to establish a pattern for these responses during the 90-minute period that the experiments lasted.

(a) The pressor responses to stimulation of the spinal sympathetic outflow to noradrenaline and angiotensin in the intack pithed rat at oestrus and at dioestrus

Figures 36 a, b, c show the pressor responses during the recorded six cycles, to stimulation of the spinal sympathetic outflow, to noradrenaline and to angiotensin in the intact pithed rat at oestrus and dioestrus.

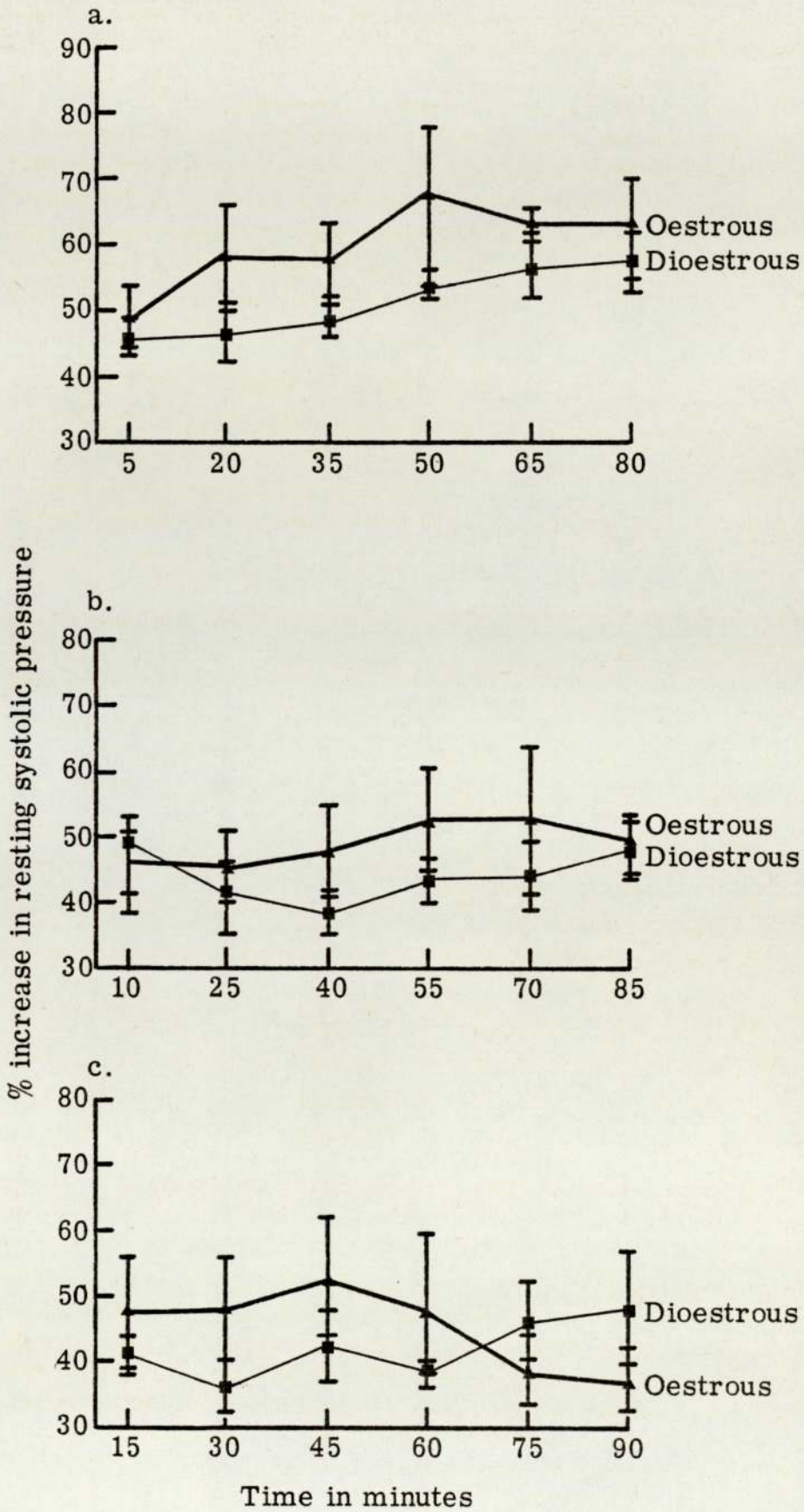
It can be seen that the responses to stimulation (Figure 36a) showed some increase with time in both oestrous and dioestrous animals. However, the pressor responses in oestrous animals showed a bigger increase (but not statistically significant), than those in dioestrous animals.

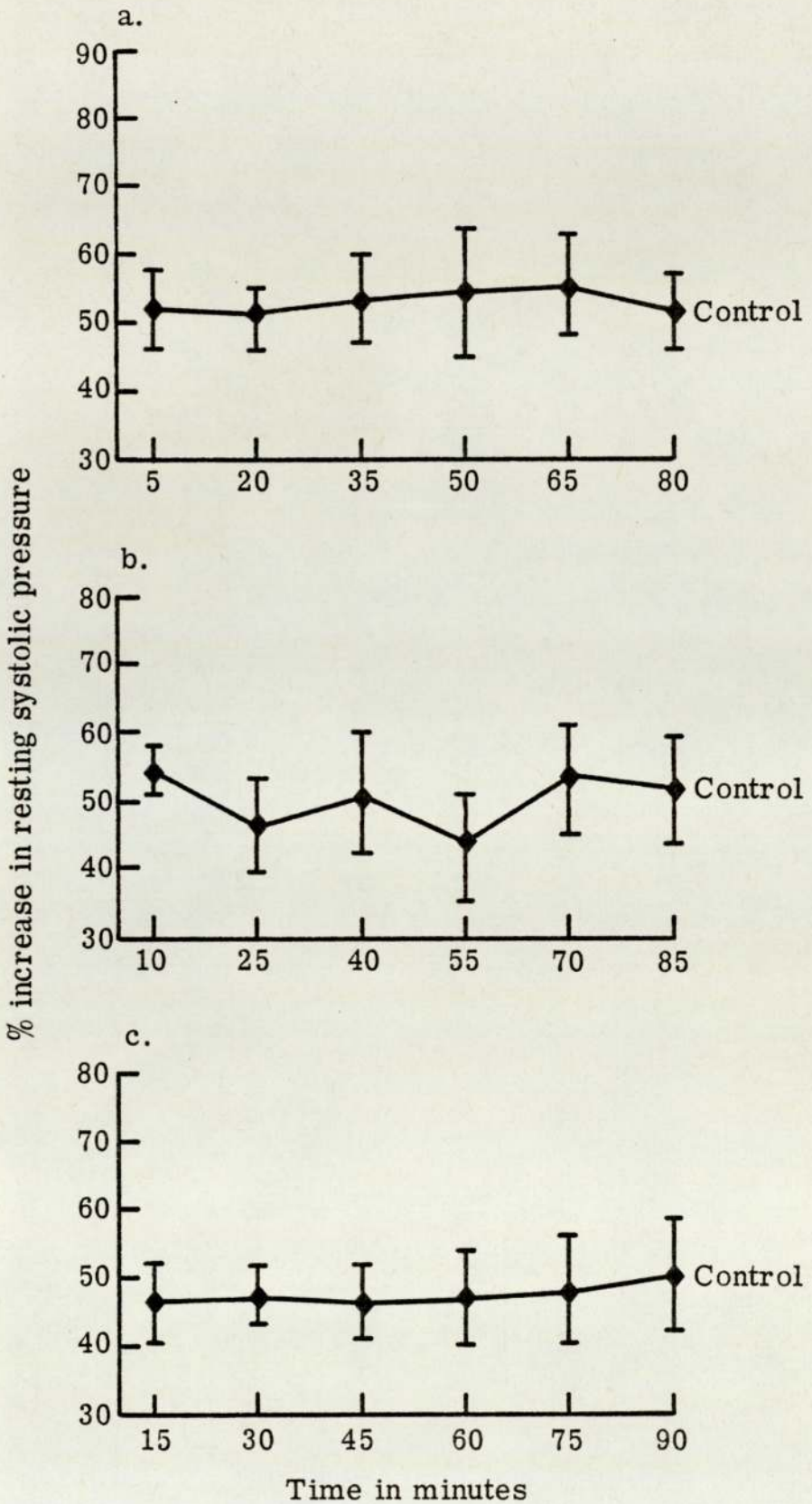
The responses to noradrenaline (Figures 36 b, c) and to angiotensin also showed some variation with time but no clear pattern can be seen. Again there was no significant difference between responses at oestrus compared with those at dioestrus.

(b) The pressor responses to stimulation of the spinal sympathetic outflow to noradrenaline and angiotensin in the ovariectomised pithed rat

Figures 37 a, b, c show the pressor responses during the recorded six cycles, to stimulation of the spinal sympathetic outflow, to noradrenaline and to angiotensin in the ovariectomised pithed rat.

It can be seen that the responses to all three stimuli remained fairly reproducible after 90 minutes. They appeared to vary less with time than the pressor responses in the intact animal (Figure 37 a, b, c).


a.
90
80
70
60
50
40
30
5
20
35
50
65
80
Oestrous
Dioestrous
(a)
b.
80
70
60
50
40
30
10
25
40
55
70
85
Oestrous
Dioestrous
(b)
c.
80
70
60
50
40
30
15
30
45
60
75
90
Dioestrous
Oestrous
(c)
% increase in resting systolic pressure
Time in minutes


a.
90
80
70
60
50
40
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5
20
35
50
65
80
Control
b.
80
70
60
50
40
30
10
25
40
55
70
85
Control
c.
80
70
60
50
40
30
15
30
45
60
75
90
Control
% increase in resting systolic pressure
Time in minutes

(a)

(b)

(c)

(c) The pressor responses to stimulation of the spinal sympathetic outflow, to noradrenaline and angiotensin in the sham-ovariectomised pithed rat at oestrus and dioestrus

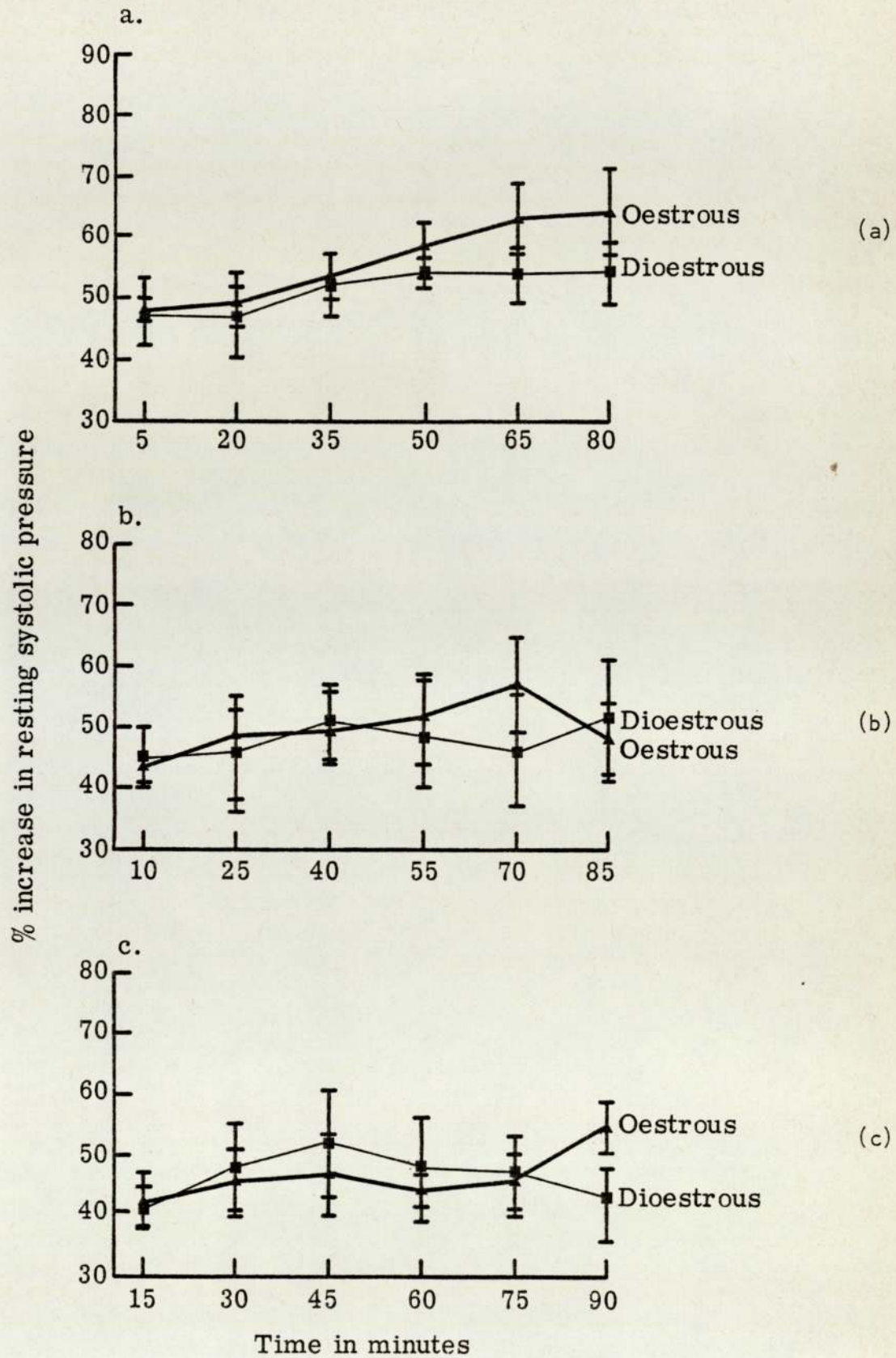
Figures 38 a, b, c show the pressor responses, during the recorded six cycles, to stimulation of the spinal sympathetic outflow, to noradrenaline and to angiotensin.

It can be seen that the responses to stimulation (Figure 38a) show a similar pattern to those seen in intact oestrous and dioestrous animals (Figure 36a, page 142) which confirmed that sham-operation did not have any significant effect on the responsiveness of the preparation.

The responses to noradrenaline and angiotensin again varied slightly with time but with no significant increase or decrease (Figures 38 b, c).

After a pattern of responses had been established during the six recorded cycles of responses in the various groups of animals, the effect of exogenous 17β-oestradiol was examined on this pattern of responses.

Initially the effect of 10 ng/kg of 17β-oestradiol was examined. This amount of 17β-oestradiol is approximately 100 times the normal circulating levels during oestrous and 25 during dioestrous. However this hormone is reduced with metabolism when it enters the circulation (Tapper and Brown-Grant 1974) and therefore such an amount may be considered physiological. It was important to chose an amount that would be considered physiological as in this series of experiments it was attempted to devise a model to study the effect of normal fluctuations of 17β-oestradiol


a.
90
80
70
60
50
40
30
5
20
35
50
65
80
Oestrous
Dioestrous
(a)
b.
80
70
60
50
40
30
10
25
40
55
70
85
Dioestrous
Oestrous
(b)
c.
80
70
60
50
40
30
15
30
45
60
75
90
Oestrous
Dioestrous
(c)
% increase in resting systolic pressure
Time in minutes

as they occur at the perimenopause (page 21) on the reactivity of the vascular smooth muscle, in order to test the 'trigger hypothesis' (see page 3).

In preliminary experiments it was found that a single dose of 10 ng/kg of 17β-oestradiol affected the pattern of the pressor responses, as it is described later in the present section. It was then examined whether a further addition of the same amount 30 minutes (ie after two cycles) after the initial addition had a further effect and it was found that it did have an effect. A further third addition after another 30 minutes had no effect. It was therefore decided to adapt the following protocol for this set of experiments:

Two cycles of control responses were recorded, and then an injection of 17β-oestradiol (10 ng/kg) was given. Another two cycles were recorded and 17β-oestradiol (10 ng/kg) was given again. Finally, another two cycles were recorded.

The responses to all three stimuli as previously discussed (pages 141, 144) did not appear to vary significantly with the stage of the oestrous cycle as seen in Figures 36, 37, 38.

However, the responses to stimulation in both intact and sham-ovariectomised animals at oestrus appear to be greater (although not statistically significant) towards the end of the experiment, when compared with those at dioestrus. When 17β-oestradiol was added (according to the protocol mentioned previously), the responses to both groups of animals did not exhibit this difference any more, and showed a very similar pattern during the whole experiment. This was not totally unexpected as the added 17β-oestradiol probably

normalised any differences in the responses due to the variation of this hormone during the oestrous cycle. Therefore, in this set of experiments oestrous and dioestrous animals were grouped together.

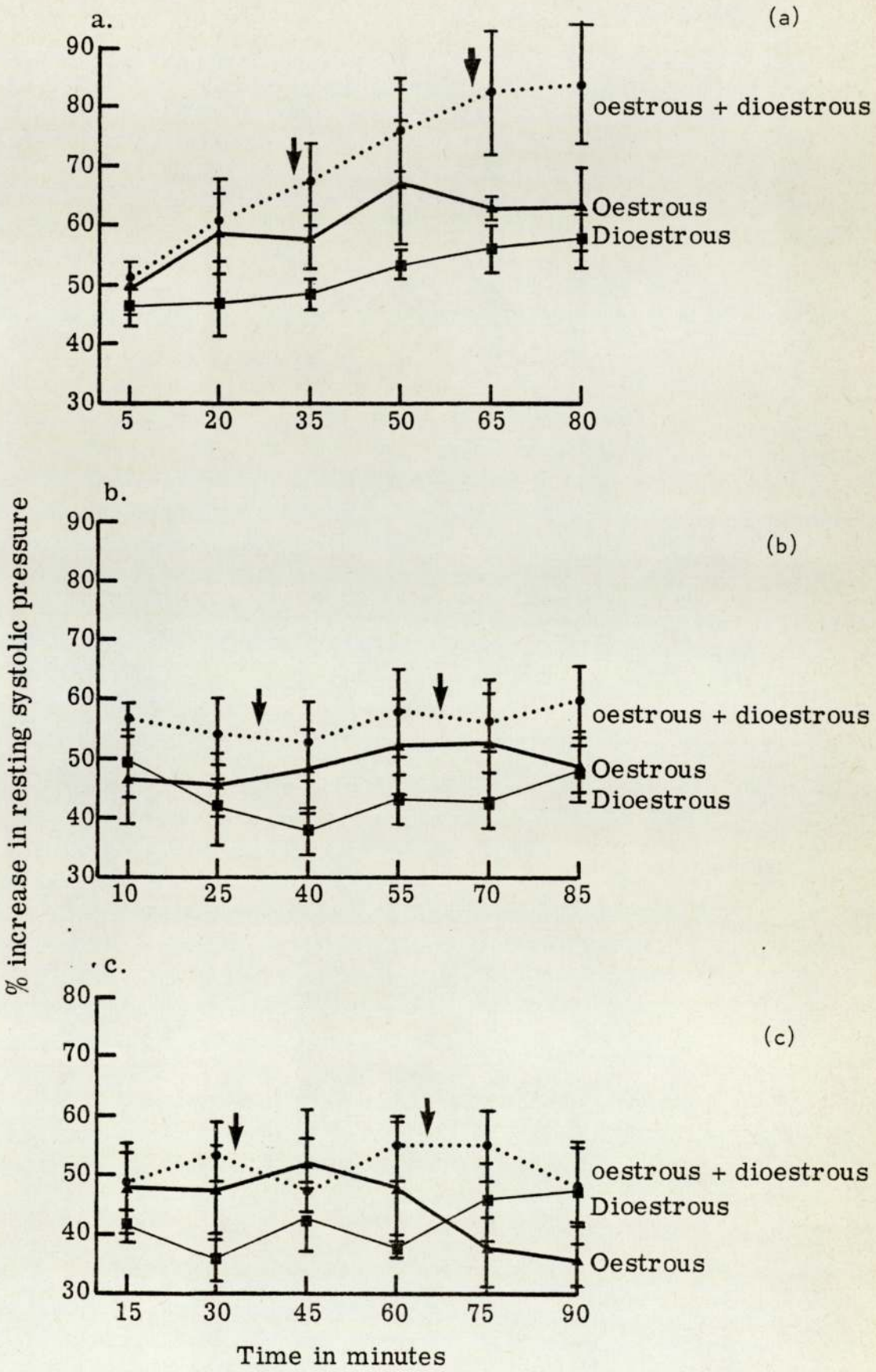
3.3: The effect of two additions of 17β-oestradiol (10 ng/kg) on the pressor responses to stimulation of the spinal sympathetic outflow, to noradrenaline and to angiotensin in:

(a) The intact pithed rat

Figures 39 a, b, c show the effect of two additions of 17β-oestradiol (10 ng/kg) on the pressor responses to stimulation of the spinal sympathetic outflow to noradrenaline and angiotensin in the intact female pithed rat (oestrous and dioestrous animals grouped together, see page 146). The pressor responses to the three stimuli at oestrus and dioestrus, in the absence of any exogneous 17β-oestradiol, are also shown for purposes of comparison.

17β-oestradiol caused an increase in the pressor responses to stimulation of the spinal sympathetic outflow compared with those seen at oestrus and dioestrus (Figure 39 a). This increase was statistically significant ($p < 0.05$) when the responses following the addition of 17β-oestradiol were compared with those seen in dioestrous animals, but not in oestrous animals, and was established immediately after the first addition of 17β-oestradiol, ie at the point corresponding to 35 minutes time as shown in Figure 39 a. The responses at this point were 66 g ± 7.2% ($n = 5$) in the oestradiol treated animals, and 48.6 g ± 2% ($n = 5$) in the dioestrous animals.

The responses to noradrenaline and angiotensin were not significantly affected by the addition of 17β- oestradiol at any point of the experiment (Figures 39 b, c) when compared with


a.
(a)
90
80
70
60
50
40
30
5
20
35
50
65
80
oestrous + dioestrous
Oestrous
Dioestrous
b.
(b)
90
80
70
60
50
40
30
10
25
40
55
70
85
oestrous + dioestrous
Oestrous
Dioestrous
% increase in resting systolic pressure
c.
(c)
80
70
60
50
40
30
15
30
45
60
75
90
oestrous + dioestrous
Dioestrous
Oestrous
Time in minutes

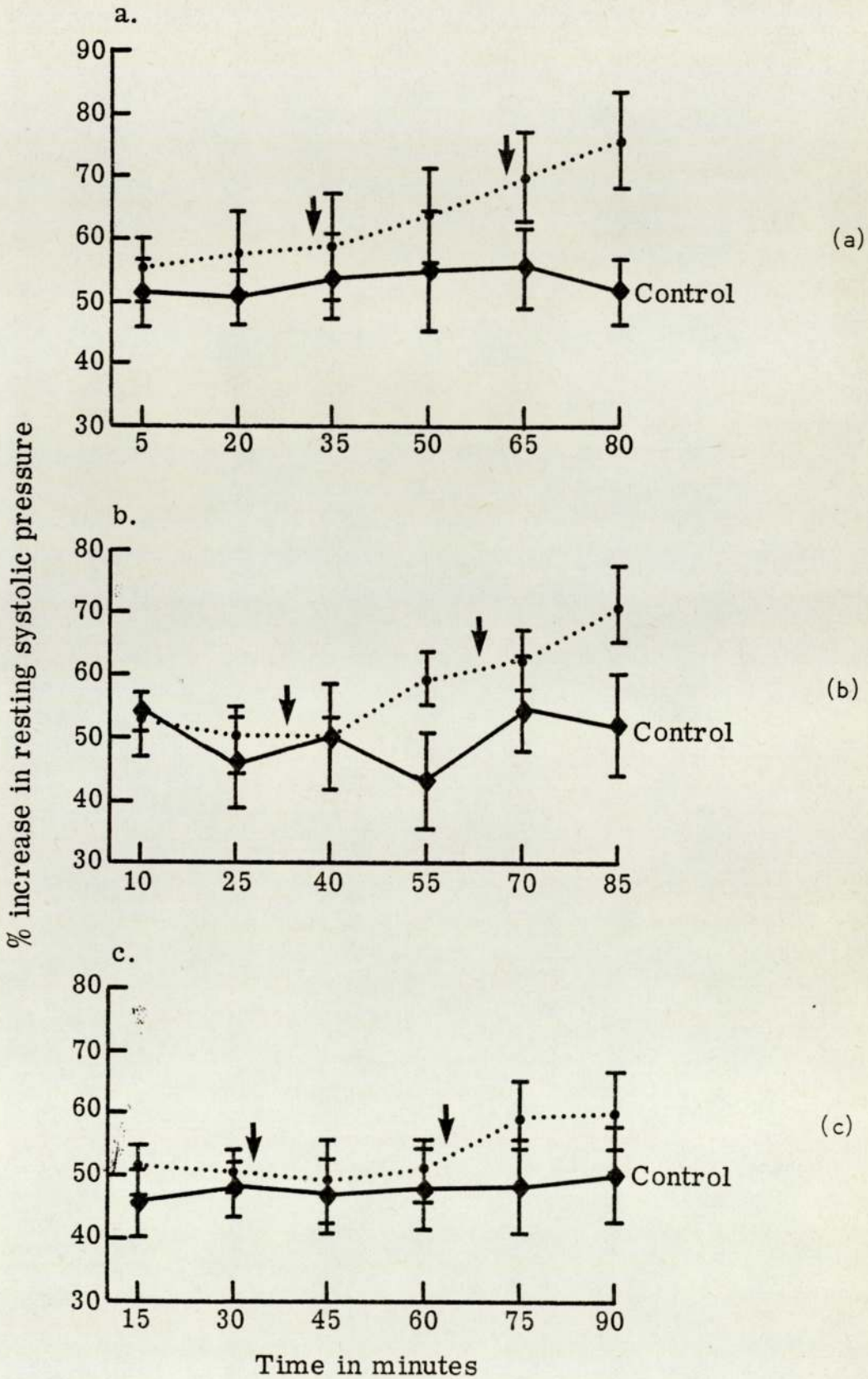
those in oestrous and dioestrous animals in the absence of any exogenous 17β-oestradiol.

(b) The ovariectomised pithed rat

Figures 40 a, b, c show the effects of two additions of 17β-oestradiol (10 ng/kg) on the pressor responses to stimulation of the spinal sympathetic outflow, to noradrenaline and to angiotensin in the ovariectomised pithed rat. The pressor responses in the absence of any exogenous 17β-oestradiol are also shown for purposes of comparison.

The pressor responses to stimulation of the spinal sympathetic outflow showed some increase after the addition of 17β-oestradiol when compared with those in the absence of any exogenous 17β-oestradiol but this increase was smaller than that seen in the intact animals. Furthermore, this increase was statistically significant ($p < 0.05$) only 15 minutes after the second addition of 17β-oestradiol, ie at the point corresponding to 80 minutes time as shown in Figure 40a. At this point, the pressor responses were $75.2 \pm 8.3\%$ ($n = 5$) in animals treated with 17β-oestradiol, and $51.5 \pm 5.5\%$ ($n = 5$) in animals in the absence of any exogenous 17β-oestradiol, of the resting systolic pressure. Thus it can be seen that an increase took considerably longer to establish in the ovariectomised animals compared with the intact. It also required two additions of 17β-oestradiol, whereas the increase was already statistically significant after the first addition of 17β-oestradiol.

The responses to noradrenaline (Figure 40 b) and angiotensin (Figure 40c) did not show any statistically significant increase at any point of the experiment.


a.
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65
80
Control
(a)
b.
80
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10
25
40
55
70
85
Control
(b)
c.
80
70
60
50
40
30
15
30
45
60
75
90
Control
(c)
% increase in resting systolic pressure
Time in minutes

The sham-ovariectomised animals responded in a similar manner to the intact animals to an addition of 17β-oestradiol which again confirmed that any difference in the biological behaviour between intact and ovariectomised animals was not due to the actual operation. It was not considered necessary to present these results in this section as they do not provide any additional or different evidence to that provided by the intact animals. Individual values for pressor responses of this group of animals are given in the Appendix.

The results presented in this section so far indicated that two additions of 17β-oestradiol (10 ng/kg) caused the pressor responses to stimulation of the spinal sympathetic outflow, to increase both in intact and ovariectomised animals, though to a different extent. It was then examined whether increasing the amount of 17β-oestradiol, added as a single dose, would have the same or a greater effect of the same direction (ie increase). The amount of 17β-oestradiol selected in this series of experiments was 100 ng/kg which is five times the total amount added in the previous series of experiments. This amount was given after two control cycles to the three stimuli. Subsequently another four cycles were recorded. Oestrous and dioestrous animals were again grouped for the same reason mentioned previously (page 146).

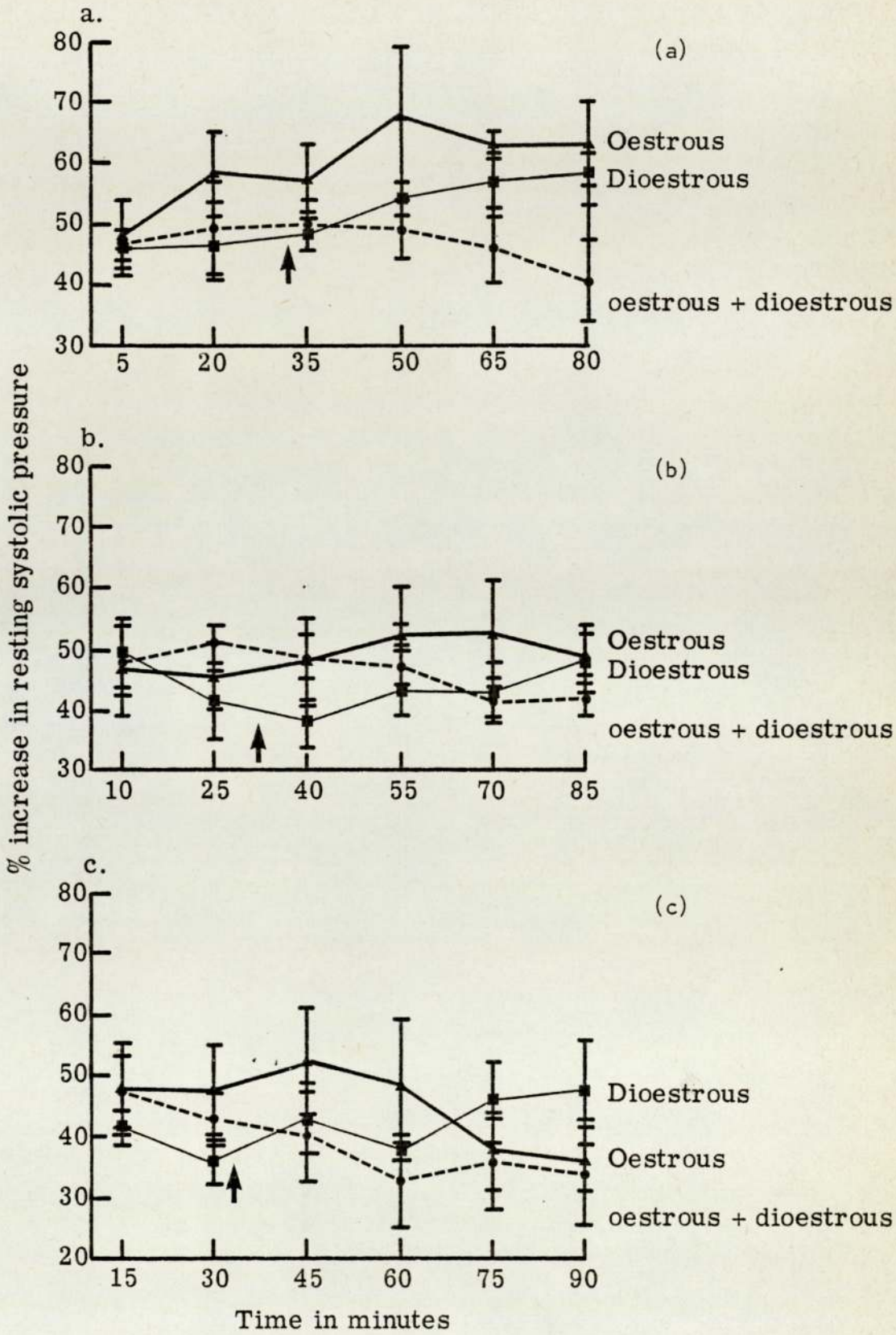
The effect of a single addition of 17β-oestradiol (100 ng/kg) on the pressor responses to stimulation of the spinal sympathetic outflow, to noradrenaline and to angiotensin in:

1) The intact pithed rat

Figures 41 a, b, c show the effect of a single addition of 17β-oestradiol (100 ng/kg) on the pressor responses to stimulation of the spinal sympathetic outflow, to noradrenaline and to angiotensin, in the intact female pithed rat. Responses at oestrus and dioestrus, in the absence of any exogenous 17β-oestradiol are also shown for purposes of comparison.

When 17β-oestradiol at 100 ng/kg was added the responses to stimulation of the spinal sympathetic outflow no longer showed any increase during the 80 minutes of the experiments (Figure 41 a). On the contrary, they showed a tendency to decrease. This decrease was statistically significant when the responses following addition of 17β-oestradiol were compared to those seen in oestrous animals, but not in dioestrous animals in the absence of any exogenous 17β-oestradiol. Thirty-five minutes after the addition of 17β-oestradiol (ie at the 65 minutes of the experiment, Figure 41 a), the pressor responses were 63.1 ± 1.0% (n = 5) in oestrous animals, and 45.8 ± 5.6% (n = 5) in 17β-oestradiol treated animals, of the resting systolic pressure. Furthermore, the responses following two additions of 17β-oestradiol (10 ng/kg) were significantly greater than those following a single addition of 17β-oestradiol (100 ng/kg), as it can be seen in Figure 41a. Statistical significance in the difference of these responses was seen immediately after additions of 17β-oestradiol at the point corresponding to 35 minutes of the experiment (for numerical values see Appendix).

Responses to noradrenaline (Figure 41b) and angiotensin (Figure 41c) were not significantly different after addition of


a.
(a)
80
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5
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35
50
65
80
Oestrous
Dioestrous
oestrous + dioestrous
b.
(b)
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60
50
40
30
10
25
40
55
70
85
Oestrous
Dioestrous
oestrous + dioestrous
c.
(c)
80
70
60
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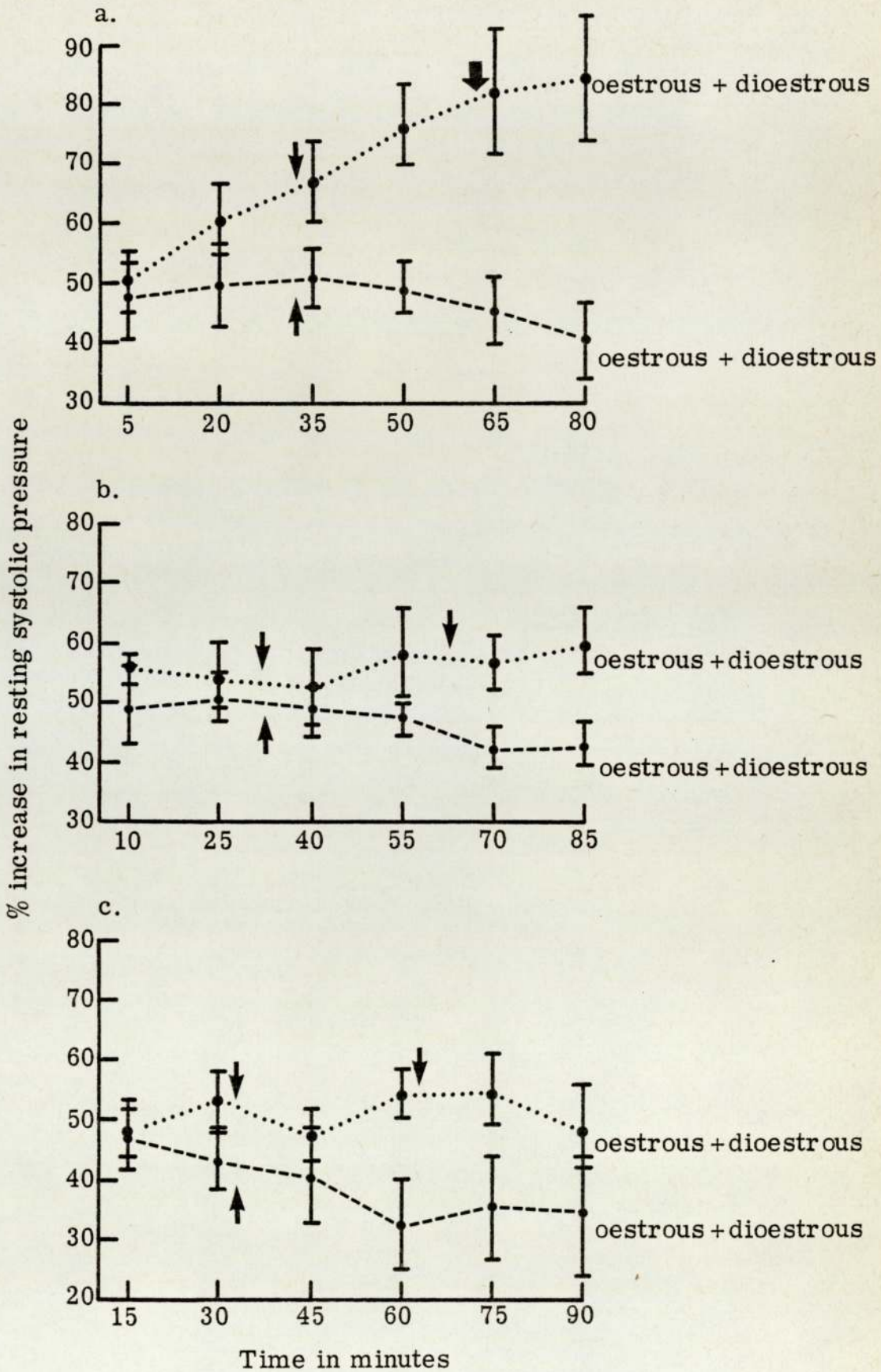
75
90
Dioestrous
Oestrous
oestrous + dioestrous
% increase in resting systolic pressure
Time in minutes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oestrous + dioestrous
oestrous + dioestrous
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85
oestrous + dioestrous
oestrous + dioestrous
c.
80
70
60
50
40
30
20
15
30
45
60
75
90
oestrous + dioestrous
oestrous + dioestrous
% increase in resting systolic pressure
Time in minutes

17β-oestradiol (100 ng/kg) compared to those in oestrous and dioestrous animals in the absence of any exogenous 17β-oestradiol. However, the pressor responses to noradrenaline following the two additions of 17β-oestradiol (10 ng/kg) were greater than those following a single addition of 17β-oestradiol (100 ng/kg) (Figure 42b) but this difference started being statistically significant ($p < 0.05$) only 40 minutes of the first addition of 10 ng/kg or of the addition of 100 ng/kg of 17β-oestradiol. At this point of the experiment which corresponded to 70 minute time the responses were $56.3 \pm 4.4\%$ ($n = 5$) for the animals treated with 2 x 10 ng/kg of 17β-oestradiol and $42.0 \pm 3.2\%$ ($n = 5$) for the animals treated with 100 ng/kg 17β-oestradiol, of the resting systolic pressure. The responses to angiotensin appeared to be higher following 2 x 10 ng/kg 17β-oestradiol compared with that following 100 ng/kg 17β-oestradiol but this difference was only significant (fig c) ($p < 0.05$) at the point corresponding to 60 minutes of the experiment. It therefore appeared that different amounts of 17β-oestradiol had produced a difference in the responses of the intact female pithed rat to noradrenaline and angiotensin but this difference was far less pronounced than that seen in the responses to stimulation of the spinal sympathetic outflow in the same group of animals.

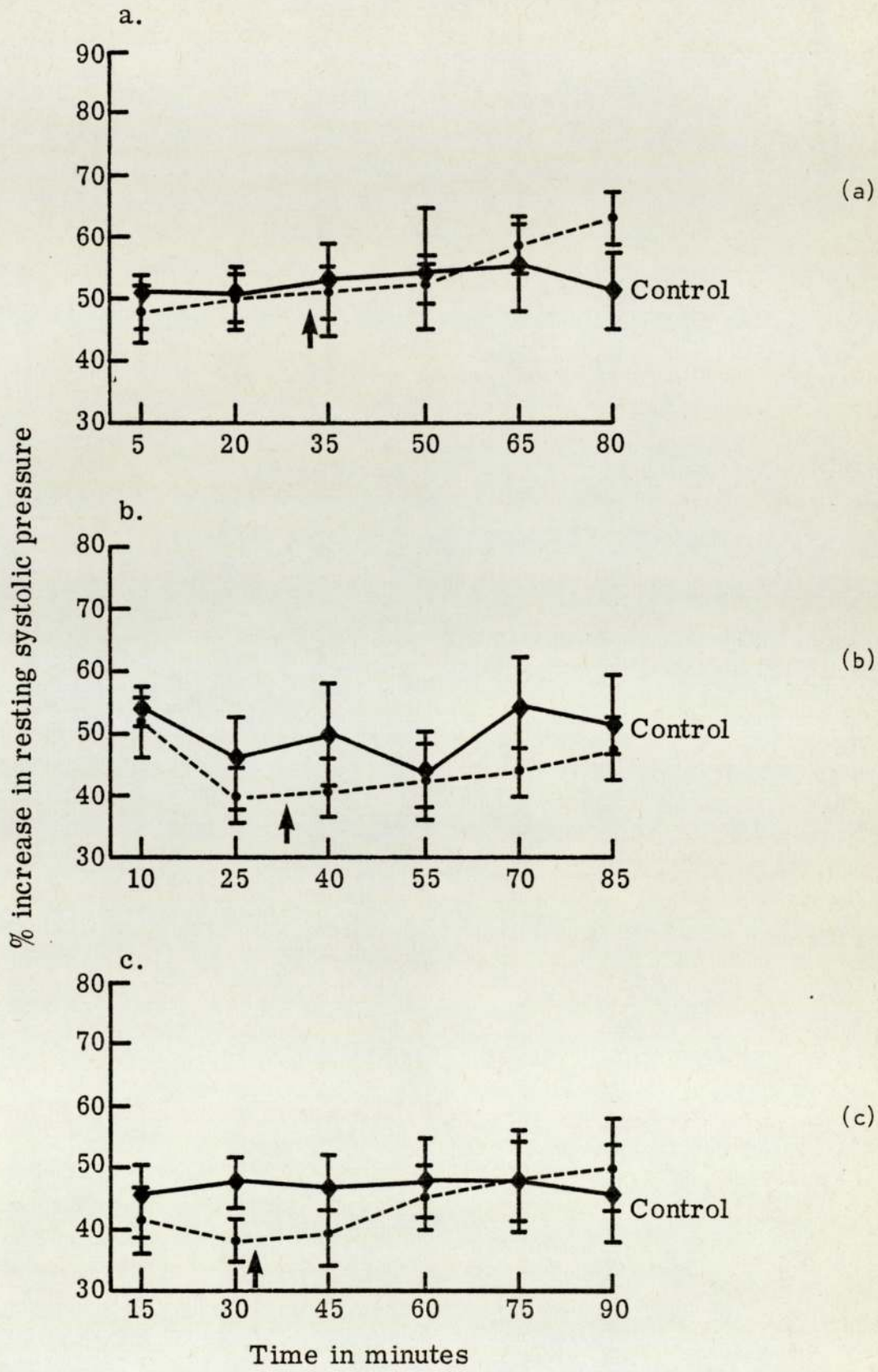
II) <u>The ovariectomised pithed rat</u>

Figures 43 a, b, c show the effect of 100 ng/kg 17β-oestradiol on the pressor responses to stimulation of the spinal sympathetic outflow, to noradrenaline and to angiotensin in the ovariectomised pithed rat. Responses in the absence of any exogenous 17β-oestradiol are also shown for purposes of comparison.

FIGURE 43a: The effect of a single injection of 17β-oestradiol (100 ng/kg) upon the pressor responses of the ovariectomised pithed rat--●-- to stimulation of the spinal sympathetic outflow (0.2 - 0.4 Hz). Pressor responses to same stimulus in the absence of any 17β-oestradiol injection —●— are shown again for purposes of comparison. ↑ Time when 17β-oestradiol was injected intravenously. Vertical bars are SE of the means (n = 5).

FIGURE 43b: The effect of a single injection of 17β-oestradiol (100 ng/kg) upon the pressor responses of the ovariectomised pithed rat--●-- to noradrenaline (50 - 100 ng/kg). Pressor responses to same stimulus in the absence of any 17β-oestradiol injection —●— are shown again for purposes of comparison. ↑ Time when 17β-oestradiol was injected intravenously. Vertical bars are SE of the means (n = 5).

FIGURE 43c: The effect of a single injection of 17β-oestradiol (100 ng/kg) upon the pressor responses of the ovariectomised pithed rat--●-- to angiotensin (50 - 100 ng/kg). Pressor responses to same stimulus in the absence of any 17β-oestradiol injection —●— are shown again for purposes of comparison. ↑ Time when 17β-oestradiol was injected intravenously. Vertical bars are SE of the means (n = 5).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Control
(b)
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45
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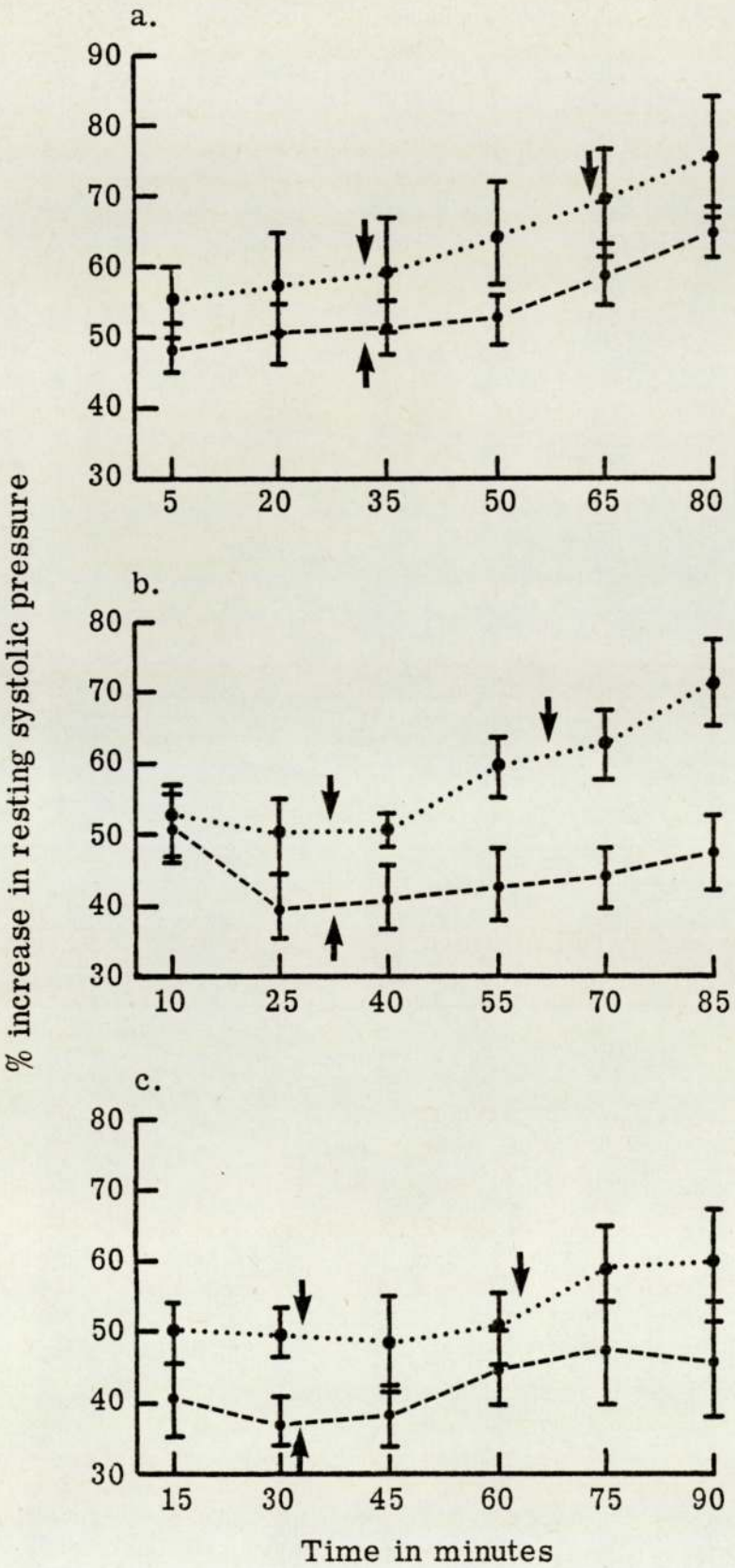
75
90
Control
(c)
% increase in resting systolic pressure
Time in minutes

The pressor responses to stimulation of the spinal sympathetic outflow were not significantly different after the addition of 100 ng/kg of 17β-oestradiol compared with those in the absence of any added 17β-oestradiol (Figure 43a). When the responses following the addition of 100 ng/kg 17β-oestradiol were compared to those following the two additions of 17β-oestradiol, it was evident that the former responses were smaller than the latter (Figure 44a) but this difference was of no statistical significance. Therefore, the ovariectomised animals appeared to respond in a different manner to addition of 17β-oestradiol either at 10 or 100 ng/kg compared to the intact animals. The responses to noradrenaline were not significantly different after addition of 17β-oestradiol (100 ng/kg) compared with those in the absence of any exogenous 17β-oestradiol. However, the former responses were smaller compared to those following the two additions of 17β-oestradiol (10 ng/kg) (Figure 44b). This difference became statistically significant ($p < 0.05$) 40 minutes after the first addition of 10 ng/kg of 17β-oestradiol or the single addition of 100 ng/kg of 17β-oestradiol. At this point which corresponded to the 70 minutes time of the experiment, the responses were $63.7 \pm 4.8\%$ ($n = 5$) following 2 x 10 ng/kg 17β-oestradiol, and $44.3 \pm 4.3\%$ ($n = 5$) following 100 ng/kg 17β-oestradiol, of the resting systolic pressure. Thus, it appeared that in the case of the responses to noradrenaline the ovariectomised animals behaved in a similar manner to the intact animals.

The responses to angiotensin were not significantly different after addition of 17β-oestradiol (100 ng/kg) compared with those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75
90
% increase in resting systolic pressure
Time in minutes

in the absence of any exogenous 17β-oestradiol (Figure 44c). The responses following the two additions of 17β-oestradiol (Fig c) (10 ng/kg) appeared to be higher than those following addition of 100 ng/kg 17β-oestradiol but this difference in responses was not statistically significant at any point of the experiment (for numerical values see Appendix).

Finally, the sham-ovariectomised animals responded in a similar manner to the intact animals to addition of 100 ng/kg of 17β-oestradiol. Data for the experiments performed in this group of animals are again available in the Appendix.

4. Summary

The pressor responses to stimulation of the spinal sympathetic outflow were significantly increased after the two additions of 10 ng/kg 17β-oestradiol when compared with responses in dioestrous animals in the absence of any exogenous 17β-oestradiol.

These responses were also increased in ovariectomised animals after the two additions of 17β-oestradiol but this increase was less pronounced than the one seen in intact animals.

A single addition of 100 ng/kg of 17β-oestradiol significantly decreased responses to stimulation when compared with responses seen in oestrous animals in the absence of any added 17β-oestradiol, but had no effect on the responses to stimulation in ovariectomised animals. In addition the responses to stimulation in intact animals given 2 x 10 ng/kg of 17β-oestradiol were significantly greater than those following administration of 100 ng/kg of 17β-oestradiol. This difference was absent in ovariectomised animals.

The responses to noradrenaline and angiotensin following either the two additions of 10 ng/kg 17β-oestradiol, or 100 ng/kg 17β-oestradiol were not significantly different compared to those in the absence of any exogenous 17β-oestradiol, both in intact and ovariectomised animals.

However, the responses to noradrenaline following 2 x 10 ng/kg 17β-oestradiol were significantly greater compared with those following 100 ng/kg, both in intact and ovariectomised animals.

The responses to angiotensin were not significantly affected by addition of 17β-oestradiol either at 10 ng/kg or 100 ng/kg in intact or ovariectomised animals.

Thus, it appeared that the effect 17β-oestradiol, both at 2 x 10 ng/kg and 100 ng/kg, although of opposite direction is primarily directed towards the pressor responses to stimulation of the spinal sympathetic outflow and, to a smaller extent, towards the pressor responses to noradrenaline. No conclusive evidence concerning the effect of 17β-oestradiol on pressor responses to angiotensin was provided by these results.

DISCUSSION

1: GENERAL INTRODUCTION

The vasomotor disorders of the menopause are one of the most common features accompanying the transition to the non-reproductive years in the human female and it is therefore surprising that they have received very little investigation.

In the present study the objective was both to establish the actual changes occurring during this vasomotor disturbance termed the 'hot flush' and to investigate the possible underlying mechanism.

2: THE EPIDEMIOLOGICAL SURVEY

The average age of the post-menopausal women who participated in the survey and who were suffering from hot flushes, was similar to the age of the onset of the menopause in Caucasian women, reported by various workers (Benjamin 1960; McKinlay *et al* 1972; Treloar 1974; Benedek Jaszmann 1976; Gray 1976). This suggested that the hot flush is a symptom manifested during the early menopause. This is consistent with the reports from these women, stating that their hot flushes started simultaneously or just after the onset of the menopause.

The fact that both oophorectomised women, as well as women who experienced a natural menopause, suffered from hot flushes suggested that the sudden decline of circulating hormones which occurs during both the natural and surgical menopause (Adamopoulos *et al* 1971; Maroulis *et al* 1976) may be important for the hot flushes to occur. However, prepubertal castrates do not exhibit hot flushes (Clorinda *et al* 1976) and it therefore appears that a simple lack of ovarian hormones is not sufficient to cause hot flushes, but prior exposure to these hormones is necessary.

The number of hot flushes experienced by these women in 24 hours was variable, which was consistent with the report of Hannan (1927). However, the small variance associated with the mean suggested that the high frequency of the occurrence of hot flushes observed in this group of women was consistent. This is not unexpected as all women studied had been referred for medical treatment for hot flushes.

The inverse correlation between the time since the onset of the hot flushes and the number of hot flushes in 24 hours in oophorectomised women could be due to a progressive change in hormonal levels in these women, which contributes to the relief or reduction of the hot flushes. A peripheral conversion of androstenedione to oestradiol and oestrone (Cooke 1976) could partially compensate for the loss of ovarian function. An alternative possibility is that the hormonal status of these women becomes more stable with time, and if the vasomotor symptoms are part of a more generalised disturbance of the homeostatic environment, then a hormonal stability might lead to reduction or even aleviation of these symptoms.

In the present study, hot flushes appeared to be of equal severity both during the day and at night which is inconsistent with other reports where the hot flushes appeared to be worse at night (McKinlay 1974). This might be due to the limited number of women who participated in the present survey.

The variation in the direction of progression of the hot flushes in these women did not appear to correlate with the time since the onset of hot flushes.

This might be due to the fact that the number of flushes reported by these women only referred to the ones of greater severity and disregarded any minor or regressed ones.

The description of the sensation of the hot flush and its progression to the various parts of the body is consistent with other reports (Hannan 1927; Molnar 1975).

Sweating was reported to accompany hot flushes in some cases which is consistent with Molnar's (1975) and Jaszmann's (1976) reports. The possible mechanism for this sweating is discussed in the next section of the discussion (page 170).

The average duration of the hot flush as reported by these women indicated that the actual discomfort felt during a hot flush does not last very long. The acute onset of the hot flush indicates that a nervous mechanism might be responsible for at least the initial sensations experienced. Some of the factors that were reported to provoke a hot flush (embarrassment, excitement) might indicate that these women have a labile autonomic nervous system which would be consistent with the suggestion proposed by Cope (1976). On the other hand, alcohol and hot drinks have also been reported to cause a hot flush, which suggests that the vasculature in these women might be particularly sensitive to vasodilator stimuli.

This study revealed warning symptoms which precede a hot flush and these do not differ from the symptoms reported by Hannan (1927). The symptoms reported by Hannan were similar to those following adrenaline injection in women suffering from hot flushes which might support a sympathetic activation before and possibly during a hot flush.

The altered sensitivity to cold or heat which was reported to occur immediately after the menopause might reflect an incapability of the peripheral circulation of these women to perform the appropriate adjustments required in response to a change in the environmental temperature.

The reported ways of avoiding a hot flush was simply referring to avoidance of the precipitating factors mentioned above which again suggests a labile autonomic nervous system and altered sensitivity.

Finally, a hot flush appears to be a completely different sensation from a blush which occurs independently of age or sex, whereas the hot flush occurs exclusively in post-menopausal women.

3: PHYSIOLOGICAL CHANGES OCCURRING DURING A HOT FLUSH IN POST-MENOPAUSAL WOMEN AND DURING HEAT INDUCED VASODILATATION IN PRE-MENOPAUSAL WOMEN

1. Cardiovascular activity

The hot flushes of the menopause are often otherwise called the 'vasomotor disorders' of the menopause. A vasomotor disorder is one which may result from a dysregulation of the cardiovascular function due to a defect in either the function of the central vasomotor control mechanisms or to a defective control of the peripheral circulation.

Indeed, many of the signs and symptoms accompanying a hot flush, such as palpitations, tachycardia, rise in blood pressure, increased blood flow (Hannan 1927; Reynolds 1941) suggest an altered cardiovascular activity which is rapid in onset and development and relatively slow in recovery.

The records of the physiological variables measured during a hot flush confirmed that there is a change in the cardiovascular activity of these women.

The increased heart rate observed at the onset of the hot flush is in agreement with findings reported by previous workers (Hannan 1927; Molnar 1975) and indicated possible increase in the activity of the sympathetic nervous system, as cardiac acceleration is under the control of this system.

This increase in the heart rate cannot be attributed to a reflex mechanism due to vasodilatation as such an increase was not observed in the pre-menopausal women who were vasodilated to a comparable degree (page 87).

The lack of a change in the blood pressure before, during or after a hot flush was not consistent with Hannan's findings (1927) who reported a short rise in the blood pressure just before the onset of the hot flush and a considerable fall after the hot flush had ceased in one patient. The findings of the present study suggested that although the heart rate and the peripheral blood flow (see pages 82,85) exhibited changes during a hot flush, the mechanism responsible for maintaining the blood pressure within a restricted range remained unimpaired.

However, continuous recordings of the blood pressure would be desirable in order to confirm these observations.

The vasodilatation seen in post-menopausal women during a hot flush was extended to both the arterial and venous side as it was accompanied by an increase in the skin temperature of the hand (page 92) (indicative of an increase in the cutaneous blood flow). Such a vasodilatation could be due to:

(a) An inhibition of the basal vascular tone through inhibition of the vasoconstrictor influence on the blood vessels (Folkow and Neil 1971) similar to that known to occur in the cutaneous vessels of the hand during warming (Roddie *et al* 1957a).

(b) A direct stimulation of the sympathetic vasodilator fibre system which is thought to innervate cutaneous blood vessels of the forearm and the calf (Grant *et al* 1938; Barcroft *et al* 1956; Edholm *et al* 1956; Roddie *et al* 1956, 1957).

(c) Release of a vasodilator substance into the circulation such as prostaglandins, histamine or kinins.

Passive vasodilatation due to an inhibition of the activity of the vasoconstrictor fibres in the hand, or an active one as described in (b), does not seem to be a possible explanation for the vasodilatation seen in the hand and forearm as both mechanisms are thermoregulatory responses to heat load. However, in the present study increases in skin temperature in post-menopausal women appeared to be only a consequence of an increase in blood flow as they followed a few minutes after the vasodilatation seen in the hand. The above mentioned mechanisms could account for the vasodilatation induced thermally in the pre-menopausal women, as vasodilatation was caused by the temperature increase in this case.

The release of a vasodilator substance into the circulation appears to be a likely mechanism for the vasodilatation during a hot flush.

An alternative explanation might be an inhibition of the myogenic tone known to maintain the resistance vessels and sphincters under partial constriction. Such a mechanism might be due to factors interfering with the basic machinery of the vascular smooth muscle itself (Mellander *et al* 1968).

Both possible mechanisms were examined in the present study and are discussed on sections 5 and 6.

2. Skin temperature

The observed increases in the forehead, forearm and hand temperatures, seen during skin temperature measurements and during thermography, were probably a direct consequence of vasodilatation, in post-menopausal women.

This is supported by the fact that at least in the hand, where blood flow was monitored, the increase in temperature followed the onset of the hot flush, which is marked by a vasodilatation (see page 93).

To confirm whether temperature increases were in fact due to the vasodilatation, and not *vice versa*, it would be useful to have data concerning internal body temperatures. However, although such measurements were meant to be performed at a later stage of the study, lack of volunteers prevented us from doing so.

In pre-menopausal women the temperature increases were directly proportionate to the heating stimulus applied and only recovered after the removal of such a stimulus. Furthermore, they were slow in onset, as were the observed increases in cutaneous blood flow. The fact that in one post-menopausal women, in whom warming did not cause a hot flush, the pattern of skin temperature increases and consequent vasodilatation was similar to that seen in pre-menopausal women, confirms that a hot flush is distinguishable from thermal vasodilatation.

Finally, it appears that slight warming and a hot drink act as a 'stress stimulus' and not as a 'thermal stimulus' as it does not always cause a hot flush (see above). Furthermore, the same post-menopausal woman mentioned above reported that a 'cold stimulus' provoked hot flushes in her case. Therefore, the warming stimulus should not be considered as a facilitating factor to cause increases in temperature and vasodilatation in post-menopausal women.

3. Skin resistance

Changes in the skin resistance of post-menopausal women were first suggested by Molnar's (1975) finding that the base line of the ECG base line fluctuated during a hot flush which was attributed to a change in the conductivity of the skin due to sweat gland activity. This finding was confirmed in the present study and investigated further by direct measurement of changes in skin resistance. The skin resistance changes also confirmed the evidence provided by the ECG and indicated a possible increase in the activity of the sympathetic nervous system (the electrodermal reflex is believed to be mediated mainly by this division of the autonomic nervous system (page 60)). Almost all of the models proposed to correlate changes of the skin resistance with physiological processes mainly implicate the sweat gland activity as responsible for such changes (Adams 1966; Martin *et al* 1967).

Sweat gland activity would be expected to be higher in the pre-menopausal women as they exhibited greater temperature increases than post-menopausal women (page 94). However, no significant changes in the skin resistance were observed in the former group of women. This suggests that, assuming that skin resistance changes were due to increased sweat gland activity, in post-menopausal women such an increased activity was possibly due to a greater number of sympathetic impulses arriving to the sweat glands, independent of a thermoregulatory mechanism.

Alternatively, the non-sudorific element of the electro-dermal reflex (Darrow *et al* 1970) plays an important part in the changes of the skin resistance.

The evidence in the present study does not allow a clear distinction between the two possible mechanisms. However, the post-menopausal women reported that they experienced sweating during a hot flush, therefore the former explanation appears the most likely of the two.

4: PHARMACOLOGICAL INVESTIGATION OF THE MECHANISM OF THE HOT FLUSH

The hot flush in post-menopausal women was shown to be associated with an altered cardiovascular activity (increased heart rate, increased cutaneous blood flow, fluctuation of the ECG base line) which might be linked with the delicate hormonal balance existing in these women as a result of the menopause (Campbell 1976; Cope 1976).

Some possible mechanisms that may account for this altered cardiovascular activity have been outlined in the previous section of the Discussion. However, most of the mechanisms mentioned were related to a possible defect in the nervous regulation of the cardiovascular function.

A humoral component in the control of the circulation, providing slower and more long term adjustments than the nervous one, might partly account for the manifestations of the hot flush.

The humoral control of the circulation is exerted through metabolic and non-metabolic autoregulation of local blood flow (Rabela *et al* 1964; Guyton 1977), through the renin-angiotensin system (Davis 1971; Clayhaugh *et al* 1972; Coote *et al* 1972; Cowley *et al* 1972), through the antidiuretic hormone (Cousineau 1973; Öberg 1976) and finally through other vasoactive substances such as prostaglandins, kinins or histamine.

In the present study the possible involvement of prostaglandins, kinins or histamine in the mechanism of the hot flush

was investigated as well as the possible involvement of circulating catecholamines and acetylcholine. Most of these substances have been implicated in the flushes which occur in pathological conditions (Pernow *et al* 1957; Sandler *et al* 1960; Robertson *et al* 1962; Priestley *et al* 1963; Dollinger *et al* 1966; Zeitlin *et al* 1966; Sandler *et al* 1968; Smith *et al* 1974; Rothschild *et al* 1976) and it has been suggested that they might be responsible for the mechanism of these flushes, although their role is not very clear.

The contractile activity on the rat fundic strip of plasma samples from post-menopausal women at rest and during flushing and from pre-menopausal women at rest and during heat-induced vasodilatation were found to be similar. This might be evidence for the lack of a humoral component but alternatively it was possible that the large amounts of 5-hydroxytryptamine present in plasma (Humphrey *et al* 1954) were masking any smaller differences between individual plasma samples.

The differential blocking of the contractile activity on the rat fundic strip of plasma from post-menopausal women at rest and during flushing, is an original finding and suggests that there is a humoral component involved in the mechanism of the hot flush. A differential blocking was not observed in the case of pre-menopausal women which suggested that the observed difference could not be simply due to vasodilatation.

That this difference was unmasked after the contractile activity due to 5-hydroxytryptamine was blocked with methysergide indicated that this substance does not appear to be involved in the mechanism of the hot flush.

The question as to what accounts for the remaining activity in the plasma of pre-menopausal women remained unresolved as, although it is of interest it was considered more important in the present study to attempt an elucidation of the reason for the difference in biological activity of the plasma from post-menopausal women at rest and during a hot flush.

The present study did not provide conclusive evidence as to the humoral factor responsible for that difference. However, it appeared not to be due to histamine-, acetylcholine-, or catecholamine-like activity.

The prostaglandin E-like activity and kinin-like activity of whole blood appears to be similar at rest and during a hot flush. It is however possible that prostaglandins are involved in the hot flush. The discovery of the new member of the prostaglandin series prostacyclin which is produced mainly by the blood vessels (Moncada *et al* 1978a) and which is vasodilator and believed to be a circulating hormone involved in autoregulation offers a new possibility for the humoral factor involved in the hot flush. Intravenous infusion of this substance has been shown to produce symptoms very similar to those seen during a hot flush

However, it was only very recently that specific receptor antagonists and assays for its measurement became available. Therefore it was difficult at the time of the present study to investigate its involvement in the hot flush.

Furthermore, the measurement of total prostaglandin E-like activity which might also reflect changes in prostacyclin-like activity involved haemolysis, during which large amounts of

thromboxane A would be released (Moncada *et al* 1979) and possibly mask any differences in the amounts of other prostaglandins present.

Prostacyclin, however, remains a strong possibility as it is removed by the lungs, having therefore a longer half life in the circulation and it is a much more potent vasodilator than prostglandin E. Its involvement should therefore be investigated.

Catecholamines might also play an important role in the mechanism of the hot flush. Although they do not appear to account for the difference in biological activity of the plasma at rest compared to that during a hot flush, differences in their overflow into the circulation would reflect changes in the activity of the sympathetic nervous system which has been implicated in the mechanism of the hot flush (page 166, 3:). Measurement of plasma catecholamine levels would be a very useful index of sympathetic nerve activity and it should be within the aims of any future work attempting an elucidation of the hot flush.

5: THE EFFECT OF 17β - OESTRADIOL UPON THE RESPONSIVENESS OF THE RAT VAS DEFERENS

The innervation of the isolated vas deferens preparation is, in its major part, sympathetic adrenergic (Birmingham *et al* 1963; Swedin 1971). Nevertheless the presence of non-adrenergic nerves has not been excluded. Ambache and Zar (1971), suggested that an independent cholinergic nerve supply might exist in the rat vas deferens but its contribution to the motor response of this preparation has not been considered to be significant.

In the present study this preparation was used as a model to investigate the effect of ovarian steroids upon the adrenergically innervated non-vascular smooth muscle, *in vitro*. The effect of 17β-oestradiol upon responses of the vas deferens to transmural stimulation, to noradrenaline and to potassium chloride, was studied in order to distinguish whether this oestrogen has a presynaptic or post-synaptic effect on the sympathetic neuro-effector junction, or whether it directly affects the ability of the muscle to contract.

17β-oestradiol caused a reversible inhibition of the responses to transmural stimulation, to added noradrenaline and to potassium chloride which suggested that the effect of 17β-oestradiol is directed towards the effector cell, possibly through interference with ionic movement across the membrane. This would be supported by the fact that responses to potassium chloride, which is a non-receptor stimulant and which causes the muscle to contract through membrane depolarisation and calcium influx (Goodman and Weiss 1971; Van Breeman, Farinas, Gerba and McNaughton 1972; Marshall and

Kroeger 1973), were completely abolished and failed to recover completely.

17β-oestradiol has been shown to inhibit responses of the ileal smooth muscle to acetylcholine and histamine (Seaman, Famaey, Fontaine and Rense 1977; Famaey, Fontaine, Rense and Seaman 1978) and it is believed that it causes this effect through a non-specific action on biological membranes permeability (Ehrenkranz 1976), which would be consistent with the findings of the present study.

The fact that responses to noradrenaline and to transmural stimulation were reduced to a lesser degree than those to KCl suggested that either:

(a) The mechanism through which contractions to KCl are mediated is more sensitive to the effect of 17β-oestradiol.

(b) 17β-oestradiol might also affect the processes for the inactivation of noradrenaline post- and or presynaptically by a possible inhibition of such processes. In that case the effect of 17β-oestradiol on the responses to transmural stimulation and to noradrenaline might be a combination of an inhibition and potentiation, hence the greater responses remained in the case of transmural stimulation and noradrenaline compared to those of KCl.

6: THE EFFECT OF 17β-OESTRADIOL UPON PRESSOR RESPONSES IN THE PITHED RAT

The effect of 17β-oestradiol upon the pressor responses of the pithed rat preparation was tested in order to examine the hypothesis of a possible 'trigger' which might be based on a sympathetic vasoconstrictor mechanism. As 17β-oestradiol fluctuates during the perimenopausal years, the time when the hot flushes occur, it appeared reasonable to form the hypothesis that if a sympathetically based 'trigger' does exist, this trigger might be activated by the fluctuations of this hormone.

The results of the present study showed that there exists an interaction between 17β-oestradiol and the responsiveness of the vascular smooth muscle to various stimuli.

The possible interaction between ovarian hormones and the cardiovascular system has received early recognition and investigation. Reynolds (1941) investigated the effect of local oestrogen injection in post-menopausal women and observed changes in the peripheral blood flow similar to those occurring during a hot flush. Clemetson (1962) observed an increase in capillary strength in post-menopausal women after they had received oestrogen orally or intravenously.

Pickford and Lloyd in the late fifties and sixties studied the effect of ovarian hormones on cardiovascular responsiveness to various stimuli both in animal and in man. From these studies it was concluded that vascular responsiveness varied with the concentration of ovarian hormones in the body (Lloyd 1959a,b; Lloyd *et al* 1963) and that oestrogens might affect sympathetic nervous activity (Lloyd *et al* 1961, 1962).

Finally, Rosenfeld *et al* (1976) and Altura (1972, 1975) investigated the effect of oestrogen on the regional blood flow and on the vascular responsiveness of the rat to added catecholamines. Those workers suggested that oestrogen increases both peripheral blood flow and cardiac output and the responsiveness of blood vessels to added catecholamines.

In the present study the possible effect of oestrogen was investigated on the vasculature of the pithed female rat. In this preparation the only nervous control of the vasculature is via sympathetic outflow from the preganglionic sites (Gillespie *et al* 1967) which may be stimulated electrically. Thus the effect of oestrogen could be studied upon the presynaptic site by investigating the effect of 17β-oestradiol on the responses to stimulation of the spinal sympathetic outflow.

The fact that the resting systolic and diastolic blood pressures were similar during oestrus and dioestrus in intact, sham-ovariectomised and ovariectomised animals, suggested that endogenous oestrogen levels do not significantly affect the cardiac output or the basal vascular tone.

However the resting heart rate in ovariectomised animals was significantly lower than in intact and sham-ovariectomised animals at oestrus or dioestrus.

Since the diastolic blood pressure was similar in all animals, the decreased heart rate in ovariectomised animals could be due to (a) an increased stroke volume, (b) a decreased cardiac output and increased total peripheral resistance, in order to maintain the pressure constant. An increase in stroke volume would be consistent with an increase in the force of contraction of the heart.

Circulating oestrogens have been shown to increase myocardial contractility (Walter *et al* 1969) and in the case of ovariectomised animals, oestrogen levels are lower compared with intact and sham-ovariectomised animals. Whether lack of oestrogen would result in the opposite effect is a possibility. Therefore an increase in the force of contraction of the heart might seem unlikely in the present study.

A decrease in cardiac output might be due to low circulating levels of oestrogen after ovariectomy, since circulating oestrogens have been shown to increase cardiac output (Altura 1972). An increased total peripheral resistance might be due to a decreased elasticity of blood vessels. This would be consistent with the evidence that plasma cholesterol and lipid levels rise, with subsequent lipid deposition in the blood vessel wall, in post-menopausal women (Parrish *et al* 1967). Ovariectomy in animals might cause a similar lipid deposition possibly resulting in a decreased vessel elasticity and therefore increased peripheral resistance in these animals.

The acute effect of 17β-oestradiol was studied upon the pressor responses to stimulation of the spinal sympathetic outflow as a measure of any possible pre-synaptic action of this hormone. The effect upon the pressor responses to noradrenaline was studied as a measure of any effect upon the ability of the blood vessels to respond to the released transmitter. Finally, the effect of 17β-oestradiol was studied upon pressor responses to angiotensin as a measure of the ability of the smooth muscle of the blood vessels to contract to a vasoconstrictor stimulus which has been shown to act directly upon vascular smooth muscle (Davis 1971).

The stimulus/response relationships for the stimulation of the spinal sympathetic outflow, for noradrenaline and for angiotensin were found to be similar in intact ovariectomised and sham-ovariectomised animals and this indicated that at least in the beginning of the experiment, when these stimulus/response relationships were constructed, the cardiovascular responsiveness of these animals was not significantly affected by the circulating levels of steroid hormones.

The increase in pressor responses to stimulation of the spinal sympathetic outflow with time, which was mainly observed in the oestrous animals (both intact and sham-ovariectomised) might be attributed in the first instance to a defective inactivation of the released transmitter with consequent accumulation of this transmitter in the blood vessels during successive stimulation. The major route of inactivation for the released transmitter, which finally reaches the effector cells in the vascular wall, is metabolic inactivation since the distance between the effector cell and the nerve ending is too great for neuronal re-uptake to be an effective mechanism of inactivation (Speden 1970). This has been shown to be true particularly in the case of rat blood vessels (Maling, Fleish and Saul 1971) where neuronal uptake of noradrenaline did not modulate adrenergic responses.

An inhibition of the uptake$_2$ by steroids has been shown in the rat heart (Iversen *et al* 1970). In addition, steroid hormones have been shown to potentiate the response of arterial smooth muscle to catecholamines (Kalsner 1969) and it is believed that the mechanism of this action of steroid hormones is through inhibition of metabolic inactivation of the added catecholamines.

However the observation in the present study that responses to added noradrenaline did not exhibit the same trend, suggests that this mechanism of steroid inhibition of metabolic inactivation of catecholamines is rather unlikely.

A generalised increase in receptor sensitivity to vasoconstrictive stimuli does not appear to be a likely mechanism since it would be expected to result in an enhanced response to both noradrenaline and to angiotensin. An alternative possibility would be an increase in the release of transmitter from the nerve endings due to the presence of high circulating levels of oestrogen, but this possibility will be discussed in more detail below.

The acute administration of 17β-oestradiol at 10 ng/kg caused the pressor responses to stimulation of the spinal sympathetic outflow to increase in both intact and sham-ovariectomised (oestrous and dioestrous) animals. That these responses were significantly increased only when compared with those seen in dioestrous but not oestrous animals is not unexpected. Oestrous animals possess an already greater vascular reactivity (as previously discussed) and it is probable that this cannot significantly increase further, after the 17β-oestradiol administration. In contrast the vasculature in dioestrous animals exists in a state of responsiveness which may be further increased.

This increase in pressor responses after 17β-oestradiol administration appears to be a stronger expression of the increase in the responses of oestrous animals with time. The most likely mechanism for this increase would again appear to be an increase in the release of transmitter since the pressor responses to noradrenaline and angiotensin were unaffected.

Some of the possible mechanisms by which oestrogens might affect release of neurotransmitter by the nerve endings may be:

(a) Oestrogens might act on the presynaptic α-adrenoreceptors (Farnebo *et al* 1971; Langer *et al* 1971) thus interfering with the feedback inhibition of transmitter release.

(b) Oestrogens might interact with other substances at the neuroeffector junction, which are reported to modulate neurotransmitter release and such substances are the prostaglandins (Hedqvist 1977) and histamine (McGrath *et al* 1976).

Another possible mechanism by which 17β-oestradiol causes an increase in the pressor responses to nervous stimulation might be a decrease in the neuronal re-uptake of noradrenaline released from sympathetic neurons within the vascular wall which have been shown to exhibit re-uptake (Su and Bevan 1970). The importance of this mechanism in terminating post-synaptic effects is obviously dependent upon the distance between the nerve endings and the effector cell (Berkowitz and Spector 1975) but because of the relatively big gap existing in the blood vessels (Speden 1970) this mechanism must be important in determining the amount of transmitter that finally reaches the effector cells.

The pressor responses to electrical stimulation were also increased in the ovariectomised animals following 17β-oestradiol administration (10 ng/kg) and so these animals appeared to behave in a similar way to the dioestrous ones (intact and sham-ovariectomised). This was probably due to the fact that all these animals are in a state of low vascular reactivity possibly because of their low oestrogenic status, when compared with oestrous animals.

That this increase in pressor responses took longer to establish in the ovariectomised animals compared with the dioestrous ones may be due to a decreased responsiveness of these animals to oestrogen administration. Such a decreased responsiveness to oestrogen after a biological system has been deprived of it, subsequent to a prior exposal, is seen in the change in the sensitivity of hypothalamus to the suppressant effect to oestrogen on gonadotrophin release in post-menopausal women (Cooke 1976).

The higher dose of 17β-oestradiol (100 ng/kg) had the opposite effect, on the pressor responses to stimulation, ie it caused a decrease in the responses in intact and sham-ovariectomised animals compared with the responses in the same animals at oestrus but not at dioestrus. In addition the higher dose of 17β-oestradiol had no significant effect on the pressor responses to stimulation in ovariectomised animals. Again this could be attributed to an increased state of vascular reactivity seen at oestrus which could be significantly depressed compared with a state of low vascular reactivity in dioestrous and ovariectomised animals which could not be significantly depressed.

The pressor responses to noradrenaline and angiotensin were not significantly affected by 17β-oestradiol (100 ng/kg) administration when compared with responses in all the groups of animals in the absence of any injected 17β-oestradiol which suggested that the observed decrease in the responses to nervous stimulation may be primarily a presynaptic effect.

This effect could be exerted via a decrease in the release of transmitter from the nerve terminals and/or an increase in the

neuronal re-uptake of the released transmitter mechanisms which have been discussed previously (page 167).

It therefore appears that oestrogen might affect the circulation in a dual way depending on the amounts administered. Whether this is due to different physiological and/or pharmacological effects which are dose related or whether it is due to a bell-shaped dose-response curve similar to that suggested for prostaglandins (Horrobin 1978) needs to be determined.

That the pressor responses to noradrenaline were greater after injection of 10 ng/kg 17β-oestradiol compared with those following injection of 100 ng/kg suggested a post-synaptic action of oestrogen. This might involve a specific effect upon the noradrenaline receptors or an interference with ionic movements important for excitation contraction coupling in the smooth muscle cell. This post-synaptic effect, however, does not appear to be very pronounced since a significant difference in pressor responses to noradrenaline was only observed between animals injected with the two different amounts of 17β-oestradiol (2×10 mg/kg and 100 ng/kg).

The fact that the pressor responses to angiotensin were not significantly affected by 17β-oestradiol injection both at 10 and 100 ng/kg suggested that the observed effects of 17β-oestradiol are probably directed towards the sympathetic neuroeffector junction rather than towards the vascular smooth muscle cell directly.

These findings suggest that oestrogens affect the vascular smooth muscle in a different manner than the non-vascular one. In the non-vascular smooth muscle used in the present study, ie the rat vas deferens, oestradiol appears to inhibit responses to

transmural stimulation, to noradrenaline and potassium chloride. Furthermore, other workers (Seaman *et al* 1977; Famaey *et al* 1978) have shown that oestradiol inhibits responses of the ileal smooth muscle to acetylcholine and histamine. In contrast, oestradiol has been shown mainly to potentiate responses in vascular smooth muscle both *in vitro* (Kalsher 1969; de Felice 1973) and *in vivo* to various stimuli (Lloyd 1959a,b; Lloyd *et al* 1961a,b; Haigh *et al* 1965; Lloyd *et al* 1967a,b; Altura 1975). In addition, in the present studies oestradiol was shown both to potentiate and reduce the responses of the vascular smooth muscle *in vivo*, to stimulation of the spinal sympathetic outflow and to noradrenaline.

The proposed possible mechanisms responsible for the effects of oestradiol on vascular smooth muscle are believed to be:

Firstly, a possible inhibition of the $uptake_2$ mechanism (Kalsner 1969) which involves metabolic inactivation of the catecholamines by monoaminoxidase and catechol-O-methyl-transferase.

Secondly, a possible interference with binding and transport of calcium in the vascular smooth muscle cells.

The suggested possible mechanism responsible for the effects of oestradiol on non-vascular smooth muscle is believed to be mediated through a non-specific effect of oestrogen upon the permeability of biological membranes (Ehrenkranz 1976).

Whether this difference between the effect of oestradiol upon vascular and non-vascular smooth muscle is due to structure and/or functional differences between these types of muscle is not yet established.

The biphasic effect of 17β-oestradiol (potentiation and depression) upon the responsiveness of the vascular smooth muscle

in vivo seen in the present study is, to our knowledge, a novel finding. The possibility ought to be therefore considered as to whether this effect becomes obvious when oestrogen is given acutely and not in the form of maintenance or pretreatment dose as given in the *in vivo* studies performed by other workers.

Therefore, the results of these series of experiments suggested that there is an interaction between the sympathetically innervated vascular and non-vascular smooth muscle and 17β-oestradiol. The fact that 17β-oestradiol increases the responses to stimulation of the spinal sympathetic outflow in the pithed rat might be considered as evidence in favour of the 'trigger' hypothesis. That this effect is manifested after injection of a low dose of 17β-oestradiol may be consistent with the hypothesis that the fluctuations in the oestrogen levels in post-menopausal women might increase release of noradrenaline from the sympathetic nerve endings. As a consequence of this effect, vasodilatation might occur initially as part of the activation of the compensatory mechanisms to counteract a possible vasoconstriction in some vascular bed. That both the high dose on the pithed rat depressed the responses to stimulation of the spinal sympathetic outflow and the dose of 17β-oestradiol used in the vas deferens study depressed the responses to transmural stimulation, to noradrenaline and to KCl, may be considered as evidence for a non-specific effect of 17β-oestradiol on the neuroeffector junction. This action of 17β-oestradiol might be the base of the mechanism by which hormone replacement therapy relieves hot flushes, ie by counteracting the possible fluctuations of oestrogen during the early menopause and also by reducing the responsiveness of the vascular smooth muscle to various stimuli.

CONCLUSIONS

The vasomotor symptoms of the menopause, otherwise termed as the 'hot flush' have received very little investigation, although they occur in most women at the menopause and are generally recognised as distressing and unpleasant. In the past these symptoms tended to be regarded as 'minor complaints' and unworthy of much attention. In recent years, however, the severity of the hot flushes has been acknowledged and this has resulted in the introduction of hormone replacement therapy to relieve these symptoms. However, this has not elucidated their mechanism especially since a direct correlation between hot flushes and circulating oestrogens has yet to be established (Campbell 1976).

The present study was both a physiological and pharmacological approach to examine the mechanism of the hot flush.

The problem was approached physiologically, by monitoring changes in physiological parameters, such as cardiac activity blood pressure, blood flow, skin resistance and skin temperature; and pharmacologically, by screening the plasma of post-menopausal women for vasoactive substances which could possibly play a role in the hot flush. In the present study, the hot flush appeared to be characterised by an altered cardiovascular and sympathetic activity. Furthermore, it occurs at a time when the oestrogen levels in post-menopausal women are altered. For this reason the effect of oestrogen was investigated upon both vascular and non-vascular smooth muscle and its innervation.

The epidemiological survey suggested that post-menopausal women are a group of women possessing a rather labile autonomic nervous system with an altered sensitivity to thermal stimuli

which occurred simultaneously with the onset of the menopause. The symptoms associated with, preceding, or accompanying a hot flush appeared to be symptoms of a generalised sympathetic activation (palpitations, tachycardia, sweating, faintness etc). In accordance with these symptoms were the signs of a sympathetic activation shown in the physiological recordings during a hot flush, ie an increased heart rate, undulations in the ECG base line and decreased skin resistance.

This increased activity might have been due to a gradual rise in sympathetic outflow which possibly reached a critical level at the onset of the hot flush, with a possible subsequent explosive activation of other local compensatory mechanisms to counteract this state of imbalance. It could be the activation of the local compensatory mechanisms that was responsible for the development of the hot flush.

The changes in physiological recordings in pre-menopausal controls during heat induced vasodilatation appeared to be distinctly different in onset and recovery, compared with those seen in post-menopausal women. This suggested that the changes seen during a hot flush are not simply due to vasodilatation. Both post- and pre-menopausal women were vasodilated to a comparable degree.

The temperature increase in post-menopausal women is best explained as secondary to vasodilatation. In contrast the temperature increase in the pre-menopausal women appeared to be the first event, with vasodilatation secondary to this event. The steep increase in the skin temperature in post-menopausal women, compared to the gradual and heat stimulus-dependent increase in

pre-menopausal women, again supports the suggestion that the two processes (hot flush, thermal vasodilatation) are based on different mechanisms. This was also supported by the fact that slight warming failed to cause a hot flush in one post-menopausal women, but simply resulted in a vasodilatation comparable to that seen in pre-menopausal women.

The lack of correlation between the magnitude of the actual temperature increases and the extreme heat distress experienced by post-menopausal women is probably due to an increased sensitivity to temperature changes after the menopause.

Finally, the onset, development and duration of the hot flush as seen through the physiological recordings, ie the objective duration of the hot flush, appears to be much greater than the subjective duration reported by the post-menopausal women. The reported duration appears to correlate better with the time elapsing between the onset of the hot flush and attainment of maximal changes in the physiological recordings.

The pharmacological investigation into the mechanism of the hot flush revealed that there is a humoral component involved in this mechanism, and this has not been reported before. This humoral component only became obvious after blocking the contractile activity due to 5HT, in the plasma samples from post-menopausal women taken at rest and during flushing, which suggests that 5HT is not the causative factor. The experimental evidence in the present study suggested that this factor does not appear to be acetylcholine, histamine, catecholamines or kinins. Most of the above substances have been suggested to play a role in the flushes

of other pathological conditions, where a humoral component is also thought to be involved, but no conclusive evidence of a definite participation of these substances in the flushes is yet available.

Total-prostaglandin E-like activity was found to be similar in post-menopausal women at rest and during flushing. However, individual prostaglandins ought to be determined before any conclusions are reached concerning their involvement in the hot flush.

The involvement of catecholamines in the mechanism of the hot flush should also receive further investigation since the results of the physiological study, together with the observed effects of oestrogen upon the innervation of the vascular smooth muscle, strongly suggest that:

Firstly, the sympathetic nervous system is involved in the mechanism of the hot flush.

Secondly, some of the functions of the sympathetic nervous system may be modified by oestrogen. Unfortunately, progress in the present study was severely limited by a lack of volunteers.

However, the existing experimental evidence, provided by the present study, may lead to the hypothesis that the vasomotor balance in post-menopausal suffering from hot flushes appears to be rather sensitive, possibly as part of a more general imbalance of the hormonal status of these women. This delicate balance often results in an extremely snstable condition which reaches a threshold just prior, or simultaneously with the onset of the hot flush. Beyond this threshold the compensatory mechanisms may be explosively activated in order to re-establish normality.

The study on the effect of 17β-oestradiol upon the responsiveness of vascular smooth muscle was performed in order to investigate whether the altered cardiovascular activity, observed during a hot flush, is due to a change in the hormonal environment in the blood vessels. As there was an indication that the sympathetic nervous system might be involved in the mechanism of the hot flush, the effect of oestrogen was also investigated upon other than vascular sympathetically innervated smooth muscle.

The results of this study suggested that 17β-oestradiol affects both vascular and non-vascular smooth muscle but in a different manner.

In non-vascular smooth muscle 17β-oestradiol appears to depress responses to transmural stimulation, to added noradrenaline and to potassium chloride, which was probably due to both a pre-synaptic effect and a direct effect upon the ability of the muscle to contract.

In vascular smooth muscle 17β-oestradiol has a biphasic effect. In low doses it enhances responsiveness to the endogenously released sympathetic transmitter noradrenaline.

This enhanced sympathetic responsiveness, which was shown to result from administration of small doses of oestradiol, might explain the increased sympathetic activity seen during a hot flush as being due to a peak reached in circulating oestrogen during fluctuations which are known to occur in post-menopausal women (Campbell 1976).

Furthermore, in higher doses oestradiol depresses responsiveness of the vascular smooth muscle to endogenously released

noradrenaline. This may offer a possible explanation for the mechanism whereby oestrogen replacement therapy relieves the hot flushes. It may be that pharmacological doses of oestrogen, as given in hormone replacement therapy, depress sympathetic activity and therefore counteract the possible increase in this activity that might result from oestrogen level fluctuations.

Oestradiol administration was also found to alter the pressor responses to added noradrenaline, but this effect was not as pronounced as the effect on the release of endogenous noradrenaline. However, in view of these results the possibility of a post-synaptic effect may not be excluded.

The effect of oestrogen upon the responses of vascular smooth muscle to the endogenously released transmitter requires further investigation.

This will involve a study of the influence of oestrogen upon noradrenaline release from and re-uptake into the nerve terminals, the enzymatic metabolism of noradrenaline and the receptor interaction of noradrenaline at the post-synaptic site.

It is hoped to continue these studies of the effect of oestrogen upon the sympathetically innervated vascular and non-vascular smooth muscle with the objective of further elucidating the mechanism of the hot flush.

The observation that the contractile activity of plasma samples from post-menopausal women during a hot flush differs from that of the plasma samples taken at rest, provides the first conclusive evidence that a humoral component is involved in the mechanism of the hot flush.

Furthermore, the observation that oestrogen affects the responsiveness of vascular smooth muscle to various stimuli, provides evidence that changes in the hormonal environment of post-menopausal women might be a causative factor in the development of these vasomotor symptoms.

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APPENDIX

Digital blood volume pulse at rest

Subjects	Pen excursion in cm	Hand temperature at rest (°C)
POST-MENOPAUSAL		
1	2.0	33.5
2	1.8	32.8
3	1.5	28.5
4	1.7	32.0
5	2.5	34.4
6	1.5	28.6
7	1.5	28.5
PRE-MENOPAUSAL		
1	0.5	25.8
2	0.5	25.7
3	1.5	29.7
4	1.0	31.4
5	2.5	34.5
6	2.0	32.9

Digital blood volume pulse (pen excursion in cm)

At rest	During a hot flush	Recovery
POST-MENOPAUSAL *(n = 7)*		
1.80 ± 0.14	4.6 ± 0.22	1.7 ± 0.11
At rest	**During warming**	**Recovery**
PRE-MENOPAUSAL *(n = 6)*		
1.33 ± 0.33	3.76 ± 0.60	1.4 ± 0.40

Digital blood volume pulse at rest

Subjects	Pen excursion in cm	Hand temperature at rest (°C)
POST-MENOPAUSAL		
1	2.0	33.5
2	1.8	32.8
3	1.5	28.5
4	1.7	32.0
5	2.5	34.4
6	1.5	28.6
7	1.5	28.5
PRE-MENOPAUSAL		
1	0.5	25.8
2	0.5	25.7
3	1.5	29.7
4	1.0	31.4
5	2.5	34.5
6	2.0	32.9

Digital blood volume pulse (pen excursion in cm)

	Post-menopausal (n = 7)	Pre-menopausal (n = 6)
At rest	1.80 ± 0.14	1.33 ± 0.33
During a hot flush	4.60 ± 0.22	-
During warming	-	3.76 ± 0.60
Recovery	1.70 ± 0.11	1.40 ± 0.40

Skin temperature (in ^{o}C)

POST-MENOPAUSAL

Subjects	At rest:			During a hot flush:			Recovery:		
	forearm	forehead	hand	forearm	forehead	hand	forearm	forehead	hand
1	31.7	33.1	33.6	32.0	34.4	35.3	31.8	33.0	33.5
2	30.7	32.6	31.9	31.6	33.7		30.6	32.7	31.9
3	31.0	34.3	28.5	31.4	34.6	28.8	31.0	34.2	28.6
4	28.1	33.4	32.0	30.5	34.0	31.7	28.0	33.4	32.0
5	31.0	34.4	34.5	31.3	35.0	35.0	30.9	34.3	34.3
6	30.1	35.6	26.6	31.6	35.8	28.9	30.1	35.7	26.7
7	32.15	34.0	28.6	32.9	35.9	29.7	32.1	34.1	28.8

PRE-MENOPAUSAL

Subjects	At rest:			During warming:			Recovery:		
	forearm	forehead	hand	forearm	forehead	hand	forearm	forehead	hand
1	30.0	34.6	34.5	33.7	36.2	34.8	30.1	34.6	34.4
2	31.0	34.6	25.8	33.0	35.3	30.6	31.0	34.5	26.0
3	30.0	33.8	25.7	33.5	34.4	26.2	30.0	33.9	26.2
4	32.2	33.8	29.7	33.9	35.3	33.5	32.1	33.6	29.6
5	31.2	33.6	31.4	34.4	36.2	35.5	30.9	33.5	31.4
6	32.6	35.2	32.9	35.4	36.0	35.6	32.4	35.0	32.9

Heart rate in beats/minute

Subjects	At rest	During a hot flush	Recovery
POST-MENOPAUSAL			
1	60.0	73.5	60.0
2	63.7	93.3	85.0
3	85.0	92.0	80.0
4	77.5	92.5	75.0
5	75.0	80.0	75.0
6	75.0	92.7	67.5
7	60.0	75.0	60.0
PRE-MENOPAUSAL		During warming	
1	75.0	75.0	75.0
2	77.5	77.5	77.5
3	62.5	62.5	62.5
4	77.5	77.5	77.5
5	87.5	87.5	87.5
6	85.0	85.0	85.0

Galvanic skin resistance in K^{Ω}

Subjects	At rest	During a hot flush	Recovery
POST-MENOPAUSAL			
1	179	87.9	180
2	189	150	180
3	160	100	150
4	380	90	380
5	195	105	190
6	183	139	180
7	600	170	560
PRE-MENOPAUSAL		During warming	
1	140	100	150
2	160	150	150
3	60	30	30
4	200	210	210
5	70	60	60
6	30	75	75

Effect of methysergide (MS) 10^{-7}M upon contractions of rat fundic strip to 5-hydroxytryptamine (5HT) and to plasma

Plasma	Pen excursion in mm: in the absence of MS	Pen excursion in mm: in the presence of MS	% response remained
POST-MENOPAUSAL (n = 7)			
At rest	27.0 ± 1.6	7.2 ± 2.0	26.7 ± 4.1
During a hot flush	29.0 ± 3.5	16.4 ± 2.2	56.7 ± 9.0
5HT	26.4 ± 2.3	3.9 ± 0.8	14.9 ± 4.5
PRE-MENOPAUSAL (n = 6)			
At rest	24.5 ± 2.7	9.8 ± 1.8	40.0 ± 1.2
During warming	26.3 ± 3.2	11.1 ± 2.1	42.3 ± 4.1
5HT	28.0 ± 4.2	2.5 ± 0.7	9.1 ± 2.3

Effect of 17β-oestradiol (15 µg/ml) upon contractions of the rat vas deferens to:

	In the absence of 17β-oestradiol	In the presence of 17β-oestradiol	Recovery
(n = 5)	[1]*FIELD STIMULATION*		
	33.8 ± 4.7	7.8 ± 2.0	34.6 ± 3.9
(n = 5)	[1]*NORADRENALINE*		
	28.6	8.2 ± 2.3	27.0 ± 4.1
(n = 5)	[1]*POTASSIUM CHLORIDE (KCl)*		
	37.9 ± 2.4	0.0	26.3 ± 4.8

[1]pen excursion in mm

Stimulus pressor response relationship in the female pithed rat (in mm Hg) (n = 5)

		Intact:		Ovariectomised	Sham-ovariectomised:	
		at oestrus	at dioestrus		at oestrus	at dioestrus
	ELECTRICAL STIMULATION					
Hz	0.1	14.0 ± 2.6	16.0 ± 1.0	13.5 ± 3.5	13.5 ± 1.0	14.0 ± 2.1
	0.2	24.0 ± 2.7	23.2 ± 3.8	19.1 ± 1.9	20.3 ± 3.6	20.3 ± 3.6
	0.4	34.0 ± 3.0	40.0 ± 4.4	34.6 ± 1.9	36.0 ± 4.9	36.0 ± 4.9
	0.8	54.8 ± 1.9	63.0 ± 5.9	52.9 ± 1.9	55.6 ± 3.4	55.6 ± 3.4
	NORADRENALINE					
ng	25	15.0 ± 2.8	18.8 ± 2.1	11.6 ± 1.6	13.6 ± 5.0	10.8 ± 2.2
	50	22.2 ± 2.3	26.0 ± 2.3	15.5 ± 1.3	29.0 ± 2.5	19.5 ± 4.0
	100	34.0 ± 3.5	38.8 ± 2.8	27.0 ± 2.7	33.5 ±13.9	28.7 ± 4.5
	200	47.4 ± 1.7	50.5 ± 1.1	36.0 ± 2.6	42.5 ± 6.5	38.5 ± 6.4
	ANGIOTENSIN					
ng	25	15.8 ± 4.0	18.0 ± 4.0	17.6 ± 1.9	14.0 ± 2.6	12.0 ± 3.9
	50	19.6 ± 2.8	29.5 ± 3.9	25.3 ± 3.5	26.0 ± 2.0	16.5 ± 3.0
	100	30.0 ± 2.5	39.0 ± 2.8	28.4 ± 2.4	32.4 ± 5.4	26.0 ± 4.4
	200	44.0 ± 2.23	47.5 ± 4.5	38.8 ± 1.5	41.6 ± 3.7	37.5 ± 6.4

The effect of time and of 17β-oestradiol upon pressor responses of the female pithed rat to electrical stimulation (in mm Hg) (n = 5)

INTACT ANIMALS

Time in min	In the absence of 17β-oestradiol: at oestrus	at dioestrus	In the presence of 17β-oestradiol (2 x 10 ng/kg)	In the presence of 17β-oestradiol (100 ng/kg)
5	32.4 ± 3.3	32.4 ± 3.5	34.6 ± 3.4	31.7 ± 4.1
20	37.4 ± 8.4	33.2 ± 3.9	38.2 ± 2.3	31.5 ± 4.0
35	36.4 ±10.4	34.8 ± 2.1	47.0 ± 4.0	34.7 ± 2.4
50	40.8 ± 6.4	40.8 ± 2.8	51.8 ± 1.7	26.7 ± 4.3
65	38.0 ± 7.8	42.0 ± 3.2	54.0 ± 4.2	23.7 ± 5.0
80	36.0 ±16.3	38.8 ± 5.1	55.0 ± 5.1	22.5 ± 4.0

OVARIECTOMISED ANIMALS

Time in min	In the absence of 17β-oestradiol	In the presence of 17β-oestradiol (2 x 10 ng/kg)	In the presence of 17β-oestradiol (100 ng/kg)
5	29.6 ± 2.4	33.0 ± 3.8	32.2 ± 2.7
20	27.2 ± 2.2	32.0 ± 4.7	34.0 ± 5.2
35	27.4 ± 2.7	35.0 ± 7.8	35.4 ± 5.5
50	28.2 ± 3.4	39.8 ± 8.0	34.8 ± 3.8
65	29.0 ± 5.6	41.8 ± 8.0	40.4 ± 5.6
80	30.6 ± 2.8	44.0 ± 9.7	43.8 ± 5.7

SHAM-OVARIECTOMISED ANIMALS

Time in min	In the absence of 17β-oestradiol: at oestrus	at dioestrus	In the presence of 17β-oestradiol (2 x 10 ng/kg)	In the presence of 17β-oestradiol (100 ng/kg)
5	27.4 ± 1.9	31.5 ± 5.9	36.5 ± 3.7	32.8 ± 3.7
20	28.0 ± 1.3	31.5 ± 5.6	36.0 ± 2.2	31.5 ± 3.4
35	27.2 ± 4.6	35.0 ± 6.1	40.5 ± 4.8	32.0 ± 3.6
50	36.5 ± 4.6	36.2 ± 4.9	49.5 ± 2.5	33.6 ± 4.2
65	40.0 ± 4.6	37.0 ± 4.4	51.0 ± 4.6	32.8 ± 5.4
80	40.3 ± 5.5	37.7 ± 5.8	54.5 ± 6.3	33.0 ± 7.1

The effect of time and of 17β-oestradiol upon pressor responses of the female pithed rat to noradrenaline (in mm Hg) (n = 5)

INTACT ANIMALS

Time in min	In the absence of 17β-oestradiol: at oestrus	at dioestrus	In the presence of 17β-oestradiol (2 x 10 ng/kg)	In the presence of 17β-oestradiol (100 ng/kg)
10	31.4 ± 4.4	33.8 ± 4.1	39.2 ± 2.5	34.8 ± 4.1
25	32.8 ± 5.6	28.0 ± 4.3	42.6 ± 1.9	31.5 ± 1.7
40	30.8 ± 2.6	26.8 ± 4.0	42.2 ± 2.0	29.5 ± 2.5
55	32.4 ± 4.0	30.4 ± 6.5	41.0 ± 2.9	31.5 ± 3.5
70	31.2 ± 2.6	33.5 ± 6.2	39.4 ± 2.9	24.0 ± 2.1
85	26.6 ± 3.0	38.0 ± 5.0	46.7 ± 2.6	23.3 ± 3.7

OVARIECTOMISED ANIMALS

Time in min	In the absence of 17β-oestradiol	In the presence of 17β-oestradiol (2 x 10 ng/kg)	In the presence of 17β-oestradiol (100 ng/kg)
10	34.4 ± 3.3	29.8 ± 3.8	33.8 ± 2.6
25	27.6 ± 4.4	28.5 ± 5.1	25.2 ± 2.3
40	30.8 ± 5.7	29.5 ± 4.5	26.0 ± 3.1
55	27.3 ± 5.1	36.5 ± 6.4	27.2 ± 2.9
70	34.2 ± 6.2	29.3 ± 1.0	29.2 ± 4.3
85	34.0 ± 6.6	39.3 ± 6.6	31.0 ± 4.2

SHAM-OVARIECTOMISED ANIMALS

Time in min	In the absence of 17β-oestradiol: at oestrus	at dioestrus	In the presence of 17β-oestradiol (2 x 10 ng/kg)	In the presence of 17β-oestradiol (100 ng/kg)
10	29.0 ± 3.5	23.5 ± 4.8	32.4 ± 3.5	32.0 ± 3.7
25	33.5 ± 2.6	24.4 ± 6.3	33.2 ± 2.6	27.0 ± 3.4
40	35.5 ± 5.0	27.2 ± 6.5	34.0 ± 2.5	28.6 ± 2.7
55	38.3 ± 5.9	29.6 ± 7.1	37.6 ± 2.8	28.8 ± 4.4
70	41.6 ± 5.3	33.0 ± 8.6	39.5 ± 7.0	30.0 ± 4.4
85	41.0 ± 2.0	32.0 ± 7.2	42.8 ± 7.9	28.0 ± 3.3

The effect of time and of 17β-oestradiol upon pressor responses of the female pithed rat to angiotensin (in mm Hg) (n= 5)

INTACT ANIMALS

Time in min	In the absence of 17β-oestradiol: at oestrus	at dioestrus	In the presence of 17β-oestradiol (2 x 10 ng/kg)	In the presence of 17β-oestradiol (100 ng/kg)
15	29.6 ± 3.6	29.7 ± 2.7	35.2 ± 1.9	34.0 ± 3.7
30	28.0 ± 2.6	26.3 ± 3.9	36.0 ± 2.5	31.8 ± 4.6
45	31.0 ± 4.0	32.5 ± 5.6	33.2 ± 3.5	29.0 ± 5.4
60	26.4 ± 4.5	28.5 ± 1.6	38.2 ± 4.8	23.3 ± 5.3
75	23.8 ± 1.8	34.0 ± 6.4	35.8 ± 5.6	22.8 ± 4.4
90	18.3 ± 2.0	32.0 ± 1.6	30.3 ± 4.2	24.0 ± 4.0

OVARIECTOMISED ANIMALS

Time in min	In the absence of 17β-oestradiol	In the presence of 17β-oestradiol (2 x 10 ng/kg)	In the presence of 17β-oestradiol (100 ng/kg)
15	27.4 ± 4.4	26.8 ± 3.6	26.0 ± 2.1
30	27.8 ± 4.8	26.8 ± 4.5	26.0 ± 3.8
45	26.6 ± 3.9	27.4 ± 7.3	26.8 ± 5.3
60	28.4 ± 5.7	25.3 ± 4.6	32.2 ± 6.9
75	29.8 ± 6.0	34.0 ± 7.9	31.2 ± 6.4
90	31.6 ± 7.1	28.8 ± 7.0	32.0 ± 8.1

SHAM-OVARIECTOMISED ANIMALS

Time in min	In the absence of 17β-oestradiol: at oestrus	at dioestrus	In the presence of 17β-oestradiol (2 x 10 ng/kg)	In the presence of 17β-oestradiol (100 ng/kg)
15	27.3 ± 4.2	29.6 ± 7.0	28.5 ± 2.0	28.7 ± 3.7
30	27.5 ± 5.6	32.4 ± 7.4	33.0 ± 2.8	29.3 ± 3.3
45	31.3 ± 4.6	30.4 ± 7.7	32.0 ± 5.9	29.5 ± 7.4
60	30.0 ± 1.8	31.6 ± 7.6	34.5 ± 7.6	27.5 ± 5.2
75	40.0 ± 1.6	37.6 ± 9.3	34.0 ± 7.1	29.0 ± 7.4
90	40.0 ± 1.6	35.0 ± 8.0	40.0 ± 9.0	30.0 ± 7.5

Stimulus pressor response relationship in the female pithed rat (% increase in resting systolic pressure) (n = 5)

		Intact:		Ovariectomised	Sham-ovariectomised:	
		at oestrus	at dioestrus		at oestrus	at dioestrus
	ELECTRICAL STIMULATION					
Hz	0.1	21.4 ± 3.7	24.5 ± 2.6	20.5 ± 4.3	18.3 ± 1.2	18.8 ± 2.4
	0.2	38.1 ± 4.1	34.2 ± 3.9	29.4 ± 3.4	31.5 ± 1.5	35.0 ± 6.4
	0.4	52.4 ± 3.0	53.0 ± 6.2	53.8 ± 2.1	52.9 ± 7.6	50.8 ± 5.6
	0.8	89.5 ± 5.0	84.2 ± 8.4	77.6 ± 4.0	81.2 ± 7.9	91.0 ± 9.7
	NORADRENALINE					
ng	25	23.9 ± 4.1	31.3 ± 6.8	21.2 ± 6.0	19.7 ± 6.4	19.4 ± 3.8
	50	37.6 ± 3.8	42.9 ± 7.3	25.4 ± 2.7	38.6 ± 7.3	30.3 ± 6.2
	100	55.5 ± 4.0	55.8 ± 5.6	43.4 ± 6.5	49.0 ± 9.3	43.3 ± 6.6
	200	69.0 ± 4.0	69.0 ± 3.0	61.4 ± 5.2	60.8 ± 9.9	66.5 ± 8.2
	ANGIOTENSIN					
ng	25	25.3 ± 3.5	26.4 ± 4.1	30.9 ± 1.0	23.2 ± 4.7	21.4 ± 5.7
	50	31.3 ± 3.3	41.9 ± 5.1	34.7 ± 4.3	37.1 ± 3.3	28.3 ± 4.4
	100	48.7 ± 5.3	53.4 ± 4.9	43.9 ± 2.6	40.9 ± 6.0	44.3 ± 5.6
	200	73.9 ± 3.5	64.8 ± 4.3	64.1 ± 6.6	62.3 ± 7.9	67.3 ± 8.4

The effect of time and of 17β-oestradiol upon pressor responses of the female pithed rat to electrical stimulation (% increase in resting systolic pressure) (n = 5)

INTACT ANIMALS

Time in min	In the absence of 17β-oestradiol: at oestrus	at dioestrus	In the presence of 17β-oestradiol (2 x 10 ng/kg)	In the presence of 17β-oestradiol (100 ng/kg)
5	49.2 ± 4.6	45.6 ± 3.3	50.8 ± 5.5	47.3 ± 6.3
20	58.4 ± 7.3	46.8 ± 4.5	60.2 ± 6.4	49.5 ± 7.5
35	57.2 ± 5.4	48.6 ± 2.0	66.9 ± 7.2	51.2 ± 5.7
50	67.6 ± 11.0	54.6 ± 1.6	76.6 ± 7.1	49.6 ± 4.3
65	63.0 ± 2.0	57.2 ± 4.1	82.8 ± 10.6	45.8 ± 5.6
80	63.0 ± 7.0	58.0 ± 4.0	83.4 ± 10.5	40.3 ± 6.4

OVARIECTOMISED ANIMALS

Time in min	In the absence of 17β-oestradiol	In the presence of 17β-oestradiol (2 x 10 ng/kg)	In the presence of 17β-oestradiol (100 ng/kg)
5	51.9 ± 5.2	55.2 ± 4.6	47.4 ± 3.1
20	51.4 ± 4.4	57.7 ± 6.7	50.3 ± 3.9
35	53.2 ± 7.2	58.4 ± 7.9	51.2 ± 3.9
50	54.2 ± 9.7	64.2 ± 7.3	52.8 ± 3.4
65	55.1 ± 7.5	69.4 ± 7.5	58.9 ± 4.5
80	51.0 ± 5.5	75.2 ± 8.3	64.1 ± 3.6

SHAM-OVARIECTOMISED ANIMALS

Time in min	In the absence of 17β-oestradiol: at oestrus	at dioestrus	In the presence of 17β-oestradiol (2 x 10 ng/kg)	In the presence of 17β-oestradiol (100 ng/kg)
5	48.1 ± 1.8	47.9 ± 5.5	50.2 ± 3.0	47.6 ± 3.1
20	49.4 ± 2.7	46.7 ± 7.0	50.9 ± 1.7	45.9 ± 3.9
35	53.5 ± 2.7	52.7 ± 5.1	56.6 ± 4.7	45.8 ± 3.1
50	58.8 ± 4.8	54.2 ± 2.2	61.1 ± 3.0	48.7 ± 3.3
65	62.9 ± 5.5	53.0 ± 4.2	69.2 ± 5.5	47.5 ± 3.9
80	63.8 ± 6.7	53.7 ± 5.2	77.5 ± 7.3	45.1 ± 5.6

The effect of time and of 17β-oestradiol upon pressor responses of the female pithed rat to noradrenaline (% increase in resting systolic pressure) (n = 5)

INTACT ANIMALS

Time in min	In the absence of 17β-oestradiol: at oestrus	at dioestrus	In the presence of 17β-oestradiol (2 x 10 ng/kg)	In the presence of 17β-oestradiol (100 ng/kg)
10	46.3 ± 7.3	49.6 ± 5.0	56.2 ± 2.2	48.7 ± 6.4
25	45.6 ± 5.3	41.4 ± 5.4	54.1 ± 5.4	50.8 ± 3.4
40	48.0 ± 6.8	37.8 ± 3.6	52.7 ± 6.4	48.5 ± 3.5
55	52.4 ± 7.8	43.7 ± 4.0	58.2 ± 7.2	47.7 ± 2.1
70	52.6 ± 10.2	43.7 ± 5.2	56.3 ± 4.4	42.0 ± 3.2
85	49.0 ± 4.1	48.1 ± 4.3	59.9 ± 5.6	43.0 ± 3.1

OVARIECTOMISED ANIMALS

Time in min	In the absence of 17β-oestradiol	In the presence of 17β-oestradiol (2 x 10 ng/kg)	In the presence of 17β-oestradiol (100 ng/kg)
10	54.3 ± 3.1	53.0 ± 4.9	51.8 ± 4.9
25	45.8 ± 6.9	50.1 ± 5.1	39.4 ± 3.9
40	50.5 ± 8.5	50.2 ± 2.3	41.0 ± 4.5
55	43.4 ± 8.0	60.0 ± 3.5	43.0 ± 5.2
70	54.1 ± 8.3	63.7 ± 4.8	44.3 ± 4.3
85	52.1 ± 8.1	71.5 ± 6.1	47.7 ± 5.0

SHAM-OVARIECTOMISED ANIMALS

Time in min	In the absence of 17β-oestradiol: at oestrus	at dioestrus	In the presence of 17β-oestradiol (2 x 10 ng/kg)	In the presence of 17β-oestradiol (100 ng/kg)
10	43.9 ± 4.4	45.1 ± 4.4	53.5 ± 4.7	40.7 ± 4.3
25	49.2 ± 7.2	45.6 ± 9.0	48.1 ± 6.4	39.6 ± 1.6
40	49.5 ± 5.9	50.6 ± 6.0	53.5 ± 5.0	42.3 ± 4.0
55	51.9 ± 7.5	48.6 ± 9.7	52.8 ± 4.2	42.7 ± 3.7
70	56.9 ± 7.7	45.4 ± 8.8	56.7 ± 8.9	48.6 ± 4.8
85	48.7 ± 5.3	51.9 ± 9.5	66.3 ± 10.0	42.4 ± 2.5

The effect of time and of 17β-oestradiol upon pressor responses of the female pithed rat to angiotensin (% increase in resting systolic pressure) (n = 5)

INTACT ANIMALS

Time in min	In the absence of 17β-oestradiol: at oestrus	at dioestrus	In the presence of 17β-oestradiol (2 x 10 ng/kg)	In the presence of 17β-oestradiol (100 ng/kg)
15	47.6 ± 8.5	41.4 ± 2.4	47.8 ± 4.5	48.0 ± 5.8
30	47.4 ± 7.9	35.3 ± 4.0	53.7 ± 4.9	43.0 ± 4.5
45	52.2 ± 9.2	42.7 ± 5.4	47.6 ± 4.3	40.3 ± 7.5
60	48.0 ±10.5	37.9 ± 2.1	54.9 ± 4.3	32.0 ± 7.0
75	37.4 ± 5.6	45.9 ± 6.3	54.8 ± 5.8	35.3 ± 8.2
90	34.5 ± 5.2	47.3 ± 8.6	48.4 ± 7.0	34.0 ± 9.0

OVARIECTOMISED ANIMALS

Time in min	In the absence of 17β-oestradiol	In the presence of 17β-oestradiol (2 x 10 ng/kg)	In the presence of 17β-oestradiol (100 ng/kg)
15	46.6 ± 5.4	50.6 ± 3.3	40.1 ± 5.8
30	47.8 ± 4.4	50.0 ± 3.1	36.9 ± 2.9
45	45.6 ± 5.3	48.4 ± 6.1	38.7 ± 3.9
60	47.0 ± 6.8	50.5 ± 4.8	45.1 ± 5.2
75	47.8 ± 7.6	59.0 ± 5.4	47.2 ± 6.9
90	50.3 ± 8.7	60.6 ± 8.3	45.9 ± 7.6

SHAM-OVARIECTOMISED ANIMALS

Time in min	In the absence of 17β-oestradiol: at oestrus	at dioestrus	In the presence of 17β-oestradiol (2 x 10 ng/kg)	In the presence of 17β-oestradiol (100 ng/kg)
15	42.7 ± 4.6	41.6 ± 3.9	40.7 ± 1.3	38.7 ± 3.0
30	45.7 ± 5.2	48.3 ± 7.5	48.9 ± 4.9	40.1 ± 3.2
45	46.9 ± 6.4	52.4 ± 9.0	43.4 ± 6.1	38.8 ± 3.3
60	44.2 ± 2.8	47.7 ± 8.8	46.4 ±10.0	30.0 ± 4.1
75	46.2 ± 4.3	46.9 ± 6.6	42.5 ± 9.0	33.2 ± 4.0
90	54.8 ± 4.3	42.8 ± 5.8	49.8 ±10.0	46.1 ±10.0