# THE PHARMACOLOGY OF VASODILATOR AGENTS ON VASCULAR MUSCLE.

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The aims of this study were; i) to evaluate rat vascular muscle preparations as model systems for predicting or assessing vasodilator efficacy on human vasculature; ii) to use rat vascular muscle to classify vasodilators by their spectrum of activity and to investigate the mechanisms of action underlying this empirical division. Rat portal vein and aortaic preparations were used and the effects of vasodilator agents upon the isometric tension developed in response to various stimuli was assessed.

Four parameters of portal vein reactivity were measured; spontaneous activity and contractions induced by KCl, noradrenaline or electrical field stimulation. It was found that not all vasodilators inhibited the parameters of reactivity in the portal vein to a similar degree; some agents (NP, GTN, and TOLM) caused a selective suppression of spontaneous activity, it was upon this basis that the vasodilators were classified. Similar experiments were carried out for selected vasodilators on responses of the rat aorta. When the results obtained from these experiments werecompared with published data from human vessels it was concluded that; i) the action of vasodilators on the spontaneous activity of the portal vein most closely resembled the actions of these agents on human hand veins; ii) the rat aorta was a poor model of the action of vasodilators on human arterioles.

The mechanistic basis of selectivity of inhibition of portal vein spontaneous activity by NP, was investigated. Kreye (1981) has suggested NP acted by causing vascular muscle hyperpolarization and consequently the action of hyperpolarizing treatments on the portal vein was investigated. Hyperpolarizing events caused a selective suppression of portal vein spontaneous activity similar to that seen with NP, consequently it may be that selective suppression of spontaneous activity of the portal vein is indicative of membrane hyperpolarization. Possible mechanisms by which NP, and other agents, caused hyperpolarization by alteration of $Cl^-$ and $K^+$ handling were also investigated.

Key Words.
1) Vascular Muscle. 2) Vasodilators.
3) Excitation-Contraction Coupling.
4) Membrane Hyperpolarization.

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To learn the age old lesson day by day,
It is not in the bright arrival planned,
But in the dreams men dream along the way,
They find the Golden Road to Samarkhand.

Omar.

B) Excitation Contraction Coupling in Vascular Muscle.



MATERIALS AND METHODS.



RESULTS AND DISCUSSION.

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## Introduction.

Vasodilators are agents which dilate blood vessels by relaxing the vascular smooth muscle. Early documentary evidence of the therapeutic usefulness of vasodilators was provided in 1867 by Brunton who used amyl nitrite to relieve the pain of angina pectoris. To this day angina remains one of the conditions for which vasodilators are used to provide effective relief. Other established uses of vasodilator therapy include hypertension and peripheral arterial diseases. Recently it has become apparent that vasodilators may be of considerable benefit in the management of cardiac failure and in reducing the eventual magnitude of cardiac damage following acute myocardial infarctions (AMI). In recent years, clinical experience with vasodilators has shown that for angina and AMI it is probable that agents with selective venodilator actions would be of most benefit, whereas in heart failure arteriodilators or non-selective agents may be of more use. Thus a clinical interest in vasodilator selectivity has seeded renewed scientific interest in the mechanisms of action of vasodilators of which this thesis forms but a small part.

A1. Vasodilator Classification.

Vascular muscle tone is the main determinant of vascular resistance which in turn is the main determinant of distribution of blood throughout the body. Consequently any drug altering tone will also effect distribution. Drugs can modulate tone by acting at a variety of sites ranging from the central nervous system to the vascular muscle cell. One diverse group of drugs which can interact with this system are the vasodilators. Since vasodilatation, i.e. a reduction in vascular tone, may be induced in a variety of ways it is useful to attempt to classify vasodilator drugs by their site of action. A possible classification is;

1) Alpha-adrenoceptor antagonists
2) Beta-adrenoceptor agonists
3) Ganglion blocking drugs
4) Directly acting vasodilators
5) Calcium antagonists
6) Angiotensin II inhibitors
7) Central Alpha-adrenoceptor agonists
8) Drugs acting on vascular endothelium

(Brown, 1980; Opie, 1980)

This study is primarily involved with the investigation of the modes of action of directly acting vasodilators. Directly acting vasodilators, as the name suggests, are those drugs which act at the level of the vascular muscle cell without interaction at a known specific receptor. Using this definition it is possible to classify calcium

antagonists as directly acting vasodilators. They have, however been placed in a separate class as they have a well defined selective site of action on the channel of the slow inward calcium current.

Vasodilator drugs are used in the treatment of several pathological conditions among which are; congestive heart failure, angina pectoris, "variant" or Prinzmetal's angina, hypertension, and myocardial infarction. To appreciate their therapeutic actions it is necessary to understand the haemodynamic consequences of vasodilatation in the patho-physiological states present in these conditions.

## A2. The Effects of Vasodilators on Cardiovascular Parameters.

The myocardial insufficiency seen in congestive heart failure may be brought about by many pathological conditions; e.g. ischaemic heart disease, primary cardiomyopathy and chronic pressure or volume overload seen in patients with long standing hypertension or valvular disease. All these conditions bring about a fall in cardiac performance which can to some extent be relieved by vasodilator therapy (Chatterjee & Parmley, 1980; Cohn & Franciosa, 1977).

With an intact circulation cardiac output (CO) is determined by four major factors;

1. Contractile State.
2. Pre-load.
3. After-load.
4. Heart rate.

(Cohn, 1973).

When stroke volume (SV) falls due to myocardial failure the heart tends to dilate. The dilatation of the left ventricle causes an increase in left-ventricular end diastolic volume (LVEDV; pre-load) in an attempt to maintain forward stroke volume. An increase in SV is brought about by an increase in left-ventricular filling pressure (LVFP) secondary to the increase in LVEDV. There may also be an increase in the adrenergic stimulation of the heart as a result of raised sympathetic tone and an increase in

circulating catecholamines (Chidsey,*et al.*, 1965). The resulting enhanced adrenergic state will increase heart rate and contractile state thereby tending to increase CO.

When CO is low (as is usual during myocardial insufficiency) total systemic vascular resistance (TSVR) tends to rise to maintain arterial pressure. Arterial pressure may be considered to be the product of CO and TSVR therefore an increase in TSVR which may induce a rise in aortic impedance may in turn produce a state of forward heart failure further reducing CO. A positive feedback loop may then be set up which will lead to a decreasing CO associated with an inappropriatly high TSVR. If vasodilator therapy can reduce TSVR it may be possible to break this loop and maintain or improve CO.

These compensatory haemodynamic changes have other deleterious effects. An increase in pre-load (LVEDV) gives rise to an increase in left-ventricular end diastolic pressure (LVEDP), this in turn may cause a passive rise in left atrial pressure and pulmonary venous pressure which are the primary determinants of pulmonary congestion and oedema. Furthermore because of the increase in left ventricular size (secondary to raised LVEDV) wall stress will increase and since La Place's relationship predicts that the proportion of energy required to maintain tension will rise, an increase in myocardial oxygen requirements and a decrease in the pumping efficiency of the heart will ensue. Myocardial oxygen demand may also by elevated by the enhanced

sympathetic state of the heart. This overall increase in the oxygen demand could have serious effects in patients with ischaemic heart disease. The effects of vasodilators on myocardial oxygen demand is discussed more fully in the next section.

The predominant mechanism by which vasodilators increase CO is by decreasing systemic arteriolar tone and thus lowering afterload. In isolated heart muscle reduction of the load against which the muscle must shorten increases the degree and rate of shortening (Sonnenblick, 1962). In an intact heart a similar reduction in load (resistance to ejection) can improve rate and degree of myocardial shortening and increase SV due to improved contraction. In terms of the left ventricular function curve (the Frank-Starling curve) this constitutes a displacement upwards and to the left in response to vasodilatation. The reduction in the aortic impedence brought about by a fall in TSVR should allow a greater SV to be expelled for a given left ventricular filling pressure (LVFP) dependent upon the initial level of LVFP. The two paramters, aortic impedence and TSVR, are closely related but not equivalent. The aortic input impedence spectrum is a complex relationship between the pulsatile flow and the pulsatile pressure but is reasonably approximated to the aortic pressure divided by the aortic flow at the same instant. Total systemic vascular resistance, on the other hand, can be thought of as an analogous relationship between mean flow and mean pressure (Brown, 1980; Milnor, 1975).

Many vasodilators relax systemic veins, increasing the volume of these capacitance vessels, thus redistributing blood volume. This may give rise to a transient fall in venous return which then rapidly returns to a steady state. The pooling of blood is however, effective in reducing filling pressure and intracardiac volume of both the right and left sides of the heart. The reduction of right and left filling pressures is beneficial in relieving pulmonary and systemic venous congestion. The resultant effect on SV is dependent on the initial LVFP and may rise, remain unchanged or fall. If SV falls there may be a compensatory reflex tachycardia. Similar changes in SV due to vasodilators which are equipotent on arteries and veins are also determined by initial LVFP.

Vasodilators may also improve cardiac function of patients with heart failure by increasing left ventricular compliance. The pressure-volume relationship of the left ventricle is curvilinear, such that at low LVEDV, a volume increment will produce only a small rise in LVEDP. At higher LVEDVs the same increment will produce a much greater increase in LVEDP (Chatterjee & Parmley, 1980).

As LVEDV is a major determinant of SV (via LVFP), a shift in the left ventricular pressure-volume curve will also influence the relationship between LVFP and SV. Thus if the left ventricular pressure-volume curve is moved to the right (an increase in compliance) a leftward shift of of the

ventricular function curve (LVFP vs. SV) will occur, indicating an improvement in cardiac performance (Parmley, 1976).

Recent studies (Glantz *et al.*, 1978, Glantz & Parmley, 1978) have suggested that some vasodilators cause an acute increase in left ventricular compliance. The mechanism of this change remains unclear, however several suggestions have been made; reduction of ischemia and relief of ischaemic contracture, increased ventricular relaxation and changes in the interaction of the right and left ventricles in a confined pericardial space. Whatever the mechanism of vasodilator action on ventricular compliance it can be seen that an increase in compliance will improve a pathologically reduced cardiac function.

A3. Effects of Vasodilators on Myocardial Oxygen Demand.

Myocardial oxygen demand is largely determined by four factors; heart rate, contractile state, arterial pressure and heart size. Vasodilators are not generally inotropic or chronotropic and cannot therefore alter oxygen demand via these mechanisms. However during vasodilator therapy arterial pressure may well fall, especially in the presence of an arterio-selective vasodilator. LVEDV may also be reduced causing a reduction in heart size, this effect being seen to a greater extent with veno-selective agents. Vasodilators, by their action to decrease heart size and lower arterial pressure, are likely to have a favourable action in lowering myocardial oxygen demand. The effect of the decreased arterial pressure may be counteracted to some extent by a reflex tachycardia which will tend to increase myocardial oxygen demand. The overall effect of the decrease in pressure will depend on the extent of the tachycardia. However in the clinic the raised heart rate is unimportant as it can be readily blocked by a beta-adrenoceptor antagonist. Nitrate vasodilators also redistribute blood flow along collateral channels and thus alter oxygen supply, increasing perfusion of the endocardial region. This action is probably due to the ability of nitrates, especially GTN, to preferentially dilate large coronary arteries. (Schnaar & Sparks, 1972, Opie, 1980) These actions of nitrates on oxygen supply are not universal amongst vasodilators and some agents worsen the situation by diverting blood away from the ischaemic area (dilating epicardial vessels) a so called 'coronary steal' effect. Overall any decreased oxygen

consumption may contribute to an improved cardiac performance and is likely to be important in myocardial ischaemia.

## A4. Vasodilators in Angina Pectoris.

The haemodynamic changes during an anginal attack are well documented (Parker, 1972) and include; elevated pulmonary perfusion pressure, pulmonary wedge and left ventricular end diastolic pressures. LVEDP can rise to four times its normal resting levels during excercise in anginal patients. The increase in LVEDP causes an elevation in LVFP and LVEDV also resulting in part from a decreased ventricular compliance, these changes are not accompanied by an increased CO. A situation where these conditions exist would indicate a depressed left-ventricular function curve. From the above information it could be concluded that angina is a readily reversible acute form of left ventricular failure. Another result of the increased filling pressure is a reduction in the blood flow to the endocardial regions of the left ventricular wall. This flow usually occurs when intra-ventricular pressure is at its lowest during diastole. When LVEDP is elevated the pressure gradient across the ventricular wall which induces flow to occur is reduced. This reduction in gradient being known as 'diastolic crunch' (Parratt, 1975). As discussed previously myocardial oxygen demand is elevated when LVEDP is increased due to a rise in ventricular wall tension. The anginal pain is experienced because of the increased oxygen demand, normally due to exercise, which cannot be supplied by a rise in coronary blood flow consequently producing areas of myocardial ischaemia. The inability of the coronary vasculature to increase supply is generally due to atherosclerotic obstruction of the epicardial arteries.

For many years it was thought that vasodilator therapy increased myocardial perfusion directly and consequently improved myocardial oxygen supply and thus relieved anginal pain (Lewis, see Opie, 1980). Work carried out by Gorlin and co-workers (Gorlin *et al.*, 1959) showing that GTN, in anti-anginal doses, had no effect on total coronary flow, went some way to refuting this theory. Further evidence in support if the theory of a peripheral site of action was gained by studies that compared systemic intravenous administration of vasodilators with direct injection into the coronary circulation (Ganz & Marcus, 1972). These experiments were carried out in patients who's coronary arteries had been catheterised for purposes of angiography. Coronary sinus outflow was measured, by a heat dilution method, during anginal attcks induced by pacing the heart. The anginal pain was relieved by systemically administered vasodilator (Glyceryl Trinitrate, GTN) but not by the locally administered drug despite the fact that intracoronary injection increased the total coronary flow. It was noted that systemic administration of vasodilator (GTN) actually reduced total coronary flow, probably due to a reduction in myocardial oxygen demand. The fall in demand was probably caused by the same mechanisms which reduce oxygen demands during vasodilator therapy of congestive heart failure (See above.). It would therefore appear that for the therapy of angina pectoris the systemic actions of vasodilators are more important than any coronary dilatation they may cause.

The ability of some vasodilators (notably GTN) to increase total coronary flow has been implicated in their usefulness in the treatment of Printzmetal's (variant) angina. It is thought that variant angina is largely due to spasms of the coronary arteries (Maseri & Chierchia, 1980). It is characterised by; chest pain at rest, S-T segment elevation in the standard electrocardiograph leads, ectopic ventricular activity and atrioventricular block. GTN has been shown to bring about an increase in the perfusion of the inner (endocardial) layers of the left ventricular wall. It has been suggested that this is due to its unusual ability to preferentially dilate large coronary arteries. Many such arteries penetrate the myocardium at right angles to the wall from the superficial epicardial vessels (Schnaar & Sparks, 1972, Winbury *et al.*, 1969). The increase in endocardial perfusion brought about in this manner would improve localised oxygen supply and relieve anginal pain. It is possible that such preferential relaxation of the large coronary arteries is due to the blockade of slow inward $Ca^{2+}$ currents by GTN (Harder *et al.*, 1979). This hypothesis is supported, to some extent, by the fact that calcium entry blockers have also been found to be highly effective in the treatment of variant angina (Muller & Gunther, 1978; Yasue, 1980). On the other hand this redistribution of blood flow could be due to an increase in the perfusion gradient to the endocardium as a direct result of a reduction in wall stress and LVEDP which would in turn result from systemic and not coronary vasodilatation (Parratt, 1975; Winbury, 1971).

## A5. Directly Acting Vasodilators.

Clinicaly used vasodilator drugs cover a wide variety of chemical types and spectra of action. A short review of the known mechanisms of action of the individual agents used in this study is given below.

### A5i. Glyceryl Trinitrate.

Glyceryl trinitrate (GTN) was one of the first clinically used vasodilators, its effects as an anti-anginal agent being discovered over one hundred years ago. (Murrel, 1879)

GTN has been shown to be veno-selective *in vitro* (Mackenzie & Parratt, 1977) and to reduce venous return *in vivo* (Ferrer, *et al.* 1966). The reduction in venous return is thought to be due to venous pooling as there is no reduction in circulating blood volume (Ferrer *et al.* 1966) or any change in transcapillary exchange (Ablad & Mellander, 1963). The sites of pooling are unknown, however both skin and skeletal vascular beds have been suggested (Mason & Braunwald, 1965). It is this increase in pooling which reduces LVEDP and thus myocardial oxygen demand by the mechanisms outlined earlier. While this is the major route of action by which GTN relieves the symptoms of angina it has several other interesting haemodynamic effects.

GTN has the unusual ability to dilate large coronary

arteries which can be put to considerable clinical use in the treatment of variant angina. This action has been discussed more fully above. (See, A4. Vasodilators in Angina Pectoris.) GTN has also been shown to reduce the extent and severity of the ischaemic injury in canine hearts where the major coronary artery has been occluded, probably by improvement of the collateral supply (Hirshfeild, *et al.*, 1974).

GTN, unlike most vasodilators, is positivly inotropic. This action has been shown both *in vivo* (Weiner *et al.*, 1969) and *in vitro* (Korth, 1975). However this action can be prevented by propranolol or by pretreatment with reserpine (Weiner *et al.*, 1969, Korth, 1975). It therefore appears that this inotropic action is due to the release of endogenous myocardial catecholamines. It has also been suggested that GTN may have some action in raising the ventricular fibrillation threshold during myocardial ischaemia. It is unlikely however, that any of these actions are of clinical significance (Hirshfeild, 1974).

GTN has also been used in the treatment of cardiac failure due to myocardial infarction. GTN is of particular use in patients with elevated pulmonary wedge and left ventricular filling pressure as these are markedly reduced by peripheral venodilatation. A secondary effect of the reduction of LVFP is a decrease in the degree of any pathological mitral and aortic regurgitation. It is not clear however, how useful GTN is in myocardial infarction

not associated with left-ventricular failure.

The pharmacological basis of GTN's ability to relax vascular, especially venous, muscle is both complex and poorly understood. The biotransformation of organic nitrates has been implicated both in their duration of action and their mode of inactivation. Oberst and Snyder (1948) showed that there were two mechanisms by which GTN was removed from the circulation of rabbits. One hepatic system which could be inactivated by high temperatures and a system found in blood and muscle as well as in the liver which was temperature independent. The temperature independent GTN metabolism was shown to be due to a non-enzymatic reaction between GTN and glutathione (GSH) to form nitrite and oxidised GSH (Heppel & Hilmoe, 1950). Heppel and Hilmoe (1950) also showed that the temperature dependent inactivation was due to a reaction between GTN and GSH enzymatically catalysed by glutathione-organic-nitrate reductase. It was later shown by Di Carlo & Melger (1970) that the non-enzymatic breakdown of GTN by GSH did not play a significant role in the removal of GTN *in vivo*.

Needleman, Lang and Johnson (1972) showed that the parent organic nitrate compound was necessary for vasodilatation. When injected via the jugular vein GTN has a short lived but marked vasodilator action. A dose of GTN producing a marked vasodepression by the jugular route was completely inactive when injected directly into the hepatic circulation. They also showed that the transient life of the

parent organic nitrate ester was matched directly with the duration and extent of the vasodilatation. On the other hand the long lived metabolites of hepatic origin are without any vasodilator action. These findings explain the lack of efficacy of orally administered GTN as the active compound would pass directly from the gut to the liver, via the portal circulation, where it would be inactivated without ever reaching the systemic vasculature.

Needleman & Johnson (1973) have suggested that in order to bring about vascular relaxation organic nitrates must react with a "receptor". GTN is supposed to do this by reacting with sulphydryl groups at the putative receptor site. They have also proposed a mechanism for nitrate tolerance based on a nitrate-"nitrate receptor" interaction. They argue that the nitrate oxidises a critical sulphydryl group in the receptor to form a disulphide moeity which then has a lower affinity for the nitrate. Their hypothesis is supported by the fact that GTN tolerance has been located at a vascular level. Aortae from GTN tolerant rats have been shown to exhibit markedly smaller relaxations to GTN *in vitro* than aortae from non tolerant rats. They have also shown that tolerance induced both *in vitro*, by incubation of vascular muscle with GTN in alkaline pH, and *in vivo* by chronic administration of GTN can be reversed by agents which can reduce disulphide groups, e.g. dithiothreitol.

GTN has also been shown to increase levels of cyclic-GMP by a factor of two in rat ductus deferens (Bohme, 1977). Mackenzie and Parratt (1977) have suggested that GTN,

as well as other organic nitrates, may stimulate adenyl cyclase. Further credence has been given to this suggestion by the finding that GTN induced relaxation is potentiated by the phosphodiesterase inhibitor theophylline (Levy & Wilkenfeld, 1968). It is as yet unclear whether alterations in cyclic nucleotides of this order could be significant in mediating relaxation. Similar experiments have been carried out where changes in cAMP in response to GTN have been measured (Andersson, 1973). In this case, in the bovine mesenteric artery, there was a twofold increase in cAMP levels apparently due to phosphodiesterase inhibition. Again it as unknown if this has a significant role to play in the relaxant effect of GTN. More recently it has been suggested that GTN has its relaxant actions on vascular muscle by a non-selective suppression of $Ca^{2+}$ mobilization from storage sites without a notable change in membrane properties (Ito *et al.*, 1980).

Levin, Weksler & Jaffe (1980) have recently suggested that GTN may release prostacyclin ($PGI_2$) from vascular endothelium. $PGI_2$ is a potent vascular muscle relaxant (Moncada & Vane, 1979) and could in some part be responsible for GTN's vasodilator action. Lippton and co-workers (Lippton *et al.*, 1981) have investigated the action of indomethacin, a cyclo-oxygenase inhibitor, on the vasodilator acions of GTN in cat mesenteric and hind-limb vascular beds. They showed that indomethacin, in a dose which inhibited the vascular action of arachidonic acid, did not attenuate the vasodilator response to GTN. It would

therefore appear that there is some doubt as to the role of prostaglandins in mediating vasodilator responses to GTN, this may in part be due to species and experimental differences.

A5ii. Sodium Nitroprusside.

Nitroprusside's (NP) hypotensive action was first extensivly studied by Johnson (1929) who suggested a therapeutic use for NP in hypertension. However, it was not until roughly 30 years later that NP found widespread clinical usage (Page *et al.*, 1955). Sodium nitroprusside (NP) is a potent, short acting, intravenously active vasodilator drug. It is used to a limited extent in hypertensive crisis, congestive heart failure, valvular regurgitation and in the deliberate production of hypotension to reduce bleeding during surgical procedures (Macrae & Hillis, 1980; Mehta *et al.*, 1981). The use of NP is limited largely by the necessity for intravenous administration and the prudence of monitoring cyanide levels in patients undergoing prolonged infusion.

NP produces considerable alterations in haemodynamic parameters, the most marked being a reduction in mean arterial pressure associated with a reduction in total peripheral resistance (Rowe & Henderson, 1974). There is, however, still some controversy as to the arterial or veno-selectivity of NP. Robinson, Collier and Dobbs (1979) have suggested that NP is approximately 10 times more effective on veins than on arteries whereas other authorities (Opie, 1980) have reported an equipotent action. The haemodynamic consequences will be largely dependent upon the balance of arterial and venous dilatation and any alteration of coronary responses will depend on the initial

state of the heart ( See: A4 The Effects of Vasodilators on Cardiovascular Parameters.). The cardiovascular effects of NP are partially antagonised by a reflex tachycardia and NP induced hypotension can be greatly increased by concurrent use of ganglion blocking agents, beta-adrenoceptor antagonists or surgical sympathectomy (Kyncl, 1971).

The mechanism of NP action on vascular muscle is as yet unclear. NP has been shown to have no action in electro-mechanical uncoupling in rat portal vein (Jetley & Weston, 1980). A specific alpha-adrenoceptor antagonist action of NP has been ruled out as it caused a marked relaxation of rat aorta contracted with Angiotensin II or $Phe^2$-$Lys^2$-vasopressin (Kreye *et al.*, 1975). Verhaege & Shepherd (1976) have also shown that there is no alteration in transmitter release from nerve endings after treatment with NP by measuring NA efflux from electrically stimulated saphenous veins relaxed by NP.

It has been suggested (Kreye *et al.*, 1975; Hausler & Thorens, 1976) that NP might interfere with $Ca^{2+}$ metabolism, either by inhibiting $Ca^{2+}$ entry into the vascular muscle cell or by affecting its handling after it has crossed the cell membrane. The exact action of NP on $Ca^{2+}$ movements and handling is confused, Kreye and co workers (1975) showed NP inhibited $^{45}Ca$ influx during $K^+$ depolarization while Hausler and Thorens (1975) found no change in $Ca^{2+}$ movements. Under similar, though not identical, conditions, Van Breemen (1976) found that NP increased calcium uptake into $K^+$

depolarized rabbit aortae. The weight of evidence is now favouring a mechanism other than inhibition of $Ca^{2+}$ influx. NP has been shown to inhibit NA induced contractions induced in calcium free media in the presence of verapamil or lanthanum chloride. Furthermore, it has been shown (Kreye & Luth, 1976) that NP can increase the rate of relaxation of $K^+$ depolarized rat aorta independently of extracellular $Ca^{2+}$ concentration. These studies cannot however, show any alteration of internal $Ca^{2+}$ metabolism induced by NP, e.g. increased intra-cellular sequestration or cellular extrusion.

Hausler and Thorens (1976) have reported that NP causes a dose-dependent hyperpolarization of rabbit main pulmonary artery. This hyperpolarization could be brought about in four ways:- activation of an electrogenic Na pump; increasing $K^+$ permeability; reducing $Na^+$ permeability or reducing $Cl^-$ permeability (Kreye, Kern & Schleich, 1977). Reduction of $Cl^-$ permeability would cause hyperpolarization in vascular muscle because the equilibrium potential $E_{Cl}$ differs considerably from the resting potential $E_m$ (-20 to -30mV compared with -50 to -60mV for $E_m$ ) · Siegel *et al.*, 1976).

Kreye and co-workers (Kreye, Kern & Schleich, 1977) have shown that NP has no action on $^{24}Na$ efflux or permeability. Rapoport and Murad (1983) have suggested that the hyperpolarization was caused by stimulation of the

electrogenic $Na^+$ pump. However, Karaki *et al.* (1984) have shown NP to remain effective in $K^+$ depolarized rat aorta in the presence of ouabain. Kreye *et al.* (1977) have also suggested that as NP induced relaxation is unaffected by ouabain, it is therefore unlikely that any Na pumping mechanism is involved. In the same study Kreye *et al.*(1977) showed that NP reduced $Cl^-$ efflux induced by NA, this supports the hypothesis that the hyperpolarization seen with NP is due to a decrease in $Cl^-$ permability. Further evidence on the effects of $Cl^-$ transport on membrane polarization has recently been published by Kreye, Bauer & Villhauer (1981). They suggest that there is a furosemide sensitive active chloride transport mechanism in rabbit aorta and that the action of furosemide on the transport of $Cl^-$ is such as to induce a hyperpolarization in the vascular muscle cell membrane. None of the above observations rule out the possiblity that NP may increase $K^+$ permeability in such a way as to cause hyperpolarization.

NP has been shown to increase levels of cGMP in many vascular and non-vascular preparations (Bohme *et al.*, 1977; Schultz *et al.*, 1978) by stimulation of guanylate cyclase. Furthermore the finding that 8-bromo-cGMP could relax rat aorta coupled with the fact that the NP induced changes in cGMP were large and generally associated with relaxation suggested that there could be a causal link between the two observations. The role of cGMP in mediating the relaxant effects of NP is further supported by the recent observation that agents which inactivate $NO^-$ moieties released

intracellularly by NP, inhibited both the relaxation and the stimulation of guanylate cyclase caused by NP (Gruetter *et al.*, 1979). The action of bromo-cGMP and the phosphodiesterase inhibitor M&B 22 948 in increasing the relaxant effects of NP while increasing the levels of cGMP also support their causal linkage (Kukovetz *et al.*, 1979).

There are however, some findings which call this hypothesis into question, most notable among these are the observations of Diamond and Janis (1978) and Kreye and co-workers (Kreye,*et al.*, 1975) that NP could increase cGMP levels in rat vas deferens without causing relaxation. Lincoln and Keely (1980) have also shown that in rat heart NP can raise cGMP levels with no aparant action on force of contraction. The cause of this action they propose is that cGMP production induced by NP was not coupled to protein kinase activation and thus end organ response.

Whatever the underlying mechanism of action of NP, it is obvious that it is complex and may combine more than one of the above hypotheses as they may not be mutually exclusive.

A5iii Tolmesoxide (RX71107).

Tolmesoxide is an aryl sulphoxide derivative which has been shown to be hypotensive (Doxey, 1978). This action is due to a direct relaxing effect on vascular muscle (Doxey, 1978). Tolmesoxide has been shown to be equipotent in relaxing human venous and arterial smooth muscle (Collier *et al.*, 1972). Consequently any haemodynamic changes would be due to a mixture of reduction in arterial impedence and LVEDV. However, as no clinical data is available it is impossible to predict if tolmesoxide would be of any therapuetic use in hypertension.

The mechanism by which tolmesoxide has its direct vasodilator action is unknown. Mikkelssen and Lederballe-Pedersen (1981) have shown in isolated human crural vessels that tolmesoxide has only a slight inhibitory action on contractions induced by the addition of $Ca^{2+}$. Furthermore $^{45}Ca$ flux studies have shown little inhibition of the inward $^{45}Ca$ flow during depolarization in the presence of tolmesoxide. These results tend to suggest that there is little or no blockade of calcium entry exhibited by tolmesoxide, however they do not rule out the possibility that the drug is in some way altering $Ca^{2+}$ handling within the cell.

A5iv Hydrallazine.

Hydrallazine was amongst the first synthetic compounds to be used clinicaly as a hypotensive agent (Page, 1951). It has been used extensively in combination with reserpine and hydrochlorothiazide for the treatment of hypertension and still retains a place in the modern clinic (Gross, 1977).

Hydrallazine produces a marked vasodilatation limited almost exclusively to the arteriolar resistance vessels. As a consequence of this it is a potent hypotensive agent and its major use has been in the control of hypertension. The hypotensive effects of hydrallazine have a set pattern, being slow in onset, of marked, but not profound, extent and of prolonged duration. When adminstered to patients with cardiac insufficency its action in increasing CO is due largely to a reduction of arterial impedence (Fries *et al.*, 1953). Thus, hydrallazine therapy may be of considerable use in the treatment of patients with hypertensive congestive heart failure. It should be noted that the reflex tachycardia exhibited in normotensive subjects treated with hydrallazine is rarely seen in patients suffering from heart failure. Hydrallazine has also proved useful in the treatment of mitral and aortic regurgitation again due to its ability to reduce arterial impedence (Greenburg *et al.*, 1978). The reduction in arterial impedence leads to an increase in left ventricular ejection fraction and a decrease in regurgitation volume. Kharti and co-workers (1977) have suggested that hydrallazine has a positive

inotropic action but this effect has only been seen *in vitro* with doses that would be difficult to attain *in vivo*.

Hydrallazine is metabolised by both the gut wall and the liver and forms several metabolites. Notably the acetone, pyruvate and alpha-ketogluterate hydrazones. The pyruvate and alpha-ketogluterate metabolites appear to be as active as the parent compound in relaxing smooth muscle *in vitro* (Haegle *et al.* 1978). The degree to which any patient can metabolise hydrallazine is determined by liver levels of N-acetyltransferase which are genetically determined. This metabolic change may cause difficulties in maintaining level concentrations of active compounds *in vivo*. Hydrallazine appears to be actively bound to vascular muscle in both arteries and veins with a preference for arteries (Wagner & Hedwall, 1972). The duration of vascular wall binding of hydrallazine and/or its metabolites correlates with the duration of the hypotensive effect. However, no correlation between concentration in the wall (measured by radio-isotope binding) and degree of hypotension has been found (Wagner & Hedwell, 1975).

The mode of action of hydrallazine is as yet unclear. It has been shown to have little effect on cGMP levels in non-vascular smooth muscle (Diamond & Janis, 1978; 1980). It has been suggested that hydrallazine may have its vasodilator action by interfering with the entry of $Ca^{2+}$ into cells, reflecting some effect of hydrallazine at a cellular level (McLean, 1978). Another mode of action

suggested for hydrallazine is an interaction with a smooth muscle receptor sensitive to endogenous purines (Chevillard *et al.* 1980). Trapani and co-workers (1980) have shown a hydrallazine induced hyperpolarization in the rat caudal artery which can be partally blocked by purines. They concluded that the hyperpolarization may have a significant role to play in the vasodilatation induced by hydrallazine and that the antagonism between hydrallazine and purines may be due to a direct membrane effect rather that the receptor interaction envisaged by Chevillard (1980). At a sub-cellular level, hydrallazine has been shown to inhibit several enzymes either by direct action or by chelating heavy metal ions needed as enzymatic co-factors (Liu *et al.*, 1974). It is as yet uncertain whether this action of hydrallazine is related to its vasodilator properties. However, which, if any of these models is the predominant mechanism is unknown.

A5v Verapamil.

Verapamil is a $Ca^{2+}$ entry blocker which relaxes vascular muscle by a direct action on the transmembrane $Ca^{2+}$ fluxes (Henry, 1980). It is a potent cardiodepressive agent with negative chronotropic and inotropic effects (Singh & Roche, 1977). Verapamil prolongs atrio-ventricular conduction time in a dose dependent manner and because of this is widely used in the treatment of re-entrant supraventricular arrhythmias. Verapamil is used both in arrhythmias where re-entry is within the A-V node and also where it occurs via an accessory pathway (The Wolff-Parkinson-White syndrome) (Opie, 1980). Verapamil also has some mild anti-anginal activity although its anti-arrythmic action is of far greater clinical significance (Krikler, 1974). Fortunately the negative chronotropic and inotropic actions of verapamil are seen at doses in excess of the therapuetic range (Mangiardi, *et al.*, 1977) and so have not limited the clinical usefulness of this agent.

The stereochemistry of verapamil may have some bearing on its mode of action (Bayer, *et al.*, 1975). The two enantiomers of verapamil have been shown to have different effects on the cardiac action potential. The (+)-enantiomer depresses the rate of rise of the rapid phase of the action potential, suggesting an action on the fast channels (A local anaesthetic like action.). While the (-)-enantiomer depresses the plateau phase of the action potential,

suggesting a slow channel blocking action.

The action of verapamil on vascular muscle $Ca^{2+}$ movements are complex. Thorens and Haeusler (1979) showed that verapamil, but not GTN or NP, caused a dose-dependent inhibition of $^{45}Ca$ influx. It has been suggested that as $K^+$ induced contractions are largely due to an increase in vascular muscle $Ca^{2+}$ uptake that verapamil should have a preferential inhibitory action on these contractions. Some doubt as to this action of verapamil still remains, Massingham (1973) has shown a preferential reduction of $K^+$ responses while Mikkelsen and co-workers (1979) have showen verapamil to be equipotent against $K^+$ and NA contractions. This apparant conflict in results may be due to species differences.

Verapamil has also been shown to be effective in inhibiting $Ca^{2+}$ dependent action potentials in canine coronary arteries induced by electrical stimulation in the presence of TEA (Henry, 1980). Care must be taken when interpreting such results as the experiments are frequently carried out in abnormal ionic environments.

A5vi Diazoxide.

Diazoxide which has been shown to be a potent hypotensive agent is a derivative of the benzothiadiazine diuretics (Rubin, Roth & Winbury, 1961). Diazoxide lowers blood pressure both in normotensive and hypertensive experimental animals although some workers have reported a more pronounced effect on elevated pressures (Stanton & White, 1973). Ramaswamy and Richardson (1968) have shown an increase in stroke volume and CO which may in part be due to diazoxide's ability to release catecholamines as this increase in CO can be inhibited by propranolol. Diazoxide has found little use in the general treatment of hypertension because of its hyperglycaemic action, it is however used in the managment of hypertensive crisis.

Diazoxide has its direct relaxant effect on vascular muscle independent of any action on beta-adrenoceptors (Naylor, *et al.*, 1968). Taylor and Green (1970) suggested that diazoxide may have some action in antagonising the effects of phenylephrine at the alpha-adrenoceptor. Wohl and co-workers (1968) have shown that there was a non-competitive antagonism between diazoxide and NA in the rat aorta. Constantine (1974) also showed that the vasodilator action of diazoxide, unlike that of prazosin, was unaffected by pretreatment with phentolamine, again suggesting an action not mediated by alpha-adrenoceptors.

Diazoxide has been found to have a competitive

interaction with barium chloride induced contractions of the rat aorta (Wohl, *et al*, 1968). Janis and Triggle (1973) have suggested that diazoxide alters the reactivity of vascular muscle to $Ca^{2+}$ stimulation. It is therefore possible that the major mechanism of action of diazoxide in relaxing vascular muscle is by altering $Ca^{2+}$ metabolism.

One other possible mechanism by which diazoxide could relax vascular muscle is by increasing cAMP levels. Moore (1968) found that diazoxide could inhibit phosphodiesterase in a manner similar to that of theophyllne. It was later noted that diazoxide could relax NA induced contractions of mesenteric arteries while the contractions were undiminished by isoprenaline, another agent which raises cAMP levels (McNeill *et al.*, 1969). This finding cast some doubt as to the probability that cAMP mediates diazoxide's vasodilator response.

A5vii Papaverine.

Papaverine is an opiate alkaloid which has a long history of use as a smooth muscle relaxant. In large doses *in vivo*, it reduces TSVR and has been shown to cause a marked coronary vasodilatation in experimental animals. However, the large doses needed to induce these actions have also been shown to cause myocardial ischaemia, probably due to an unfavourable redistribution of coronary blood flow in conjunction with the positive inotropic actions of the drug. At these high doses considerable cardiac arrhythmias can occur probably as a result of depressed intraventricular conduction. Largely because of these side effects papaverine has been superceded in clinical use by agents with higher therapeutic indeces and fewer side effects.

Papaverine was thought to have its smooth muscle relaxant action by means of its ability to raise cAMP levels. This increase in cAMP concentration is caused by an inhibition of phosphdiesterase, the enzyme resposible for the breakdown of cAMP (Andersson, 1973a, 1973b; Kukovetz & Poch, 1970). However, a blockade of the slow inward $Ca^{2+}$ current has been suggested as a possible mechanism of relaxant action (Schneider *et al.*, 1975). This observation on the action of papaverine on $Ca^{2+}$ fluxes has been made in cardiac muscle and it is as yet unknown if a similar action exists in vascular muscle. It is possible that the vasodilator actions of papaverine are due to a combination of its actions on cAMP and $Ca^{2+}$ (Anderson *et al.*, 1980).

A5viii Isoprenaline.

Isoprenaline is a noradrenaline derivative which acts as a beta-adrenoceptor agonist. The receptor stimulation in turn activates the enzyme adenyl-cyclase which produces the cyclic nucleotide cAMP from adenosine triphosphate. It is thought that isoprenaline has its vasodilator action by directly relaxing vascular muscle via an increased cAMP level in the muscle cells. The major pharmacological responses to beta-adrenoceptor stimulation are; vasodilatation, bronchodilatation and an increase in CO. Isoprenaline was used for some time in the treatment of asthma but it has been largely superceded in this role by salbutamol. The reason for its lack of clinical use in asthma is its high incidence of cardiac side effects. By stimulating the heart directly isoprenaline increases the oxygen consumption while simultaneously lowering blood pressure by vasodilatation. The resultant hypotension reduces oxygen supply to the heart in an environment of increased oxygen demand and cardiac ischaemia so that angina can easily be induced.

Another major problem associated with the clinical use of isoprenaline is its short period of action. Isoprenaline is degraded by catechol-O-methyl-transferase (C-O-MT) both in the neuroeffector junction and in the effector cell. Isoprenaline can be metabolised by effector cell C-O-MT becuase it acts as a substrate for the uptake$_2$ mechanism and is consequently rapidly taken up by the cell. One of the

products of isoprenaline breakdown by this mechanism is 3-O-methyl isoprenaline which has been shown to act as a weak antagonist at the beta-adrenoceptor. Therefore the situation may arise where isoprenaline is metabolised and acts as an antagonist against itself.

## B. Excitation Contraction Coupling in Vascular Muscle.

### B1. Vascular Muscle Classification.

Although the term vascular muscle is widely used it is clear that this type of smooth muscle cannot be considered a homogeneous group. Several attempts to sub-divide vascular, and other smooth muscles into distinct functional types have been made.

Bolton (1979) has suggested that smooth muscle may be divided into two major types, those which generate action potentials (APs) and those which do not. Vascular muscle of the former type may spontaneously disharge APs associated with contractions. An example of this type of muscle being that of the rat portal vein (Funaki, 1966; Axelsson *et al.*; 1967; Casteels, 1982). In AP generating smooth muscle it is generally true that agents that cause contractions increase the rate of AP firing while agents that decrease AP frequency cause relaxation. This type of smooth muscle can also contract in the absence of APs as is evident during a maintained tone generated by high concentrations of $K^+$, which depolarizes the membrane completely and abolishes APs (Bolton, 1979).

In smooth muscles which do not generate APs substances that increase tension can do so by depolarizing the cell membrane. Alternatively, changes in membrane permeability may allow $Ca^{2+}$ or $Na^+$ to enter the cell and initiate

contraction. A third possible mechanism for stimulation of contraction without generation of APs is by release of intracellular stores of activator $Ca^{2+}$ secondary to a ligand-receptor interaction. Substances may relax non-AP generating muscle by causing hyperpolarization of the cell membrane or by interfering with a variety of other mechanisms regulating levels of activator $Ca^{2+}$ (*vide infra*).

Vascular muscles may also show differences in the populations of ion channels admitting activator $Ca^{2+}$. There are at least two different types of $Ca^{2+}$ channel operating in vascular muscle, one channel sensitive to changes in the membrane potential and the second group dependent upon specific ligand receptor interactions. The first of these channel types is the so called potential sensitive calcium channel (PSC) the second is the receptor operated channel (ROC) (Bolton, 1979).

The PSCs are a population of ion channels which admit $Ca^{2+}$ when the membrane potential falls. In AP generating vascular muscle PSCs are thought to be responsible for the initial part of the AP (the upstroke). In non AP generating vascular muscle PSCs are presumed to open in response to a graded reduction in membrane potential. During stimulation of vascular muscle with high $K^+$ concentrations, increased influx of $^{45}Ca$ has been demonstrated (Carrier, *et al.*, 1976, Van Breemen & Lesser, 1971). This $Ca^{2+}$ influx is presumably due to the opening of PSCs.

Contractions of vascular muscles induced by high concentrations of $K^+$ have been sub-divided by their form into so called 'fast' and 'slow' components (Godfraind & Kaba, 1972). This classification of contractions is by form of the response only and it is not thought to be indicative of mobilization of different $Ca^{2+}$ pools. However, Godfraind and Kaba (1972) have shown that contractions of a similar form induced by high concentrations of NA can also be divided into 'fast' and 'slow' components. The initial fast component is not dependent on $Ca^{2+}$ in the extracellular fluid and only prolonged $Ca^{2+}$ depletion inhibits this contraction. The slow phase of the contraction is dependent upon extracellular $Ca^{2+}$, and is severely attenuated by removal of $Ca^{2+}$ from the bathing medium. Godfraind and Kaba (1972) have termed these components 'phasic' and 'tonic' respectively, these should therefore not be confused with the 'fast' and 'slow' components of $K^+$ contractions.

Both the initial fast component of contraction and the slow, maintained contraction produced by high-$K^+$ concentrations are susceptible to inhibition by the calcium entry blocking drugs verapamil and D600 (Peiper, *et al.*, 1971; Bilek, *et al.*, 1974). Similarly the ionic $Ca^{2+}$ antagonist $Mn^{2+}$ inhibits both components of contraction induced by $K^+$ (Collins, *et al.*, 1972; Sullivan & Briggs, 1968). However it appears that $Mn^{2+}$ has a somewhat non-selective action since it has also been shown to block tonic components of NA induced contractions in arterial muscle (Keene, *et al.*, 1972; Steinsland, *et al.*, 1973). The

fast and slow components of $K^+$-induced contractions are also inhibited by $La^{3+}$ (Freeman & Daniel, 1973; Goodman & Weiss, 1971). $La^{3+}$, as with $Mn^{2+}$, has some action in inhibiting the tonic component of agonist induced contractions of vascular muscle (Freeman & Daniel, 1973).

The ROC is an ion channel operated by a receptor for a specific stimulant substance, it is postulated that there are different ROCs associated with each receptor type. This may provide some explanation for the varying maxima produced by different agonists, it may be that each receptor type does not have the same effect on $P_{Ca}$ when maximally stimulated because of differences in its associated ROCs. Agents which open ROCs following an agonist-receptor interaction, stimulate vascular muscle by increasing calcium influx and consequently raising $[Ca^{2+}]_i$. As more receptors are occupied by the agonist then more ROCs will be opened and calcium influx will increase (Bolton, 1979). In some tissues ROCs can bring about contraction when the muscle is depolarized by the presence of high $K^+$ concentrations, which suggests that at least some of these channels are operated in a potential independent manner (Golenhofen, *et al.*, 1973). Agonists can also produce contractions in smooth muscle where PSCs have been inhibited by calcium entry blockers (Boev, *et al.*, 1976), which suggests that ROCs are less susceptible to calcium entry blockers. Bolton (1979) has suggested a mechanistic scheme in which PSCs and ROCs combine to bring about contraction (Fig.1.).

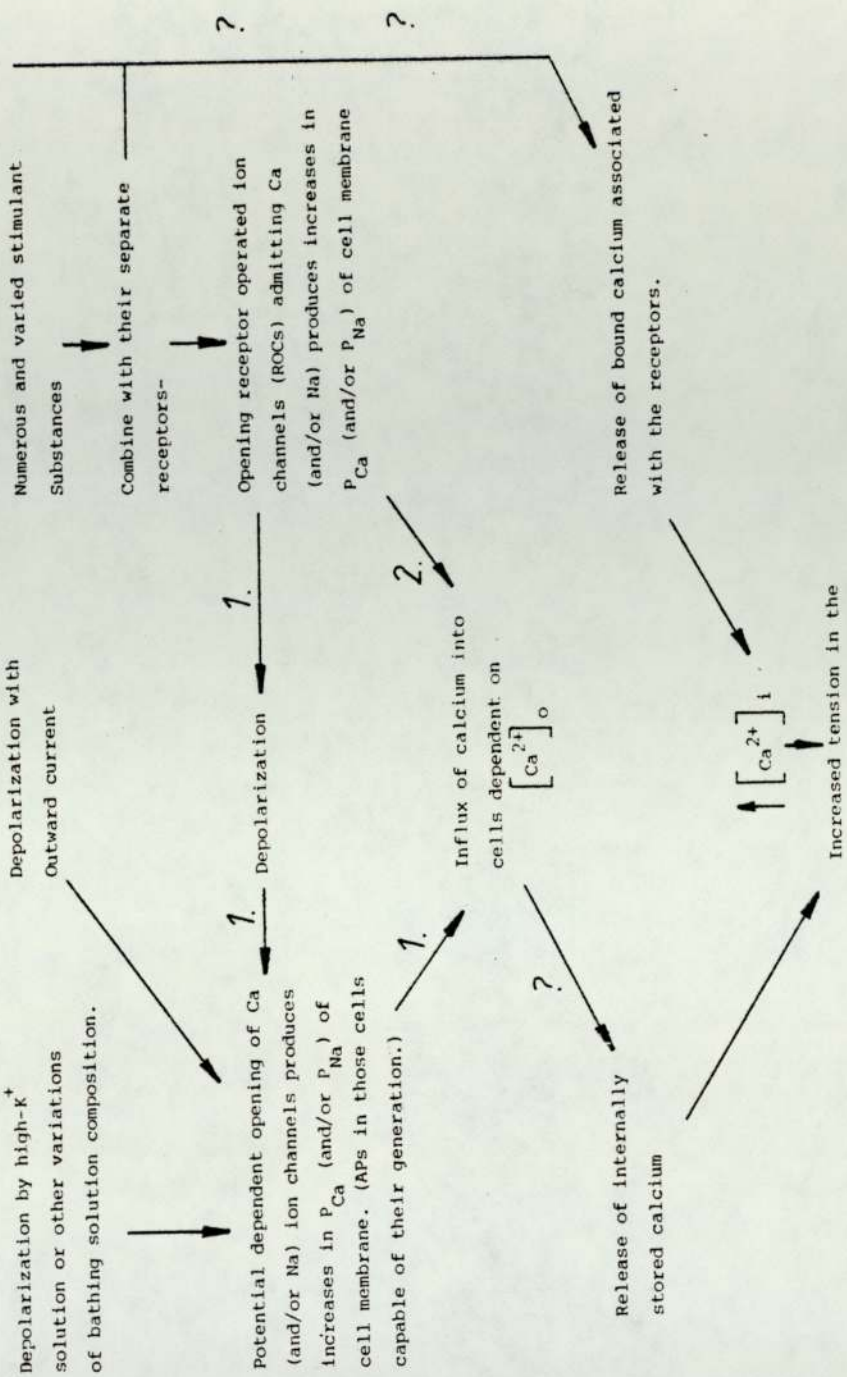



Fig. 1. A putative scheme for the roles of $Ca^{2+}$ and its channels in coupling stimulation to contraction in smooth muscle cells. Low concentrations of stimulants have their actions via pathways labelled 1: small depolarization, increased AP discharge, opening of some PSCs. High concentrations of stimulants have their actions via pathways labelled 2: large depolarization inhibits APs in muscles capable of their generation or in $K^+$ depolarized tissues. (Bolton, 1979).

Calcium entry blockers are frequently seen to have a differential effect on agonist induced contractions leaving the initial 'phasic' component intact but blocking the slower 'tonic' part of the contraction (Godfraind & Kaba, 1972). In tissues stimulated by NA this differential effect of calcium entry blockers varies from preparation to preparation dependent upon the degree of utilization of intracellular $Ca^{2+}$ stores. For example the rat portal vein is more sensitive to calcium entry blockers than the rat aorta because it is almost entirely dependent upon extracellular or loosely bound stores of activator $Ca^{2+}$ (Sigurdsson, *et al.*, 1975). The rat aorta, on the other hand, produces a biphasic contraction the initial phasic component of which is resistant to $Ca^{2+}$ depletion and calcium entry blockers and is ascribed to release of intracellular $Ca^{2+}$ stores (Godfraind & Kaba, 1972).

Golenhofen and his co-workers (Golenhofen, 1973,1976; Golenhofen & Neuser, 1974; Golenhofen & Weston, 1976) have differentiated smooth muscle contractions into two types, either tonic (T) or phasic (P), dependent upon their form and timecourse. Golenhofen claims that these types of activity are caused by differing activation mechanisms which have specific and dissimilar sensitivities to antagonists. Although the two types of activity can, and often are, exhibited in the same tissue and even within the same contraction, some tissues can be defined as being

predominantly of one form or the other. The calcium entry blocking drugs verapamil, nifedipine and D600 are selective in their inhibition of the system stimulating phasic activity. On the other hand NP has been put forward as an antagonist which preferentialy inhibits the system responsible for tonic contractions. One problem with this division of 'P' and 'T' activation systems is that the supposedly specific antagonists do have actions on the alternate type of activity.

B2. $Ca^{2+}$ and Generation of Tension.

In vascular muscle it is generaly accepted that the contractile proteins are stimulated to increase tension by a rise in $[Ca^{2+}]_i$. Recent work carried out in chemically skinned vascular muscles has shown these preparations to be very sensitive to increases in $Ca^{2+}$ concentration in the bathing medium which is closely related to $[Ca^{2+}]_i$ in these tissues (Endo, *et al.*, 1977; Gordon, 1978; Blaustein, 1977).These studies have confirmed earlier findings which had shown $Ca^{2+}$ to be essential for normal contractions of vascular muscle (Filo, *et al.*, 1972).

The routes by which $Ca^{2+}$ may enter the vascular muscle cell have been discussed above. $Ca^{2+}$ influx is controlled by the state (open or closed) of PSCs, ROCs or other changes in $P_{Ca}$. Having thus increased $[Ca^{2+}]_i$ the form of the response will be dependent on the handling of the activator $Ca^{2+}$ within the cell. To induce or maintain a contraction it has been suggested that the $[Ca^{2+}]$ in the sarcoplasm must be maintained above a threshold level (Hurwitz & Suria, 1971; Somlyo & Somlyo 1968; Blaustein, 1977) at which it can interact with the contractile machinery. Gordon (1978) has shown in glycerinated hog carotid artery preparations, that the threshold $[Ca^{2+}]$ is approximately 0.2μM. Endo and co-workers (1977) have found threshold $[Ca^{2+}]$ of the order of 0.1μM in chemically skinned rabbit main pulmonary artery.

If $[Ca^{2+}]_i$ is the major determining factor in vascular

muscle contraction then any circumstance which affects it will be of significance when producing an 'end organ response'. One of the major mechanisms by which $[Ca^{2+}]_i$ can be reduced is the binding of $Ca^{2+}$ to intracellular organelles. Many organelles have been suggested as possible sites of $Ca^{2+}$ sequestration: nucleus, nuclear and other intracellular membranes and the mitochondria (Jonas & Zelck, 1974; Somlyo & Somlyo, 1971, 1976a, 1976b). The methods used in attempts to deliniate the importance of each organelle to alteration of $[Ca^{2+}]_i$ leave some doubt as to the validity of the results to the 'physiological' situation. For example such experiments often necessitate sub-cellular fractionation and measurement of $^{45}Ca$ binding.

With this methodological consideration in mind the two organelles which appear to be able to bind sufficient $Ca^{2+}$ to significantly alter $[Ca^{2+}]_i$ are mitochondria and the cellular microsomal fraction. The microsomal fractions, which probably contain some plasma and intracellular membranes, provides a high affinity site for $Ca^{2+}$ sequestration (Allen, 1977; Aoki, *et al.*, 1976; Ford, 1976; Ford & Hess, 1975). The uptake of $Ca^{2+}$ by microsomal fractions is energy requiring (Moore, *et al.*, 1975).

It has been suggested that some relaxant agents can stimulate $Ca^{2+}$ uptake into microsomal and other stores (Baudolin-Legross & Meyer, 1973; Baron & Kreye, 1973). The actions of cAMP on the binding of $Ca^{2+}$ is in some doubt since Baudouin-Legross & Meyer (1973) have suggested that in

rabbit aorta dibutaryl-cAMP increases $Ca^{2+}$ binding as does adrenaline. Whereas Allen (1977) has found no effect of cAMP on canine aortic microsomal binding. Conversely some stimulant agents have been reported to decrease microsomal binding of $Ca^{2+}$, for example angiotensin and NA in the rabbit aorta (Baudouin-Legross & Meyer, 1973), however evidence from vascular muscle is sparse. On the whole the situation is too confused to consider any major role for microsomal $Ca^{2+}$ in the control of vascular muscle contraction.

The other major sites of $Ca^{2+}$ binding within vascular muscle cells appear to be the mitochondria (Allen, 1977; Ford & Hess, 1975) and sarcoplasmic reticulum (Devine, *et al.*, 1972). Once again the physiological significance of this binding is unknown but despite their lower affinity the mitochondrial sites have a larger capacity for $Ca^{2+}$ (Ford & Hess, 1975).

Another putative method of $[Ca^{2+}]_i$ regulation is via a $Ca^{2+}$ extrusion pump. Assuming that maintenance of tone is dependent upon $[Ca^{2+}]_i$ any fall in concentration induced by $Ca^{2+}$ extrusion could be important. One suggested method by which $Ca^{2+}$ could be removed from the sarcoplasm is by a Na-Ca exchange mechanism. Na has been implicated because of the observation that Na removal from the bathing solution induced contractions associated with an increased Ca influx and a decreased efflux (Reuter, *et al.*, 1973). Leonard (1957) has shown that raising intracellular Na

concentration, using cardiac glycosides, induced a contraction of arterial muscle. This suggests that lowering external Na and/or increasing intracellular Na, tends to increase $[Ca^{2+}]_i$ and thus cause a contraction. Blaustein (1977) has suggested that there is a Na-Ca exchange mechanism in vascular muscle, which derives energy from the Na electrochemical gradient, which plays a role in regulating the Ca gradient across the sarcolemmal membrane.

B3. Calmodulin.

Calmodulin is an intracellular protein which binds $Ca^{2+}$ with high affinity and specificity. Cheung (1980) and Means and Dedman (1980) have suggested calmodulin may act as an intracellular $Ca^{2+}$ receptor mediating cellular functions triggered by an increase in $[Ca^{2+}]_i$, for example vascular muscle contraction. Klee (1977) has shown that there are four $Ca^{2+}$ binding sites on calmodulin and that binding to any of these sites produces a conformational change in the molecule. Cheung (1980) has suggested that the new conformation of calmodulin that arises from the binding of one or more $Ca^{2+}$ ions produces an activated form of calmodulin which then interacts with the enzyme system it is regulating.

The role calmodulin plays in vascular muscle contraction is far from clear. There are three main areas in which $Ca^{2+}$-calmodulin interactions could effect reactivity in vascular muscle: 1) Regulation of $[Ca^{2+}]_i$; 2) Interaction with the machinery of contraction; 3) Alteration of vascular muscle cyclic-nucleotides. Calmodulin may alter $[Ca^{2+}]_i$ in several ways, it acts as a direct $Ca^{2+}$ sink by virtue of its own $Ca^{2+}$ binding properties. Calmodulin has also been shown to stimulate $Ca^{2+}$ transport across plasma membranes (Larsen & Vincenzi, 1979) which may be important in $Ca^{2+}$ sequestration and efflux, however these observations have not been demonstrated in vascular muscle and so must be treated with caution.

In vascular muscle contraction is dependent on an actin-myosin interaction which is in turn dependent upon the light chain myosin being phosphorylated by myosin-light-chain-kinase. The phosphorylation which is necessary for contraction to occur requires the presence of an activator protein which has been identified in bovine aortic preparations as being calmodulin (Hidaka, *et al.*, 1979). A more recent study (Hirita, *et al.*, 1980) has suggested that another $Ca^{2+}$ binding protein, leiotonin C, is responsible for the activation of myosin light chain kinase. It can therefore be seen that at the present time the role of calmodulin in regulation of contractile machinery is still in considerable doubt.

Hidaka and co-workers (1979) showed that calmodulin can activate cyclic nucleotide phosphodiesterase in bovine aorta and thus could have a role in the regulation of cAMP levels. Since the activation of cyclic nucleotide phosphodiesterase by calmodulin occurs at physiological levels of $Ca^{2+}$, then if $[Ca^{2+}]_i$ is high enough to induce contractions a fall in the level of cyclic nucleotide would be expected. No reduction in cGMP or cAMP levels associated with contractions has been found in vascular muscle and this must cast some doubt on any proposed cyclic nucleotide regulatory function for calmodulin.

It can be seen that calmodulin has been implicated in several possible mechanisms for regulation of contraction

induced by $[Ca^{2+}]_i$ at the most fundamental level. Further research into calmodulin and calcium interaction could lead to a better understanding of the process of vascular muscle contraction itself. If calmodulin does have a role to play in vascular muscle contractions it is theoretically possible that some of the 'directly acting' vasodilators may interact with calmodulin in such a way as to result in a lower $[Ca^{2+}]_i$.

## B4. Vascular Muscle Contraction and Ionic Environment.

It has been generally accepted for a number of years that changes in the ionic mileu of vascular muscle can greatly alter vascular reactivity. The role of $Ca^{2+}$ in excitation contraction coupling has been discussed previously, however many other inorganic ions are capable of inducing large changes in vascular muscle activity.

### B4i. The $H^{+}$ Ion.

Vanhoutte and Clement (1968), using dog saphenous vein, showed that changes in pH and $PCO_2$ could alter the reactivity of venous muscle to electrical stimulation. Alkalosis enhanced the isometric contraction while acidosis depressed reactivity. Tobian and co-workers (1959) performed similar experiments in rat aortic strips stimulated with NA and likewise found that alkalosis enhanced the contractions while acidosis caused relaxation. Changes in pH over the range of 6.8 - 7.8 have been shown to cause linear changes in vascular muscle membrane potential in dog common carotid artery (Siegel, *et al.*, 1976a, 1976b), alkalosis causing depolarization and contraction and acidosis causing hyperpolarization and relaxation.

Somlyo and co-workers (1968, 1976) have suggested that changes in membrane potential induced by changes in pH could be due to changes in the membrane properties or in the local concentrations of ions around the membrane. Siegel and

co-workers (1978) have investigated changes in membrane binding of several ions in response to altered pH. They showed that $K^+$-binding in dog carotid artery increases with increased pH over the range 4 to 6.8 and then falls from its maximum as the pH becomes more basic.

An explanation put forward to account for this effect (Siegel, *et al.*, 1978) is that there is a competitive binding for the same sites on connective tissue between $K^+$ and $Ca^{2+}$. At basic pH, Ca-binding is greatly increased and since $Ca^{2+}$ has a higher affinity for the sites, $K^+$ is displaced. Thus at pH from 7.4 to acidity $K^+$ is bound to the connective tissue, when the pH rises then $K^+$ is released and $Ca^{2+}$ is bound. If this is the case then in alkalosis the extracellular $[K^+]$ will increase causing a depolarization and contraction, during acidosis the $[K^+]$ will be reduced causing a hyperpolarization and relaxation (Siegel, *et al.*, 1976a).

Siegel and co-workers have also suggested that acidosis might cause release of $Ca^{2+}$ ions from connective tissue. This action of acidosis would increase the concentration of $Ca^{2+}$ in close proximity to the cell membrane. This could give rise to the so called 'dual effect' of calcium (Bohr, 1963) causing membrane stabilization and hyperpolarization and/or an upward shift in the threshold level of depolarization necessary for initiation of contraction. The binding curve for $Ca^{2+}$ in dog carotid artery connective tissue is steep, such that a small change in pH could result in a much larger change in $Ca^{2+}$ binding (Siegel, *et al.*,

1978) allowing for significant local alterations in $Ca^{2+}$ concentration.

B4ii. The $K^+$ Ion.

Changes in the extracellular concentration of $K^+$ have been shown to alter vascular reactivity in a number of different ways. High concentrations of $K^+$ depolarize vascular muscle membranes and cause tonic contractions (Su, *et al.*, 1964; Axelsson, *et al.*, 1967). Konold and co-workers (1968a, 1968b) have shown alterations in tone of bovine facial artery with changes in $[K^+]_o$ over a physiological range of 2 - 6mM, a decrease in $[K^+]_o$ increased tone in vascular muscle while increased in $[K^+]_o$ cause relaxation. Nguyen-Duong and collegues (1977) have suggested that the changes in tension observed under these conditions could be due to a number of factors: changes in membrane polarization; release of endogenous noradrenaline; alterations in the activity of the $Na^+$-$K^+$ ATPase and/or changes in the electrogenic outward transport of $Na^+$. Experiments carried out to test these hypotheses (Nguyen-Duong, *et al.*, 1977) have shown that in bovine facial artery the most likely cause of altered muscle tone in response to changes in $[K^+]_o$ is mediated via changes in membrane potential.

Siegel and Schneider (1981) have shown, using the dog carotid artery, a relationship between $[K^+]_o$, degree of polarization and tension developed by the muscle. They noted that the lowest tension was found at levels of $[K^+]_o$ which produced the greatest hyperpolarization (Siegle, *et al.*, 1978) and that both increasing or decreasing the $[K^+]_o$ from this level caused a depolarization and contraction. This

observation went some way to explaining the long standing experimental observation which has shown that placing vascular muscle in nominally $K^+$ free solutions causes a contraction.

Siegel and Schnieder (1978) have shown, in dog carotid artery, that tight electro-mechanical coupling exists. The practical upshot of this being, because of the steep nature of the stationary activation curve, small changes in $E_m$ can cause significant changes in tension. Siegel and Schnieder quote a figure of 8.7mV being equivalant to a change in tension of 1g. Small alterations of $[K^+]_o$ may be capable of changing $E_m$ in a passive manner to such an extent as to cause notable changes in tension. This effect of altered tension with changed polarization would only occur in tissues that are electro-mechanically coupled. Tissues which are pharmaco-mechanically coupled would not be as susceptible to changes in $[K^+]_o$ which in turn may alter tissue polarization and mechanical activity. If tissues are truly pharmaco-mechanically coupled then presumable not only could contraction occur without depolarization but also relaxation could occur without hyperpolarization. Perhaps it may be a better test of coupling type to look at the effects of hyperpolarization on relaxation, if hyperpolarization causes no relaxation then the tissue is truly pharmaco-mechanicaly coupled.

B4iii. The $Mg^{2+}$ Ion.

Over the last twenty to thirty years changes in $Mg^{2+}$ levels have become implicated in various pathological conditions. In essential hypertension and other diseases often associated with raised blood pressure e.g. eclampsia, atherosclerosis, and alcoholism, depressed levels of $Mg^{2+}$ are often apparent. The converse is often true, disease states that produce raised levels of $Mg^{2+}$ are frequently accompanied by depressed blood pressures (Altura, 1982).

Increases in $[Mg^{2+}]_o$ have been shown to produce vasodilatation in isolated vascular beds and in intact circulations (Hazard & Wurmser, 1932; Altura & Altura, 1974). Reduction in the plasma levels of $Mg^{2+}$ have been shown to raise the blood pressure of the intact dog (Emerson, *et al.*, 1970) and the perfusion pressure of isolated vascular beds (Haddy & Scott, 1973). Altura (1982) has suggested that the alterations in vascular reactivity seen with changes in $[Mg^{2+}]_o$ are due to some action of $Mg^{2+}$ at the cell membrane because of the rapidity of the vascular response (Altura & Altura, 1978a, 1978b) and the insensitivity of $[Mg^{2+}]_i$ to changes in $[Mg^{2+}]_o$ (Palaty, 1974).

Several possible mechanisms have been put forward to explain the action of changes in $[Mg^{2+}]_o$, Sigurdsson and Uvelius (1977) have suggested that the inhibitory action of elevated $Mg^{2+}$ levels on the spontaneous myogenic activity of the rat portal vein may be due to hyperpolarization. Using

the sucrose gap method of electrical recording Sigurdsson and Uvelius showed that reduced mechanical activity induced by increasing $[Mg^{2+}]_o$ is associated with reduced electrical activity. The evidence to suggest hyperpolarization is circumstantial, in that slight depolarization induced by raised $[K^+]_o$ inhibited the effect of elevated $[Mg^{2+}]_o$. Direct measurement of membrane potential using microelectrode puncture techniques could elucidate any changes in membrane potential.

Altura and Altura (1976) have suggested that reduction in $[Mg^{2+}]_o$ may have its stimulant action by altering $Ca^{2+}$ availability. Addition of EGTA, a $Ca^{2+}$ chelating agent, or divalent cations thought to inhibit transmembrane $Ca^{2+}$ fluxes, e.g. $Mn^{2+}$ or $Cd^{2+}$, caused relaxation of contractions induced by a nominally $Mg^{2+}$-free bathing solution (Altura & Altura, 1976). Further evidence for the hypothesis that $Mg^{2+}$ can effect $Ca^{2+}$ permeability in vascular muscle has been gathered by looking at the effect of altered $[Mg^{2+}]_o$ on $Ca^{2+}$ induced contractions of depolarized muscle (Weiner, *et al.* 1979). Weiner and co-workers have shown that elevated $[Mg^{2+}]_o$ inhibited $Ca^{2+}$ induced contractions of $K^+$ depolarised muscle while reduced $[Mg^{2+}]_o$ enhanced $Ca^{2+}$ contractions. Recent studies using $^{45}Ca$ flux measurements (Goldstein & Zsoter, 1978; Turlapaty & Altura, 1978) have shown that $^{45}Ca$ influx is increased when $[Mg^{2+}]_o$ is lowered. Raised $[Mg^{2+}]_o$ appears to reduce membrane exchangable $Ca^{2+}$ and it may be that $Mg^{2+}$ and $Ca^{2+}$ are competing for a membrane binding site. Altura and Altura (1982) have also shown that reduced $[Mg^{2+}]_o$ can

enhance the $^{45}Ca$ efflux and raised $[Mg^{2+}]_o$ can reduce $^{45}Ca$ efflux, this may play some role in the regulation of activator $Ca^{2+}$ in the cell. The physiological relevance of these observations in control of excitation-contraction coupling is as yet unclear, it does appear however, that $Mg^{2+}$ can profoundly alter $Ca^{2+}$ handling.

B4iv. The $Cl^-$ Ion.

Very little is known about the role of anions in the control of vascular muscle reactivity or in the maintenance of membrane potential in these tissues. The anion on which most work has been performed is the $Cl^-$ ion. Early work on the $Cl^-$ ion centered around determination of intracellular ion content and the method of transmembrane distribution. Dodd and Daniel (1960) and Headings and co-workers (1960) in separate studies, using rabbit aorta and dog carotid artery respectively, showed that the intracellular concentration of $Cl^-$ was too high to be accounted for by a passive Donnan distribution.

Subsequent studies by Haljamae and colleagues (1970) showed that in the rat portal vein the level of measured intracellular $[Cl^-]$ was not consistent with a simple two compartment model of intra- and extra-cellular distribution. Similar work carried out by Villamil and co-workers (1968a) using dog carotid artery also found a $[Cl^-]_i$ higher than predicted, both studies suggested three possible explanations for these results; 1) intracellular binding of $Cl^-$; 2) active uptake of $Cl^-$; 3) complex $Cl^-$ distribution in more than two compartments. Villamil and co-workers (1968b) showed that $Cl^-$ uptake in the dog carotid artery unlike that in taenia coli was unaffected by DNP or oubain. Furthermore a reduction in temperature only changed uptake after a reduction of 20°C. These results led Villamil to suggest that there was no active uptake of $Cl^-$ in the dog carotid

artery muscle.

In recent studies Somlyo and colleagues (1979) have shown, using electron probe techniques, that the $[Cl^-]_i$ is largely found in the cytoplasm suggesting no important role for $Cl^-$ sequestration by intracellular organelles. These results led to a more intensive search for an active $Cl^-$ uptake mechanism. Recent work by Kreye and co-workers (1981) has shown the existence of a furosemide sensitive $Cl^-$ transport mechanism in rabbit aorta. Furosemide is known to inhibit $Cl^-$ transport in non-vascular preparations, (Ludens, *et al.*, 1980), and Kreye has shown that vascular $Cl^-$ efflux is increased in the presence of furosemide. Electrophysiological measurements carried out in these studies showed a hyperpolarization in the presence of furosemide suggesting that $Cl^-$ distribution is important for determination of resting membrane potential. In tissues incubated with furosemide, $[Cl^-]_i$ decreased to a level consistent with a Donnan type distribution and the membrane potential rose. These results support the hypothesis that; $[Cl^-]_i$ is higher than predicted by passive diffusion because of the operation of an inwardly directed $Cl^-$ pump; and that $Cl^-$ distribution plays a role in the determination of the resting membrane potential in vascular muscle.

The role of $Cl^-$ in the maintenance of membrane potential is still controversial, Wahlstrom (1973) showed that, in the rat portal vein, membrane potential could be determined by the permeabilities and distributions of $K^+$ and

$Cl^-$ ions by use of the Goldman equation. Mekata and Niu (1972) on the other hand, found that substituting sulphate for $Cl^-$ had no effect on membrane potential in rabbit carotid artery, similar results were obtained by Harder and Sperelakis (1978) in the guinea-pig mesenteric artery. These results suggest that $Cl^-$ distribution had no role in determining $E_m$. The disparity between studies may to some extent be due to inter-tissue variation and also to the apparent complexity of the relationship between $Cl^-$ ions and membrane potential.

If $Cl^-$ distribution is a factor in determining $E_m$ then agents which can alter $Cl^-$ fluxes can then change transmembrane distribution and thus membrane potential. Wahlstrom (1973) showed that in the rat portal vein NA caused a dose-dependent increase in the $^{36}Cl$ efflux which was not secondary to the NA induced depolarization. When he applied his data to the Goldman equation, Wahlstrom considered that the change in $Cl^-$ was sufficient to explain the NA induced depolarization. Paradoxicaly Droogmans and co-workers (1977) showed, in the rabbit ear artery, that $Cl^-$ and $K^+$ effluxes were stimulated by NA without any measured changes in membrane potential. These findings may merely demonstrate an inability to correlate changes in membrane fluxes of ions with depolarization and contraction. In a recent study (Casteels, *et al.*, 1977) has shown that caffeine can alter $Cl^-$, $K^+$, and $Ca^{2+}$ fluxes and will depolarize the membrane of the rabbit main pulmonary artery without having any effect on the contractile state.

Changes in the $Cl^-$ fluxes in vascular muscle have been implicated in the hyperpolarization caused by nitroprusside (NP) and has been put forward as a possible mode of vasodilator action (Kreye, 1980; Kreye, *et al.*, 1977). Kreye and co-workers (1977) have also suggested that GTN can alter $Cl^-$ fluxes and cause hyperpolarization, however later work (Ito, *et al.*, 1980) suggested that this hyperpolarization was only seen at high concentrations and was due to increased $K^+$ conductance of the membrane. Whatever the importance of changed $Cl^-$ fluxes to the vasodilator action they have been shown not to be secondary to changes in cell stress induced by relaxation as no difference in $Cl^-$ movement is noted in cells relaxed by omission of $Ca^{2+}$ from the bathing medium.

## Aims of the Present Study.

The overall aim of this study was to investigate the pharmacological acions of vasodilators on vascular muscle *in vitro*. Experiments were designed in order to gain some insight into the mode of action of the agents chosen. To this end the following investigations were attempted:

i) The inhibitory actions of vasodilators on rat vascular preparations were investigated in order that the sensitivity of preparations to vasodilators could be assessed and compared with the sensitivity of human vasculature to the same agents, as reported by other workers.

ii) The spectra of activity of vasodilators on rat vascular preparations were compared with each other in order to construct a functional classification of these agents.

iii) The underlying mechanism of action of each vasodilator was investigated in an attempt to determine whether the functional classification obtained had a basis in cellular actions.

iv) The results of the study were discussed against a background of current knowledge of excitation-contraction coupling processes in vascular muscle in order to identify the many ways in which pharmacological vasodilatation may be brought about.

Methods.

1. Animals.

All animals used in this study were specific pathogen free male Wistar rats within the weight range 200-280 grams.

2. Dissection and Tissue Preparation.

The dissection to remove the portal vein of the rat was carried out in a manner similar to that used by Axelssen and co-workers (1967). 15 to 20 mm of the portal vein was tied off, cut from underlying connective tissue and placed *in vitro* in normal Krebs' solution. If during the subsequent experiment the portal vein was to undergo field stimulation the vessel was mounted in a perspex and platinum electrode shown in FIG. 2. Stimulation was provided by a square wave stimulator (SRI 6050) at supramaximal voltage (100V) with a pulse width of 2ms and of variable frequency. If no electrical stimulation was to be carried out then the vein was mounted on a normal stainless steel tissue holder. (FIG. 3a.)

The dissection to remove the thoracic aorta was carried out by a method similar to that of Cohen & Wiley. (1977) The aorta was cut just above the the level of the diaphragm and dissected free from the chest wall for a length of 25 to 35 mm, removed and placed *in vitro* in normal Krebs' solution. Any remaining fat and connective tissue was then removed. The artery was then cut transversly to form tubes of

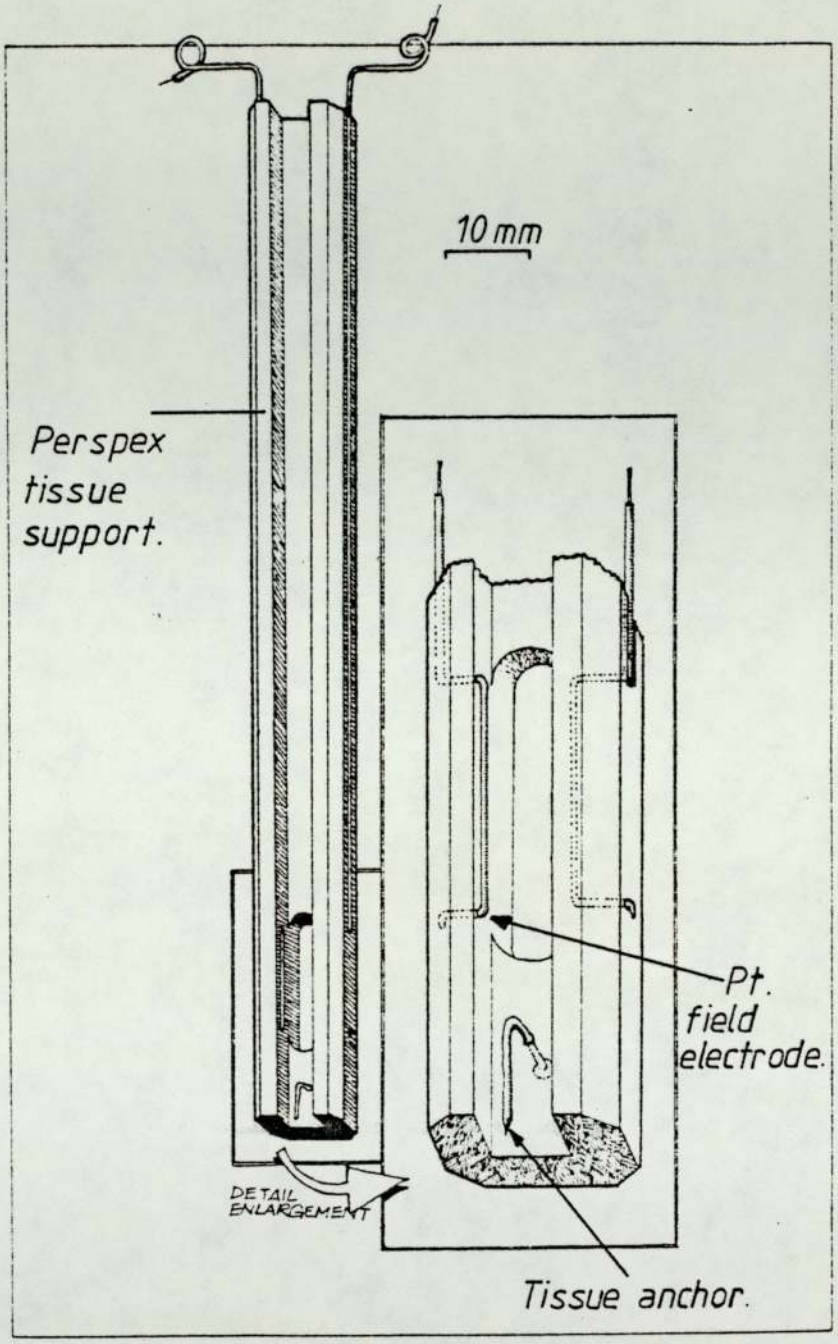



Fig. 2. The perspex tissue holder used in experiments with the portal vein. The detail shows the platimun field stimulation electrodes.

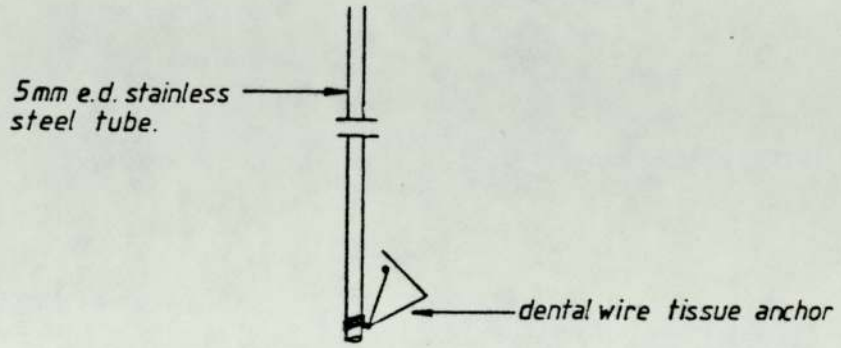



Fig. 3a. The stainless steel tissue holder used in experiments with the aorta.

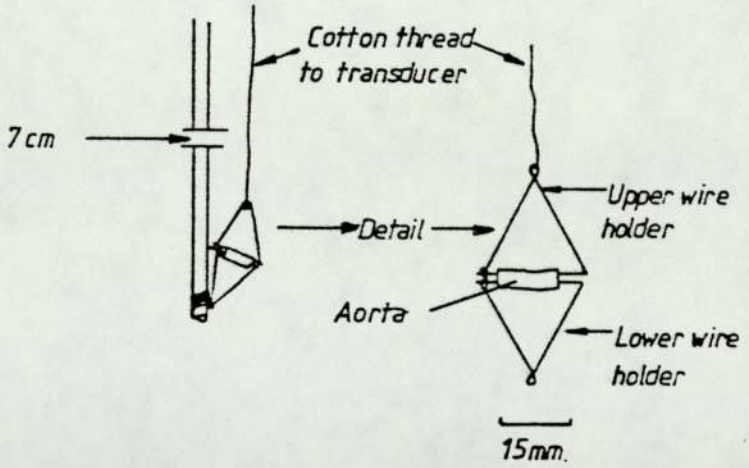



Fig. 3b. Diagram showing a circular preparation of the rat thoracic aorta mounted on stainless steel wires.

approximately 12 to 15 mm in length which were then mounted on wires. (Fig. 3b.) The wire used to mount the tissues was stainless steel dental wire of 0.4mm diameter. (Evans and Sons, Cardiff.)

The portal veins and aortae were suspended under 0.5 and 2 grams tension respectively in normal Krebs' solution and allowed to equilibrate for one hour. During the equilibration period the solution bathing the tissues was changed every fifteen minutes and the tension was reset once after thirty minutes.

3.Recording System.

Isometric recordings of tissue contractions were made with UF1 transducers (Pye-Ether) coupled to a pre-amplifier (Ormed 3559) and displayed on an Ormed MX4 chart recorder.

Aortic contractions were quantified by measuring peak tensions, each recording channel was calibrated at the start of every experiment. The spontaneous contractions exhibited by the portal vein render peak tension measurement unsuitable as a method of measuring portal vein responses. To overcome this problem the mechanical responses of the portal vein were integrated electronically, initially using Ormed integrators (3630) and subsequntly using integrators of our own manufacture to the design of Illingworth and Naylor. (1980)

3i. Electrical Recording.

Extracellular electrical recordings were made from isolated portal vein preparations by the perfused capillary method. (Golenhofen & von Loh, 1970) Electrical activity was recorded from platinum ring electrodes and amplified by an a.c. amplfier (Devices, 3160) with a time constant of 0.2 s and a rise time of <0.01 ms. Simultaneous mechnical recordings were made (as above) and both these and the output from the a.c. amplifier were displayed on a Devices M2 chart recorder. The capillary bath with platinum ring electrodes is shown in Fig.4.

4. Physiological Salt Solutions.

Throughout the experiments tissues were bathed in Krebs' bicarbonate solution of the following composition: NaCl 118.4 mM; KCl 4.75 mM; $CaCl_2$ 5 mM; $MgSO_4$ 1.18 mM; $KH_2PO_4$ 1.19 mM; $NaHCO_3$ 24.9 mM; Glucose 11.66 mM. Under normal conditions tissues were maintained at 37°C by a circulating heated water pump (Conair-Churchill) and gassed with 5% $CO_2$ in $O_2$ (Carbogen, BOC).

Some experiments involving changes in the constituents of the Krebs' solution were carried out, the changes were as follows.

1. Alteration of the $Ca^{2+}$ concentration.

The $CaCl_2$ concentration was altered between 0.0 and 10mM without alteration to any other constituent of the solution.

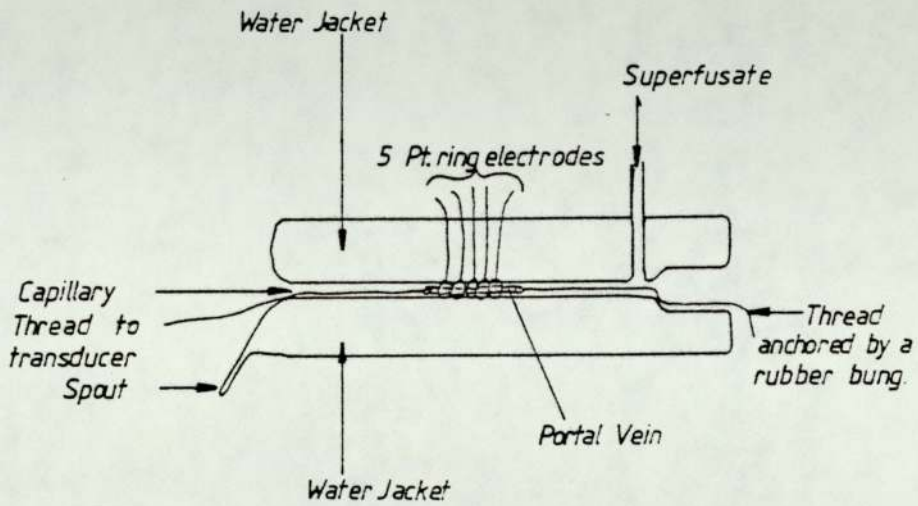



Fig. 4. Diagram showing the capillary bath with platinum ring electrodes used for recording extracellular electrical activity of the rat portal vein.

Concentrations in excess of 10mM were not used because in these concentrations a white precipitate of Ca $PO_4$ formed rendering the true $Ca^{2+}$ levels unknown.

2. Alteration of the $Mg^{2+}$concentration.

The concentration of $MgSO_4$ was alterd between 0.0 and 5 mM without alteration to any other constituent of the solution.

3 Alteration of $Cl^-$ concentration.

The concentration of $Cl^-$ was altered, 72 mM or 16 mM (from 144.5 mM) by reducing the NaCl concentration and substituting this with an equivalant ammount of Na Isethionate to maintain Na levels. (Kreye, Kern & Schleich, 1977)

5. Alteration of the pH of the Krebs' Solution.

Alterations in the pH of the Krebs' solution bathing the tissues was made by altering the gas mixture bubbled through the solution (Tobian, Martin & Eilers, 1959, Vanhoutte & Clement, 1968). A decrease in pH from 7.4, the normal pH of Krebs' solution gassed with carbogen, to pH 7 was achieved by increasing the percentage of $CO_2$ in he gassing mixture. The increase in $PCO_2$ was induced by simultaneously gassing the Krebs' in the organ bath with carbogen and pure $CO_2$. The course of the pH change was followed in an adjacent organ bath with a pH electrode and meter (Corning-EEL, Model 12 pH Meter). The meter output was displayed concurrently with the mechanical responses of the tissues on the chart recorder. The flow of $CO_2$ to each bath

was adjusted so as to produce the same pH change in each bath. This was checked by measuring the pH in each bath in response to a test gas mixture at the start and end of each experiment.

It was found that increasing $PCO_2$ in this manner caused a reduction in the $PO_2$ of the Krebs' solution. The alterations in sensitivity caused by changes in the pH could therefore have been due to changes in $O_2$ availability. The $PO_2$ was measured using an oxygen electrode (Rank Bros.) and was found to have been reduced by 20% when gassing with carbogen and pure $CO_2$ to produce a pH of 7. In order to determine the effect of a 20% reduction in $O_2$ availability on tissue reactivity a mixture of $O_2$ (75%), $N_2$ (20%), and $CO_2$ (5%) was used. (BOC, Special Medical Gasses Devision.) This gas mixture produced the same $PO_2$ found during $CO_2$ gassing (pH7) while retaining the normal pH (7.4) of the Krebs' solution.

Increases in the pH of the Krebs' solution bathing the tissues were achieved by gassing the solution with pure $O_2$ instead of carbogen. Using this method the pH could be raised from 7.4 to 7.9. Increases in pH were measured and displayed in the same manner as pH decreases (see above).

6. Drugs.

The drugs used in this study were as follows; Noradrenaline (l-arterenol Bitartrate, Sigma); Isoprenaline

(dl-Isoproterenol Sulphate, Sigma); Na Nitroprusside (BDH); Tolmesoxide (RX71107, Reckitt & Colman Ltd.); Hydrallazine HCl (Ciba); Papaverine HCl (Hopkin & Williams Ltd.); Verapamil HCl (Abbott Laboratories Ltd.); Glyceryl Trinitrate (SDM); Tetrodotoxin (Sankyo); Prazosin HCL (Pfizer); Diazoxide (Eudemine, Allen & Hanburys Ltd.); Ethylenediaminetetra-acetic acid, disodium salt (BDH); l-Ascorbic acid (BDH); Strychnine HCl (BDH); Tetraethylammonium Br (Sigma); 4-aminopyridine (Sigma).

All drugs were made up in distilled water and concentrations calculated from the molecular weight of the salts.

Glyceryl Trinitrate (GTN) was not readily available at the beginning of this study so solutions of GTN were prepared from tablets by disolving the tablets in ethanol. Ethanol itself relaxes vascular muscle preparations (Meckenzie & Parratt, 1977) so precautions were taken to ensure that ethanol played no part in the relaxation induced by GTN. The minimum concentration of ethanol which caused a relaxation was found, and at no time during the addition of GTN was the amount of ethanol vehicle allowed to exceed 10% of this value. (Mackenzie & Parratt, 1977). Subsequently GTN solid (SDM, 10.11% GTN in lactose) became available and was found to be soluble in water at the concentrations required. No difference was found betweeen the two preparations of GTN solutions.

NA and Isoprenaline were made up in $10^{-5}$M Ascorbic acid solution and stored on ice throughout the experiment in order to reduce drug oxidation.

7. Statistics.

Results were expressed as the mean of 'n' results plus and minus the standard error of the mean. Statistical evaluation was made using Student's t-test, p values $<0.05$ were considered to be significant.

The Responses of the Rat Portal Vein.

The rat portal vein was stimulated in a number of ways; directly by depolarization with $K^+$ ions, by the addition of an exogenous agonist or by release of endogenous neuroeffector agents induced by electrical stimulation. The actions of vasodilators on these parameters and on the spontaneous myogenic contractions of the portal vein were investigated in an attempt to elucidate possible mechanisms of vasodiltor induced vascular muscle relaxation.

The exogenous agonist used as a portal vein stimulant in these experiments was NA. To ensure that the effects of the vasodilators on the three induced perameters were comparable, log concentration - response and log frequency - response curves were constructed so that concentrations and a frequency could be chosen to give equi-effective degrees of stimulation. In the cases of NA and KCl a five minute period of control, unstimulated myogenic activity was recorded prior to the addition of the stimulating agent. The agent was left in contact with the tissue for one minute and the organ bath was then washed out with fresh Krebs' solution at least twice. The next concentration of NA or KCl was not added until the myogenic activity of the vessel had returned to pre-stimulation levels. A similar protocol was used when the vessels were stimulated electrically, however in this case the period of stimuation was 30 seconds and no post-stimulation wash out was carried out.

Representative traces of stimulation of the portal vein by NA; KCl or electrical field stimulation (FIG. 5.) are shown to demonstrate the form of the responses. Log concentration - response curves for stimulation of the portal vein by NA and KCl and a log frequency - response curve for electrical stimulation were constructed (figure 6, 7 and 8 respectively). The response for each concentration (or frequency) was expressed as a percentage of the maximum response induced by that form of stimulation. Concentrations of NA, KCl and a frequency of electrical stimulation which produced approximately 70% of their respective maxima were chosen as control responses upon which the actions of vasodilators were tested. The concentrations chosen were 60mM and 3μM respectively for KCl and NA and the frequency of field stimulation used was 6Hz.

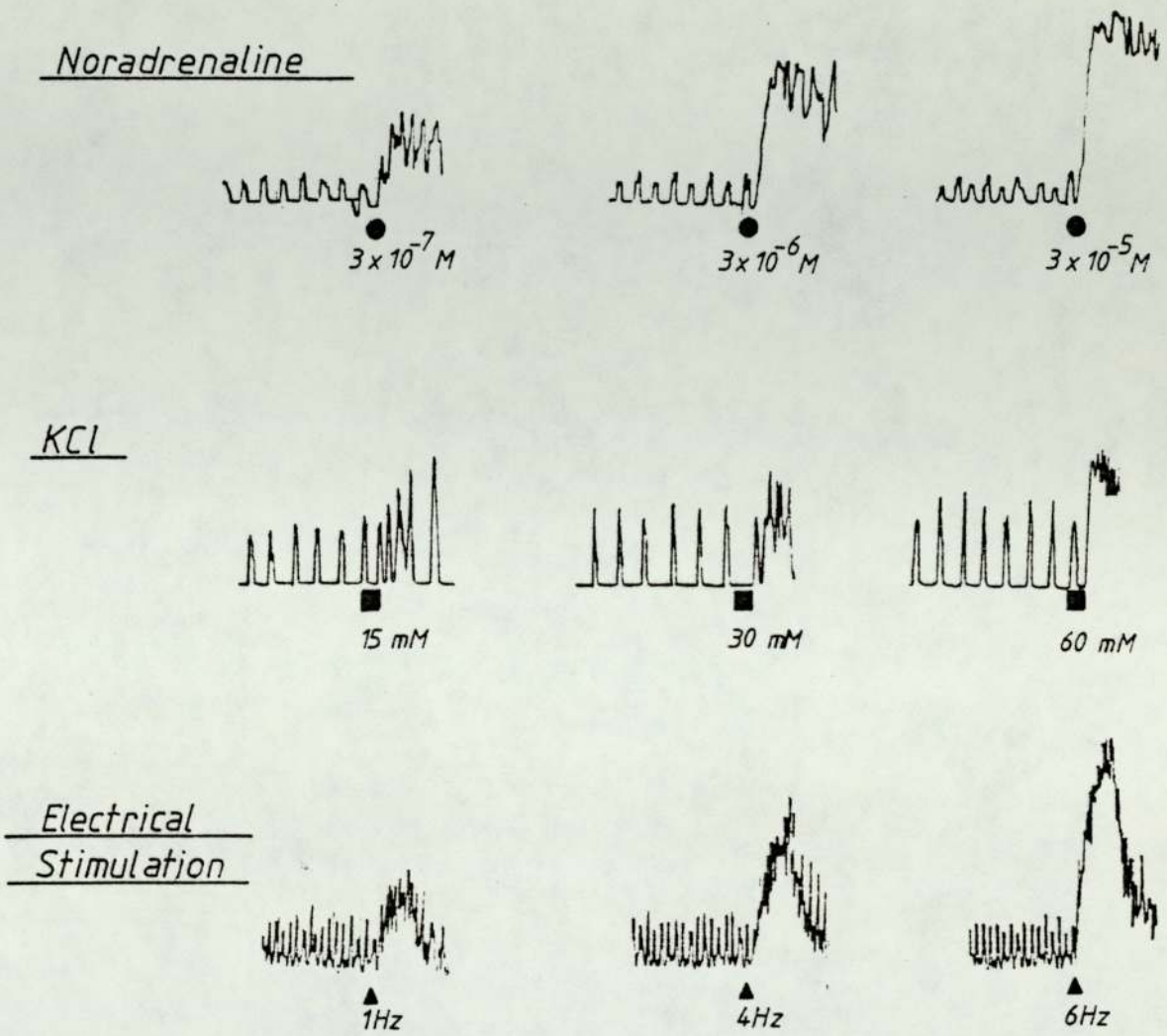



Fig. 5. Representative traces showing contractions of the portal vein induced by NA (●); KCl (■) and electrical field stimulation (▲).

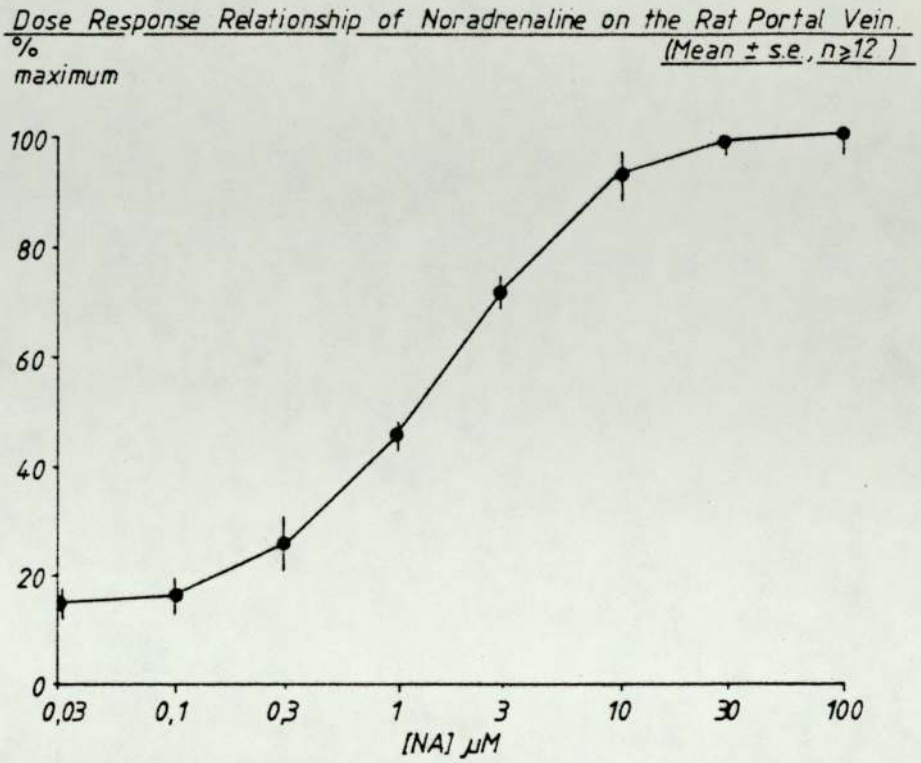



Fig. 6. Dose response relationship of noradrenaline, in the concentrations shown, on the rat portal vein. Vertical bars represent the s.e. mean.

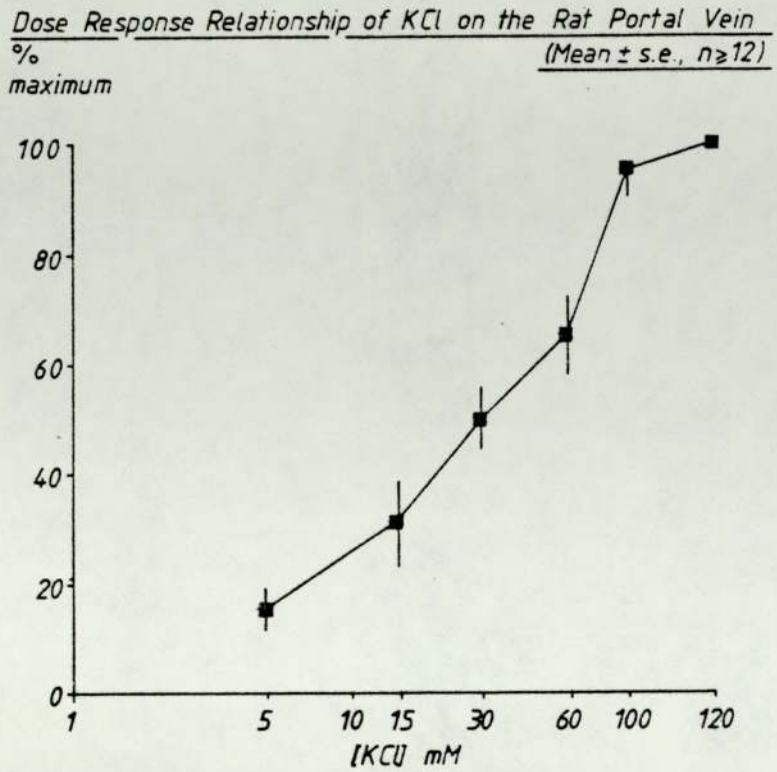



Fig. 7. Dose response relationship of KCl, in the concentrations shown, on the rat portal vein. Vertical bars represent the s.e. mean.

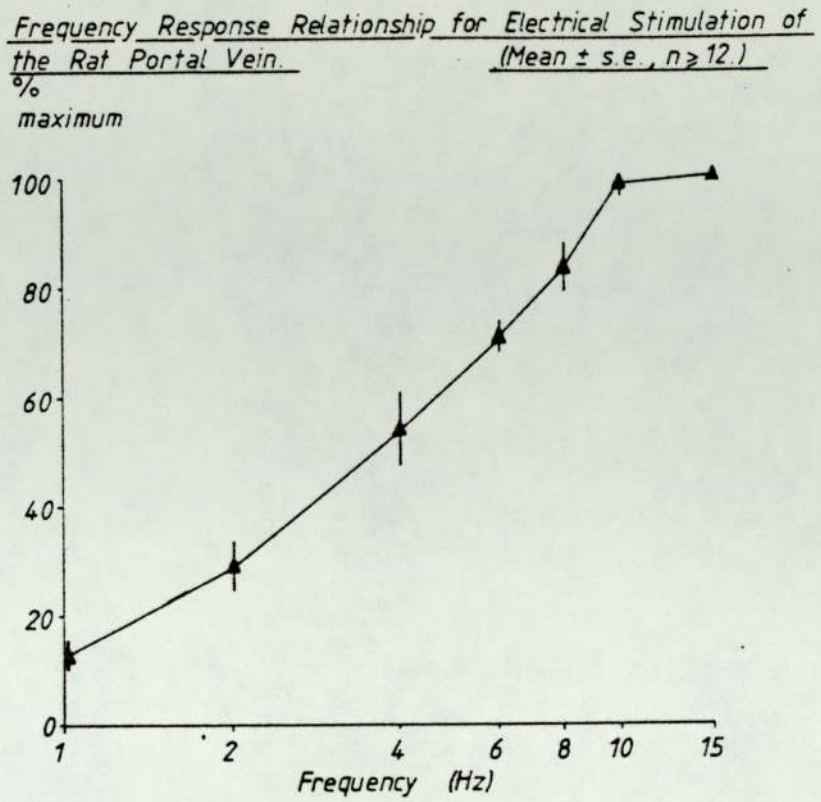



Fig. 8. Frequency response relationship of electrical stimulation, at the frequencies shown and supramaximal voltage, square wave stimulation of pulse width 1msec, on the rat portal vein. Vertical bars represent the s.e. mean.

The Responses of Circular Preparations of the Rat Thoracic Aorta.

The rat thoracic aorta, in common with the rat portal vein, may be stimulated directly by depolarizing concentrations of $K^+$ or by the addition of NA. Unlike the portal vein, the aorta is largely unresponsive to electrical field stimulation and consequently only NA and KCl induced responses were investigated. By investigating the actions of selected vasodilators on responses of the rat aorta it was hoped that arterio-venous comparisons would be possible thus providing information on any selectivity of action of the dilator agents.

As previously discussed for responses of the portal vein, it was necessary to construct log concentration vs. response relationships for both agents to allow equi-effective concentrations of NA and KCl to be chosen. The action of vasodilators on the equi-effective concentrations of NA and KCl could then be directly compared. The stimulant agent was added to the preparation after an initial equilibration period (as with portal vein preparations) and the contraction was allowed to continue until a constant peak tension had been attained. When peak tension was reached the preparation was washed out with fresh Krebs' solution at 10 minute intervals until it had regained the original tension and only then was a subsequent concentration of stimulant added.

Representative traces of contractions of the aorta induced by NA and KCl are shown (FIG. 9.) to demonstrate the form of the response. Log concentration-response curves for stimulation of the aorta by NA and KCl were constructed (FIGs. 10 and 11 respectively) by a similar method used for the responses of the portal vein. Concentrations of NA and KCl which produced approximately 70% of their respective maxima were chosen as control responses upon which the actions of vasodilators were tested. The concentrations chosen were 60mM for KCl and 1μM for NA.

Apart from the tonic contractions of the aorta mentioned above, large phasic contractions could be induced by the addition of low concentrations of NA (0.03μM) in the presence of EDTA (10μM) (Biamino and Thron, 1969 ). A representative trace showing the induced phasic activity of a circular preparation of the rat aorta can be seen in FIG. 12. This phasic activity was assessed by multiplying the frequency of contraction by the mean amplitude during a five minute period. No attempt was made to quantify these responses by integration because the phasic activity was frequently superimposed on a changing basal tone which in itself would have altered the integrated result. Changes in the induced phasic activity were calculated by the method previously described for the portal vein.

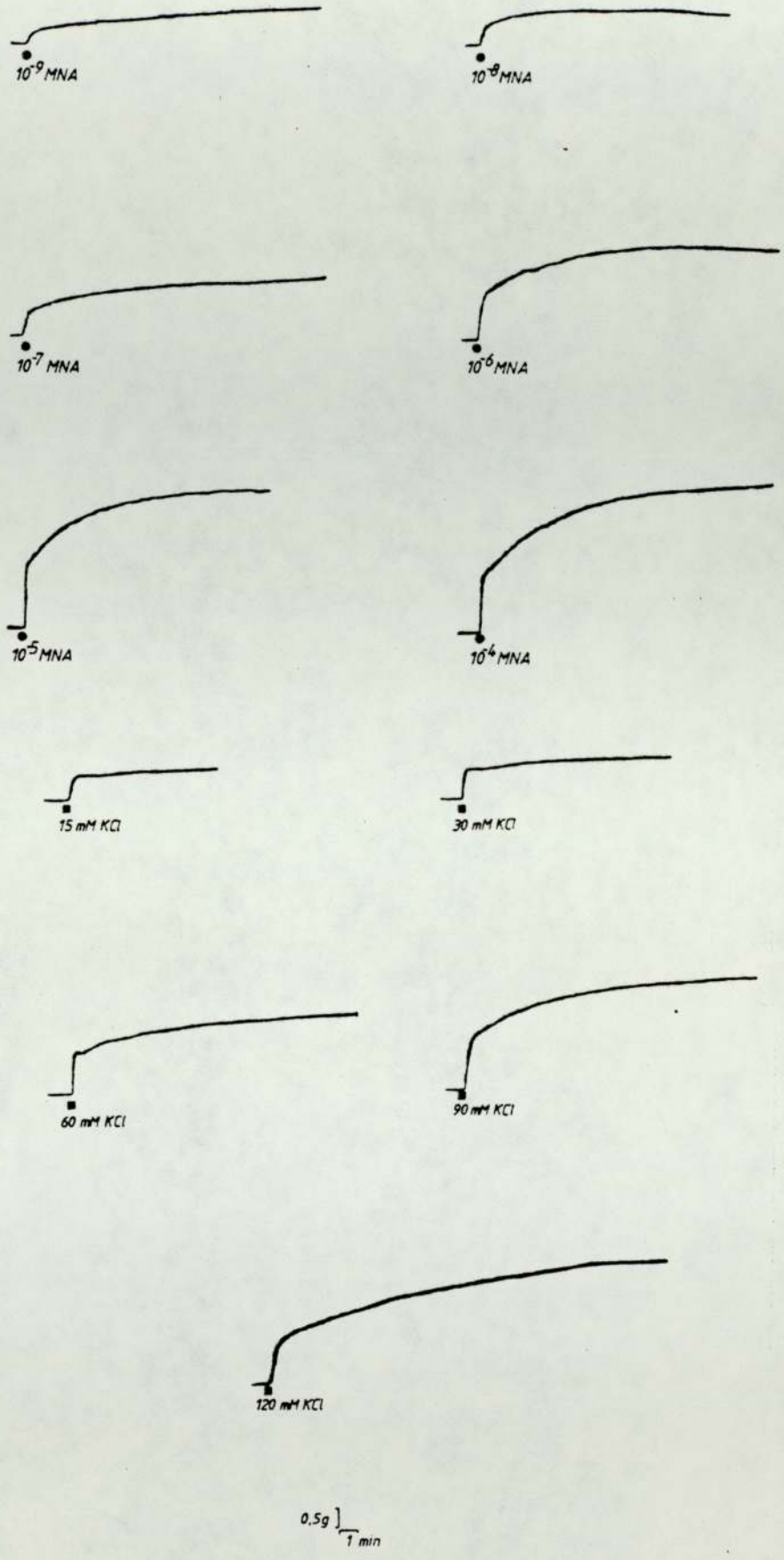



Fig. 9. Representative traces showing contractions of the rat aorta induced by noradrenaline (●) and KCl (■) in the concentrations shown.

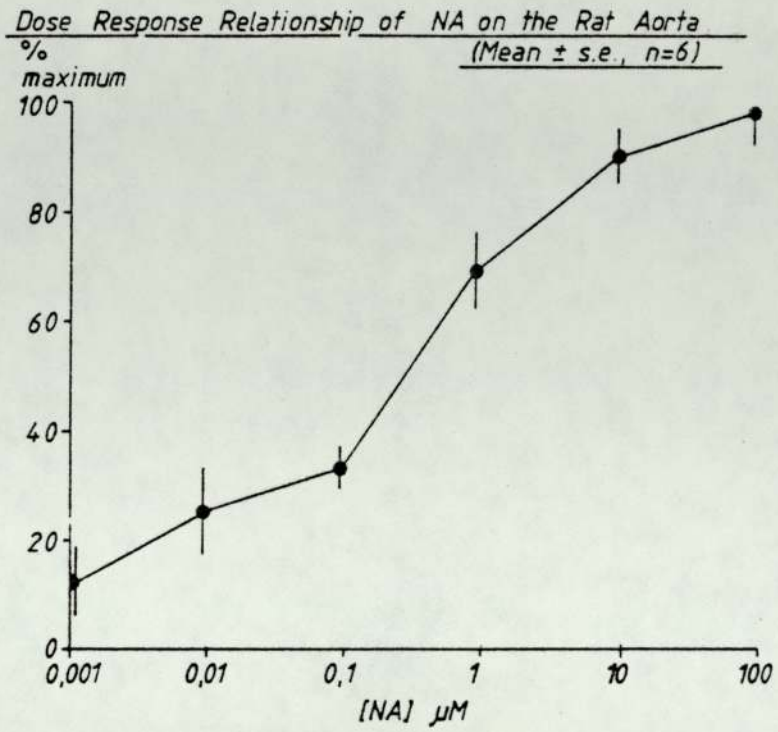



Fig. 10. Dose response relationship of noradrenaline, in the concentrations shown, on the rat aorta. Vertical bars represent the s.e. mean.

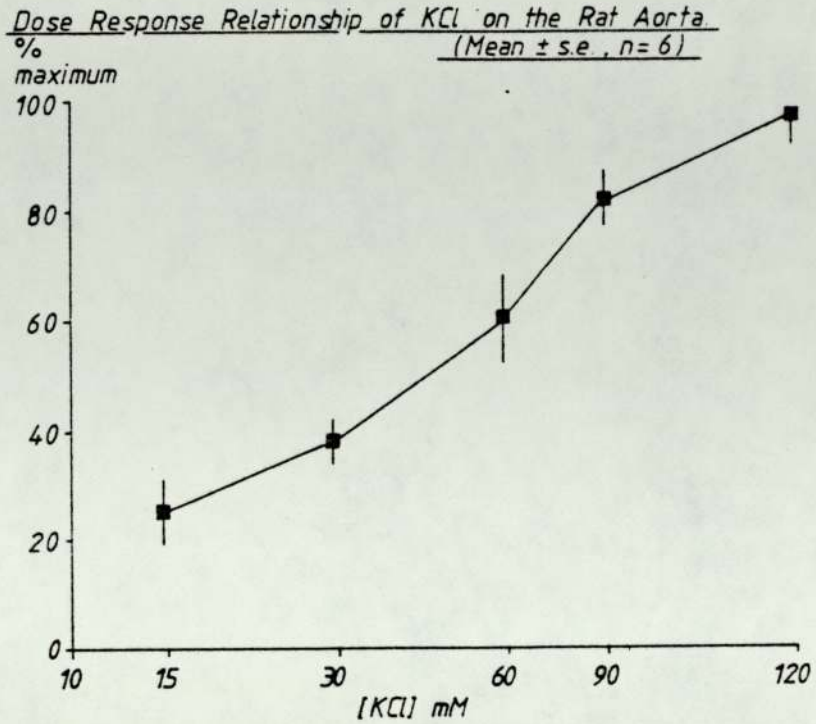



Fig. 11. Dose response relationship of KCl, in the concentrations shown, on the rat aorta. Vertical bars represent the s.e. mean.

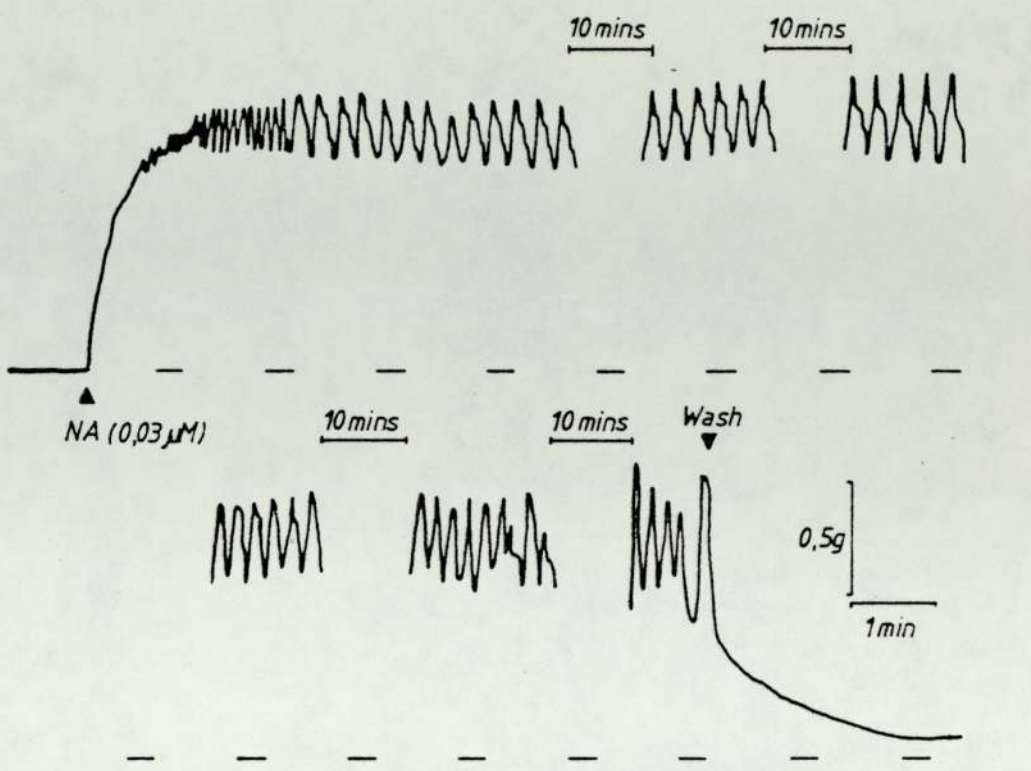



Fig. 12. Representative trace showing phasic activity of circular preparations of the rat aorta induced by 0.03 µM noradrenaline in the presence of EDTA (10 µM).

## Actions of Altered $Ca^{2+}$ and $Mg^{2+}$ on Reactivity of the Rat Portal Vein.

### Introduction.

As previously discussed in the introduction, alteration of the ionic composition of the Krebs' solution bathing smooth muscle can radicaly alter its ability to contract. For this reason the actions of raising and lowering the concentrations of $Ca^{2+}$ and $Mg^{2+}$ on responses of the rat portal vein were investigated.

The initial experiments carried out during this study of the action of altered $Ca^{2+}$ concentration were also used to determine the $Ca^{2+}$ levels of the Krebs' solution used for the rest of the study. Various $Ca^{2+}$ concentrations have been used by different workers in this field (Jetley & Weston 1980, Altura, 1982; Ebiegbe,1982) The early experiments in this study were quantified by multiplying the frequency of spontaneous contractions by their mean force calculated over a five minute period. It can be seen that a $Ca^{2+}$ concentration which rendered this process as simple and reliable as possible was an important factor in reducing the degree of error. The concentration of $Ca^{2+}$ chosen as the 'normal' level in these experiments, for the reasons stated above, was 5mM. Whenthe experimental procedure was altered to include integration of responses this level of $Ca^{2+}$ was maintained because it generaly provided a stable baseline which increased the accuracy of integration.

Results.

1. Action of Altered [$CaCl_2$].A. Lowered $Ca^{2+}$ Representative traces of portal vein contractions in 5mM $CaCl_2$ and the effect of reduced $CaCl_2$ are shown in Fig.13. The effect of lowering [$CaCl_2$] on spontaneous activity was to reduce the force of contraction and increase the frequency at which the contractions occured. There was an overall reduction in activity as measured by integration. At lowered [$CaCl_2$] the contractions induced by KCl, NA and electrical stimulation altered their form. At 2.5mM [$CaCl_2$] the initial fast rising phase of contraction was reduced and when $CaCl_2$ concentration was further lowered to 1.25mM this part of the response was lost completely.

B. Raised $Ca^{2+}$. Representative traces of the action of increased [$CaCl_2$] on the responses of the portal vein are shown in Fig.14. In this case the spontaneous activity was reduced both in frequency and force of contraction. The induced contractions of the portal vein in raised [$CaCl_2$] were of reduced magnitude, however there was no apparant change in the form of these contractions except in the case of KCl where some loss of the initial rising phase of contraction was noted.

As can be seen from figure 15 both increasing and decreasing the [$CaCl_2$] from the initial level of 5mM resulted in a reduction in all the parameters tested. The points shown in FIG. 15 ( and those in FIG. 18 in the case

of altered $Mg^{2+}$) were calculated as a percentage change for each paramter from control value elicited in 5 mM $CaCL_2$ (or 1.18 mM $MgSO_4$ in the case of $Mg^{2+}$). The effects of altered [$CaCl_2$] were, however, not uniform for each parameter. At the lowest concentration of $CaCl_2$ tested (1.25mM) electrical stimulation and spontaneous activity were reduced to a greater extent than were responses to KCl and NA ($p<0.05$, $n=6$). These results gave a rank order of sensitivity to reduced [$CaCl_2$] of electrical stimulation>spontaneous acvtivity>>KCl>NA.

As with reductions in [$Ca^{2+}$] there was a variation in the sensitivity of each parameter to increased [$Ca^{2+}$], furthermore the rank order was different to that seen on lowering [$Ca^{2+}$] . When [$CaCl_2$] was increased the rank order or reduction in response was spontaneous activity>electrical activity>NA>KCl.

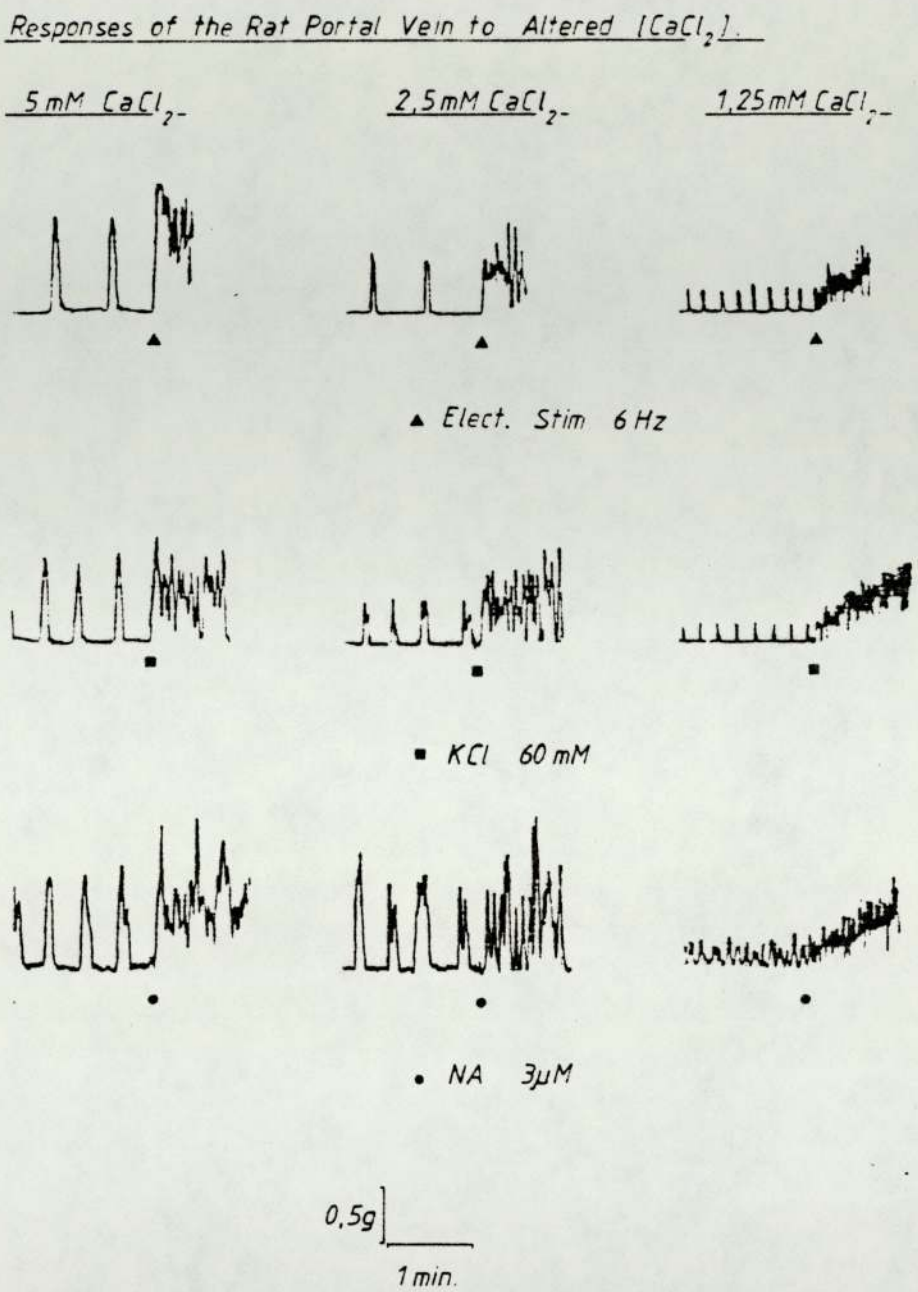



Fig. 13. Representative traces showing the effects of reduced [$CaCl_2$] on responses of the rat portal vein to electrical field stimulation, 6 Hz ( ▲ ); noradrenaline ( ● ); and KCl ( ■ ) in the concentrations shown.

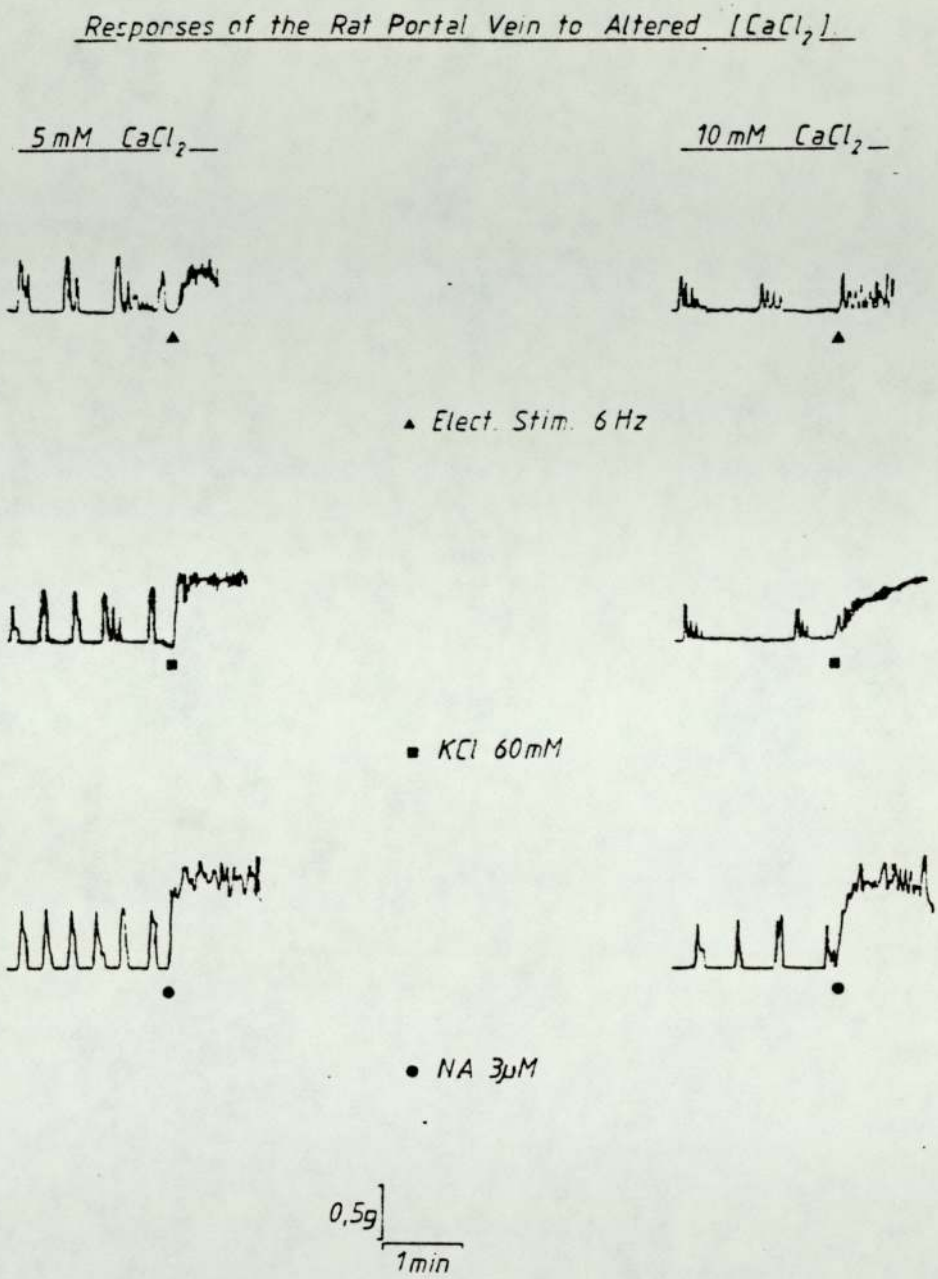



Fig. 14. Representative traces showing the effects of increased [$CaCl_2$] on responses of the rat portal vein to electrical field stimulation, 6 Hz (▲); noradrenaline (●); and KCl (■) in the concentrations shown.

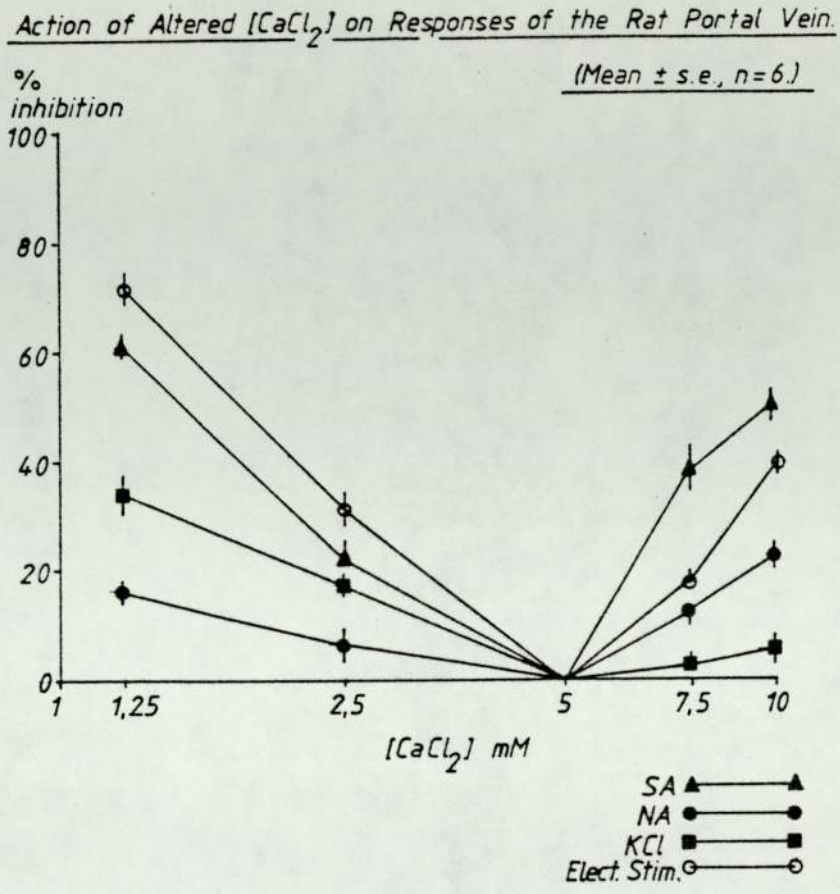



Fig. 15. Effects of altered [$CaCl_2$] on responses of the rat portal vein; spontaneous activity (SA, ▲ ); contractions induced by noradrenaline (NA, 3 μM, ● ); KCl (60 mM, ■ ) and field stimulation (6Hz, ○ ). Verticle bars represent s.e. mean.

## 3. Action of Altered [$MgSO_4$].

### A. Lowered $Mg^{2+}$.

Alteration of the [$MgSO_4$] in the Krebs' solution bathing the portal vein produced a diverse range of responses. Reduction of [$MgSO_4$] below normal control levels of 1.18mM generally induced an increase in responsiveness of the portal vein. One exception to this pattern of activity was the action of reduced [$MgSO_4$] on the responses to electrical stimulation. In this case reduction in [$MgSO_4$] below control levels (1.18mM) caused a small, but significant ($p<0.05$, $n=6$), reduction in response.

Representative traces of the action of decreased [$MgSO_4$] on responses of the portal vein are shown in figure 16. At a [$MgSO_4$] of 0.6mM the spontaneous contractions of the portal vein were generally of a slower frequency but a larger amplitude and gave a small overall increase in integrated activity. On the other hand at 0mM [$MgSO_4$] the frequency of spontaneous contractions was greatly increased and was sometimes accompanied by a reduced force of contraction. There was also a change in the form of the spontaneous contractions in $MgSO_4$-free solutions in that there was an increase in the number of contractions showing smaller second and third peaks during the relaxation phase of the original contraction.

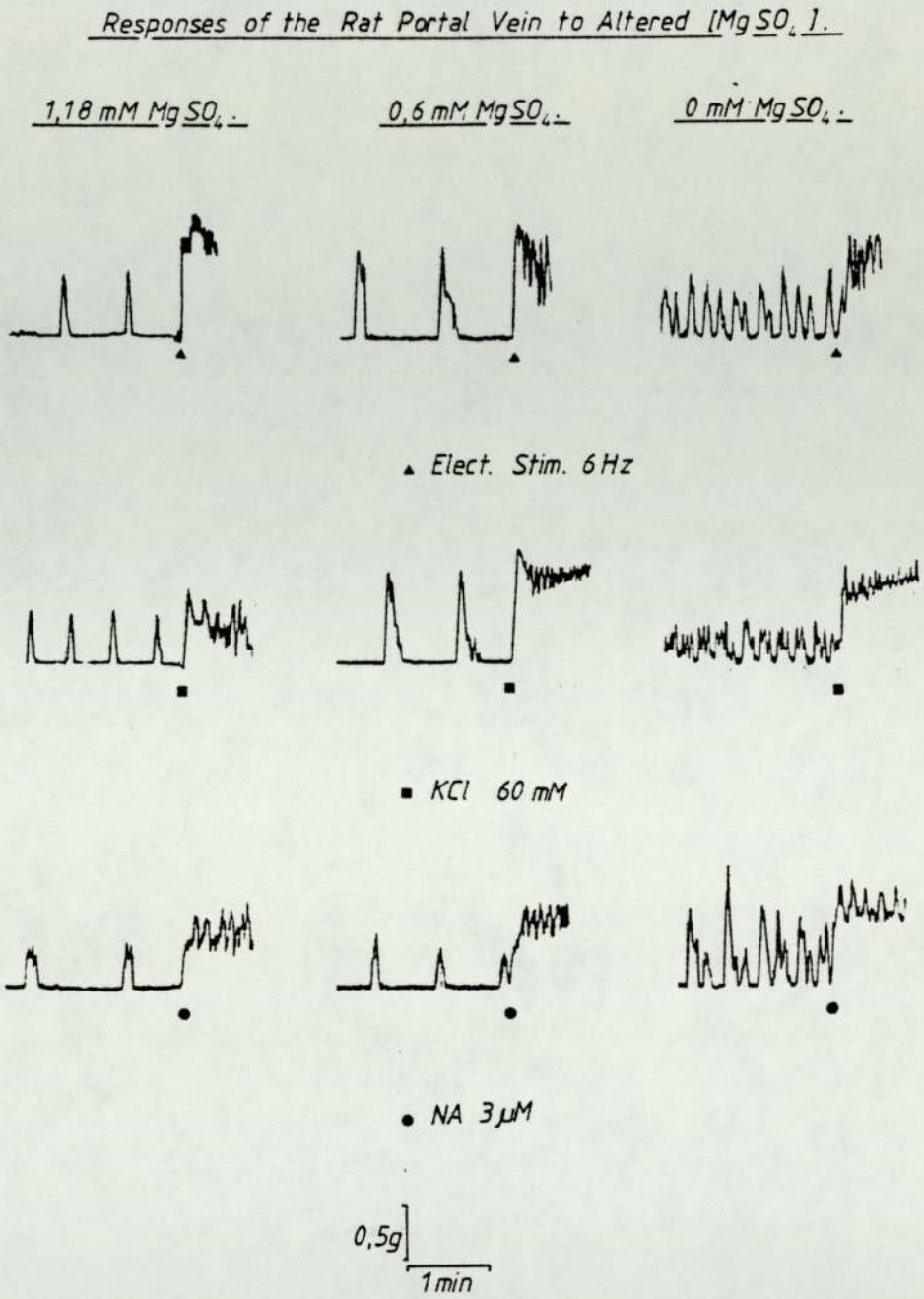



Fig. 16. Representative traces showing the effects of reduced [$MgSO_4$] on responses of the rat portal vein to electrical field stimulation, 6 Hz ( ▲ ); noradrenaline ( ● ); and KCl ( ■ ) in the concentrations shown.

The form of contractions induced by NA and KCl appeared unchanged, except in their increased magnitude. The shape of the contraction induced by electrical stimulation was altered in that there was a slower onset of contraction (at 0mM $MgSO_4$) associated with a slightly reduced peak tension.

B. Raised $Mg^{2+}$.

The effect of increases in the [$MgSO_4$] on responses of the portal vein are shown in FIG. 17. As [$MgSO_4$] was increased there was generally a reduction in both the rate and size of the spontaneous contractions. In some cases (NA panel of FIG. 19.) the reduction in the rate of spontaneous contractions was associated with a small increase in their tension. However in these cases the reduction in frequency was so marked as to produce an overall decrease in integrated activity.

In general the only alteration to induced contractions seen in increased [$MgSO_4$] was a diminution of the peak tension attained. In some experiments carried out using NA there was no reduction of the initial peak contraction but tension was not maintained throughout the response as was normal at these [NA] in unaltered Krebs' solution (NA panel, FIG, 19.).

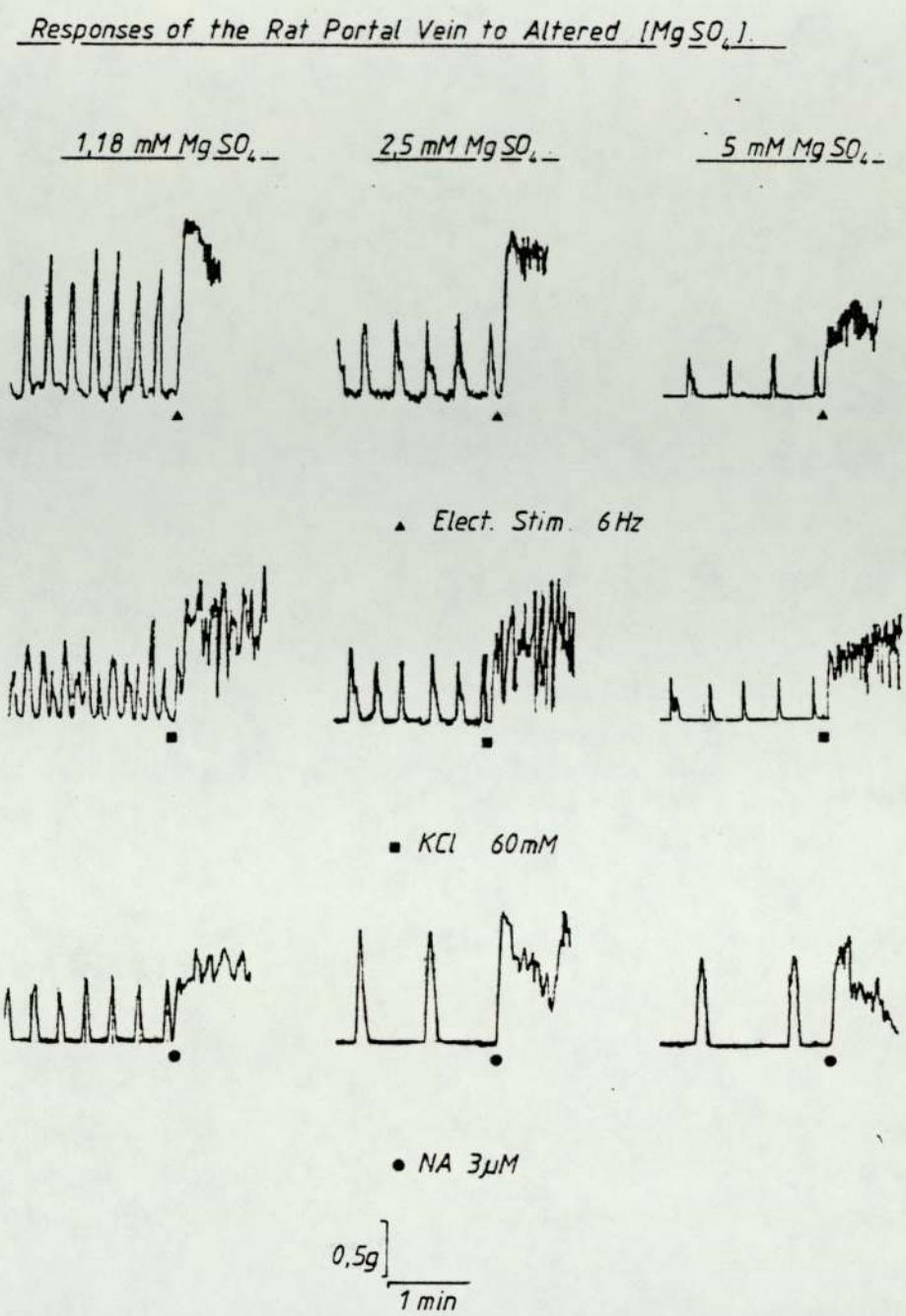



Fig. 17. Representative traces showing the effects of increased [$MgSO_4$] on responses of the rat portal vein to electrical field stimulation, 6 Hz (▲); noradrenaline (●); and KCl (■) in the concentrations shown.

A graph of [$MgSO_4$] plotted against percentage inhibition of control values (elicited in 1.18mM $MgSO_4$) is shown in FIG. 18, a negative inhibition is representative of an increase in activity. The rank order of sensitivity of responses to altered [$MgSO_4$] was spontaneous activity>NA>KCl>electrical stimulation irrespective of whether $MgSO_4$ was raised or lowered. In the case of zero $MgSO_4$ spontaneous activity was increased to a far greater extent than the induced responses ($p<0.05$, $n=6$).

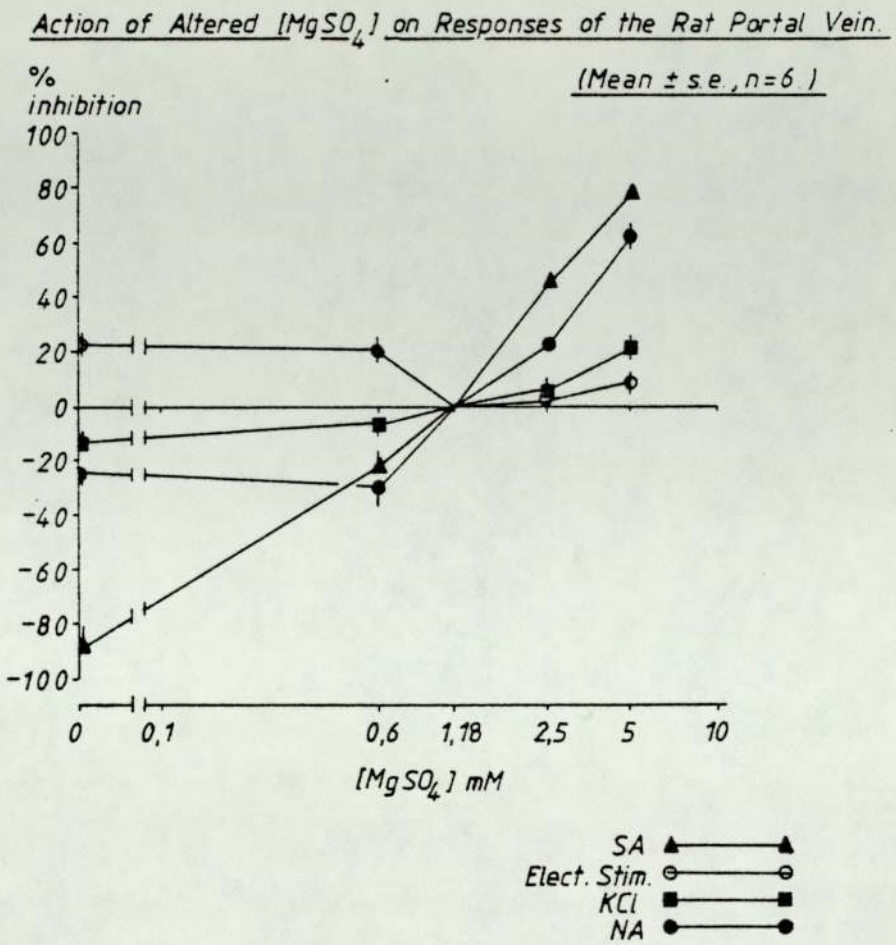



Fig. 18. Effects of altered [$MgSO_4$] on responses of the rat portal vein; spontaneous activity (SA, ▲ ); contractions induced by noradrenaline (NA, 3 μM, ● ); KCl (60 mM, ■ ) and field stimulation (6Hz, ○ ). Verticle bars represent s.e. mean.

## DISCUSSION.

### Actions of Altered $Ca^{2+}$.

Johansson *et al.* (1967) have shown that the spontaneous myogenic contractions of the portal vein could be completely inhibited by the removal of extracellular $Ca^{2+}$. Sigurdsson and co-workers (1975) repeated this observation and also showed that in tissues where $[Ca^{2+}]_o$ was zero and spontaneous activity was inhibited, the addition of KCl (122mM) produced no contractile response. A response to this depolarizing concentration of KCl was elicited when $Ca^{2+}$ and KCl were simultaneously readministered, however the form of the response was dependent upon the duration of $Ca^{2+}$ depletion. From their results Sigurdsson and collegues (1975) concluded that there was a superficially bound $Ca^{2+}$ store but that it alone was incapable of producing a response to depolarization and that it required some $[Ca^{2+}]_o$ to trigger its release. Ebeigbe (1982) has shown that in portal vein with zero $[Ca^{2+}]_o$ , stimulation with NA and KCl produced responses of different time course and he has suggested that this may be due to utilization of different $Ca^{2+}$ pools. The responses to NA in $Ca^{2+}$-free, $K^+$ depolarized portal vein was inhibited by ryanodine which Ebeigbe has suggested demonstrates the presence of an intracellular store of $Ca^{2+}$ accessible to release by NA. However, it should be noted that ryanodine had no inhibitory action on NA responses in the presence of extracellular $Ca^{2+}$ (Ebiegbe, 1982) and this may infer a relative unimportance of stored $Ca^{2+}$ in mediating responses of the portal vein in the

presence of extracellular $Ca^{2+}$.

In the present study the degree to which the response of each parameter was inhibited by reduction in the $[Ca^{2+}]_o$ may be a measure of the dependence of that parameter on extracellular $Ca^{2+}$ to produce its contractions. The results from the experiments performed here cannot cast any light on the question of utilization of intracellular stores by any of the forms of stimulation. It is interesting to note however, that the rank order of sensitivity to decreased $[Ca^{2+}]_o$ is electrical stimulation > spontaneous activity >> KCl > NA which is similar to the order of spontaneous activity > KCl > NA found by Ebeigbe (1982) in $Ca^{2+}$ depletion experiments.

Increasing the concentration of calcium has long been known to inhibit contractions of vascular muscle (Cow,1911; Bohr,1963). Bohr (1963) has suggested that an inhibitory response to raised $[Ca^{2+}]_o$ is due to membrane stabilization while the excitatory action of elevated $[Ca^{2+}]_o$ seen in some tissues is due to an action on excitation-contraction coupling (EC-coupling). Bohr (1963) has proposed that the overall effect of increased $[Ca^{2+}]_o$ is dependent upon whether membrane stability or EC-coupling is the limiting factor in the production of response in any given smooth muscle and that an interplay between the two might exist.

Altered levels of $[Ca^{2+}]_o$ could inhibit the various responses of the portal vein differently by several other

mechanisms. It is possible that raising or lowering $[Ca^{2+}]_o$ may change the sensitivity of potential and receptor operated $Ca^{2+}$ channels differently. If this were the case then the degree to which a response was inhibited would be dependent upon the profile of its activator channels. A differential action of altered $[Ca^{2+}]_o$ on so called 'electromechanical' and 'pharmacomechanical' coupling of vascular muscle is an alternative possibility to explain the effects observed on changing $[Ca^{2+}]_o$ . It is also possible that changes in $[Ca^{2+}]_o$ may produce their actions on electrical stimulation by an effect on transmitter release. Thus explaining the difference in sensitivity between contractions induced by field stimulation and those by NA addition.

Actions of Altered $[Mg^{2+}]_o$ .

Altura and Altura (1978a) have previously shown that decreased $[Mg^{2+}]_o$ caused a rapid increase in spontaneous activity of the portal vein whereas increased $[Mg^{2+}]_o$ inhibited such activity. Furthermore they have shown that such alterations in $[Mg^{2+}]_o$ did not elicit these effects by altering the osmolarity of the Krebs' solution. Altura has also suggested (1978a), based largely on the rapidity of the onset of the responses, that such actions as seen with altered $[Mg^{2+}]_o$ are due to membrane and not intracellular phenomena. This view was supported by the work of Palaty (1974) who suggested that $[Mg^{2+}]_i$ was largely insensitive to changes in $[Mg^{2+}]_o$ under experimental conditions similar to those used by Altura (1978a).

The inhibitory effect of increased $[Mg^{2+}]_o$ on the spontaneous acivity of the portal vein has been explained by Sigurdsson and Uvelius (1977) as being due to membrane hyperpolarization. Altura (1978a), on the other hand, has explained the observed actions of altered $[Mg^{2+}]_o$ on vascular muscle by a direct action on the $Ca^{2+}$ handling of the muscle cells. Another possible action of $Mg^{2+}$, which may be a facet of the two previous hypotheses or an independent mechanism, is the possibility that altered $Mg^{2+}$ may stabilise the cell membranes by changing their ion permeabilities.

The results presented here are not in disagreement with either of the main hypotheses which are in themselves not mutualy exclusive. In the '$Ca^{2+}$ rich' Krebs' solution used in these experiments increased $[Mg^{2+}]_o$ still produced quite a marked inhibition of responses. As previously discussed, the portal vein was largly dependent on extracellular sources to provide activator $Ca^{2+}$ and thus any competition between $Mg^{2+}$ and $Ca^{2+}$ for sites in superficially bound membrane stores would be rendered less important. One explanation advanced by Altura to account for the actions of altered $[Mg^{2+}]_o$ on arterial muscle was by a competition for binding sites in membrane bound stores. In the portal vein however, if $Mg^{2+}$ can compete with $Ca^{2+}$ for entry during contraction, rather than binding, a similar situation of competition between the two ions might exist in this vessel. The form of inhibiton exhibited by increasing $[Mg^{2+}]_o$ is of

interest, as selective suppression of spontaneous activity may be an indication of membrane hyperpolarization (Downing, *et al.*;1982). This possibility will be discussed at greater length later.

The increased responsiveness of the portal vein seen with decreased $[Mg^{2+}]_o$ may also be due to a competitive interaction between $Mg^{2+}$ and $Ca^{2+}$ as discussed above. The inability of decreased $[Mg^{2+}]_o$ to enhance responses to electrical stimulation was surprising. This observation was not in accord with the known actions of altered levels of $Mg^{2+}$ on nervous transmission (Altura, 1982) and no adequate explanation may be derived for this phenomena from the experimental data presented here.

SUMMARY.

Alteration of $[Ca^{2+}]$ and $[Mg^{2+}]$ on Portal Vein Reactivity.

Alteration of $[Ca^{2+}]_o$ and $[Mg^{2+}]_o$ has been shown to profoundly alter the spontaneous and elicited activity of the portal vein. The reduction in $[Ca^{2+}]_o$ had an inhibitory effect on responses of the portal vein probably due to the reliance of this vessel on the influx of extracellular $Ca^{2+}$ to provide activator $Ca^{2+}$. An increase in $[Ca^{2+}]_o$ has also been shown to reduce responses of the portal vein possibly via a mechanism of membrane stabilization as outlined by Bohr (1963).

Reduction in the $[Mg^{2+}]_o$ caused an enhancement of the responses of the portal vein, with the exception of contractions due to electrical stimulation. This stimulatory action of lowered $[Mg^{2+}]_o$ may be due to a competitive interaction between $Mg^{2+}$ and $Ca^{2+}$ where a fall in $[Mg^{2+}]_o$ would result in an increase in the availability of activator $Ca^{2+}$. The inhibitory action of increased $[Mg^{2+}]_o$ may be due to the converse situation arrising in a putative $Mg^{2+}$-$Ca^{2+}$ interaction. Another possible mechanism by which raised $[Mg^{2+}]_o$ may inhibit portal vein responses is by causing hyperpolarization of the vascular muscle membrane.

It is perhaps pertinent to consider that any directly acting vasoconstrictor or vasodilator agent may have a component of action which could involve an alteration in the

handling of either $Ca^{2+}$ or $Mg^{2+}$ by the smooth muscle cells.

## The Action of a Series of Vasodilators on the Responses of the Rat Portal Vein.

### Introduction.

A series of vasodilator drugs were investigated by comparing their effects upon the portal vein in order to find any similarities or differences in their actions which might give a clue to their *modus operandi*. By comparing their spectrum of activity on the portal vein with published data on human veins it was hoped that these experiments would allow an assessment of the usefullness of the portal vein for predicting human veno-dilator efficacy.

The effects of each vasodilator agent was investigated on spontaneous activity and contractions induced by electrical field stimulation (FS, 6Hz), NA (3μM) and KCl (60mM). The log concentration-response relationships for the inhibition of each parameter were expressed as a percentage reduction from control responses in the same preparation. The results from these experiments are plotted as log concentration of vasodilator against the percentage reduction observed. For all agents the inhibition of electrical field stimulation was not significantly different from that seen with NA induced contractions and consequently for the sake of clarity these results have been omitted from the figures.

### Results.

The vasodilators investigated in these experiments

were papaverine (PAP); isoprenaline (ISOP); Na nitroprusside (NP); tolmesoxide (TOLM); glyceryl trinitrate (GTN); verapamil (VER); diazoxide (DIAZ) and hydrallazine (HYD). To allow comparison between agents the percentage inhibition of each parameter caused by the maximum concentration of the agent is shown in TABLE 1.

PAP caused a concentration dependent inhibition of all the parameters over a concentration range of 0.1 - 100μM. In contrast to most of the vasodilators PAP did not produce reductions in both frequency and amplitude of spontaneous contractions. As the concentration of PAP was increased there was a marked reduction in the force of the spontaneous contractions but there was also a considerable increase in their frequency. A representative trace of this action is shown in figure 19a; the actions of verapamil on spontaneous contractions are shown in the lower panel for comparison. There was no significant difference between the degree of inhibition produced by PAP on any of the induced responses of the portal vein. At concentrations of less than 2.5μM the inhibition of spontaneous activity was significantly less ($p<0.05$, $n=6$) than the inhibition of the induced contractions (FIG. 20.) The inability of PAP to produce more than approximately 60% inhibition of any parameter may be a consequence of its apparent stimulant action seen with higher concentrations (FIG. 19a.).

In an attempt to determine whether the spectrum of action of PAP was due to its known ability to elevate cAMP

| | | Maximum % Inhibition of Responses | | |
|---|---|---|---|---|
| AGENT | Maximum (μM) Concentration. | Spontaneous Activity. | NA (3μM) | KCl (60mM) |
| PAP | 100 | 53 ± 4 | 66 ± 4 | 60 ± 3 |
| ISOP | 0,3 | 100 | 60 ± 12 | 64 ± 7 |
| NP | 100 | 95 ± 6 | 54 ± 5 | 3 ± 5 |
| TOLM | 1000 | 89 ± 3 | 53 ± 9 | 25 ± 7 |
| GTN | 100 | 90 ± 4 | 68 ± 6 | 57 ± 4 |
| VER | 100 | 91 ± 4 | 97 ± 5 | 93 ± 3 |
| DIAZ | 300 | 100 | 93 ± 2 | 94 ± 3 |
| HYD | 3000 | 70 ± 3 | 81 ± 5 | 76 ± 4 |

Table 1. Comparative effects of all the vasodilators tested on spontaneous activity, and responses induced by NA and KCl in the rat portal vein. Maximum percentage inhibition of each response and the concentration at which it occured are shown to allow some comparison of relative potency.

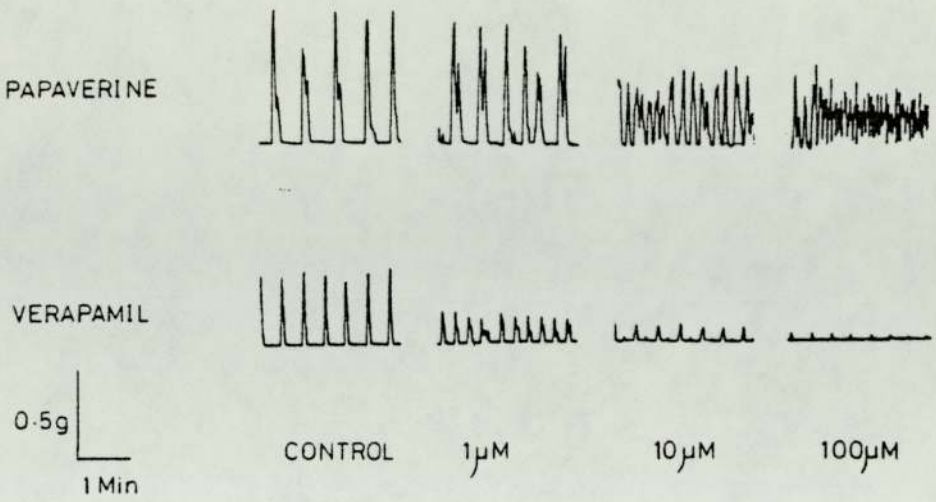



Fig. 19a. Representative traces showing the action of PAP and VER on spontaneous activity of the rat portal vein in the concentrations shown.

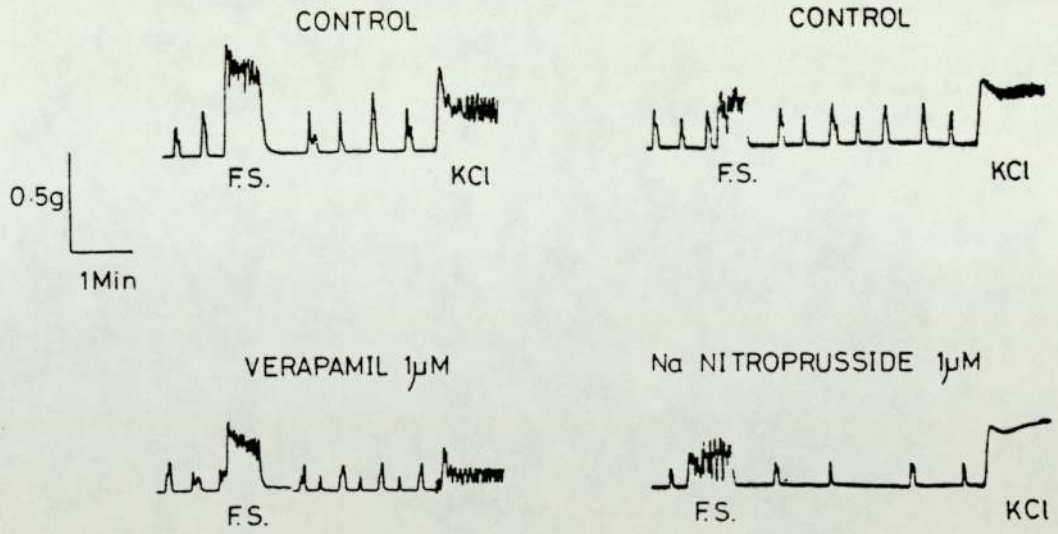



Fig. 19b. Representative traces showing the action of VER and NP (both 1 uM) on spontaneous activity and contractions induced by electrical fiels stimulation (FS, 6 Hz) and KCl (60 mM). The upper trace shows control responses and the lower trace responses in the presence of VER and NP.

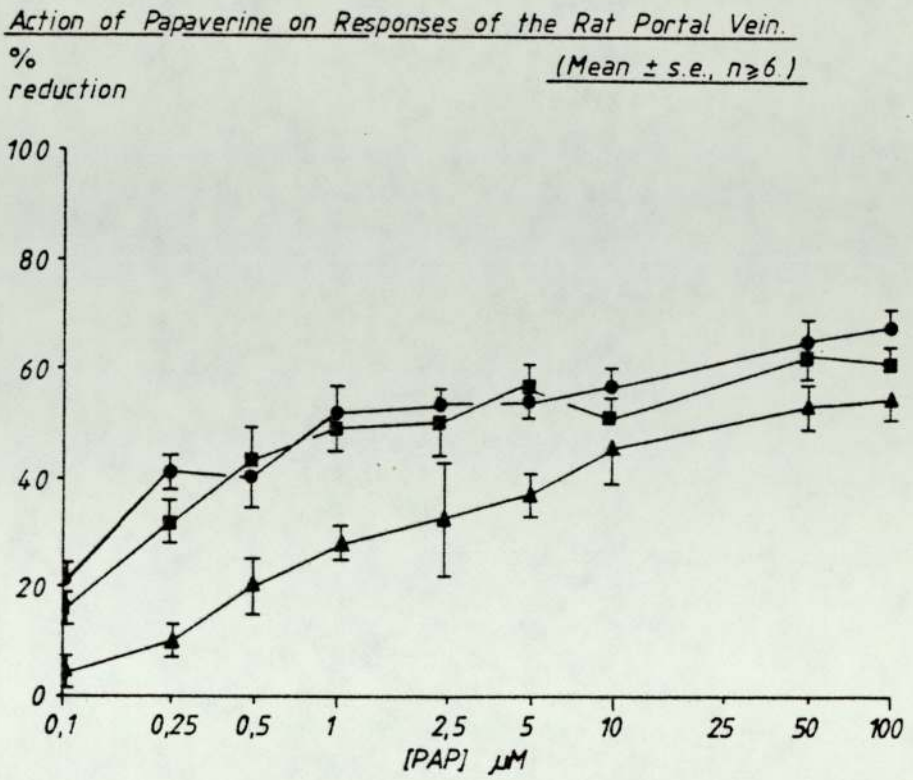



Fig. 20. Effect of papaverine on spontaneous activity (▲) and responses induced by NA (3 µM, ●) and KCl (60 mM, ■) in the rat portal vein. Verticle bars represent the s.e. mean.

levels (Kukovetz & Poch, 1970; Andersson, 1973a) another agent known to raise [cAMP], isoprenaline (ISOP) was tested. Unlike PAP which raises [cAMP] by inhibition of the phosphodiesterase enzyme (Kukovetz & Poch, 1970), ISOP raises [cAMP] by stimulating adenyl cyclase (Crawford, 1973). ISOP was a potent inhibitor of all the parameters of portal vein reactivity tested (FIG. 21.) causing a dose dependent reduction over the concentration range of 0.001 - o.3μM. In direct contrast to the actions of PAP, ISOP produced a significantly greater inhibition of spontaneous activity compared with induced responses at concentrations in excess of 0.03μM ($p<0.05$, $n=6$). It may be that raised [cAMP] was responsible for the spectrum of action of either PAP or ISOP but from the results presented here it is unlikely that the agents share a common sole mechanism of action.

A selective inhibition of spontaneous activity and a complete lack of effect on KCl contractions were the most marked features of the action of NP on the portal vein. Representative traces showing the action of NP in comparison with VER are shown in figure 19b. The concentration response curve for NP against each parameter is shown in figure 22. At concentrations of NP below 0.3μM there was no significant difference between the inhibition of each paramter. At concentrations of NP greater than 1uM spontaneous activity was inhibited to a significantly greater extent ($p<0.01$, $n=6$) than NA or electrical stimulation which were in turn significabtly reduced ($p<0.01$, $n=6$) when compared with

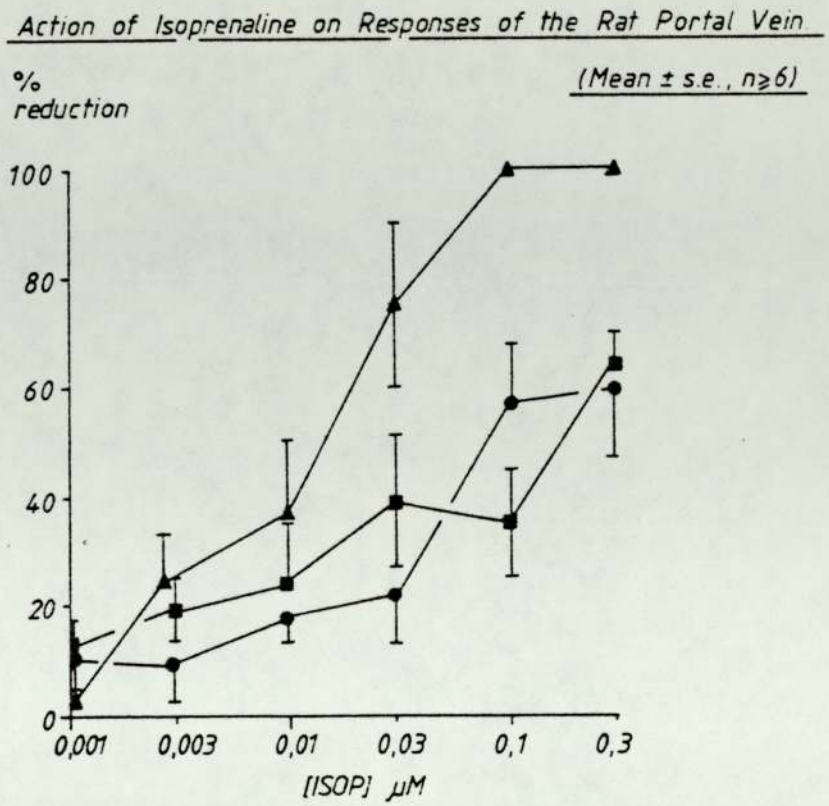



Fig. 21. Effect of isoprenaline on spontaneous activity ( ▲ ) and responses induced by NA (3 µM, ● ) and KCl (60 mM, ■ ) in the rat portal vein. Verticle bars represent the s.e. mean.

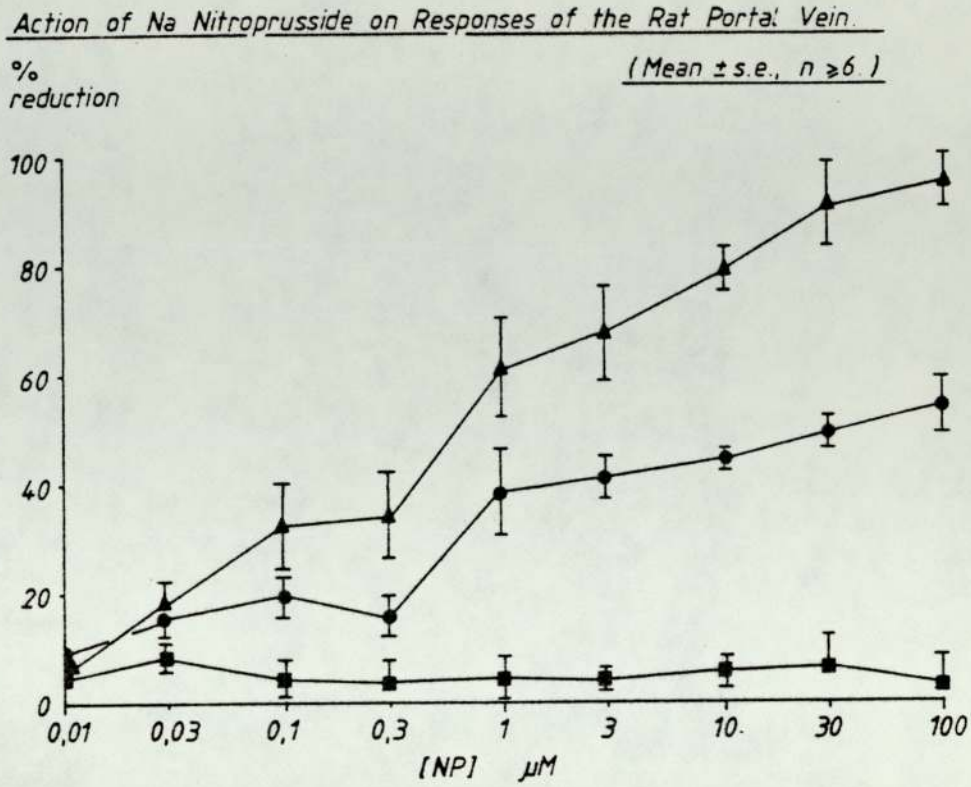



Fig. 22. Effect of Na Nitroprusside on spontaneous activity ( ▲ ) and responses induced by NA (3 µM, ● ) and KCl (60 mM, ■ ) in the rat portal vein. Verticle bars represent the s.e. mean.

responses to KCl. Contractions of the portal vein induced by KCl showed no significant reduction from control levels even in the presence of a concentration of NP (100μM) which inhibited spontaneous activity completely.

A spectrum of action similar to that exhibited by NP was seen with concentrations of tolmesoxide (TOLM) of 30μM and above (FIG. 23.). The inhibition of the spontaneous activity was significantly ($p<0.05$, $n=6$) greater than 'induced' responses. In common with NP, TOLM exhibited least effect on contractions induced by KCl. It should be noted however that NA and electrical stimulation were only significantly reduced ($p<0.05$, $n=6$) to a greater extent than KCl induced contractions at the highest concentration of TOLM used (1mM). The effect seen at 1mM TOLM were therefore similar to those seen with 1μM NP.

When the actions of GTN on the responses of the portal vein were investigated a similar selective suppression of spontaneous activity was noted. But in contrast to the results obtained with NP and TOLM the selective inhibition of spontaneous activity by GTN was more pronounced at lower concentrations (0.3 - 3μM; FIG. 24.). Furthermore GTN was able to depress the KCl induced contraction and as the concentration of GTN was increased above 3μM the differences between suppression of each parameter were no longer significant ($p,0.05$, $n=6$).

The $Ca^{2+}$ entry blocker verapamil (VER) produced a

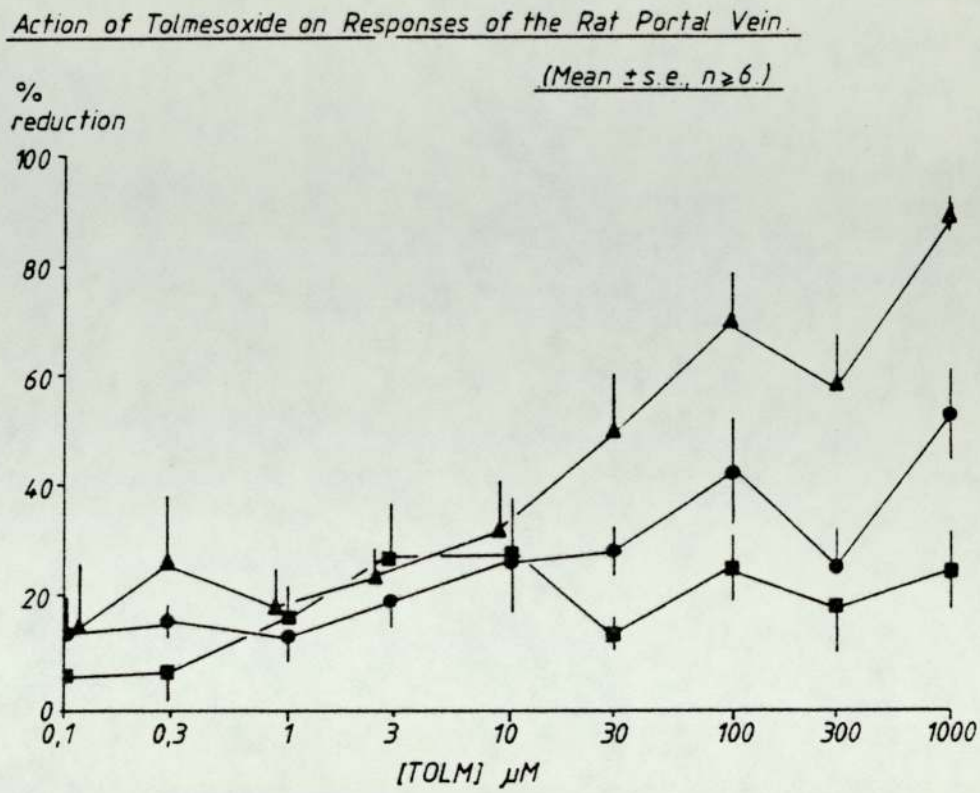



Fig. 23. Effect of tolmesoxide on spontaneous activity (▲) and responses induced by NA (3 µM, ●) and KCl (60 mM, ■) in the rat portal vein. Verticle bars represent the s.e. mean.

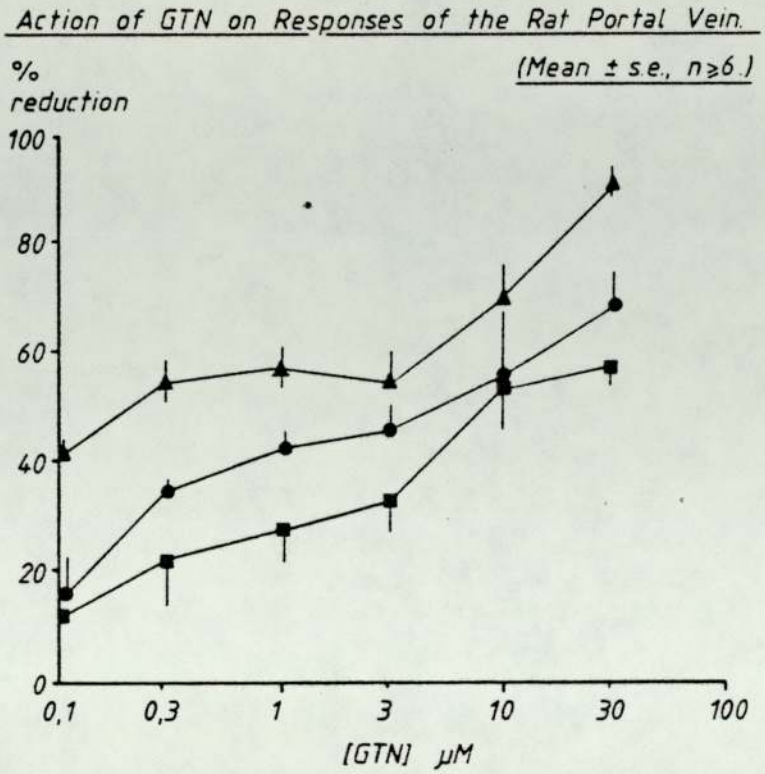



Fig. 24. Effect of glyceryl trinitrate on spontaneous activity ( ▲ ) and responses induced by NA (3 µM, ● ) and KCl (60 mM, ■ ) in the rat portal vein. Verticle bars represent the s.e. mean.

concentration dependent inhibition of all parameters over the range of 0.01 - 100μM (FIG. 25.). There was no selectivity of action; all parameters were reduced to approximately the same extent by a given concentration of VER. The 'uniform' inhibition of responses by VER may be due to the predominant dependence of the portal vein on extracellular $Ca^{2+}$ as a source of activator $Ca^{2+}$ (Sigurdsson, *et al.*, 1975).

Diazoxide (DIAZ) produced a profile of action similar to that of VER in that it inhibited equally all the parameters of portal vein reactivity in a concentration dependent manner (FIG. 26.). The concentration-response curves for DIAZ were much steeper than those of VER a full range of effects were seen over the concentration range of 1 - 300μM. As previously mentioned DIAZ was obtained as an injectable solution at pH 11.2.

The final vasodilator used in this series was hydrallazine (HYD) whose clinical efficacy is believed to be dependent almost solely on its selective relaxation of arteriolar resistance vessels (see introduction). There was no differential pattern of selectivity in the inhibition of the portal vein contractions by HYD, each parameter was inhibited to approximately the same extent by a given concentration (FIG. 27.) The arterial selectivity of this agent was apparant by the large concentration required to produce significant effects on this preparation (TABLE 1.).

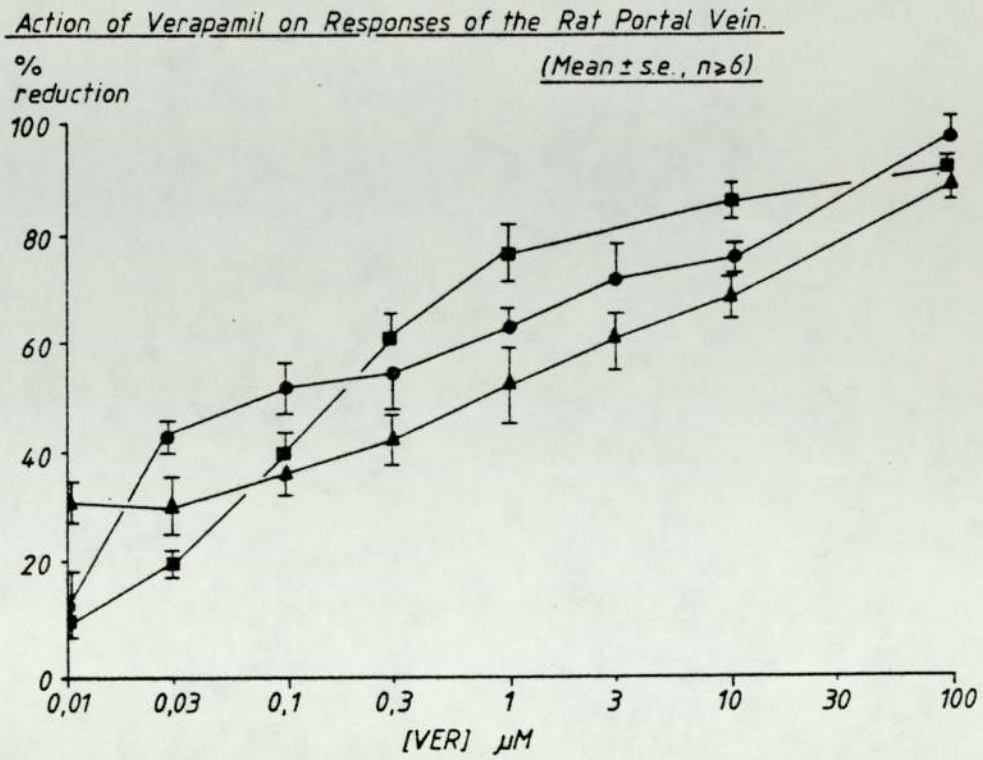



Fig. 25. Effect of verapamil on spontaneous activity ( ▲ ) and responses induced by NA (3 µM, ● ) and KCl (60 mM, ■ ) in the rat portal vein. Verticle bars represent the s.e. mean.

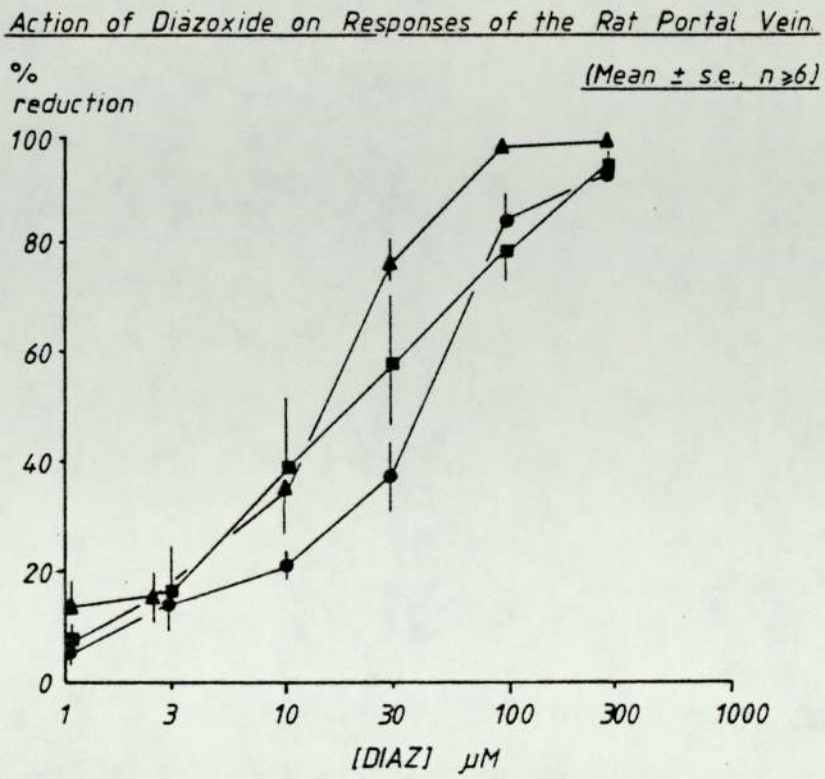



Fig. 26. Effect of diazoxide on spontaneous activity ( ▲ ) and responses induced by NA (3 µM, ● ) and KCl (60 mM, ■ ) in the rat portal vein. Verticle bars represent the s.e. mean.

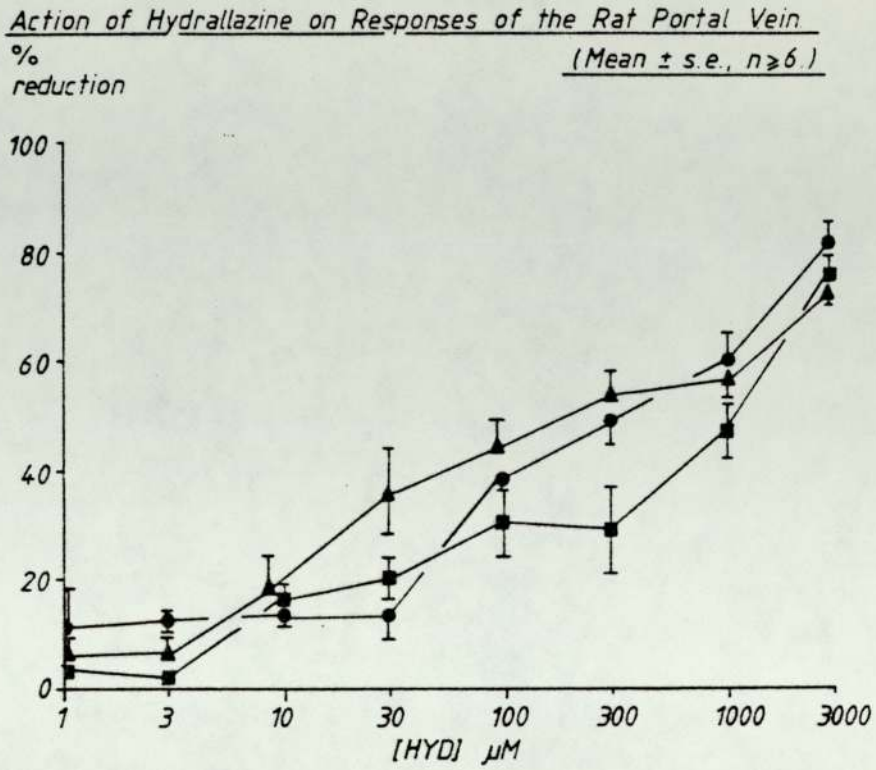



Fig. 27. Effect of hydrallazine on spontaneous activity (▲) and responses induced by NA (3 µM, ●) and KCl (60 mM, ■) in the rat portal vein. Verticle bars represent the s.e. mean.

In addition to the activity of these vasodilators, the action of dopamine on the portal vein was investigated. It has been shown (Armstrong, *et al.*, 1982) that dopamine has some vasodilator activity in peripheral vasculature and it was therefore considered worthwhile to investigate any similar activity in the portal vein. As can be seen in figure 28 a concentration dependent contraction was the response of the portal vein to the addition of dopamine. The maximum response of the preparation to dopamine was 0.33g +/- 0.04g (n=6) whereas the maximum response to NA was 1.6g +/- 0.18g (n=12) therefore dopamine caused only 20.6% of the maximum response to NA.

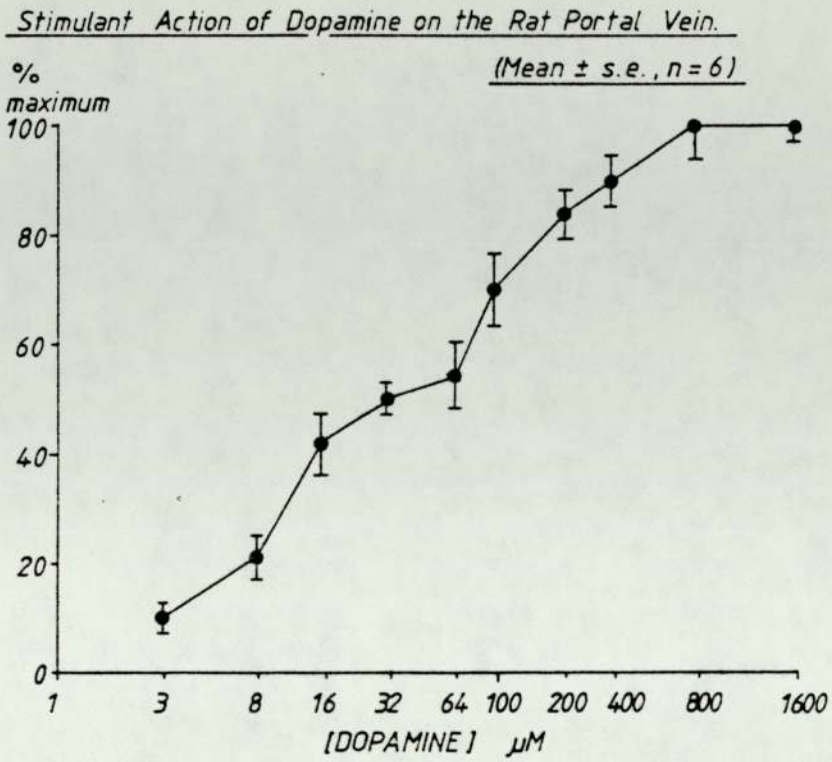



Fig. 28. Dose response relationship for dopamine, in the concentrations shown, on the rat portal vein. Verticle bars represent s.e. mean.

Classification of Vasodilators by their Actions on the Rat Portal Vein.

From the log concentration versus response relationships of the directly acting vasodilators a classification of the drugs by their spectra of action is possible. Initially the drugs tested have been separated into two broad groups, those which are equi-effective on the different types of contraction of the portal vein and those which show some selectivity of inhibition. The spectra of activity of each agent, its concentration range and the range over which it was selective can be seen in Table 2. From the information given in Table 2., it may be seen that ISO, NP, TOLM, and GTN all selectively inhibited spontaneous activity whereas VER, DIAZ, and HYD were non-selective. PAP was unusual in that it does not appear to fall into either category.

Since PAP showed some evidence of a stimulant action on the portal vein (FIG. 19a.) it may be argued that in terms of its vasodilator activity PAP should be classed as non-selective and therefore grouped together with VER, DIAZ, and HYD since it might be expected that if this stimulant activity were absent, then its depressant action on spontaneous activity would have been greater. This argument may only partially explain the action of PAP as its apparent 'selectivity' of action was seen at low concentrations (0.1 -10μM) While the stimulant actions of PAP were only seen at

the higher end of this concentration range.

| DRUG | Spectrum of Action. | Concentration Range for Selectivity of Action (μM) | Overall Concentration Range (μM) |
|---|---|---|---|
| ISOP | SA > NA ≒ ELEC ≒ KCl | 0,01 - 0,3 | 0,001 - 0,3 |
| PAP | NA ≒ ELEC ≒ KCl > SA | 0,1 - 10 | 0,1 - 100 |
| NP | SA ≫ NA ≒ ELEC ≫ KCl | 1 - 100 | 0,01 - 100 |
| TOLM | SA > NA ≒ ELEC > KCl | 30 - 1000 | 0,1 - 1000 |
| GTN | SA > NA ≒ ELEC > KCl | 0,3 - 3 | 0,1 - 30 |
| VER | NON-SELECTIVE | — | 0,01 - 100 |
| DIAZ | NON-SELECTIVE | — | 1 - 300 |
| HYD | NON-SELECTIVE | — | 1 - 3000 |

Table 2. The spectra of activity, dose range and selectivity of inhibition of the vasodilators tested in the rat portal vein. Spopntaneous activity (SA); noradrenaline (NA); electrical field stimulation (ELEC) and potassium chloride (KCl)

## Action of Selected Vasodilators on Responses of the Rat Aorta.

### Introduction.

The actions of NP, GTN, VER and HYD on induced tonic and phasic activity of circular preparations of the rat thoracic aorta were investigated. These agents were chosen as representatives of drugs having different spectra of activity on the rat portal vein as outlined previously. NP and GTN showed selective inhibition of spontaneous activity in the portal vein while VER and HYD were not selective in that preparation. These experiments were performed in order to determine whether agents which were selective in inhibiting spontaneous activity of the portal vein would exhibit a similar selectivity for the induced phasic activity of the aorta. Differences in potency between venous and arterial muscle were also expected to be determined from the results of these experiments by comparison with the portal vein.

### Results and Discussion.

Representative traces of the action of VER and NP on induced phasic activity of the aorta can be seen in FIG. 29. This figure shows, in both cases, a concentration dependent reduction in the phasic activity superimposed upon a fall in the basal tone of the preparations. Figures 30 and 31 show the actions of VER and NP respectively on tonic contractions of the aorta induced by 1μM NA. Similar results were

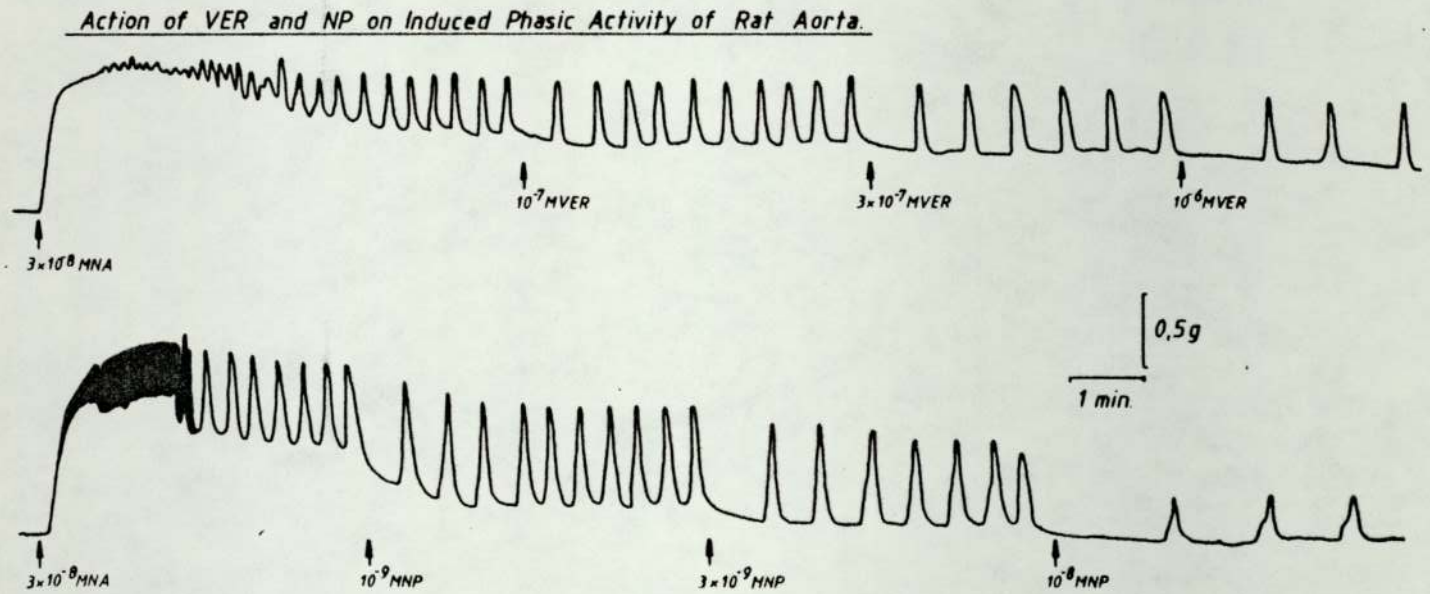



Fig. 29. Representative traces showing the action of VER (upper trace) and NP (lower trace) on phasic activity induced in the rat aorta by noradrenaline (0.03 μM) in the presence of EDTA (10 μM).

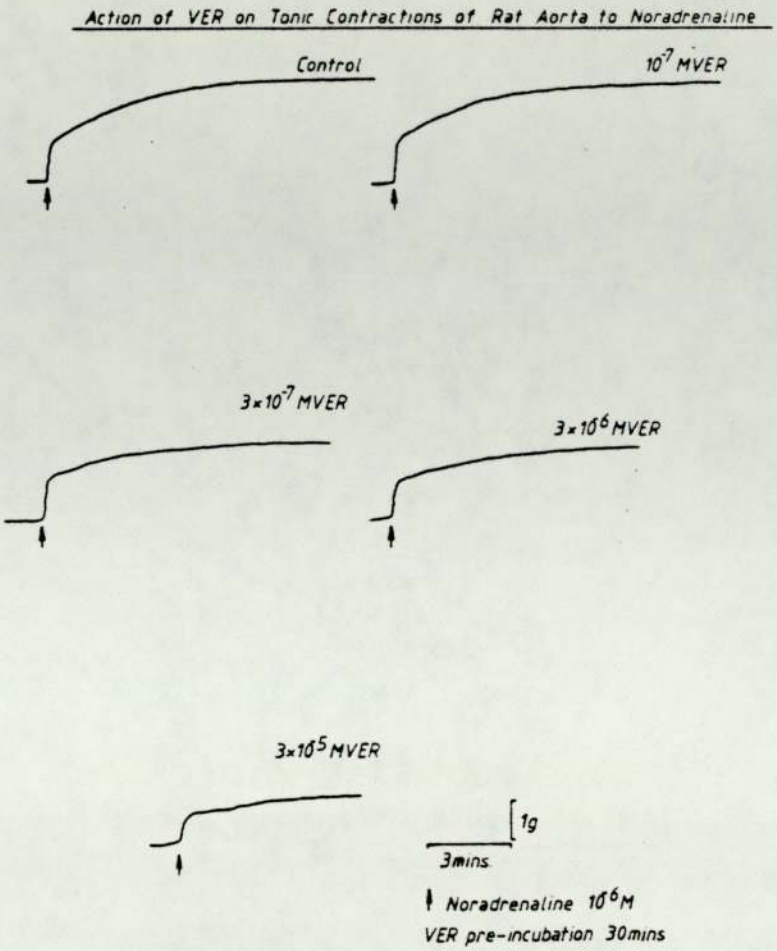



Fig. 30. Representative traces showing the action of VER on tonic contractions of the rat aorta induced by noradrenaline (1 μM). VER was pre-incubated for 30 minutes prior to the addition of noradrenaline.

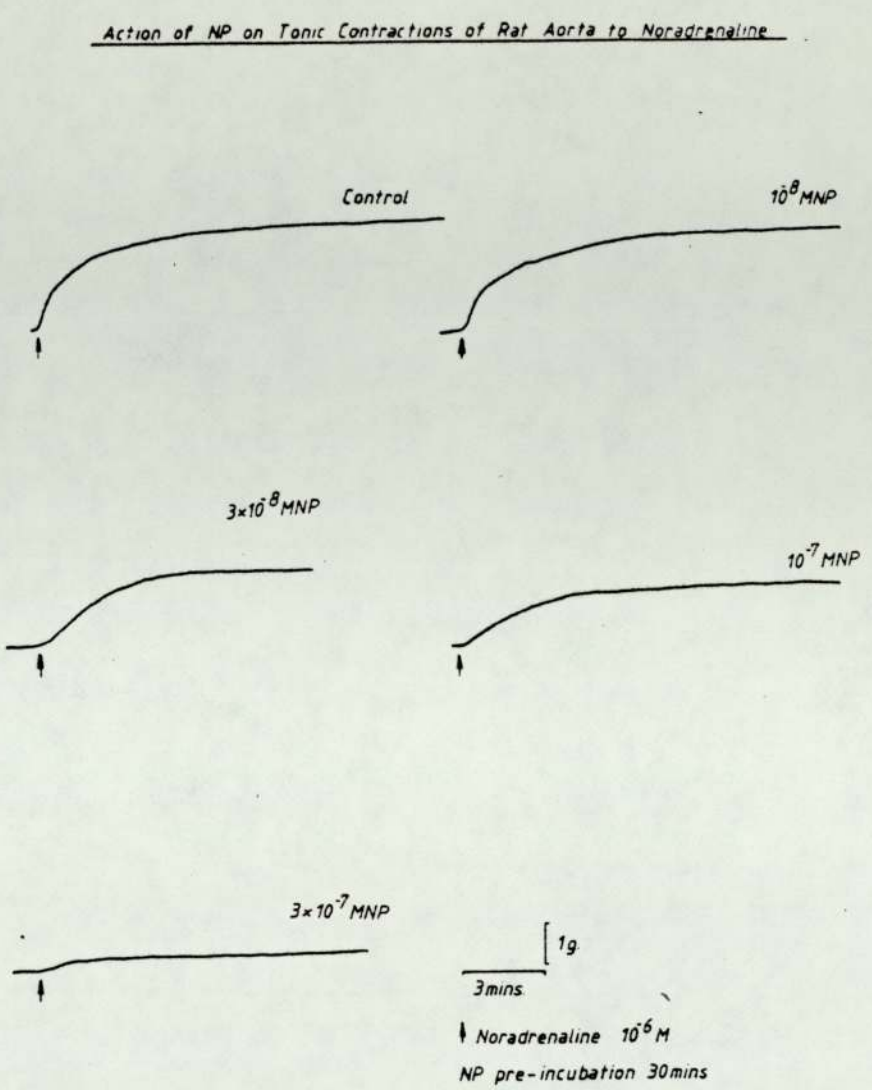



Fig. 31. Representative traces showing the action of NP on tonic contractions of the rat aorta induced by noradrenaline (1 μM). NP was pre-incubated for 30 minutes prior to the addition of noradrenaline.

obtained by the addition of GTN or HYD. Control responses for each tissue to the test concentration of 1uM NA were elicited and this response was repeated until it was constant. The tissue was pre-incubated for 30 minutes with the concentration of vasodilator being tested before the readdition of 1μM NA in the presence of the vasodilator. Similar experiments were carried out using KCl (60mM) to induce tonic contractions.

The effect of NP at different concentrations on induced tonic and phasic activity of aortic preparations can be seen in FIG. 32. This figure shows that NP was most effective in inhibiting induced phasic activity and caused a significantly greater ($p<0.01$, $n=6$) reduction when compared to NA or KCl responses throughout the concentration range used. In turn NP inhibited NA responses to a greater extent than KCl responses ($p<0.05$, $n=6$) at concentrations in excess of 0.03μM NP. It can also be seen from FIG. 32. that raising the [NP] above 0.1μM did not significantly increase the degree of inhibition of responses to KCl. Figure 33A shows the log concentration response relationship for NP, GTN, VER and HYD on tonic contractions of aortae induced by 1μM NA. Since NP was unique amongst the vasodilators tested in the aortic preparation in that it showed a differential inhibition of NA and KCl responses (all of the other agents tested inhibited tonic responses to NA and KCl equally) the action of dilators on KCl response is omitted from FIG. 33A. Figure 33B shows the action of these selected vasodilators

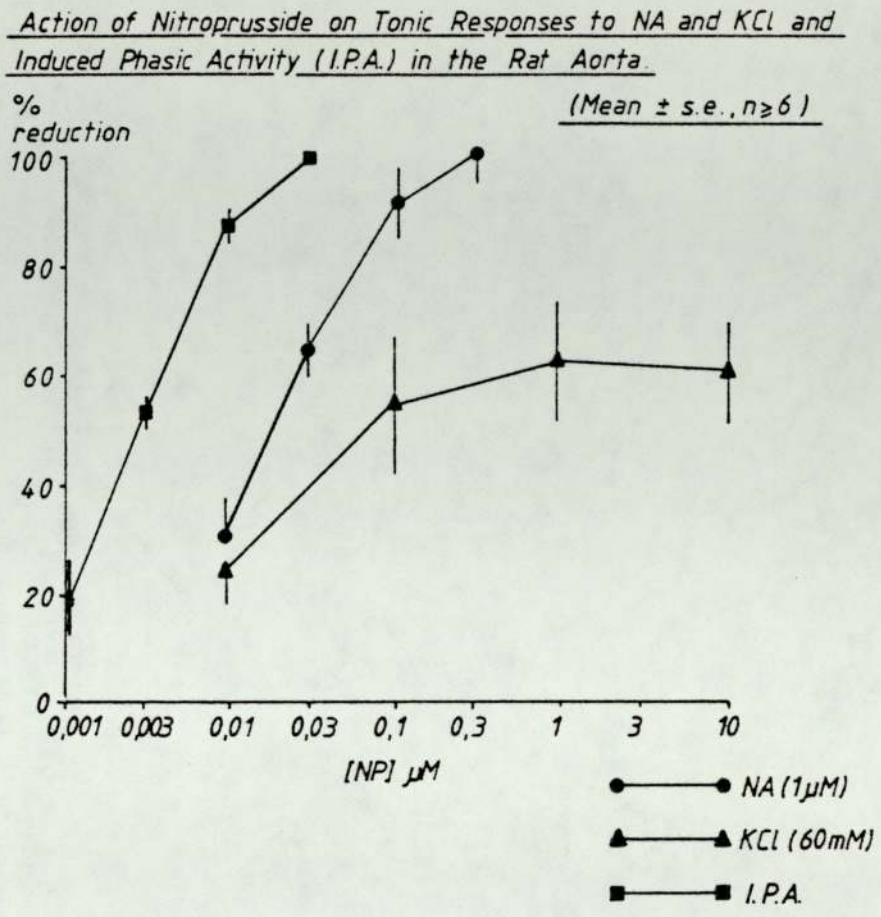



Fig. 32. Effect of NP on phasic activity induced in the aorta by noradrenaline (0.03 uM) in the presence of EDTA (10 uM) (IPA, ■ ) and tonic contractions of the aorta induced by noradrenaline, 1 µM NA ( ● ) and KCl, 60 mM ( ▲ ). Verticle bars represent s.e. mean.

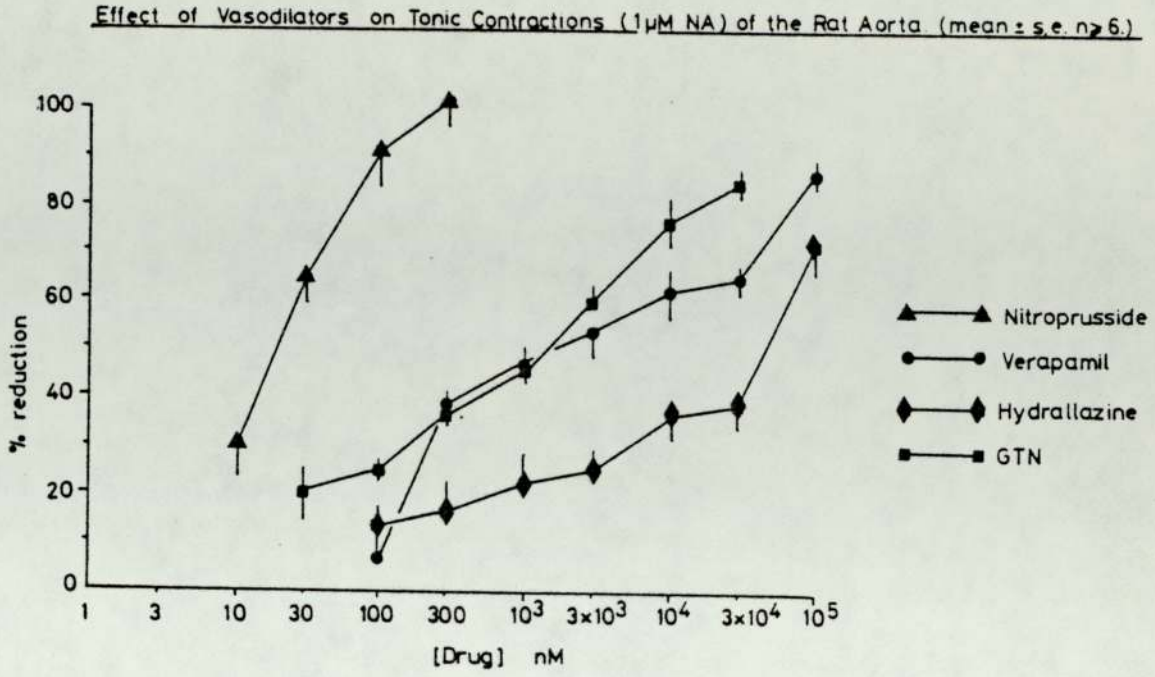



Fig. 33a. Effects of NP (▲), VER (●), HYD (♦) and GTN (■) on tonic contractions of the rat aorta induced by noradrenaline (1 uM). Verticle bars represent s.e. mean.

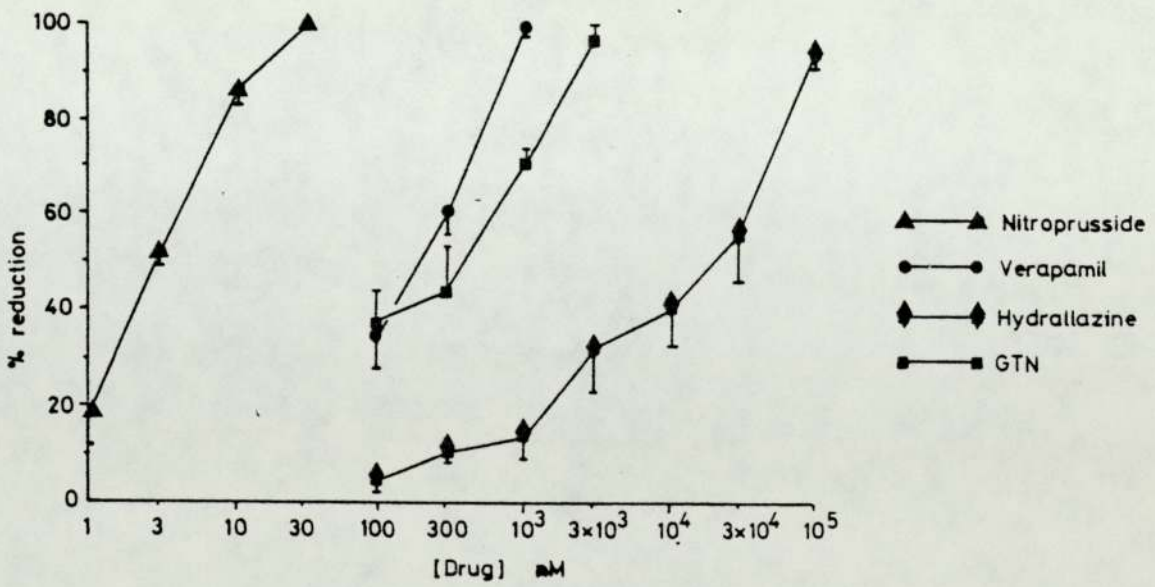



Fig. 33b. Effects of NP (▲), VER (●), HYD (♦) and GTN (■) on phasic contractions of the rat aorta induced by noradrenaline (0.03 uM) in the presence of EDTA (10 uM). Verticle bars represent s.e. mean.

on induced phasic activity of aorta. To allow easy comparison of the action of each agent $IC_{50}$ values have been calculated and are shown in Table 3.

As can be seen from Table 3. the rank order of potency for these agents on the aorta was NP>>VER=GTN>>HYD irrespective of which parameter of reactivity was used for comparison. Table 3 also shows the greater sensitivity to inhibition of induced phasic activity compared to tonic activity for all the vasodilators tested. The results presented here are further discussed in section 7.

| Drug / Activity | NP | GTN | VER | HYD |
|---|---|---|---|---|
| Induced Phasic Activity | 0,00423 ±0,0003 | 0,45 ± 0,15 | 0,237 ± 0,04 | 10,79 ± 6 |
| Induced Tonic Activity | 0,021 ±0,0018 | 2,41 ± 0,186 | 1,58 ± 0,32 | 57,8 ± 6,8 |

Table 3. $IC_{50}$ values (μM) for NP, GTN, VER and HYD on phasic and tonic activity of the rat aorta induced by 0.03 μM and 1 μM noradrenaline respectively.

The Use of Rat Portal Vein and Aorta to Determine Vasodilator Efficacy.

As has been previously discussed , in the introduction, the venodilator action of certain drugs can be of great importance in their clinical efficacy, e.g. GTN in angina (Parratt, 1975) and NP in heart failure (Cohn & Franciosa, 1977). Similarly the arteriolar-dilator properties of certain agents can be clinically significant, e,g, HYD in the treatment of mitral regurgitation (Greenburg,*et al.*; 1978). It is therefore of clinical interest to class vasodilators in terms of their relative dilator action on venous and arterial muscle. To this end the actions of the vasodilators on portal vein and aorta were compared and these results compared with published data for the same vasodilators in human vasculature.

Mackenzie and Parratt (1977) suggested the use of three vascular preparations, the dog femoral artery and saphenous vein and the rat portal vein as an initial screen to test prospective anti-anginal drugs for venodilator activity. This system would allow dilator potency on arterial and venous muscle to be compared as well as enabling any contrasting actions of agents on spontaneously active and quiescent venous muscle to be seen. The disadvantage of this protocol, particularly if it were used as a primary screening procedure, would be the great expense of using dogs.

Collier and co-workers (1978) and Robinson and collegues (1979) have studied the actions of a range of vasodilators on the responses of hand veins and forearm blood flow in volunteer subjects. In their experiments the activity of venodilators was assessed by measuring the degree of constriction of the dorsal hand veins, which had been distended by the application of a standard congesting pressure of 25mmHg to the upper arm. A butterfly needle was then inserted into the vessel under study to allow the infusion of dilator drugs and the constrictor agents (NA and KCl) necessary to produce a degree of tone from which the vessel could relax, The degree of constriction was measured by a lever system resting on the vessel. The actions of drugs on forearm arterial beds was investigated using venous occlusion plethysmography, drugs being administered intra-venously by slow infusion.

There are many problems inherent in this experimental procedure; the blood flow in the vessel under study was frequently interrupted by occlusion of the upper arm veins; the vessel was punctured thus causing damage and presumably release of endogenous substances, for example prostaglandins, which may affect the results. Although a delay between venepuncture and the start of the experiment was allowed this in no way guarantees that the action of any released substances will have ceased. Bearing in mind these possible sources of interference the results obtained using the dorsal hand vein gave the rank order of venodilator

potency of NP>GTN>VER>TOLM=DIAZ=HYD.

In order to compare the results obtained in this thesis with those of other workers it was necessary to determine rank order of potencies for the agents used. In receptor agonist-antagonist interactions this can be done by calculating log-dose ratios or log-dose shifts for each agent, however when directly acting agents are used this cannot be used as a method of comparing potency. It is possible in some cases to calculate $IC_{50}$ values, that is the concentration of substance causing 50% inhibition of a response. With the results obtained here a problem in calculating $IC_{50}$ values arrises in that certain agents do not cause 50% inhibitions of a given response (i.e. NP and TOLM on KCl induced contractions). Another difficulty encountered in the use of $IC_{50}$ values is the relatively 'flat' form of th log dose versus response relationships of many of the agents tested. This format of response produces a large spread of values when calculating the $IC_{50}$ of an agent which can call into doubt the accuracy and usefulness of these values when comparing relative potencies of agents.

A more direct method of comparing the relative potencies of the range of agents tested is by looking at the percentage inhibitions caused by a given concentration. This method has the virtue of simplicity and lacks the problem of the large errors encountered when calculating the $IC_{50}$ values for these agents. The one difficulty in comparing directly the degree of inhibition caused by a single

concentration of vasodilator is in the choice of dose. The directly acting agents are, by and large, active over approximately the same concentration range, however ISOP produced 100% inhibition of spontaneous activity at a concentration (0.1μM) at which most of the agents produced little or no action. As ISOP was included in this series mainly as a comparator for PAP it has been omitted from this part of the study.

For the reasons outlined above the calculation of $IC_{50}$ values was not initially used in the comparison of the effects of the dilator agents on the portal vein. A direct comparison of the degree of inhibition caused by a single concentration (10μM) was used to compare different drug potencies.

Portal Vein Results.

The actions of 10μM NP, GTN, VER, PAP, DIAZ, TOLM, and HYD on the parameters of portal vein reactivity can be seen in FIG. 34. The rank order of potency of the agents tested on portal vein is shown in Table 4. The rank orders were calculated at vasodilator concentrations of 10μM as shown in FIG. 34. When compared with the rank order of potency for these agents in the human hand vein preparation of Collier (NP>GTN>VER>TOLM=DIAZ=HYD) it can be seen that the best agreement on rank order between the human hand vein *in vivo* and the rat portal vein is in reduction of the spontaneous activity. Although the agreement between the hand vein results and the reduction in NA and electrically induced

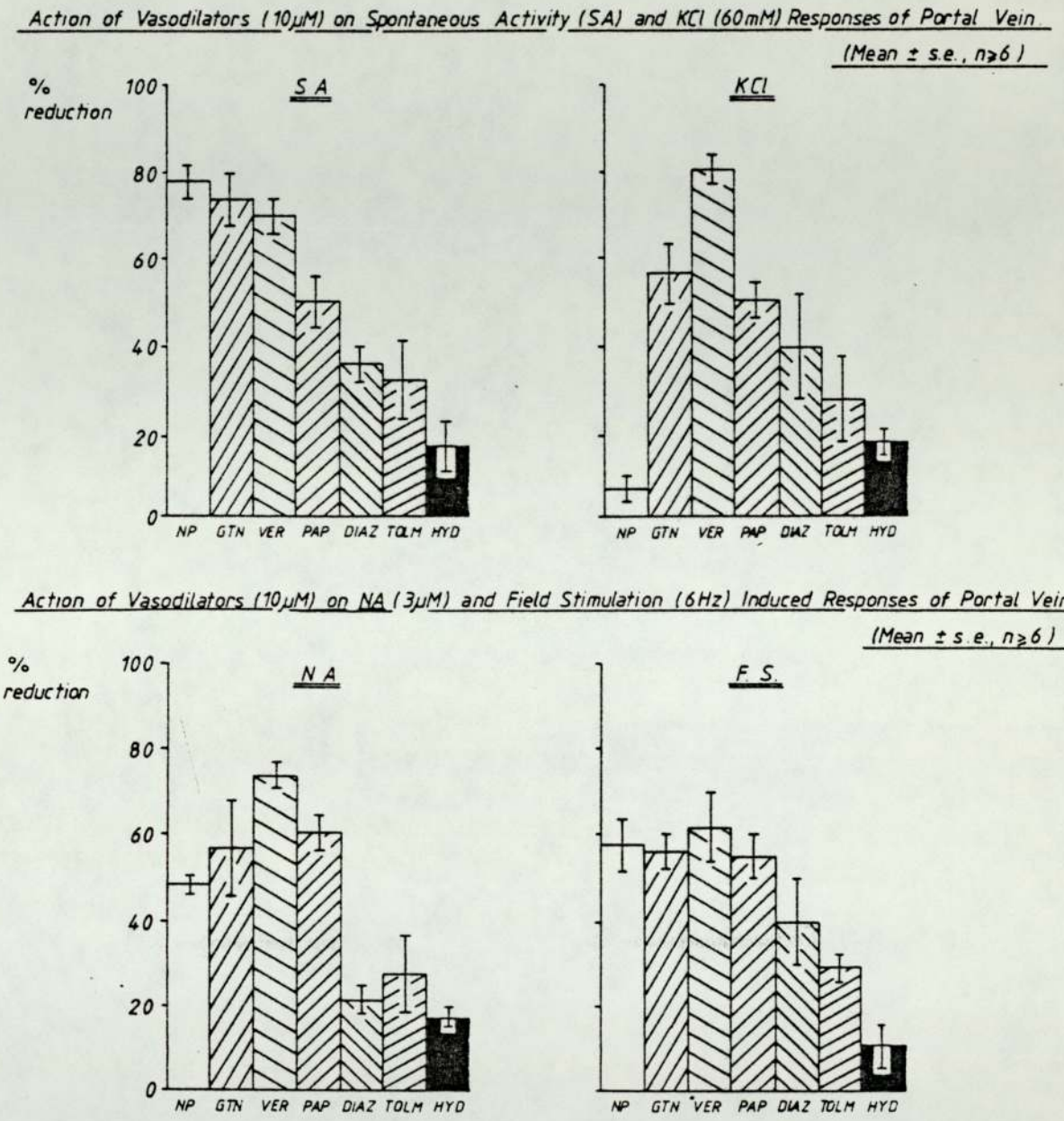



Fig. 34. Bar charts showing the effects of NP, GTN, VER, PAP, DIAZ, TOLM, and HYD (all at 10 μM) on spontaneous activity (SA) and contractions induced by KCl (60 mM), noradrenaline (NA, 3 μM) and electrical field stimulation (FS, 6 Hz).

| Parameter | Rank Order |
|---|---|
| SA | NP ≒ GTN ≒ VER ≫ DIAZ ≒ TOLM > HYD |
| NA | VER ≥ GTN ≒ NP > TOLM ≒ DIAZ ≒ HYD |
| ELEC | VER ≥ GTN ≒ NP > DIAZ ≒ TOLM > HYD |
| KCl | VER > GTN ≥ DIAZ ≒ TOLM ≥ HYD > NP |

Table 4. Rank order of potency for the vasodilators tested on the parameters of portal vein reactivity; spontaneous activity (SA), contractions induced by noradrenaline (NA, 3 μM), electrical stimulation (ELEC, 6 Hz) and KCl (60 mM).

reponses of the portal vein is less exact, the overall trend of VER, GTN and NP being more potent than the other agents tested is still shown. The refractoriness of KCl induced contractions to reduction by NP made that parameter a particularly poor indicator of dilator potency in hand veins.

When $IC_{50}$ values for NP, GTN, VER and HYD on the rat portal vein were calculated to allow comparison with results in aortic preparations (Table 5) the rank order of potency of action on spontaneous activity was GTN=NP>VER>>HYD, which was again in reasonable accord with the results of Collier *et al.* (1978). TOLM and DIAZ were omitted from this part of the study because their spectra of activity on the portal vein were similar to NP and VER respectively. The $IC_{50}$ values for vasodilator action on NA induced contractions of the portal vein gave the same rank order as that at the chosen concentration of 10μM (VER>GTN>NP>>HYD) however the differences in potency between the agents was more marked.

Aortic Preparation Results.

The results obtained when the action of vasodilators was investigated in aortic preparations are shown in section 6. These results are summarised in the form of $IC_{50}$ values in Table 5. These $IC_{50}$ values gave a rank order of potency of NP>>VER>GTN>>HYD irrespective of which parameter was used. When this order is compared with that calculated from Collier *et al.* (1978) and Robinson *et al.* (1979), ( GTN>=NP>=VER>HYD ) it can be seen that the degree of

| Parameter | Preparation | NP | GTN | VER | HYD |
|---|---|---|---|---|---|
| Spontaneous Activity | Portal Vein | 0,664 ± 0,13 | 0,287 ± 0,028 | 1,4 ± 0,3 | 329 ± 85 |
| Induced Phasic Activity | Aorta | 0,00423 ± 0,0003 | 0,45 ± 0,15 | 0,237 ± 0,04 | 10,79 ± 6 |
| NA Induced Tonic Contraction | Portal Vein | 62 ± 15 | 8,5 ± 3,3 | 0,09 ± 0,018 | 378 ± 130 |
| NA Induced Tonic Contraction | Aorta | 0,021 ± 0,0018 | 2,14 ± 0,186 | 1,58 ± 0,32 | 57,8 ± 6,8 |

Table 5. $IC_{50}$ values (μM) calculated for NP, GTN, VER and HYD on spontaneous activity and noradrenaline (3 μM) induced tonic activity of the rat portal vein and phasic and tonic activity of the rat aorta induced by 0.03 μM and 1 μM noradrenaline respectively.

agreement is poor and that the rat aorta appears especially sensitive to NP compared with human forearm arterioles.

Collier and co-workers have used their results from hand veins and forearm arteriolar beds to indicate whether a given agent is arterio- or veno-selective in man. A similar process can be carried out for the agents tested on the rat aorta and portal vein. The ratio of $IC_{50}$ values in portal vein to $IC_{50}$ values in aorta was used to give an indication of selectivity. The $IC_{50}$ values used in these calculations (Table 5) and the $IC_{50}$ vein/$IC_{50}$ artery ratios for both phasic (spontaneous activity of vein and induced phasic activity of artery) and tonic (NA) contractions are shown in Table 6.

From the data presented in Table 6 it could be infered that both HYD and NP are arterioselective. Whereas VER's selectivity was dependent upon the nature of the response inhibited (phasic or tonic). GTN appears, from the results shown here, to be approximately equi-effective on aorta and portal vein. These results are at considerable variance with those published for human vascular preparations (Robinson, *et al.*, 1979; Collier, *et al.*, 1978) which showed GTN and NP to be venoselective whereas HYD and VER were arterioselective. The differences between the apparent arterio-venous selectivity calculated here and the published human data may reflect the high sensitivity of the rat aortic preparations to vasodilators.

| Parameter | NP | VER | GTN | HYD |
|---|---|---|---|---|
| Tonic | $3,38 \times 10^{-4}$ | 17,55 | 0,25 | 0,15 |
| Phasic | $6,37 \times 10^{-3}$ | 0,169 | 1,61 | 0,031 |

Table 6. Arterio-venous comparison of $IC_{50}$values expressed as $IC_{50}$artery/$IC_{50}$vein. Tonic parameters are contractions induced by npradrenaline (1 µM in aorta and 3 µM in portal vein) and phasic activity is spontaneous activity of the portal vein and induced (0.03 µM noradrenaline) phasic activity of the aorta.

Summary.

From the results presented here it can be seen that the action of vasodilators on portal vein spontaneous activity most closely resembles the action of these agents in human hand veins. Thus if any portal vein responses were to be included in an initial screening procedure for vasodilator or anti-anginal activity (as suggested by Mackenzie and Parratt, 1977) then, from these results, spontaneous activity would appear to be the best choice. The results obtained for the action of vasodilators on rat aortic preparations tend to suggest that this rat vascular muscle would be of little use in predicting *in vivo* effects of vasodilators in humans, assuming that the observations of Collier *et al.*(1978) and Robinson *et al.* (1979) accurately reflect the overall haemodynamic profile of these agents in man.

## The Aorta and Portal Vein of the Rat and the Golenhofen 'P' and 'T' System for Smooth Muscle Classification.

As previously discussed (see Introduction), Golenhofen (1976 a & b) and co-workers (Golenhofen & Neuser, 1974) have suggested the presence of two calcium activation systems in smooth muscle. The first a so called 'P-system' which is preferentially used for producing phasic activity and is sensitive to inhibition by calcium entry blockers, and secondly a 'T-system' which is preferentially used for producing slow (tonic) contractions and is sensitive to inhibition by NP. When the action of NP on the portal vein was investigated during this study it was found, paradoxically, that NP was a more potent inhibitor of phasic (spontaneous) activity than of tonic contractions, consequently it was decided to extend the investigation to include the action of VER and NP on aortic contractions (both phasic and tonic).

From the results presented in sections 4 and 5 and summarised in the form of $IC_{50}$ values in table 5 it is possible to investigate how the hypothesis of Golenhofen related to the responses of the rat portal vein and aorta. For convenience the $IC_{50}$ values for NP and VER, extracted from Table 5, are reproduced in Table 7. The phasic and tonic responses of the aorta were induced by 0.03μM and 1μM NA respectively as outlined earlier. Tonic contractions of the portal vein were induced with 3μM NA, spontaneous

| Drug | Portal Vein | | Aorta | |
|---|---|---|---|---|
| | Phasic | Tonic | Phasic | Tonic |
| NP | 0,66 ± 0,13 | 62 ± 1,5 | 0,004 ± 0,0003 | 0,21 ± 0,002 |
| VER | 1,4 ± 0,3 | 0,09 ± 0,02 | 0,24 ± 0,04 | 1,58 ± 0,32 |

Table 7. $IC_{50}$ values (μM; mean +/- s.e., n = 6) for NP and VER on phasic (spontaneous activity) and tonic (noradrenaline induced activity, 3 μM) of the rat portal vein and phasic and tonic activity of the rat aorta induced by noradrenaline (0.03 and 1 μM respectively).

myogenic contractions of the portal vein were taken as phasic responses.

The results shown in Table 7 demonstrate that the aorta was more sensitive to the inhibitory effects of NP than was the portal vein by a factor of 165 for phasic activity and 2952 for tonic activity. It was also noted that in both preparations the phasic activity was more sensitive to NP than were the tonic contractions. In contrast to NP, VER showed no clear pattern of activity between the two preparations. On the other hand, by comparing the effect of NP and VER on tonic contractions of the two preparations it may be seen that the vein was the most sensitive to VER, whereas the aorta was the most sensitive to NP when sensitivity was expressed as a ratio of the $IC_{50}$ values for NP/VER. The NP/VER ratios being 689 and 0.013 respectively for vein and artery. Karaki, *et al.*, (1984) has also found that in spiral strip preparations of rat aorta NP was a more potent inhibitor of NA induced tonic contractions than was VER. The NP/VER $IC_{50}$ ratio calculated from the results of Karaki and co-workers (1984) is 0.0019 for contractions induced in rat aorta by 0.1μM NA. Although qualitatively similar, the quantitative difference between the values calculated above and those derived from Karaki's published results may reflect a difference between spiral and circular preparations of aortic muscle and the lower stimulant dose of NA used.

Discussion.

From the results shown above it is possible that the inhibitory effect of these agents on the tissues used was determined more by the type of tissue than the type of mechanical activity (phasic or tonic). In the rat aorta and portal vein the hypothesis of Golenhofen (1976 a & b) may have some merit in that the predominant activity seen in these vessels were tonic and phasic respectively and the supposed selectivity of NP and VER may be dependent upon the calcium activation systems responsible for the predominant activity. Thus the usefulness of classifying tissues into 'P' and 'T' types would be dependent upon the accuracy with which a tissue could be defined as having a predominantly phasic or tonic mode of activity.

## Simultaneous Electrical and Mechanical Recording of the Responses of the Rat Portal Vein.

### Introduction.

Funaki and Bohr (1964) and Funaki (1966) have shown that the spontaneous phasic contractions of the rat portal vein are associated with action potentials which may be simultaneously recorded from the preparation. Low concentrations of NA (0.001-0.1μM) have been shown to increase the duration of bursts of these action potentials and decrease the intervals between each burst (Johansson *et al.*, 1967). In the same study, high concentrations of NA (10μM), produced a depolarization and decreased or abolished action potential spikes. These electrical events were associated with a sustained contraction. Johansson *et al.* (1967) also found in the rat portal vein, that such electrical activity was dependent upon $[Ca^{2+}]_o$.

Jetley and Weston (1980) have shown that the calcium entry blocker nifedipine inhibited mechanical responses of the portal vein with little effect on the electrical activity, thus nifedipine was presumably acting as an electro-mechanical uncoupler. Jetley & Weston, (1980) also showed that D600, another $Ca^{2+}$ entry blocker, reduced both mechanical and electrical activity. Paradoxically, in the light of results presented earlier in this thesis for mechanical responses alone (See Section 4, FIGS.19b & 22), these workers found NP was inactive in reducing either

spontaneous mechanical or electrical activity of the rat portal vein.Since NP was considered by Golenhofen (1977)to be a selective inhibitor of tonic tonic responses it was considered worthwhile to determine whether NP was able to inhibit the electrical basis of the phasic activity (action potentials) in this preparation. VER and DIAZ were also included in this study.

A simultaneous electrical and mechanical recording technique similar to that used by Jetley and Weston (1980). Extracellular platinum ring electrodes mounted in a glass capillary organ bath were used to record the electrical activity of portal vein preparations superfused with Krebs' solution (See Methods Section for details).

Results.

Representative traces showing simultaneous electrical and mechanical recordings from portal vein preparations can be seen in FIG.35. This figure shows that bursts of electrical activity were associated with each spontaneous contraction and with contractions induced by low concentrations of KCl (15mM and 30mM). A high $[K^+]$ (60mM) induced a large tonic contraction which had a burst of electrical spike activity associated with the initial rising phase of contraction, during the subsequent sustained contraction no electrical activity was recorded. Similar results were obtained with NA and a representative response of the portal vein to 10μM NA is also shown in FIG.35.

Action of NA and KCl on Mechanical and Electrical Activity of the Rat Portal Vein.

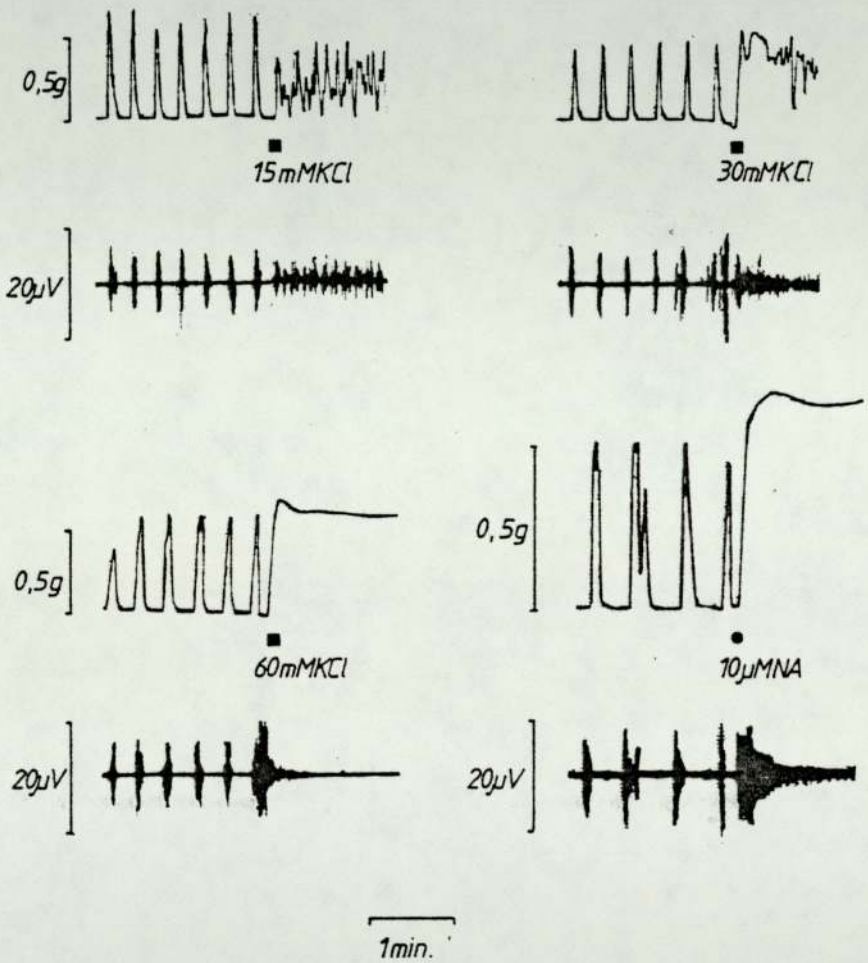



FIG. 35. Representative traces showing the actions of noradrenaline and KCl on mechanical (upper records) and electrical (lower records) activity of the rat isolated portal vein.

The effect of the removal of $[Ca^{2+}]_o$ or the addition of VER (1μM) on electrical and mechanical responses of the portal vein can be seen in FIG.36. When the tissue was superfused with calcium free Krebs' solution all mechanical and electrical activity ceased within 8 to 10 minutes. Similar results were obtained with the addition of VER, although there was no period of increased excitability with VER as was exhibited during the initial period of superfusion with $Ca^{2+}$ free solution. The action of NP (10μM) and DIAZ (100μM) on the spontaneous phasic contractions and their associated electrical activity can be seen in FIG.37. As can be seen from this figure (and from FIG. 36.) none of the agents tested appeared to cause uncoupling.

Discussion.

The results presented here for the action of NA and KCl on electrical activity of the portal vein are in accord with earlier published data (Johansson, *et al.*, 1967) obtained using a different electrical recording technique ( the 'sucrose gap' method). The inhibitory effect of $Ca^{2+}$ removal also agreed with the results of Johansson (1967) and Funaki (1964) who both found that the electrical activity of the portal vein was dependent upon $[Ca^{2+}]_o$. The increase in electrical and mechanical activity associated with the initial phase of the response to calcium removal could be due to a membrane effect of altered concentrations of

*Action of VER and $Ca^{++}$ Removal on Spontaneous Mechanical and Electrical Responses of the Rat Portal Vein.*

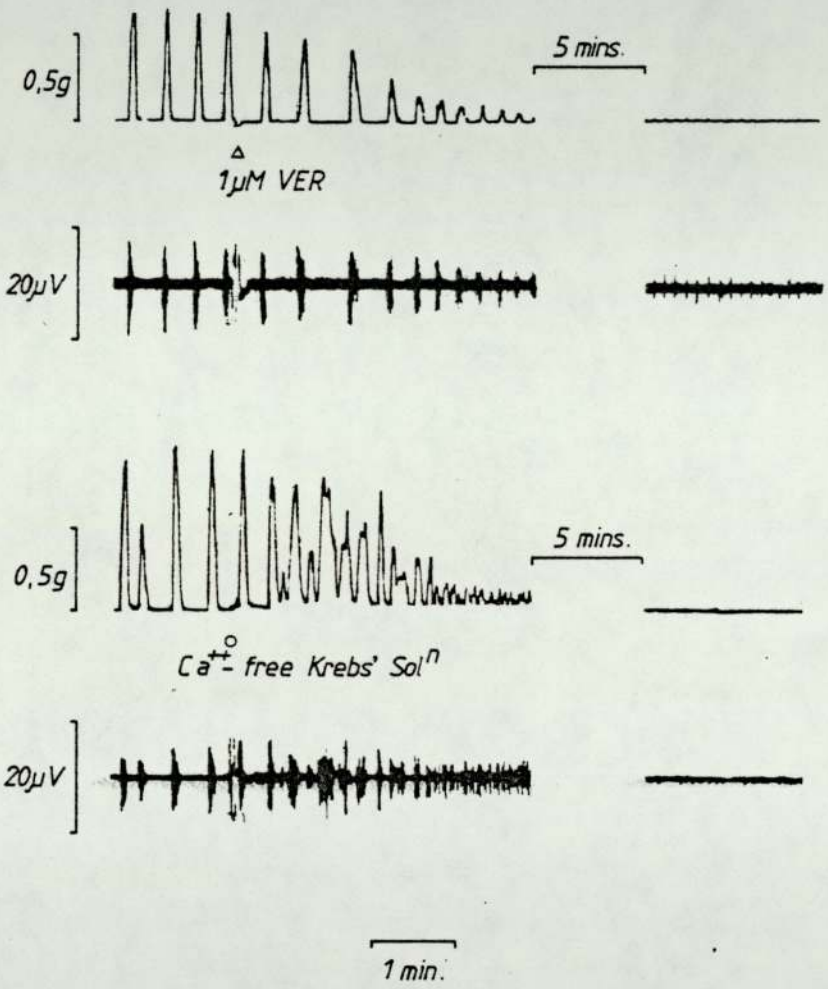



FIG. 36. Representative traces showing the actions of VER (1 uM) and $Ca^{2+}$ removal on spontaneous mechanical (upper records) and electrical (lower records) activity of the rat isolated portal vein. To acheive $Ca^{2+}$ removal the Krebs' solution superfusing the vessel was changed for one which had no added $CaCl_2$.

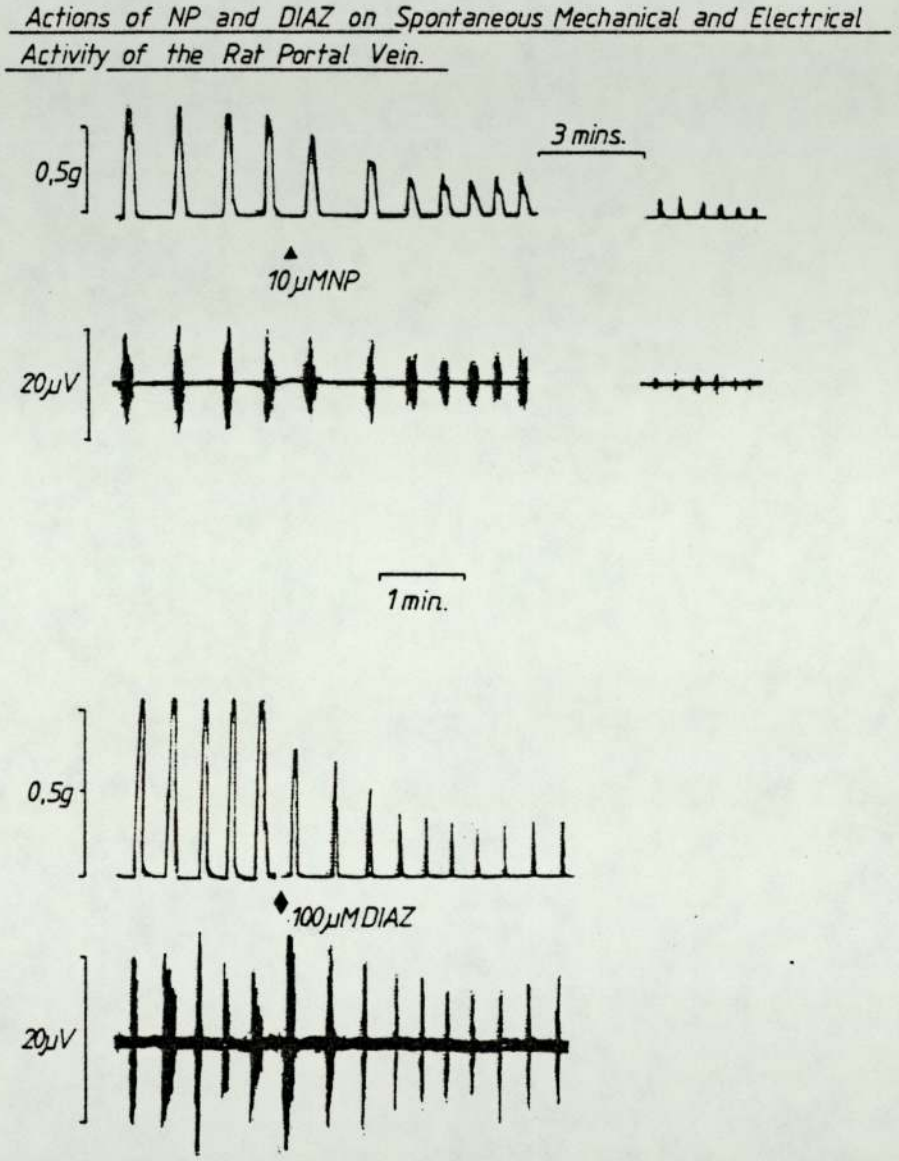



FIG. 37. Representative traces showing the actions of NP and DIAZ on spontaneous mechanical (upper records) and electrical (lower records) activity of the rat isolated portal vein.

calcium changing calcium fluxes over a short period of time. However, no conclusions can be drawn as to the cause of the initial stimulatory action $Ca^{2+}$ removal. The inhibitory action of VER on both electrical and mechanical responses of the portal vein was expected in the light of the known $Ca^{2+}$ entry blocking activity of VER and the $[Ca^{2+}]_o$ dependence of the portal vein.

As it was not possible to quantify the electrical responses of the portal vein in this study the only conclusion that can be drawn on the actions of NP and DIAZ on this system was that no electro-mechanical uncoupling appeared at the concentrations tested.

Since only one preparation at a time could be investigated using this technique, the aquisition of data was slow, consequently the simultaneous recording of mechanical and electrical activity has been performed when it was considered necessary. Helical strips of aortae induced to produce phasic activity were investigated in the same bath but no sign of electrical spike activity could be seen. This was presumably because the phasic activity of the aorta is thought to be associated with 'slow wave' depolarizations (Biamino & Thron, 1969) which would be attenuated by the amplifier used in these studies.

## Actions of DIAZ, NP and VER on the Time Course of Portal Vein Responses to Varying Concentrations of KCl and NA.

### Introduction.

It has been suggested that in certain smooth muscle preparations, e.g. guinea-pig taenia coli (Bulbring & Tomita, 1970) and rat uterus (Kao & McCullough, 1975) a major source of activator calcium is calcium entry into the cell during the upstroke of action potentials. If $Ca^{2+}$ entry during action potential spiking is a major source of activator $Ca^{2+}$ then agents which block action potential linked $Ca^{2+}$ entry may have a preferential inhibitory effect on contractions, or parts of contractions, associated with action potentials. Results presented in this study showed that electrical activity of the portal vein (as measured by extracellular electrodes) is dependent upon $[Ca^{2+}]_o$. Stimulation of the portal vein by high concentrations of KCl or NA caused a contraction during which the associated electrical spike activity waned after approximately 20 seconds (bottom panel FIG. 35.). In contrast, low concentrations of NA or KCl produced contractions during which electrical spike activity continued throughout the period of stimulation.

If the above hypothesis of selectivity of $Ca^{2+}$ entry blocker activity is correct, then such agents would be expected to cause a preferential inhibition of the initial phase of contraction of the portal vein induced by high

[NA] or [KCl] which was associated with electrical spike activity. No preferential inhibition of the initial phase of contraction would be expected of responses induced by low concentrations of NA or KCl which exhibited electrical activity throughout their time course. It was therefore considered germane to investigate the inhibitory action of agents on the time course of portal vein response to high and low concentrations of NA and KCl. VER was used in these experiments because of its known action as a $Ca^{2+}$ entry blocker which inhibited electrical spike activity in the rat portal vein. DIAZ was included in this part of the study as it has been shown to inhibit the initial phase of contractions in intestinal smooth muscle (Golenhofen, *et al.*, 1977; Golenhofen & Weston, 1976). The action of NP was also investigated in an attempt to determine if it possessed any specificity of action on action potential associated contractions. A period of 20 seconds was chosen to represent the initial phase of the response. Results were calculated as percentage inhibition of control responses elicited in the absence of the vasodilator for the first 20 seconds of contraction and for the period from 21 to 60 seconds during the remainder of the response.

Results.

The action of DIAZ on the time course of inhibition of KCl induced responses of the portal vein can be seen in FIG. 38. As can be seen from FIG. 38., Diaz caused a significantly greater ($p<0.01$, $n>7$) inhibition of the initial 20 second response than the 21 to 60 second response

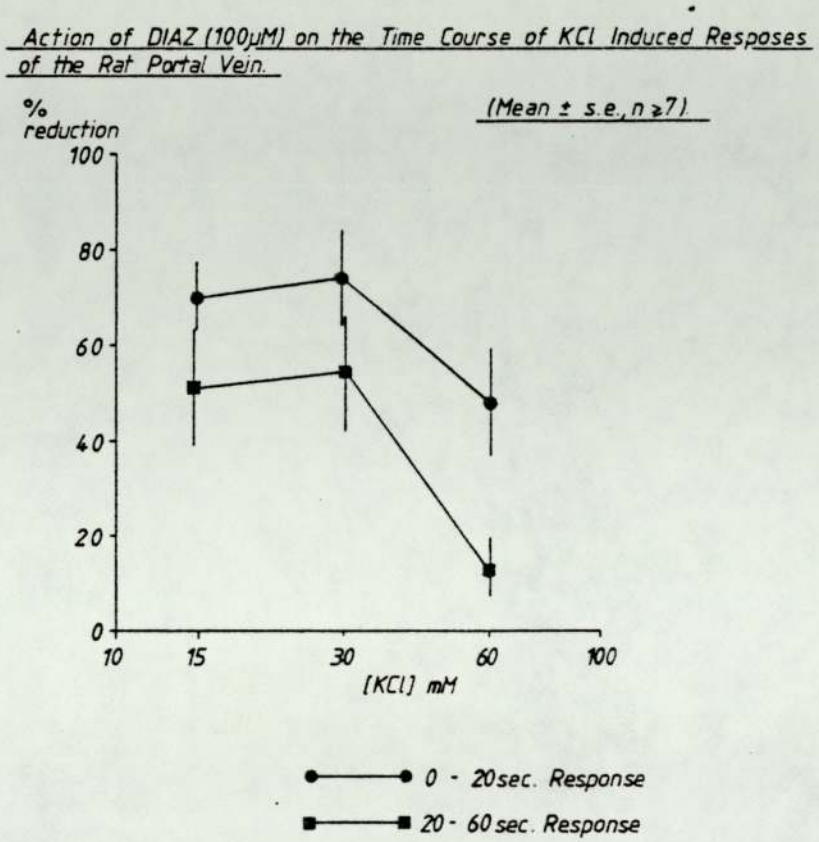



FIG. 38. Effects of DIAZ (100 μM) on the initial (0–20 sec. response, ● ) and secondary (21–60 sec. response ■ ) responses of the rat isolated portal vein stimulated with KCl. Verticle bars represent the s.e. mean.

at a [KCl] of 60mM. When contractions were induced by 15 or 30mM KCl there was no significant difference between the degree of inhibition caused by DIAZ on the initial and secondary phases of contraction. Similar results were obtained when the actions of DIAZ on the time course of NA induced responses was investigated (FIG. 39.). At a [NA] of 3μM or above the initial (20 second) response was reduced to a significantly greater extent ($p<0.01$, $n=6$) than the secondary (21 to 60 second) phase of contraction.

In the light of the results obtained with DIAZ the actions of VER and NP were investigated on only two concentrations of KCl (15mM and 60mM) and NA (0.1μM and 10μM). These concentrations were chosen to represent the 'low' and 'high' stimulant concentrations discussed above. The actions of NP and VER on the initial and secondary phases of contractions induced by 'high' and 'low' concentrations of KCl and NA can be seen in FIG. 40. As can be seen from this figure neither VER or NP exhibited any selectivity of action on initial or secondary phases of contractions induced by 'low' concentrations of NA or KCl (Fig. 40. left panel).

In preparations stimulated with 'high'concentrations of KCl or NA, VER inhibited the initial response (0-20 seconds) to a significantly greater extent ($p<0.01$, $n=6$) than the secondary response. In contrast to the results obtained for VER, NP showed no selectivity of action on initial or secondary phases of responses induced by high concentrations

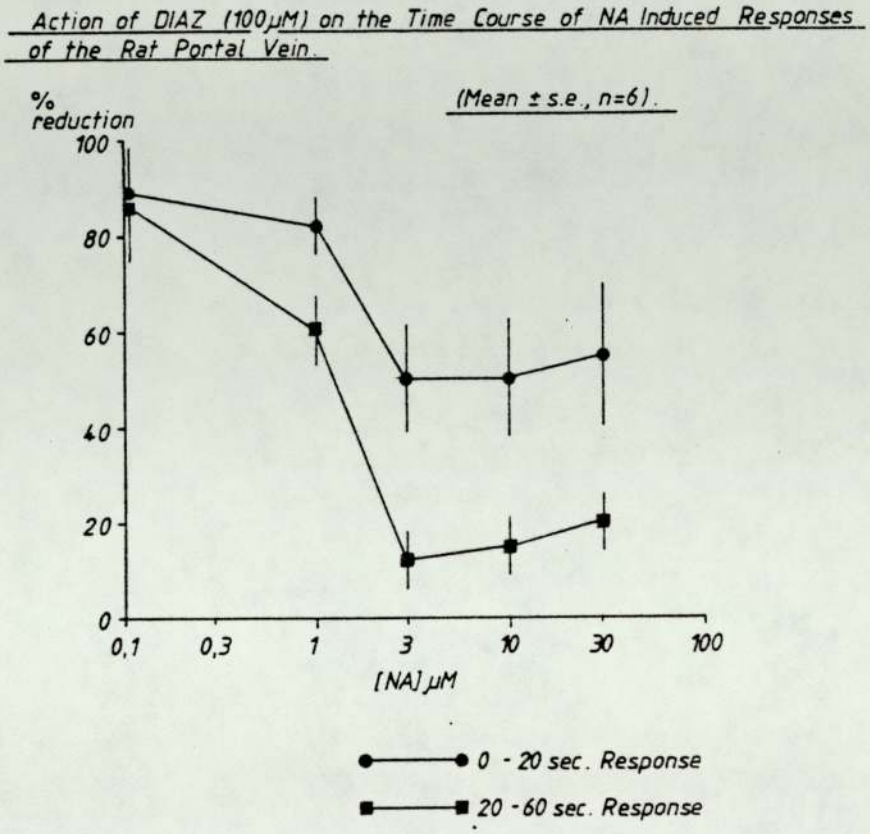



FIG. 39. Effects of DIAZ (100 μM) on the initial (0–20 sec. response, ● ) and secondary (21–60 sec. response ■ ) responses of the rat isolated portal vein stimulated with noradrenaline. Verticle bars represent the s.e. mean.

*Action of VER and NP on the Time Course of NA and KCl Induced Responses of the Rat Portal Vein.*

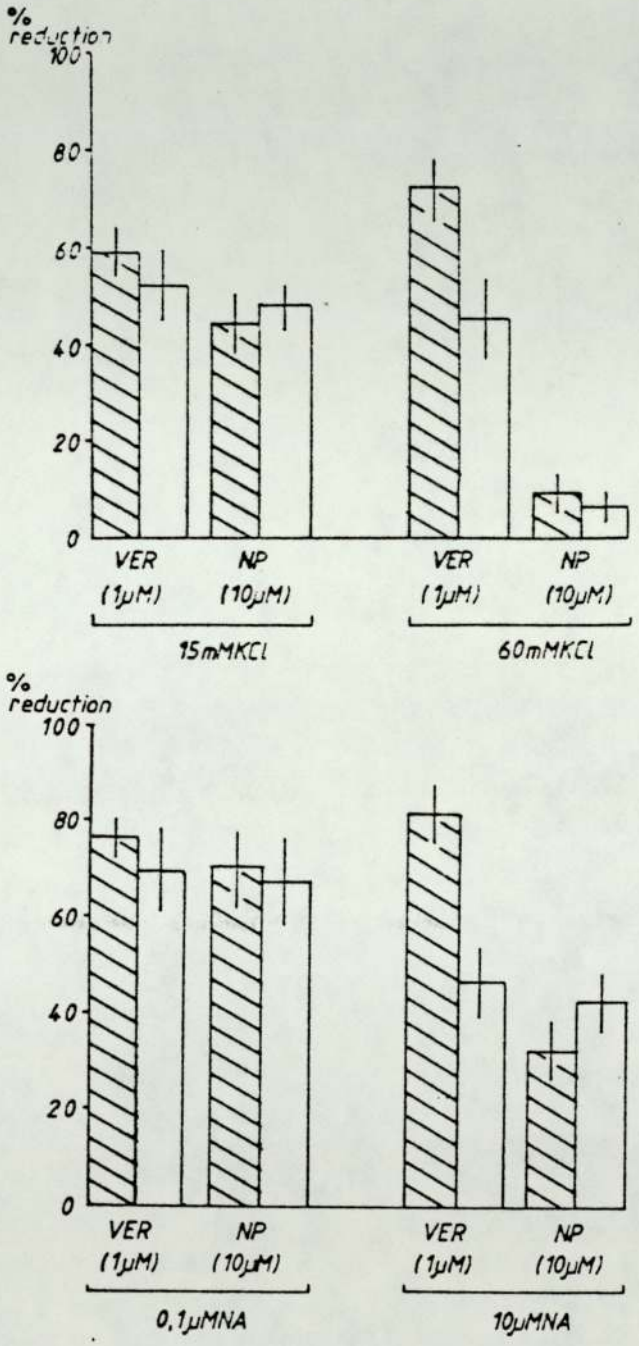



FIG. 40. Effects of VER (1 μM) and NP (10 μM) on the initial (0-20 sec. response, hatched column) and secondary (21-60 sec. response, open column) responses of the rat isolated portal vein stimulated with low and high concentrations of KCl (15 and 60 mM respectively) and noradrenaline (0.1 and 10 μM respectively) . Verticle bars represent the s.e. mean, n = 6.

of KCl or NA (FIG. 40., right panel). Although, as previously discussed, NP was less effective in inhibiting responses induced by high concentrations of stimulants than the responses to lower concentrations.

Discussion.

The results presented here are in general accord with the hypothesis outlined above. VER, a known $Ca^{2+}$ entry blocker, showed a selective action in that it inhibited the initial phase of portal vein response to 'high' [KCl] or [NA], this part of the response, in portal vein, was probably due to $Ca^{2+}$ entry as a result of electrical spike activity.

DIAZ showed a selective action, similar to that exhibited by VER, on responses to 'high' concentrations of both KCl and NA. Janis and Triggle (1973) have shown DIAZ reduced the responsiveness of the rat aorta to stimulation by $Ca^{2+}$. Wohl *et al.* (1968) have shown that, in rat aorta, DIAZ inhibited $Ba^{2+}$ induced contractions in a competitive manner. As a result of the similarity in action between DIAZ and VER and the published results of Janis and Triggle and Wohl and collegues it may be that, under the experimental conditions prevalent here, DIAZ reduced the activator $Ca^{2+}$ levels during the initial part of the contraction by inhibiting $Ca^{2+}$ entry during action potentials. This hypothesis could only be confirmed by combined ion flux and electrophysiological investigation.

NP showed no selectivity of action on intial or secondary phases of portal vein responses to either high or low concentrations of KCl or NA. Although some evidence exists to show that $Ca^{2+}$ influx, stimulated by $K^{+}$, was inhibted by NP (Kreye, *et al.*, 1975) the results were far from conclusive as Hausler and Thorens (1975) found no effect of NP on $^{45}Ca$ fluxes under similar experimental conditions. Results presented in Section 9 showed that reduced levels of spontaneous mechanical activity of the portal vein induced by NP, in common with those reduced by DIAZ and VER, were associated with lower levels of electrical spike activity. If activator $Ca^{2+}$ for the initial phase of contraction induced by high concentrations of stimulant is derived from action potential linked $Ca^{2+}$ entry then NP wopuld be expected to inhibit this in a manner similar to DIAZ or VER; assuming $Ca^{2+}$ entry during spontaneous electrical spike activity is similar to that associated with spike activity during the initial phases of stimulant induced contraction. As no preferential inhibition of either phase of contraction was observed with NP it may be that NP was more potent than DIAZ or VER in inhibiting the secondary phase of contraction during which electrical spike activity had ceased. If this were the case and NP was a more potent inhibitor of the secondary phase of contraction than VER or DIAZ, it is possible that the effect of NP was due to an action other than reduction of activator $Ca^{2+}$ by action potential inhibition; or that two PSCs may exist, one sensitive to all the agents tested and associated with action potentials and a second PSC not associated with

spike activity which was more sensitive to NP than VER or DIAZ. Such hypotheses however, must await further experimental evidence to allow their possible roles to be clarified.

It is interesting to note that both VER and DIAZ appear to be selective for contractions concurrent with action potential discharge whereas NP was not especially since Golenhofen (1976a, Golenhofen & Weston, 1976) considered VER and DIAZ, but not NP to be selective for Phasic contractions. VER and DIAZ may both be selectively blocking $Ca^{2+}$ entry which occurs during the upstroke of the action potential whereas NP is blocking $Ca^{2+}$ entry non-selectively, perhaps by an action on membrane potential.

## Hyperpolarizing Events and Vascular Muscle Reactivity.

### Introduction.

As previously outlined, one of the aims of this study was to attempt to relate the profile of action of vasodilator agents on rat vascular muscle to their possible mechanism of action. In this context it is germane to consider the action of NP which showed a marked selectivity of inhibition of spontaneous activity in the portal vein. Although as seen from the previous section it does not appear to selectively inhibit action potentials. If NP were to hyperpolarize the portal vein then it would be expected to reduce all $Ca^{2+}$ entry through PSC's, i.e. those channels associated with action potentials and those associated with steady depolarization (e.g. High- $K^{+}$) Haeusler and Thorens (1976) have shown a concentration-dependent hyperpolarization of rabbit main pulmonary artery by NP, while Ito and co-workers (1978) have shown a similar effect in rabbit portal vein, where NP induced hyperpolarization coincident with relaxation. It was therefore considered worthwhile to investigate further the effect of hyperpolarization on rat portal vein responsiveness. Since intracellular recording techniques were not available 'indirect' methods of investigation were saught.

Siegel and Schneider (1981) have presented evidence which suggests that membrane hyperpolarization is the causal link between acidosis and vascular muscle relaxation. Siegel

and co-workers (1978) have also suggested that reduction of $[K^+]_o$ of the Krebs' solution bathing vascular muscle from 5.9mM (the normal concentration in the Krebs' solution used) to 2.5mM causes a hyperpolarization. The effect of these two treatments, acidosis and reduced $[K^+]_o$, on responses of the portal vein were therefore investigated and compared with NP, the assumption was made that the two former events would induce hyperpolarization, although direct ratification of this was not attempted.

Alteration of pH.

The pH of the Krebs' solution bathing portal vein preparations was reduced by increasing the percentage of $CO_2$ in the gassing mixture aerating the tissues. (Tobian, *et al.*,1959; Vanhoutte & Clement, 1968). A reduced level of $[K^+]_o$ was acheived by reducing the concentration of KCl in the Krebs' solution.

Results.

A representative trace showing the action of reduced pH on spontaneous activity and a KCl induced contraction of the portal vein can be seen in FIG. 41. The reduction in pH from a normal level of 7.45 in the portal vein caused a selective inhibition of the spontaneous activity while it caused only a partial reduction in responses to NA and KCl. The action of acidosis at two levels, pH 7.2 and 7 on spontaneous, KCl and NA contractions (induced by 60mM and 3μM respectivly) are shown in figure 42.

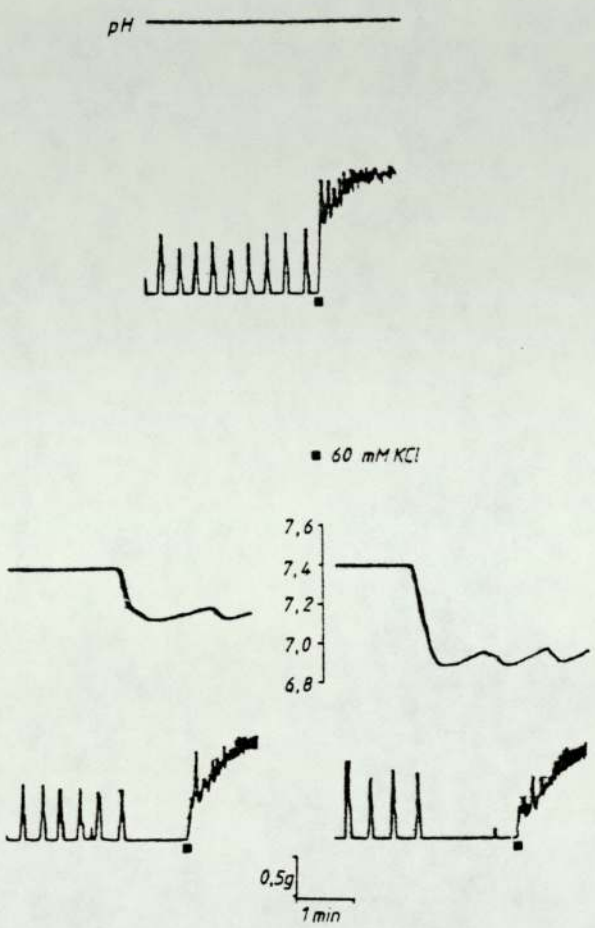



FIG. 41. Representative traces showing the effects of two levels of acidosis (pH 7.2 and 7) on spontaneous activity and KCl induced contractions of the rat isolated portal vein. Simultaneous traces of pH (upper records) and mechanical activity (lower records) are shown, the top panel shows a control response elicited in Krebs' solution at pH 7.4

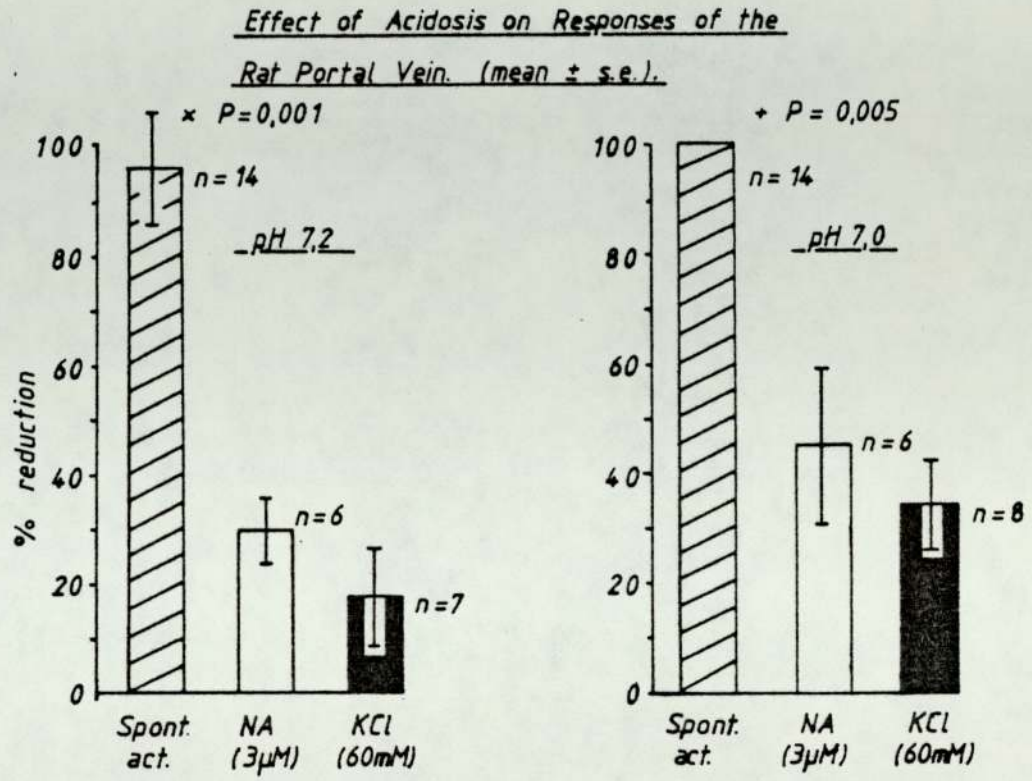



FIG. 42. Effects of two levels of acidosis (pH 7.2 and 7) on spontaneous (hatched column), noradrenaline (open column) and KCl (solid column) induced activity of the rat isolated portak vein. Verticle bars represent the s.e. mean.

As discussed in the methods section reduction of the Krebs' solution pH by increased $CO_2$ gassing also caused a reduction in the partial pressure of $O_2$ in solution. The $PO_2$ level of normal Krebs' solution, gassed with carbogen (5% $CO_2$ in $O_2$), at pH 7.45 was measured using a Rank $O_2$ electrode and found to be 59.5kPa (446mmHg). When the $PCO_2$ of the gassing mixture was increased to such an extent as to reduce the pH of the Krebs' solution to 7 the $PO_2$ level was reduced to 41.6kPa (312mmHg). In order to ensure that the reduction of activity seen in Krebs' solution at pH 7 was due to acidosis and not to reduced $PO_2$ levels experiments were carried out using a gas mixture of 70% $O_2$, 25% $N_2$ and 5% $CO_2$. Bubbling the special gas mixture through the Krebs' solution caused a reduction in $PO_2$ to 40.3kPa (302mmHg). The reduced $PO_2$ level, in the absence of any change in pH, caused no significant reduction in any of the responses of the portal vein, it must therefore be assumed that the action of increased $PCO_2$ was due to the reduction in pH it caused and not to the concomitant fall in $PO_2$.

The action of increased pH, achieved by reducing the $PCO_2$ of the gassing mixture, on responses of the portal vein was also investigated. A representative trace showing the action of raising the pH of the Krebs' solution from 7.45 to 8 on spontaneous activity and a KCl contraction is seen in Fig 43. As the pH was increased there was a slight reduction in the magnitude of the spontaneous contractions associated with an increase in their frequency which resulted in an

increased integrated response. The responses of the portal vein to increased pH were quantified as a percentage increase from control responses elicited in Krebs' solution at pH 7.45 and were spontaneous activity 29% ± 7 %, KCl (60mM) 28% ± 3%, NA (3μM) 24% ± 6% and electrical stimulation 26% ± 5% all at n=6.

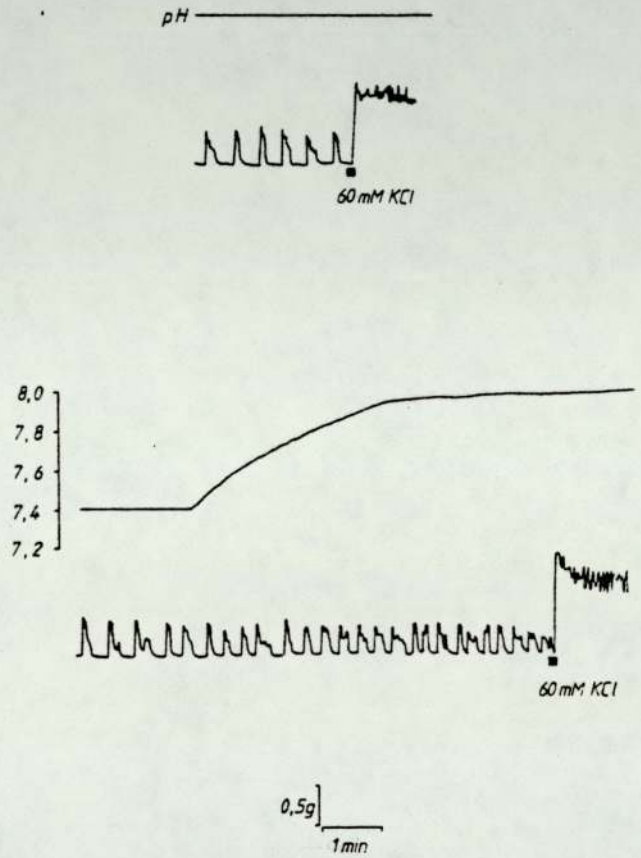



FIG. 43. Representative traces showing the effects of alkalosis (pH8) on spontaneous and KCl induced activity of the rat isolated portal vein. The top panel shows a control response elicited in Krebs' solution at pH 7.4

The Effect of NP and Acidosis on Spontaneously Occuring Phasic Activity of Circular Preparations of the Rat Aorta.

In addition to the induced phasic activity seen in the presence of NA and EDTA and discussed previously, spontaneous phasic contractions of the rat aorta were seen in only 22% of all aortae investigated.These spontaneous phasic contractions were of a similar form to those induced by NA, and occured most frequently during the intial equilibration period of each experiment. After the preparation had been stimulated by NA or KCl the spontaneous activity usually ceased. For these reasons; rarity and general lability of response, experimental investigation of this phenomenon was difficult, consequently NP was the only vasodilator fully investigated. The action of acidosis on spontaneous aortic contractions was also observed on four preparations.

Figure 44a shows a representative trace of the inhibitory action of NP on spontaneous contractions of the rat aorta. In common with the action of acidosis, NP reduced both the spontaneous activity and the basal tone of the preparation. Figure 45. shows a concentration response curve for the action of NP on spontaneous and induced phasic activity of the rat aorta (the data for induced activity was taken from FIG.32.) As can be seen in FIG.45. spontaneous phasic contractions were more sensitive to inhibition by NP than were induced phasic contractions.

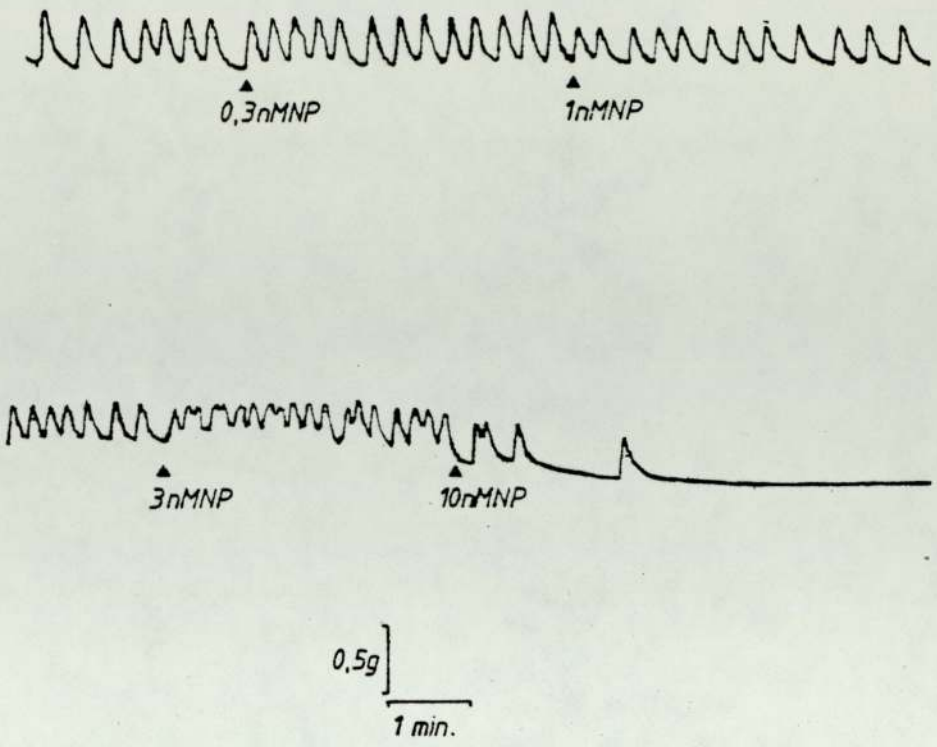



FIG. 44a. Representative trace showing the effects of NP (0.3 - 10 nM) on spontaneous phasic activity of the rat isolated thoracic aorta.



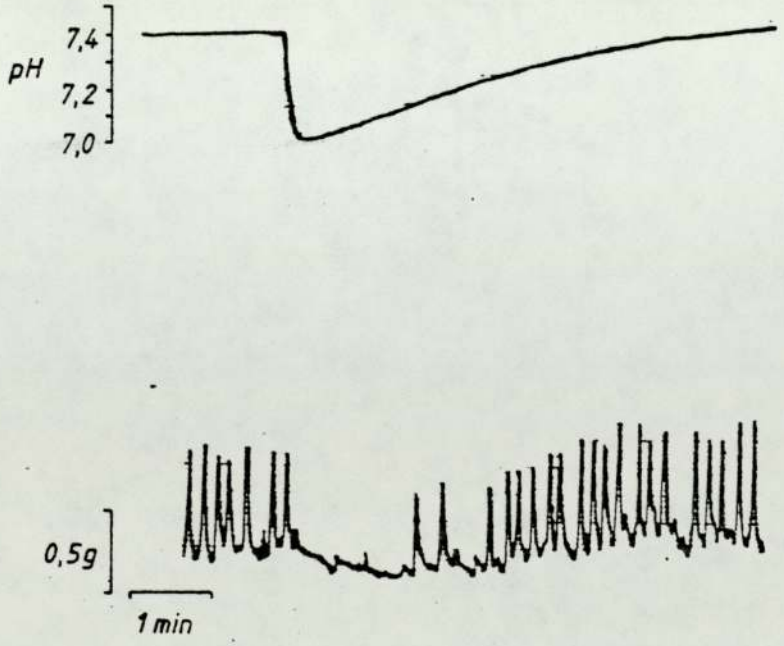



FIG. 44b. Representative trace showing the effects of acidsis (pH7) on spontaneous phasic activity of the rat isolated thoracic aorta.

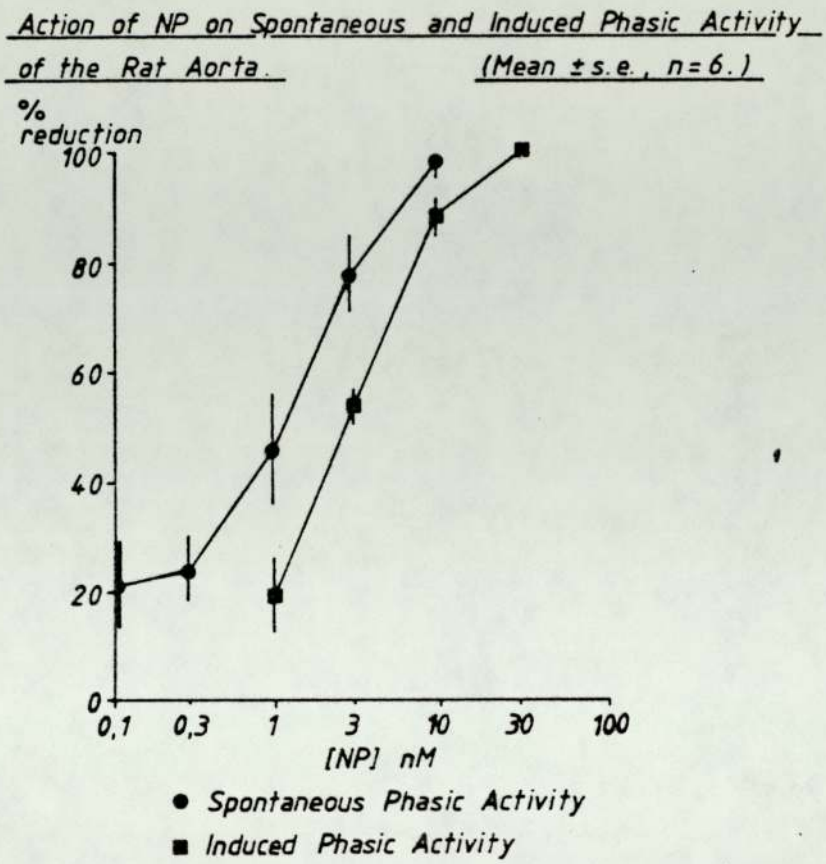



FIG. 45. Effects of NP on spontaneous ( ● ) and induced ( ■ ) phasic activity of the rat isolated thoracic aorta. Verticle bars represent the s.e. mean.

To compare the action of NP on induced and spontaneous phasic activity on a quantitative basis $IC_{50}$ values were calculated as described previously. The $IC_{50}$ values were 4.23 +/- 0.3nM and 1.3 +/- 0.26nM respectively for induced and spontaneous activity, thus NP caused a significantly greater inhibition of spontaneous activity ($p<0.05$, $n=6$).

A representative trace showing the action of acidosis on spontaneous phasic contractions of the rat aorta can be seen in FIG. 44b. This figure shows that when the pH of the Krebs' solution was reduced from 7.45 to 7 the spontaneous activity was completely inhibited and a small reduction in the basal tone of the preparation occured. Figure B also shows that as pH returned to normal levels the spontaneous activity returned and the basal tone of the preparation regained its pre-acidotic level. The actions of NP on induced phasic activity of the rat aorta are discussed in Section 6.

The results presented above are in agreement with those of Vanhoutte and Clement (1968) who showed acidosis caused relaxation of the dog saphenous vein while alkalosis enhanced contractions induced by electrical stimulation. It should also be noted that acidosis preferentially inhibited the spontaneous activity of the rat portal vein to a significantly greater extent than it inhibited contractions to NA and KCl ($p<0.01$, n-6). On the other hand, alkalosis showed no preferential action on any of the parameters tested. Thus it can be seen that acidosis has a profile of action in the portal vein similar to that exhibited by NP, although unlike NP acidosis did not inhibit NA to a significantly greater extent than KCl. It should also be noted that acidosis reduced the phasic part of the contractions induced by KCl (FIG. 41.) in a selective manner, on effect not observed with NP, but seen with VER and DIAZ (See Section 10). This suggests that acidosis may be selectively blocking a $Ca^{2+}$ entry during action potentials.

Lowering the Extracellular Concentration of $K^+$

The $[K^+]_o$ was reduced from 5.9mM to 2.5mM, a concentration which Siegel and collegues (1978) have shown to cause maximal hyperpolarization of the dog saphenous vein, and the spontaneous activity and responses to NA in the portal vein were recorded. The results from this experiment are shown in figure 46, and as can be seen from this figure the spontaneous activity of the portal vein was

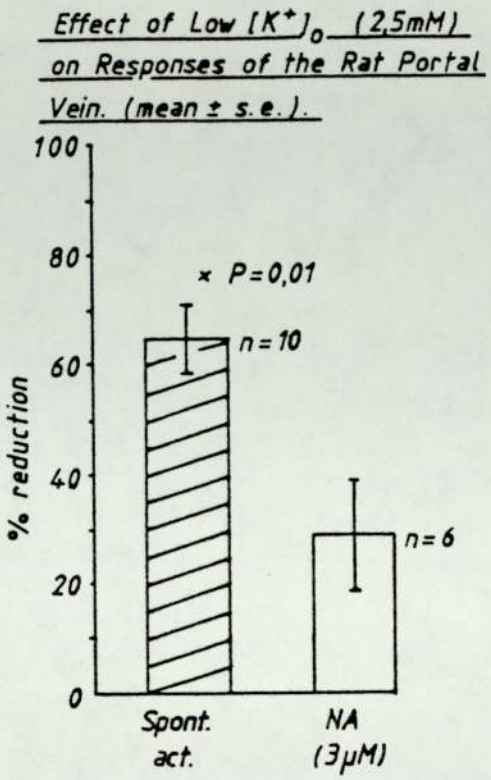



FIG. 46. Effect of reduced $[K^+]_o$ (2.5 mM) on spontaneous (hatched columns) and noradrenaline induced (3 μM, open column) activity of the rat isolated portal vein. Verticle bars represent the s.e. mean.

inhibited to a significantly greater extent ($p<0.05$, $n=6$) by reduced $[K^+]_o$ than was the response to NA. Obviously no investigation of the action of lowered $[K^+]_o$ was possible when KCl was used as a stimulant.

Discussion.

As discussed in section 3 increased concentrations of $MgSO_4$ in the Krebs' solution also selectively reduced the spontaneous activity of the rat portal vein. Sigurdsson and Uvelius (1977) have suggested that this action of raised $[Mg^{2+}]_o$ is due to hyperpolarization of the portal vein.

Thus it can be seen that the profile of action of NP on the portal vein, was mimicked by three seperate methods which are thought to induce membrane hyperpolarization in vascular muscle. From these results it is proposed that selective suppression of the spontaneous activity of the rat portal vein may indicate membrane hyperpolarization. In order to substantiate this hypothesis it would be necessary to determine the effect of all agents/procedures which selectively reduced the spontaneous activity of the portal vein on the membrane potential using electrophysiological techniques.

Actions of pH and NP on Responses Induced by Varying Concentrations Of KCl and NA.

Karaki, Hester and Weiss (1980) have shown, in canine renal arteries, that NP was more effective in inhibiting responses to low concentrations of $K^+$ and NA when compared with its action on high concentrations. This has been confirmed for the rat portal vein, see Section 10, FIGS. 38 & 39. Consequently experiments were carried out on the effects of pH and NP on responses of the aorta and portal vein to low concentrations of KCl and NA. In common with KCl induced responses of the canine renal artery (Karaki, *et al.*; 1980), when the concentration of KCl used to stimulate either the aorta or portal vein was reduced (to 30 or 15mM) then a given concentration of NP (10μM) produced a greater percentage inhibition of the response in both preparations (FIG. 47). NP was a more potent inhibitor of aortic than portal vein contractions to KCl throughout the concentration range of KCl tested ($p<0.01$, n shown in FIG. 47).

In light of the similarity of action of NP and acidosis in reducing responses of the portal vein it was considered worthwhile to investigate the action of reduced pH on lower concentrations of KCl and NA in both aorta and portal vein. In common with results obtained for NP it was found that as the stimulant concentration of KCl was reduced, a given level of acidosis (pH7) produced a greater percentage inhibition of responses (FIG. 48). In contrast to the action

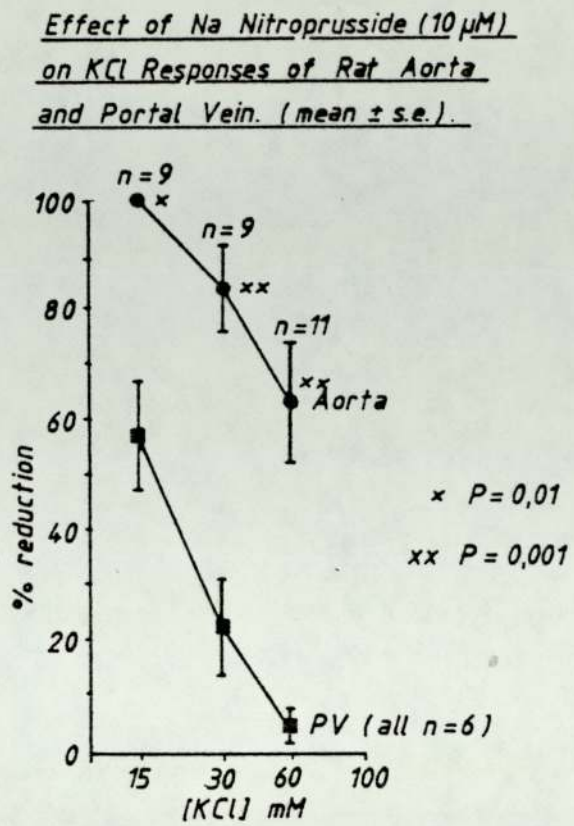



FIG. 47. Effects of NP (10 μM) on responses of the rat isolated aorta ( ● ) and portal vein ( ■ ) to various concentrations of KCl. Verticle bars represent the s.e. mean.

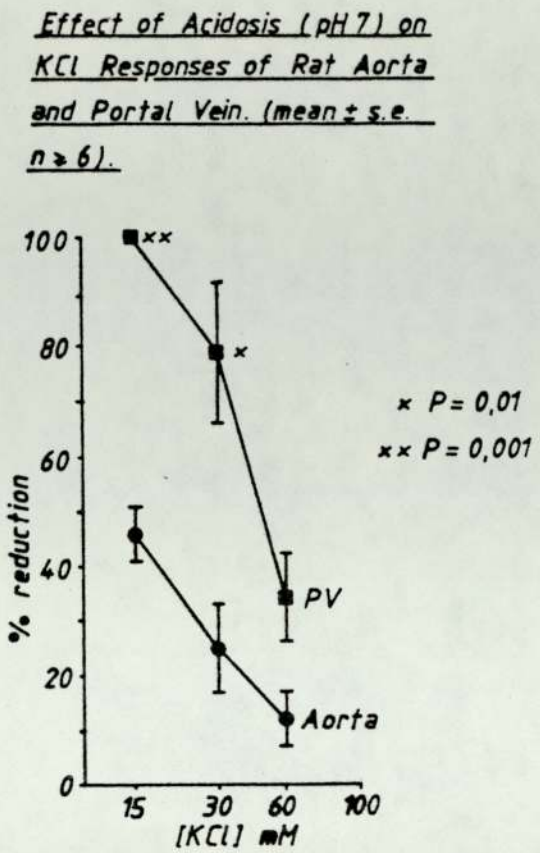



FIG. 48. Effects of acidosis (pH7) on responses of the rat isolated aorta ( ● ) and portal vein ( ■ ) to various concentrations of KCl. Verticle bars represent the s.e. mean.

of NP, acidosis was more effective in inhibiting responses of the portal vein than the aorta, throughout the concentration range of KCl tested (being significantly greater at [KCl] of 30 and 15mM, $p<0.01$, $n=6$).

Similar results were obtained when the action of acidosis (pH7) on low concentrations of NA in aortic and portal vein preparations was tested (FIG. 49.). As the stimulant concentration of NA was lowered the percentage reduction induced by acidosis (pH7) increased. As with the results obtained for stimulation with KCl, the portal vein was more sensitive to the effects of acidosis than the aorta (FIG. 49) throughout the concentration range tested.

The action of NP (10μM) on contractions induced by low concentrations of NA in the portal vein can be seen in FIG. 50. In the rat aortic preparations the effects of 10μM NP on responses to low NA concentrations were not investigated because a 100% inhibition of the response to 1μM NA was achieved by a NP concentration of 0.3μM. Although this situation means that no evidence on the form of the NA/NP concentration relationship could be presented it should be noted that throughout the concentration range of NA tested, 10μM NP caused a 100% inhibition, thus NP could still be considered a more potent inhibitor of NA responses in aorta than it was in the portal vein.

Discussion.

From the results presented above it can be seen that

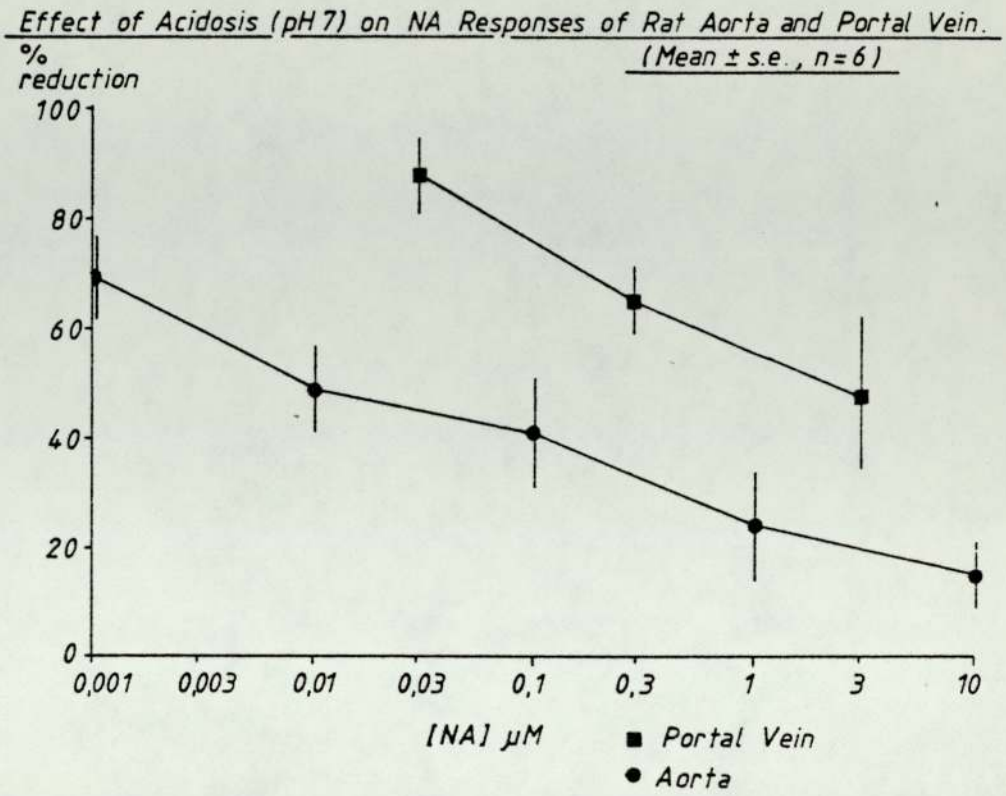



FIG. 49. Effects of acidosis (pH7) on responses of the rat isolated aorta( ● ) and portal vein ( ■ ) to various concentrations of noradrenaline. Verticle bars represent the s.e. mean.

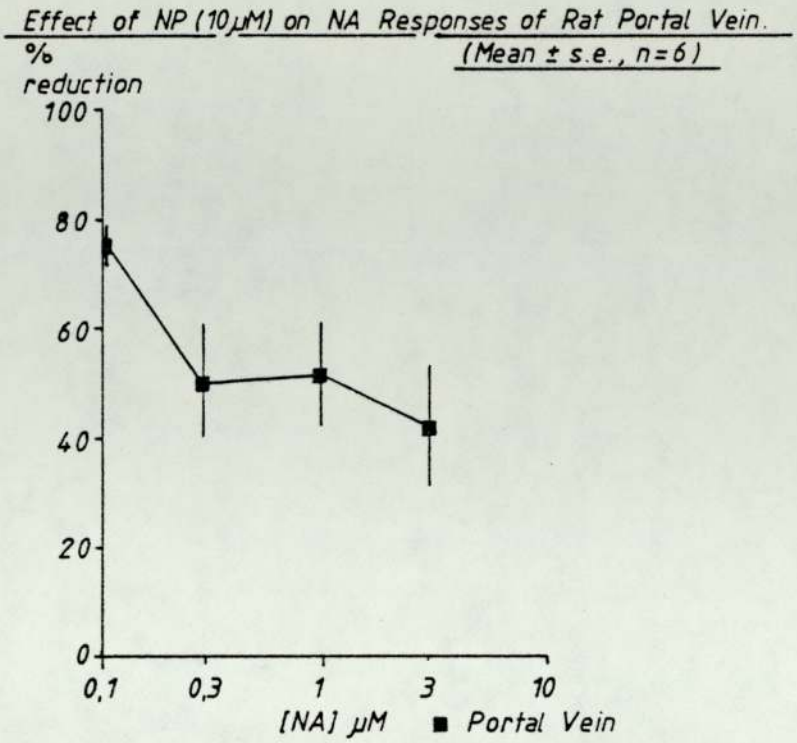



FIG. 50. Effects of NP (10 μM) on responses of the rat isolated portal vein to various concentrations of noradrenaline. Verticle bars represent the s.e. mean.

irrespective of whether NA or KCl was used to stimulate the vascular preparations NP was a more potent inhibitor of aortic responses than portal vein responses. In the case of inhibition of response by acidosis the opposite situation arose with portal vein showing greater sensitivity irrespective of which stimulant was used. Despite the differences in sensitivity of the two preparations it can also be seen that the responses to low concentrations of stimulant (NA or KCl) of both aortic and venous preparations were more suscepible to inhibition by both NP and acidosis.

Hester *et al.* (1979) has suggested that the action of NP was greater on responses to low concentrations of agonist because such responses were dependent upon a $La^{3+}$ sensitive $Ca^{2+}$ uptake mechanism which could be inhibited by NP. In the case of responses to high concentrations of agonists a $La^{3+}$ resistant $Ca^{2+}$ fraction was shown to be increased (Hester, *et al.*; 1979) and this increase was not inhibited by NP. D600, a $Ca^{2+}$ entry blocker which inhibited the $La^{3+}$ resistant $Ca^{2+}$ fraction inhibited responses to both high and low concentrations of agonist. When the action of VER on reduced concentrations of KCl was investigated in this study it was found that 0.1uM VER caused a similar reduction in responses to low and high [KCl](41% +/- 6%; 40% +/- 14%; 38% +/- 9% inhibitions for 15, 30 and 60mM KCl induced contractions of portal vein, n=6). It may therefore be that the enhanced action of NP and acidosis on low concentrations of agonist in aorta and portal vein reflects a difference in $Ca^{2+}$ handling at low concentrations which was more

susceptible to the actions of NP and pH. A selective inhibition of NP on $Ca^{2+}$ entry by action potentials, which would have accounted for this, has already been discounted on the basis of a non-differential antagonism of 'early' and 'late' responses to NA. 'See Section 10)

Hester, *et al.* (1979) have also shown that NP was more effective in inhibiting responses of canine renal artery induced by NA than those induced by KCl, a situation which was reversed in the case of the calcium entry blocker D600. Hester concluded that in contrast to D600, a known $Ca^{2+}$ entry inhibitor, NP affected $Ca^{2+}$ in the canine renal artery some way not dependant on entry of $[Ca^{2+}]_o$. From the results presented in sections 4 and 5 it can be seen that, in the rat portal vein and aorta, NP was a more potent inhibitor of NA induced contractions than KCl induced contractions (FIGS. 22. and 32. respectively for the vein and artery). It should also be noted that NP was a more potent inhibitor of KCl induced responses of the aorta than the portal vein, (FIGS. 22. & 32.) as can be seen the vein was almost entirely insensitive to NP when stimulated with 60mM KCl. Thus the overall weight of evidence suggests that NP is unable to selectivel block PSC associated with action potentials.

The differences in sensitivity to acidosis and NP between aorta and portal vein cannot be readily explained by the results presented here. It may be that the aorta was more sensitive to NP because of actions of NP in addition to hyperpolarization, for example; raised cGMP levels or

altered $Cl^-$ handling (Kreye, 1980). The greater sensitivity of the portal vein to acidosis may be due to a steeper voltage/tension relationship in the portal vein than the aorta. Hermesmeyer (1982) has measure a 14mV change in membrane potential inducing an 80% contraction in the rat portal vein, thus alterations in membrane potantial of even 1 or 2mV could have marked effects on tension. If the voltage/tension relationship for the aorta were 'flatter' then it would be less susceptible to the actions of pH than the portal vein. As no electro-physiological data on the voltage/tension relationship of the aorta was available this explanation remains conjecture.

## Actions of Altered [$Cl^-$] on Responses of the Rat Aorta and Portal Vein.

### Introduction.

As previously discussed, NP has been shown to produce a concentration dependent hyperpolarization of the rabbit main pulmonary artery (Hausler & Thorens, 1976) and portal vein (Ito,*et al.*, 1980) associated with reduced muscle tone. It has been suggested (Kreye *et al.* 1977; Kreye, 1980) that NP could cause such a hyperpolarization in at least four different ways; (i) by activating an electrogenic Na pump, (ii) increasing the $K^+$ permeability of the cell membrane or (iii & iv) by reducing membrane permeability to $Na^+$ or $Cl^-$. It was therefore considered worthwhile to investigate these hypotheses further.

Kreye *et al.* (1977) and Kreye (1980) have shown, in rat aorta, that if $[Cl^-]_o$ was reduced to zero ($Cl^-$ free Krebs') neither NP or GTN produced a relaxant action as seen in normal Krebs' solution (133.15mM $[Cl^-]$). Kreye and co-workers (1977) also showed, in rabbit aorta, that both NP and GTN caused a reduction in $^{36}Cl$ efflux from NA stimulated preparations. These results suggested that the $Cl^-$ gradient across vascular muscle membranes may be important in the actions of NP and GTN, these agents might have effects on chloride permeability or chloride transport across the membrane. Consequently, the actions of altered $[Cl^-]_o$ were investigated.

$[Cl^-]_o$ and the Portal Vein.

The $[Cl^-]$ of the Krebs' solution bathing portal vein (and aortic) preparations was altered by replacing some, or all, of the NaCl with Na Isethionate. The impermeant isethionate anion acted as a substitute for $Cl^-$ (Kreye, *et al.*, 1977; Kreye, 1980; Rangachari, *et al.*, 1982). During the investigation of the actions of reduced $[Cl^-]$ on vascular muscle responses KCl was not used as a stimulant because of the increase in $[Cl^-]$ associated with its addition, consequently throughout these experiments $KNO_3$ was used as a $Cl^-$ free source of $K^+$. A preliminary investigation showed $KNO_3$ and KCl were equi-effective in stimulating the portal vein and aorta but because of the lower solubility of $KNO_3$ high concentrations (>30mM) were only achieved by dissolving $KNO_3$ directly into the Krebs' solution, addition of $K^+$ was then achieved by replacing the Krebs' bathing the tissue with Krebs' containing $KNO_3$ at the required concentration.

Initially an attempt was made to investigate the action of $Cl^-$ free Krebs' solution on the responses of the portal vein, however under these conditions the preparation produced a large tonic contraction (FIG. 51., top panel) and subsequently no spontaneous activity was observed.

When the $[Cl^-]_o$ was reduced to 14mM a transient contraction was noted during which the spontaneous activity continued at an increased frequency. When the basal tone of the preparation had returned to control levels it was found

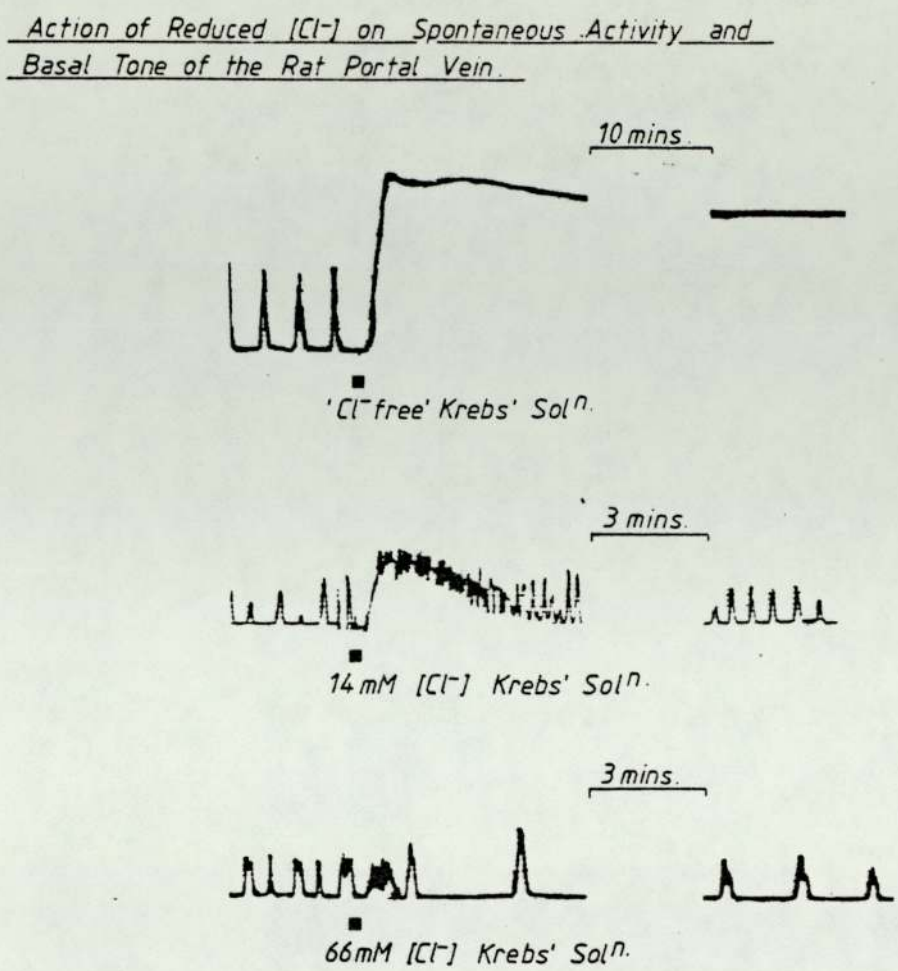



FIG. 51. Representative traces showing the actions of lowered extracellular [Cl⁻] on the spontaneous activity of the rat isolated portal vein. The Krebs' solution bathing the preparation was changed to one containing the altered [Cl⁻] at the mark (■)

by integration that spontaneous activity had been reduced. Reducing $[Cl^-]_o$ of the Krebs' solution to 50% of normal levels (66mM) reduced the spontaneous activity but little or no initial tonic contraction was seen. Representative traces showing the effects of 14mM and 66mM $[Cl^-]_o$ on portal vein can be seen in FIG.51. (middle and lower panels respectively).

During these preliminary investigations it was also found that in lowered $[Cl^-]_o$ the induced responses of the portal vein were reduced from control values in a concentration dependent manner. Spontaneous activity and responses to NA were inhibited to a significantly greater extent ($p<0.05$, $n=6$) at 14mM than 66mM $[Cl^-]_o$. On the other hand, $KNO_3$ induced contractions, although inhibited to a greater degree by 14mM $[Cl^-]$, showed no significant difference between responses in 14mM and 66mM $[Cl^-]_o$ (FIG. 52.).

<u>The Action of Vasodilators on Portal Vein Responses Elicited in Low $[Cl^-]_o$ Krebs' Solution.</u>

The actions of NP, GTN, TOLM and VER on the responses of the portal vein in the presence of reduced chloride concentration were investigated in attempt to elucidate any role of $Cl^-$ in the mode of action of these agents. A representative trace showing the inhibitory action of NP on spontaneous activity and NA induced contractions of the portal vein in 14mM $[Cl^-]_o$ can be seen in FIG.53. This figure shows that despite the alteration in chloride

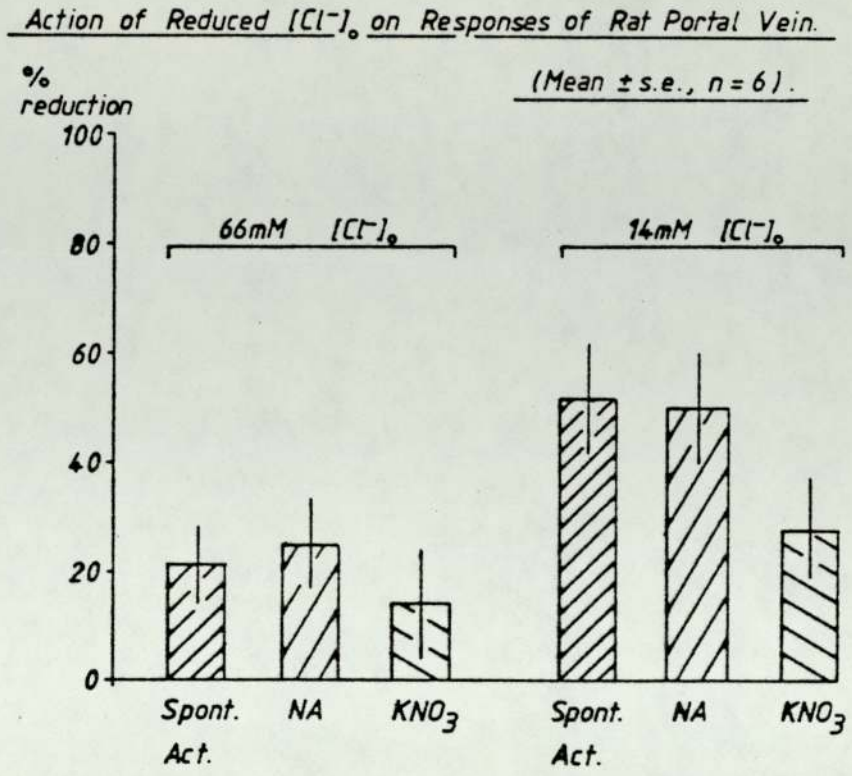



FIG. 52. Effects of reduced extracellular [Cl⁻] at 66 mM (left panel) or 14 mM (right panel) on spontaneous activity and responses of the rat isolated portal vein induced by noradrenaline (3 μM) or $KNO_3$ (60 mM). Verticle bars represent the s.e. mean.

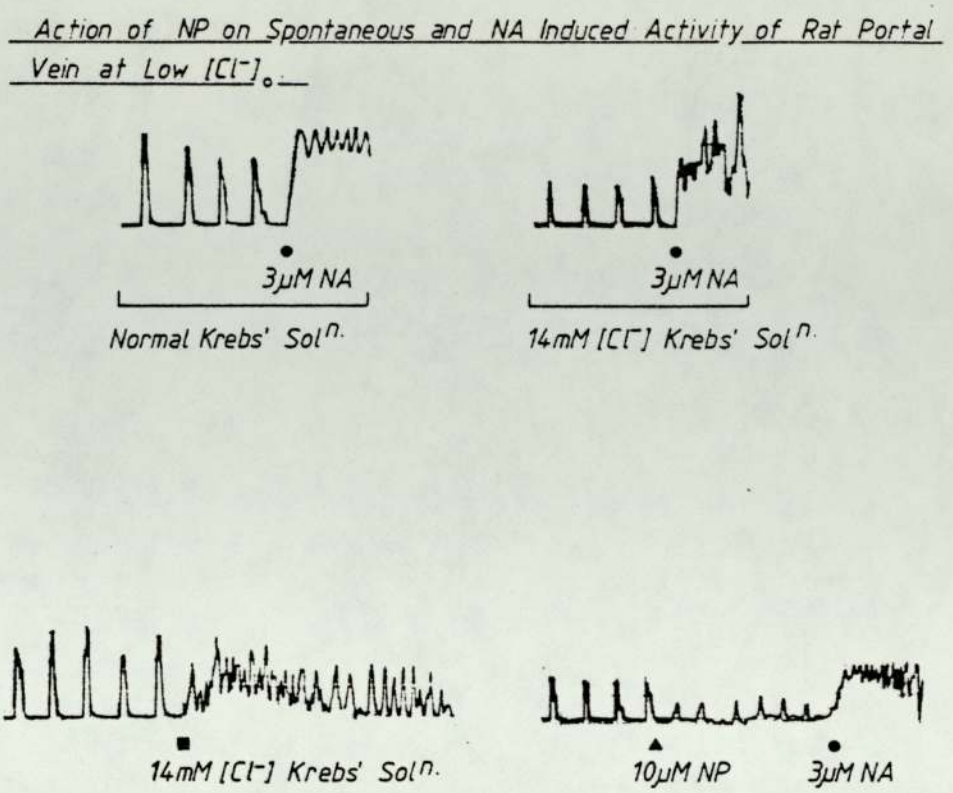



FIG. 53. Representative traces showing the actions of NP (10 μM) on spontaneous and noradrenaline induced (3 μM) activity of the rat isolated portal vein elicited in Krebs' solution woth a $[Cl^-]_o$ of 14 mM.

concentration NP was still an effective inhibitor of both phasic and tonic contractions of the portal vein.

The actions of NP, GTN, TOLM and VER on responses of the rat portal vein in 14mM $[Cl^-]_o$ can be seen in FIG.54. The percentage inhibitions in 14mM $[Cl^-]_o$ shown in this figure were calculated as a degree of inhibition of the responses which had already been reduced by the lowered chloride concentration. As can be seen from figure 54 the relaxant actions of the vasodilators were unaffected by reduction in the $[Cl^-]$ to 14 mM of the Krebs' solution bathing the preparation. A smaller reduction in $[Cl^-]_o$ to 66mM did not alter significantly the inhibitory actions of NP, GTN, TOLM and VER.

The action of 14mM $[Cl^-]_o$ Krebs' solution on the relaxant effect of acidosis was also investigated. As was found with the vasodilator agents tested, a reduced chloride level (14mM) did not alter the inhibitory action of acidosis on spontaneous or induced responses of the portal vein (FIG. 55.) This figure shows the inhibitory action of a reduced pH (7) on induced and spontaneous activity of the portal vein.

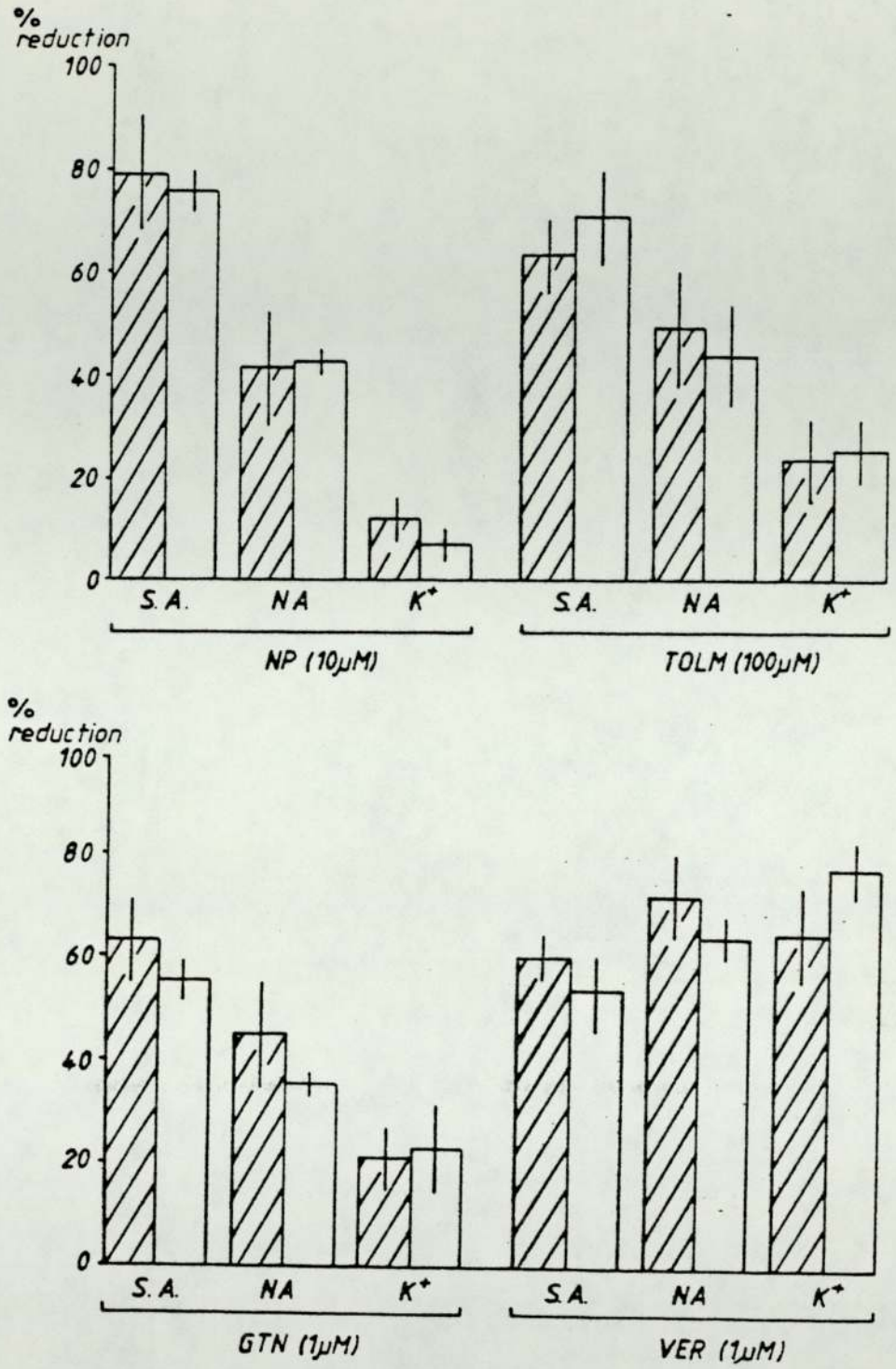



FIG. 54. Effects of NP (10 μM); TOLM (100 μM); GTN (1 μM) or VER (1 μM) on spontaneous activity and noradrenaline (3 μM) and $K^+$ (60 mM) induced responses of the rat isolated portal vein elicited in normal Krebs' solution (open columns) or 14 mM $[Cl^-]_o$ (hatched columns). Verticle bars represent the s.e. mean.

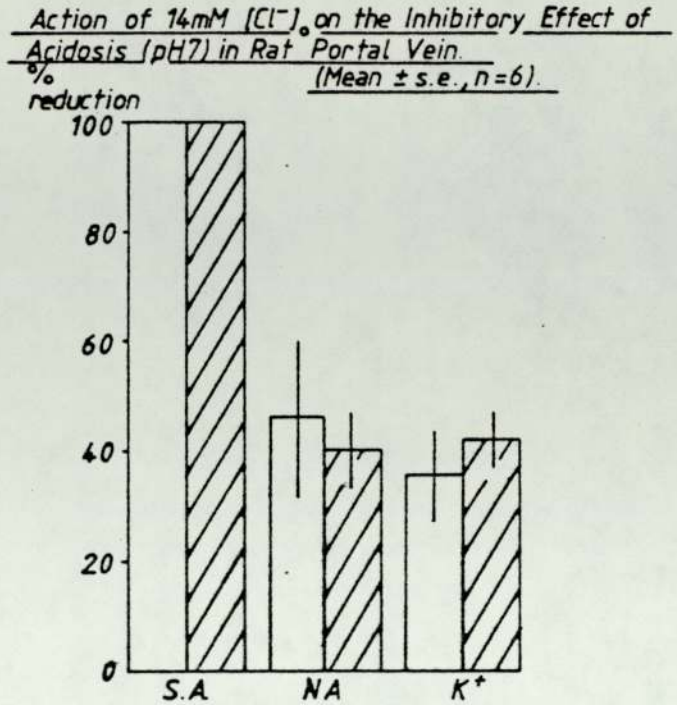



FIG. 55. Effects of acidosis (pH7) on spontaneous activity (SA), noradrenaline (3 μM) or $K^+$ (60 mM) induced responses of the rat isolated portal vein elicited in normal Krebs' solution (open columns) or 14 mM $[Cl^-]_o$ Krebs' solution (hatched columns). Verticle bars represent the s.e. mean.

## $[Cl^-]_o$ and the Rat Aorta.

In the light of the results obtained for NP and GTN in the presence of reduced $[Cl^-]_o$ in the portal vein it was considered of interest to attempt to repeat Kreye's (Kreye, *et al.*,1977) observation of the effect of chloride free solutions on the activity of NP and GTN in $K^+$ stimulated rat aorta. To maintain comparability between preparations the reduced chloride level investigated in rat aortae, stimulated with $KNO_3$, was 14mM.

In contrast to responses obtained in the portal vein, reduced levels of $[Cl^-]_o$ (14mM) had no inhibitory effect on responses of the rat aorta stimulated with $K^+$. When the Krebs' solution bathing the aortae was changed to one containing 14mM $[Cl^-]$ there was, on occasion, a transient increase in tone of a duration of less than one minute. When this transient contraction occured no subsequent alteration in the sensitivity of the preparation was observed, the results obtained with these tissues were the same as those found in tissues which had not shown this contraction.

Representative traces of the action of NP and GTN on $K^+$ induced contractions in normal and 14mM $[Cl^-]$ Krebs' solution can be seen in FIG. 56. As can be seen from this figure the inhibitory action of NP and GTN was reduced in low chloride Krebs'. The inhibitory action of NP in normal and low chloride Krebs' can be seen in FIG. 57. At a $[Cl^-]_o$

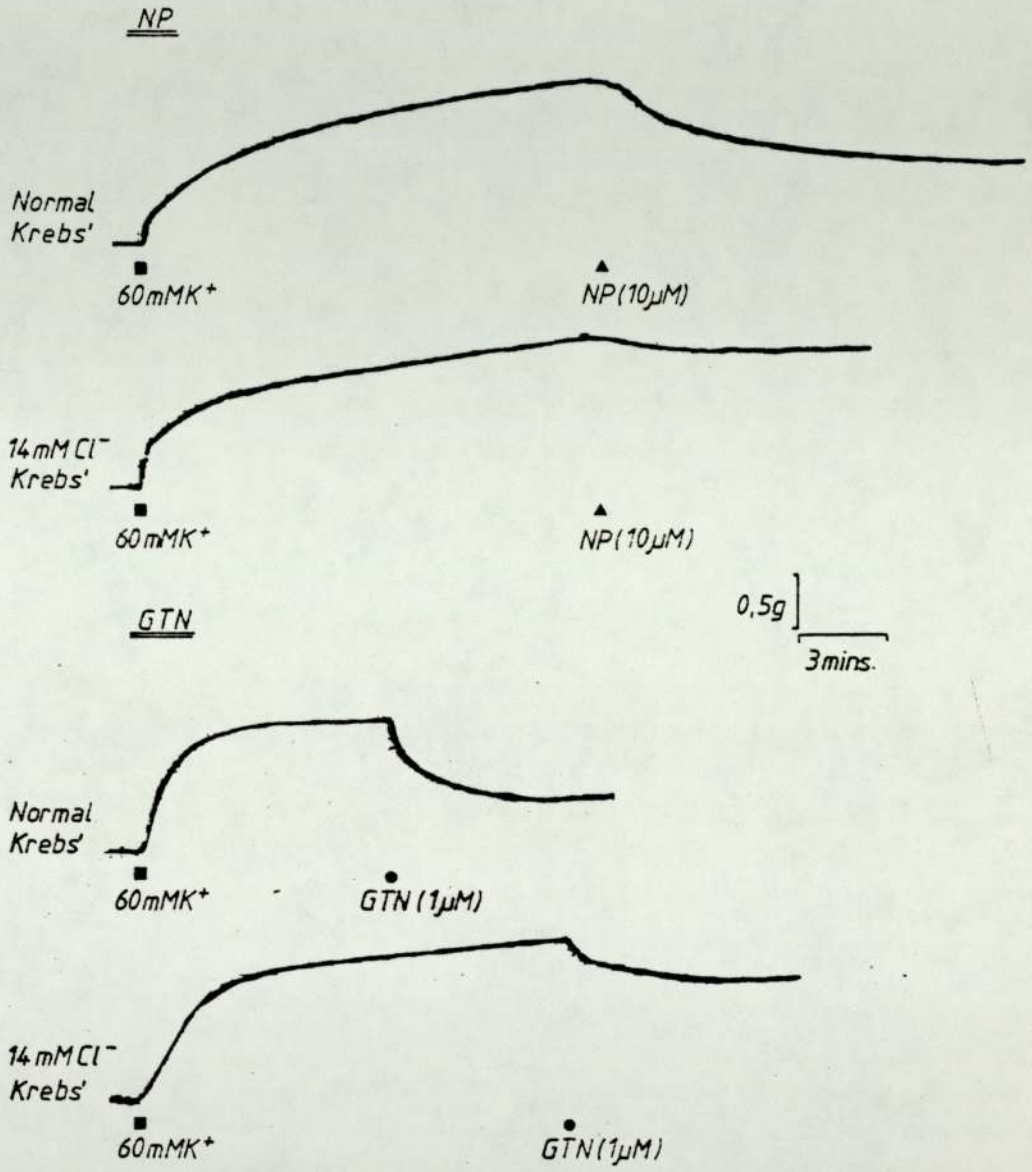



FIG. 56. Representative traces showing the effects of NP (10 μM, upper panel) or GTN (1 μM, lower panel) on $KNO_3$ (60 mM) induced contractions of the rat isolated aorta elicited in normal Krebs' solution (upper records) or 14 mM $[Cl^-]_o$ Krebs' solution (lower records).

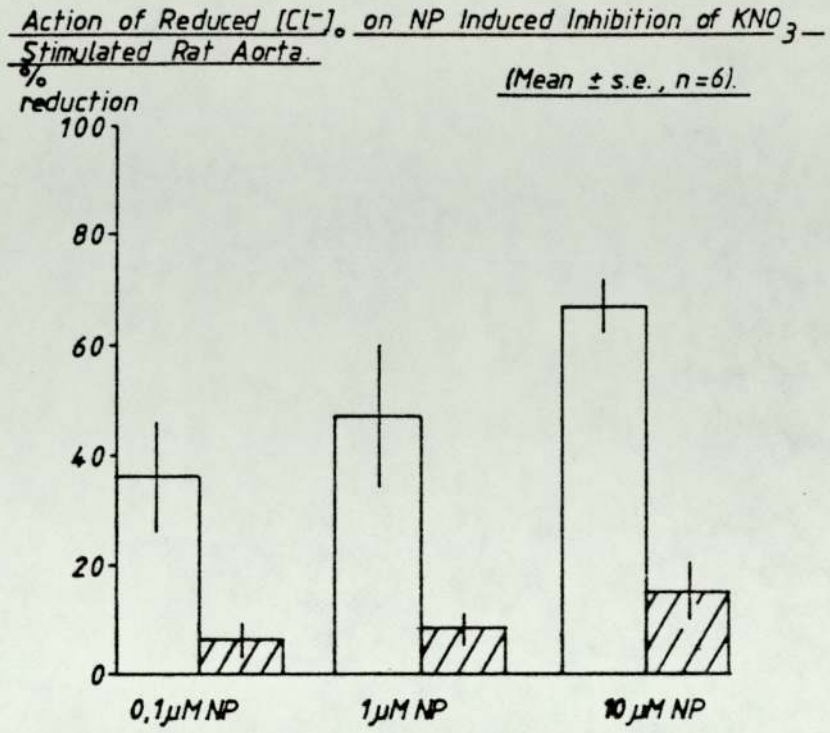



FIG. 57. Effects of NP on responses of the rat isolated aorta induced by $KNO_3$ (60mM) in normal Krebs' solution (open columns) or 14 mM $[Cl^-]_o$ Krebs' solution (hatched columns). Verticle bars represent s.e. mean.

of 14mM, NP (0.1 - 10μM) produced a significantly smaller reduction in $K^+$ contractions ($p<0.01$, n=6, hatched bars) than the inhibition seen in normal Krebs' solution. The action of GTN (1μM), TOLM (100μM) and VER (1μM) can be seen in FIG. 59. In common with NP, GTN caused a significantly smaller ($p<0.01$, n=6) reduction in response to $K^+$ in 14mM $[Cl^-]_o$ than in normal $[Cl^-]_o$ TOLM produced a lesser degree of inhibition in 14mM $[Cl^-]_o$ , but this effect was not significantly different from the relaxtion induced in control conditions. In contrast to the results obtained with NP and GTN, VER produced a similar degree of inhibition in both normal and 14mM $[Cl^-]$ Krebs' solution (FIG. 58.).

When the effect of low chloride (14mM) Krebs' solution on relaxation induced by acidosis was investigated results similar to those found with VER were obtained. At a pH of 7, in normal Krebs' solution, an inhibiton of $K^+$ induced contractions of 12% +/- 5% (n=6) was obtained by lowering pH to 7.0, at a $[Cl^-]_o$ of 14mM a reduction of 14% +/- 6% was observed. There was no significant difference between the effects of acidosis in normal and low chloride Krebs' solution.

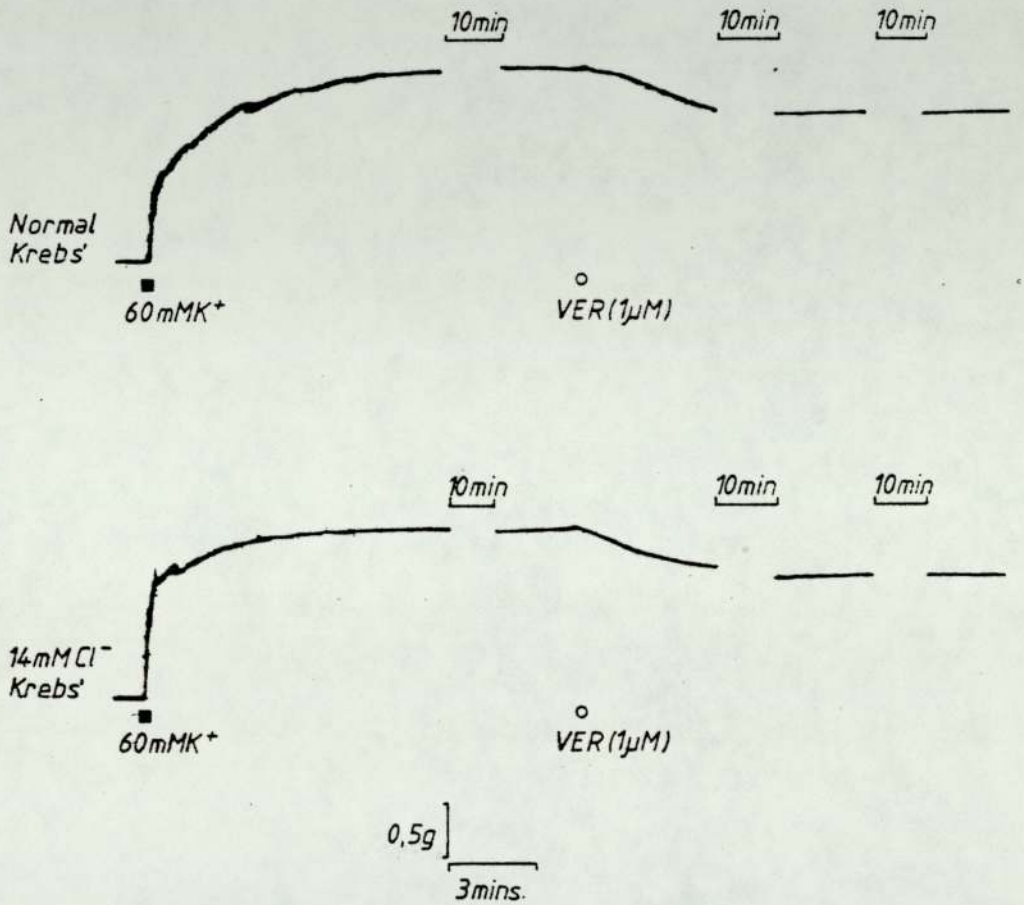



FIG. 58. Representative traces showing the action of VER on $KNO_3$ (60mM) induced contractions of the rat isolated aorta elicited in normal Krebs' solution (upper records) or 14 mM $[Cl^-]_o$ Krebs' solution (lower records).

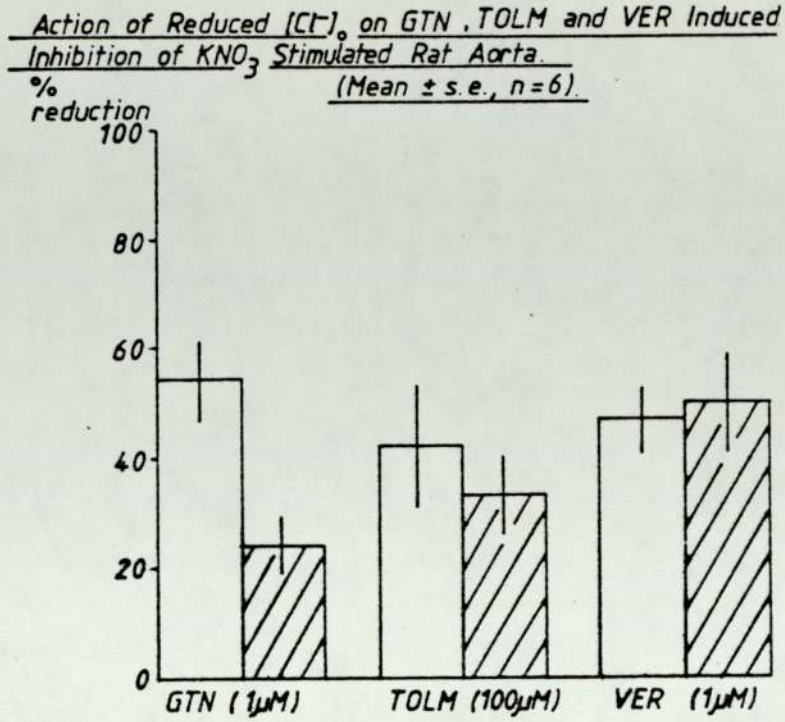



FIG. 59. Effects of GTN (1 μM); TOLM (100 μM); or VER (1 μM) on $KNO_3$ (60mM) induced responses of the rat isolated aorta elicited in normal Krebs' solution (open columns) or 14 mM $[Cl^-]_o$ Krebs' solution (hatched columns). Verticle bars represent s.e. mean.

## DISCUSSION.

### Actions of Altered $Cl^-$.

Haljamae and co-workers (1970) have shown in the rat portal vein that the measured level of intracellular $[Cl^-]$ was not consistant with a simple two compartment model of intra- and extra-cellular distribution. Haljamae *et al.* (1970) and Villamil *et al.* (1968a), who observed a similar complex $Cl^-$ distribution in dog carotid artery, suggested that these observations could be explained by three possible mechanisms; i) intracellular binding of $Cl^-$, ii) active $Cl^-$ uptake or iii) complex $Cl^-$ distribution in more than one compartment. Although the results presented here cannot bring any light to bear on which, if any, of these mechanisms operates in the portal vein it can be seen that the removal of $[Cl^-]_o$ greatly altered vascular activity. It may be that in the portal vein and other vascular muscle that the non-passive distribution of $Cl^-$ has a role to play in the determination of the resting membrane potential and thus muscle activity (see Introduction, Section B4iv.).

Haeusler and Thorens (1976) and Ito *et al.* (1980) have observed hyperpolarization associated with vascular muscle relaxation induced by NP. Kreye and co-workers (Kreye, *et al.*, 1977, 1981; Kreye, 1980) have shown in rat aorta, that removal of extracellular $Cl^-$ inhibited the relaxant effect of NP on $K^+$ induced contractions. Furthermore, Kreye *et al.* (1977) have shown that both NP and GTN reduced $^{36}Cl$ efflux from NA stimulated rabbit aorta. It has also been

demonstrated (Kreye,*et al.*, 1981) that furosemide, an inhibitor of $Cl^-$ transport, altered the steady state of $^{36}Cl$ exchange in rabbit aorta and produced a hyperpolarization. Kreye has suggested, based on these observations, that the relaxant action of NP in vascular muscle is induced by membrane hyperpolarization caused by inhibition of an active $Cl^-$ transport mechanism which is normally acting to maintain an elevated $[Cl^-]_i$.

The results presented here for the actions of NP on the portal vein are not in accord with this model of the mode of action of NP. The reduction in both spontaneous and induced activity of the portal vein, brought about by a reduced $[Cl^-]_o$ was of interest as it implies a role for $Cl^-$ in the initiation and maintenance of normal spontaneous contractions. Rangachari, Triggle and Daniel (1982) have shown in the longitudinal smooth muscle of the guinea-pig ileum that chloride removal selectively inhibited phasic contractions. They suggested this was due to alteration of a superficially bound $Ca^{2+}$ pool. Thus it may be seen that the anionic composition of the Krebs' solution can be of importance in determining the availability of $Ca^{2+}$ for excitation-contraction coupling. No reasonable explanation of the transient contraction of the portal vein response to 14mM $Cl^-$ or the maintained tonic contraction induced by complete chloride removal can be concluded from the results presented here, other than that $Cl^-$ removal had in some way altered membrane potential.

The observation that all the vasodilator agents tested were effective on responses of the portal vein induced in low chloride concentrations showed that $Cl^-$ removal was ineffective in inhibiting the relaxation thus induced. This observation suggested that $Cl^-$ was not implicated in the action of the vasodilators tested in the portal vein, in direct contrast to the observations of Kreye *et al.* (1977) in rabbit and rat aortae. The agents which were thought to cause hyperpolarization in portal vein (NP and GTN) may still act by this mechanism, but it seems unlikely that the putative hyperpolarization was induced by alteration of an active $Cl^-$ transport mechanism. Of the other suggested methods of hyperpolarization outlined by Kreye (1977) it seem unlikely that NP has its action in the portal vein by inhibiting an electrogenic $Na^+$ pump as it was found that incubating the preparation with ouabain (100μM) did not alter in any way the response to NP. However, $^{24}Na$ flux studies would provide more conclusive evidence on any involvment of $Na^+$ pumping or permeability changes. The observation that ouabain did not alter NP activity has also been demonstrated by Kreye *et al.* (1977) in the rabbit aorta.

The repetition of the observation that chloride removal in the rat aorta inhibited NP induced relaxation implied that in this preparation $Cl^-$ was linked in some way with the action of NP. The similar results obtained, but to a lesser extent, with GTN and TOLM also implicated $Cl^-$ in their mechanisms of action. VER was unaffected by reduction in

$[Cl^-]_o$ which suggested that the alteration of chloride concentration *per se* was not responsible for a general loss in vasodilator efficacy.

The ability of acidosis to induce vascular muscle relaxation both in normal and low chloride Krebs' solution suggested that if acidosis has its inhibitory action mediated by hyperpolarization then that alteration of membrane potential is not brought about by changes in $Cl^-$ handling. As acidosis produced inhibitory actions in the aorta in 14mM $[Cl^-]_o$ while the effect of NP was reduced, then if both these treatments relax vascular muscle by hyperpolarization it is unlikely that they share a common mechanism for inducing hyperpolarization.

It should also been noted that if NP and GTN produce hyperpolarizations in both arterial and venous muscle, as demonstrated in the rabbit by Haeusler and Thorens (1976) and Ito and co-workers (1980) then it may be that they produce this effect by differing mechanism in arteries and veins. It may also be the case that the differnces in activity of NP in low chloride conditions in the aorta and portal vein reflected the presence or absence of the active chloride transport mechanism which Kreye (Kreye,*et al.*, 1981) has suggested is present in the rabbit (and possibly the rat) aorta. If hyperpolarization is the mechanism of action of NP then the results presented and discussed here cannot cast any light on whether NP induced hyperpolarization could be due to alterations in $Na^+$ or $K^+$

which Kreye has suggested are the other possible mechanisms of hyperpolarization.

## The Action of Strychnine, Tetraethylammonium and 4-aminopyridine on Responses of the Portal Vein and Aorta.

### Introduction.

Since it has been shown that the spontaneous activity of the rat portal vein is selectively suppressed by agents or procedures which are thought to hyperpolarize vascular muscle and furthermore since Haeusler and Thorens (1977) have reported a hyperpolarization of the rabbit main pulmonary artery may be induced by strychnine (STRY), it was considered worthwhile to investigate the effects of STRY on rat portal vein in the expectation that it would cause a selective suppression of spontaneous activity. Surprisingly, a preliminary investigation showed STRY to enhance spontaneous activity in the portal vein. The form of the response to STRY on the portal vein resembled that previously reported for 4-aminopyridine (4-AP) by Leander *et al.* (1977) and for this reason 4-AP was included in these experiments. In addition tetraethylammonium (TEA) has been used since, like 4-AP, it has been shown to cause a blockade of potassium conductance in excitable membranes (Narahashi, 1974). The actions of these agents on the rat aorta were also investigated for the reasons outlined above. Results have been calculated and expressed in the manner described previously (Sections 1 & 2).

### Results. 1) Portal Vein.

For all three agents (STRY,TEA and 4-AP), the pattern

of activity observed was usually similar with concentrations of 0.1mM or below. This effect consisted of an increased amplitude and duration of individual phasic contractions with little change in frequency. When simultaneous electrical and mechanical recordings of portal vein activity were made by the perfused capillary method (Golenhofen & von Loh, 1970) this increase in mechanical activity was seen to be associated with a corresponding increase in electrical activity. A representative trace showing the action of STRY, TEA and 4-AP on the spontaneous mechanical and electrical activity of the portal vein can be seen in FIG. 60. As can be seen from this figure when the concentration of each agent was increased above 0.1mM then the responses to each agent became dissimilar. With TEA, there was a concentration dependent increase in amplitude and duration of contractions, as observed with lower concentrations, but the frequency of the contractions was reduced. With STRY, concentrations of up to 0.3mM gave responses similar to those seen with TEA, higher concentrations of strychnine (1mM) produced a continuous discharge of action potential associated with a small tonic contraction and a marked reduction in the phasic activity such that the integral of the mechanical response was smaller than that seen with 0.1mM (FIG. 61.) If a high concentration of STRY (1mM) was left in contact with the preparation for approximately 10 minutes, both the mechanical and electrical activities of the preparation declined to a low level. With 4-AP, concentrations in excess of 0.1mM often produced a tonic contraction as well as an increased frequency of phasic

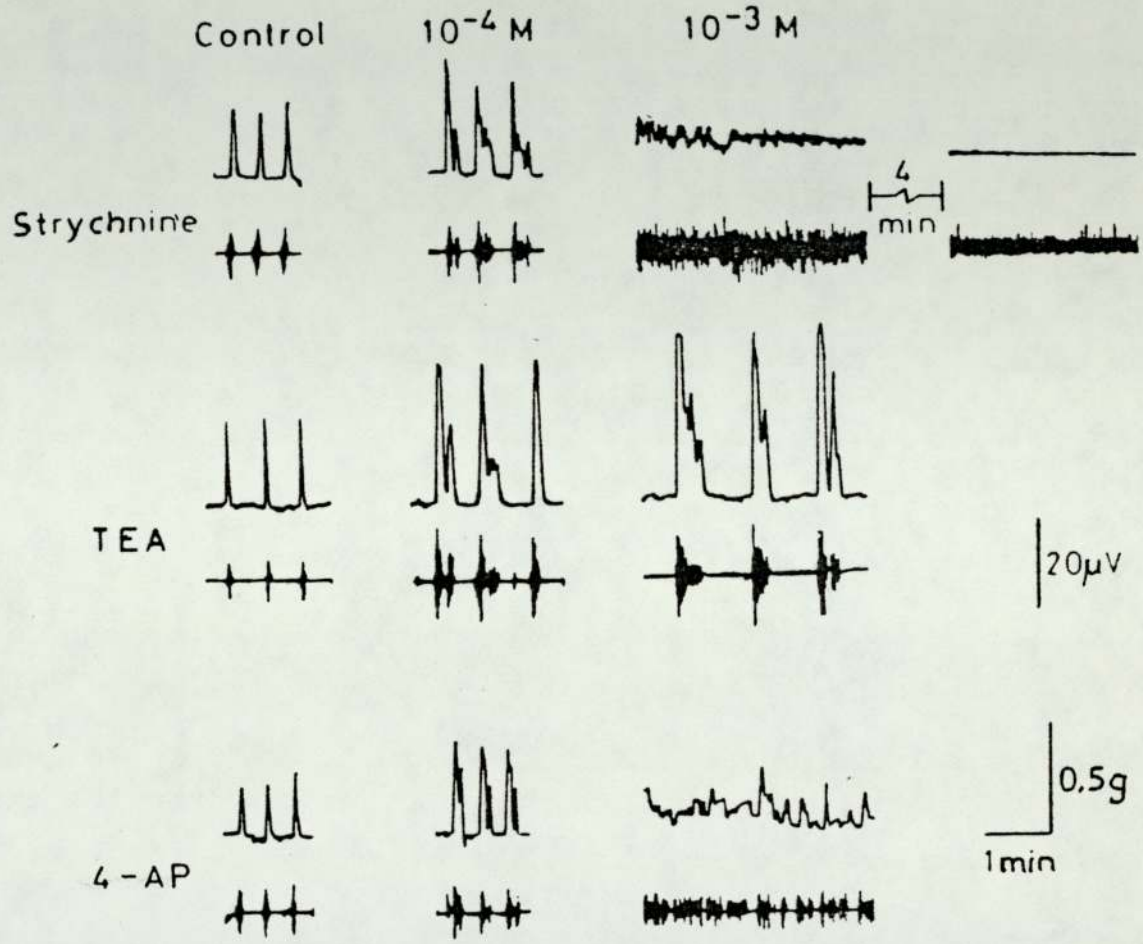



FIG. 60. Representative traces showing the actions of STRY, TEA, and 4-AP on mechanical (upper records) and electrical (lower records) activity of the rat isolated portal vein. Records shown were obtained following 2 min. contact with each agent in the concentration shown.

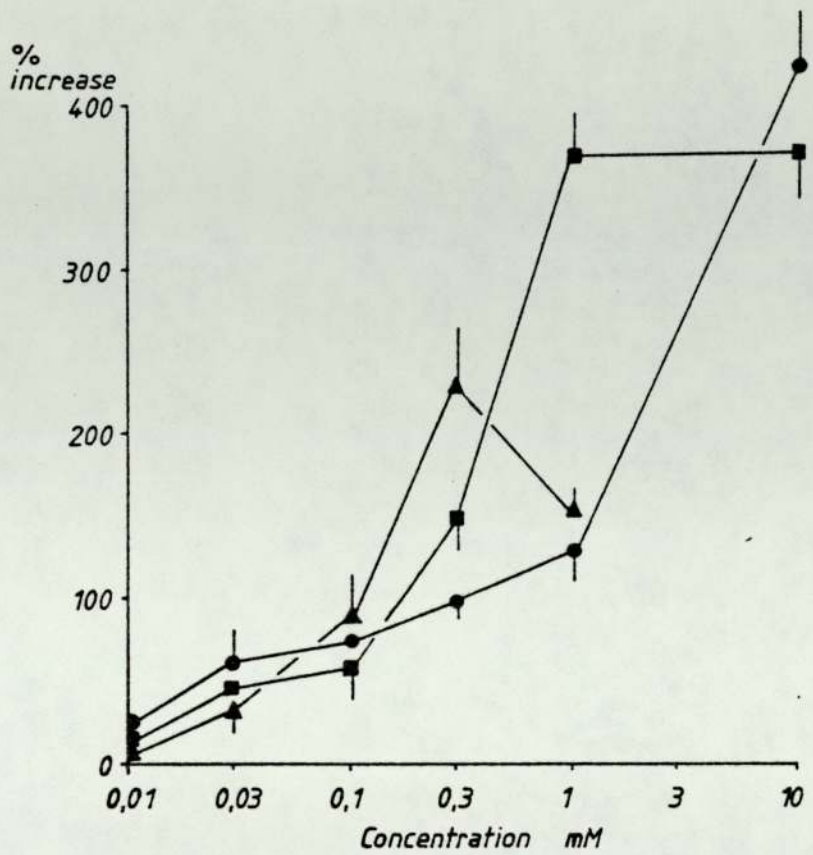



FIG. 61. Effects of STRY (▲), 4-AP (●) or TEA (■) on the percent increase in spontaneous activity of the rat isolated portal vein. Measurements were commenced 2 min. following the non-cumulative addition of each agent in the concentrations shown. Verticle bars represent s.e. mean.

contractions. A concentration vs. responses relationship for the action of these agents on the portal vein was constructed and can be seen in FIG. 61.

Preincubation with tetrodotoxin (TTX, 0.3uM) for 30 minutes was sufficient to prevent any response of the portal vein to field stimulation (6Hz) but had no significant effect on the stimulation of the myogenic activity caused by STRY (0.3mM). On the other hand, this concentration of TTX significantly reduced the stimulation seen with maximally effective concentrations of 4-AP or TEA (both 10mM) (FIG. 62.) The $alpha_1$-adrenoceptor antagonist prazosin (50nM), left in contact with the preparation for 45 minutes, did not significantly reduce the stimulant action of 0.3mM STRY (4.4% +/- 4.6% reduction, n=6) but significantly reduced by 85% +/- 3.0% (n=6) the stimulant effect of a concentration of NA (1uM) which produced an increase in spontaneous activity (integrated value) comparable to that seen with STRY.

The excitatory action of STRY on the portal vein was reduced following 30 minutes pretreatment with selective concentrations of the calcium entry blocker VER (FIG. 63.). VER produced a signifcantly greater inhibition of the excitation produced by STRY than that produced by an equi-effective concentration of NA ($p<0.05$, n=6).

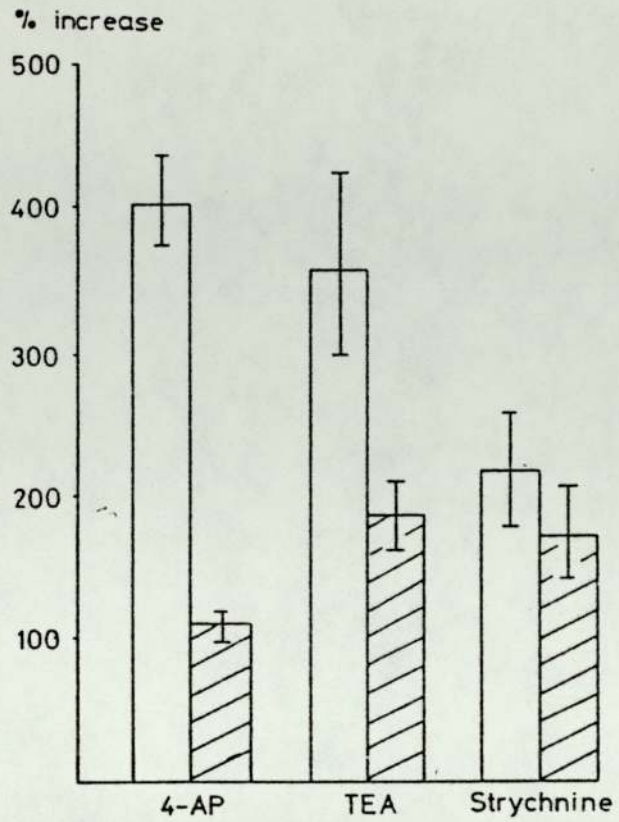



FIG. 62. Effects of tetrodotoxin on the percentage increases in mechanical activity seen with maximally effective concentrations of 4-AP (10 mM); TEA (10 mM) or STRY (1 mM). Open columns are stimulated controls; hatched columns are following 30 min. contact with tetrodotoxin (0.3 μM). Verticle bars represent s.e. mean.

Action of VER on STRY and NA Stimulated Spontaneous Activity of the Rat Portal Vein. (Mean ± s.e., n=6).

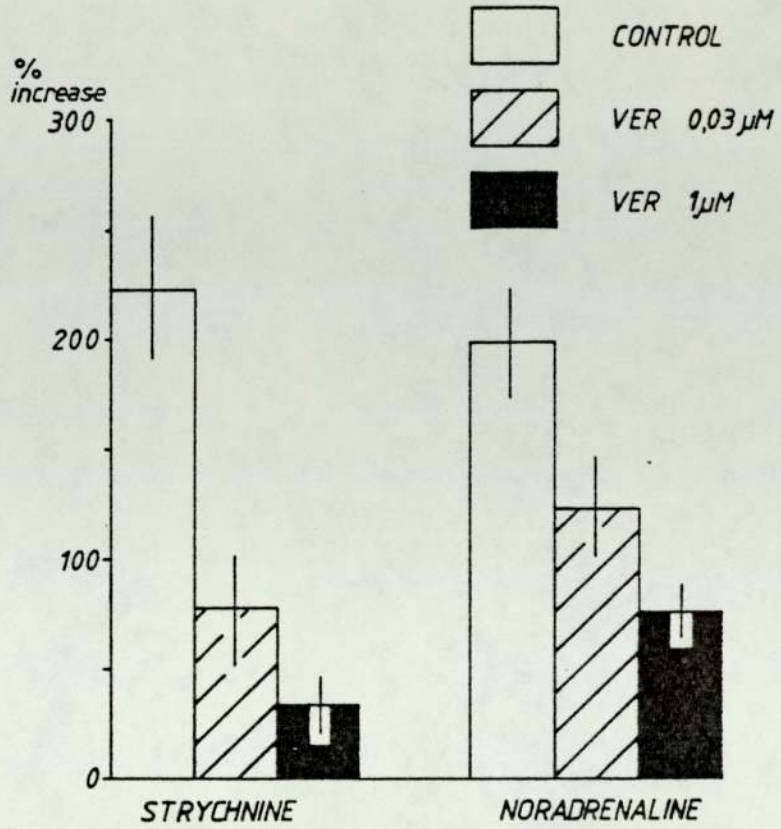



FIG. 63. Effect of VER, in the concentrations shown, on the percentage increases in mechanical activity of the rat isolated portal vein to STRY (0.3 mM) or noradrenaline (1 µM). The inhibition seen with each concentration of VER was significantly greater for STRY than noradrenaline ($p < 0.05$). Verticle bars represent s.e. mean.

Results. 2)Aorta.

STRY caused a concentration dependent inhibition of NA induced phasic activity of the aorta; 0.3mM STRY caused a complete suppression of activity whether the agent was added in a cumulative manner or as a single concentration. A representative trace of this inhibitory action of STRY can be seen in FIG. 64. Both TEA and 4-AP caused concentration dependent increases in activity, representative traces of these actions can be seen in FIG. 65. A concentration response relationship was calculated for the action of theses agents on the induced phasic activity of the rat aorta and can be seen in FIG. 66. Concentrations of 4-AP above 1mM caused a large contracture and consequent disappearance of phasic activity. When the action of these agents on tonic contractions of the rat aorta induced by KCl (60mM) were investigated a similar profile of action was discovered; STRY produced a marked relaxation of the KCl response while TEA and 4-AP both produced small increases in tone. A representative trace showing the actions if STRY, TEA and 4-AP on KCl induced contractions of aorta can be seen in FIG. 67.

*Action of STRY on Induced Phasic Activity of the Rat Aorta.*

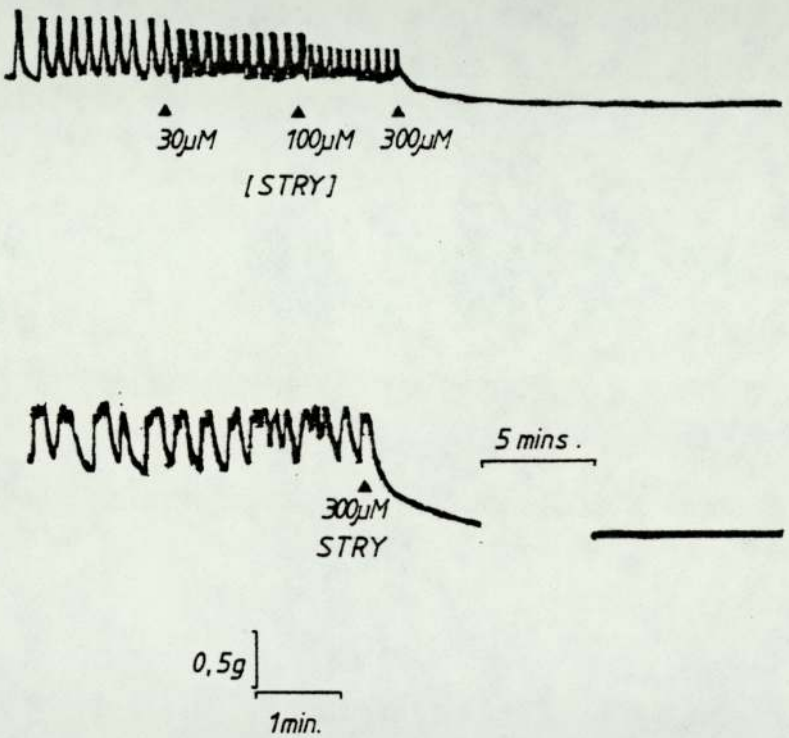



FIG. 64. Representative traces showing the effect of STRY on the noradrenaline induced phasic activity of the rat isolated aorta.

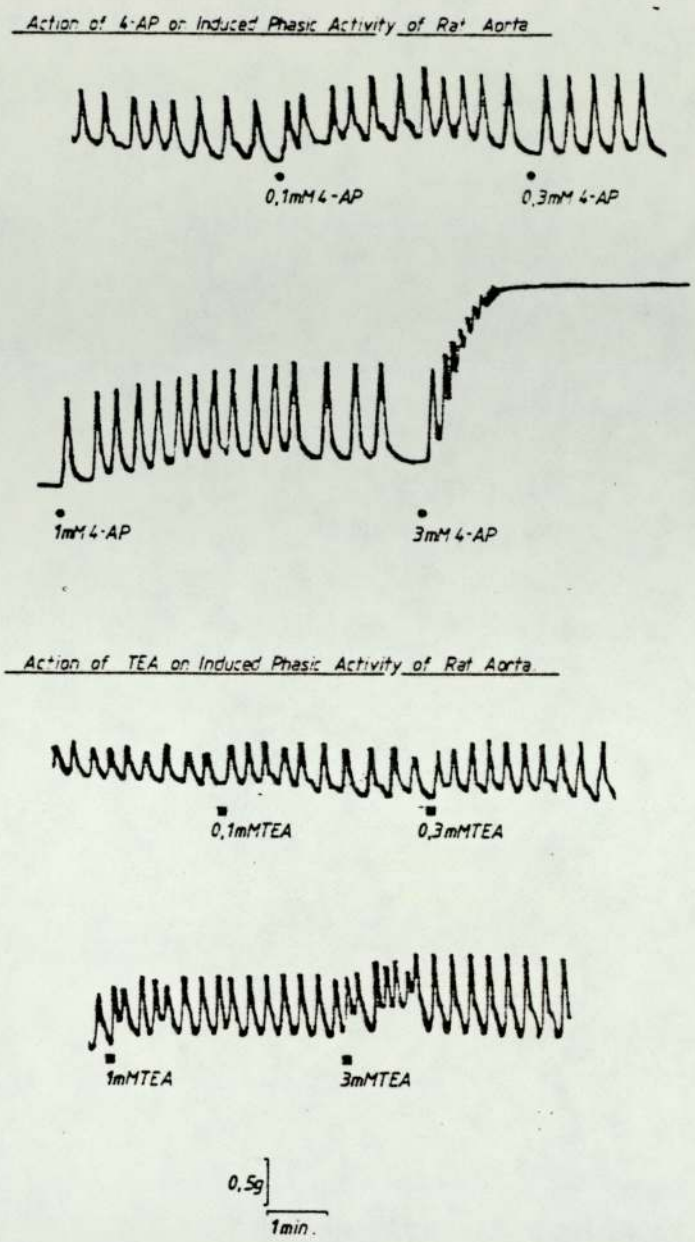



FIG. 65. Representative traces showing the actions of 4-AP (upper panel) or TEA (lower panel) on the noradrenaline induced phasic activity of the rat isolated aorta.

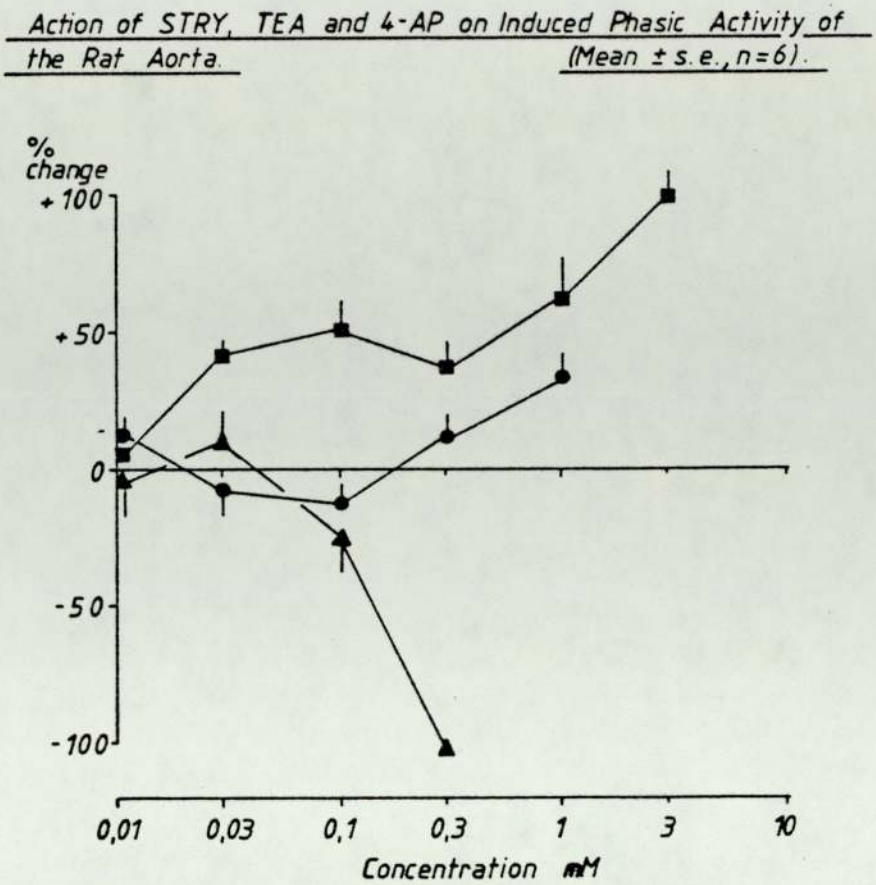



FIG. 66. Effects of STRY (▲); 4-AP (●) or TEA (■) on the percent increase or decrease in phasic activity of the rat isolated aorta induced by noradrenaline (30 nM). Measurements were commenced 2 min following the non-cumulative addition of each agent in the concentrations shown. Verticle bars represent s.e. mean.

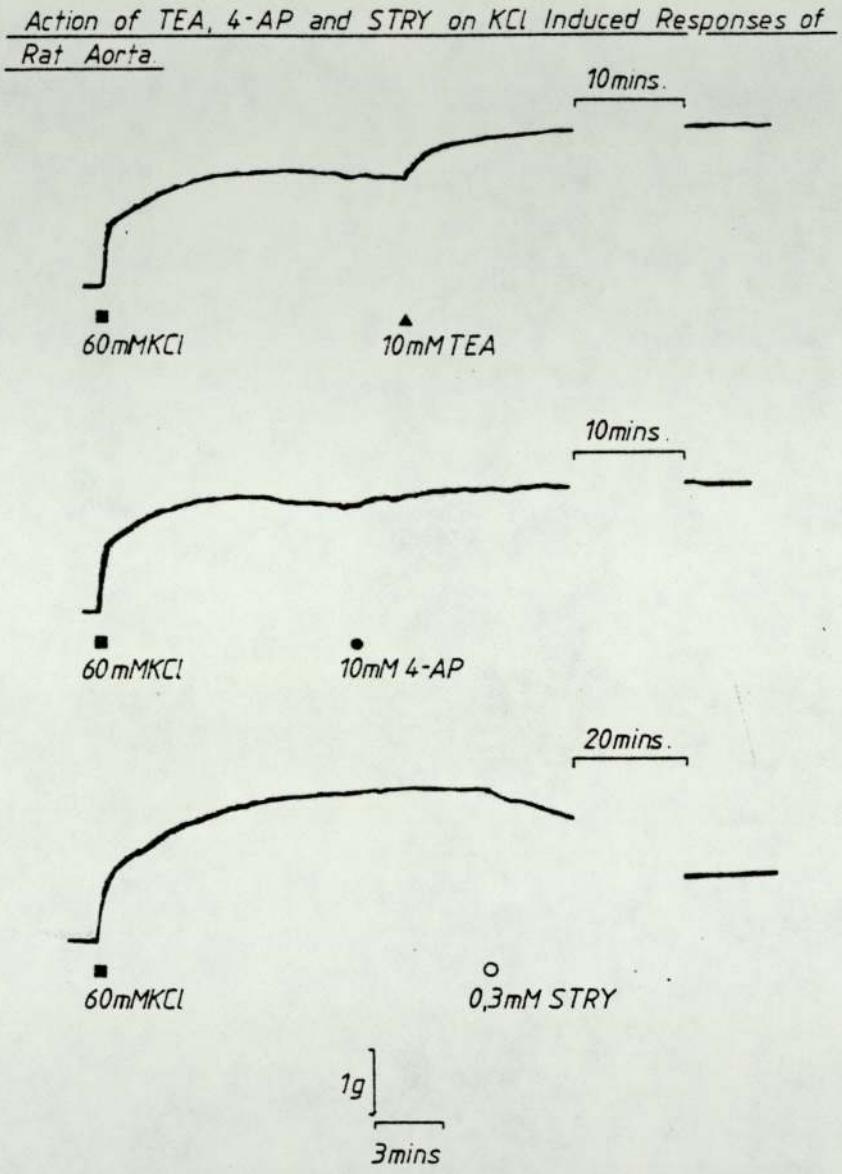



FIG. 67. Representative traces showing the stimulatory actions of TEA (upper record) or 4-AP (middle record) and the inhibitory action of STRY (lower record) on KCl induced contractions of the rat isolated thoracic aorta.

Discussion.

Only inhibitory actions of STRY on vascular muscle have been reported previously (Haeusler & Thorens, 1977; Haeusler, 1978), whereas there have been a number of reports of direct stimulant actions of both TEA and 4-AP on vascular muscle (Haeusler & Thorens, 1980; Leander, *et al.*, 1977; Mekata, 1971). These actions of TEA and 4-AP are generally considered to depend wholly or partly on their ability to selectively block potassium conductance of the cell membrane, an effect which has been the subject of detailed study in nerve and skeletal muscle (Hille, 1967; Stanfield, 1970; Yeh *et al.*, 1976). Since Shapiro and co-workers have shown that STRY blocks potassium conductance in nerve (Shapiro *et al.*, 1974; Shapiro 1977a) in a manner resembling that of TEA, it would seem likely that the excitatory action of STRY described here could reflect an ability of STRY to reduce potassium conductance in vascular muscle. On the other hand it is necessary to explain why STRY was able to stimulate the portal vein and yet, unlike TEA or 4-AP, it had only inhibitory actions on the aorta. One possibiltiy is that a hyperpolarizing action of STRY, as observed on the rabbit main pulmonary artery by Haeusler and Thorens (1977), may be responsible for the inhibition of the aortic responses. The depressant effects seen with high concentrations of STRY on the portal vein may also have been due to membrane hyperpolarization. Alternatively, these depressant actions of STRY on both preparations may be

related to an unselective membrane depression since Shapiro (1977b) has found it can reduce sodium conductance in nerves in a similar menner to procaine. Another possibility, is that the mechanism of the stimulant action of STRY may be dissimilar to that of TEA or 4-AP such that it may only be observed on muscle which normally shows spontaneous contractions.

A direct action of STRY on the smooth muscle is indicated since its excitatory actions were unaffected by TTX or prazosin. The observation that the effects of TEA and 4-AP were sensitive to TTX suggests that at least part of their stimulant actions depends upon the initiation or potentiation of action-potentials in intra-mural adrenergic nerves.

Since the excitatory action of STRY was sensitive to blockade by VER, it seems likely that STRY causes an increase in calcium conductance. In this context it is pertinent to note that Ramos (1974) has claimed an excitatory action of STRY on mammalian cerebral cortex was mediated by an increase in calcium conuctance. Some increase in calcium conductance would be secondary to the depolarization following any reduction in potassium conductance. It is interesting to note that following a detailed study of the actions of TEA on vascular muscle, Haeusler and Thorens (1980) have concluded that low concentrations of TEA increase calcium conductance more than was likely to be explained by a concomitant reduction in

potassium conductance.

The inhibitory action of STRY on both phasic and tonic activity of the rat aorta was in accord with previously reported actions of STRY on arterial muscle (Haeusler & Thorens, 1977; Haeusler, 1978). The stimulant action of TEA and 4-AP on the rat aorta was also in accord with the reported actions of these agents in vascular muscle (Haeusler & Thorens, 1980; Leander, *et al.*, 1977; Mekata, 1971). It is possible that the inhibitory action of STRY on the rat aorta was due to a membrane hyperpolarization such as that observed by Haeusler and Thorens (1977) in the rabbit main pulmonary artery. The stimulant action of TEA and 4-AP could be due to their known actions in blocking potassium conductance of the cell membrane (Haeusler & Thorens, 1980). It is apparent that the precise mode of action of the stimulant action of STRY described here must await a detailed electrophysiological study.

General Discussion.

1. Vasodilator Action and Clinical Consequences.

The directly acting vasodilators used in this study have all been seen to cause relaxation of rat vascular muscle to a greater or lesser extent. A similar direct relaxation of human vascular muscle *in vivo* is the basis of the clinical usefulness of these agents in the treatment of cardiovascular disease. The degree to which the rat preparations mimic the responses of human vascular muscle in various physiological and patho-physiological states is in considerable doubt but a comparison of vasodilator activity in the two systems has some merit.

In Section 5. (Results & Discussion) an attempt was made to classify the vasodilators used in this study on the basis of their actions on the responses of the rat portal vein. The two part classification of vasodilators made with respect to the results presented in Section 4., was i) those drugs which caused a selective suppression of the spontaneous activty of the portal vein; and ii) those drugs which showed no selectivity of inhibition. The drugs which showed a selective inhibition of the spontaneous activity were NP and GTN, both of which had previously been classified as 'nitrate vasodilators' (Needleman *et al.*, 1973) and as such considered to have similar mechanisms of action by Needleman and collegues (1973). TOLM also showed a selective suppression of spontaneous activity although it

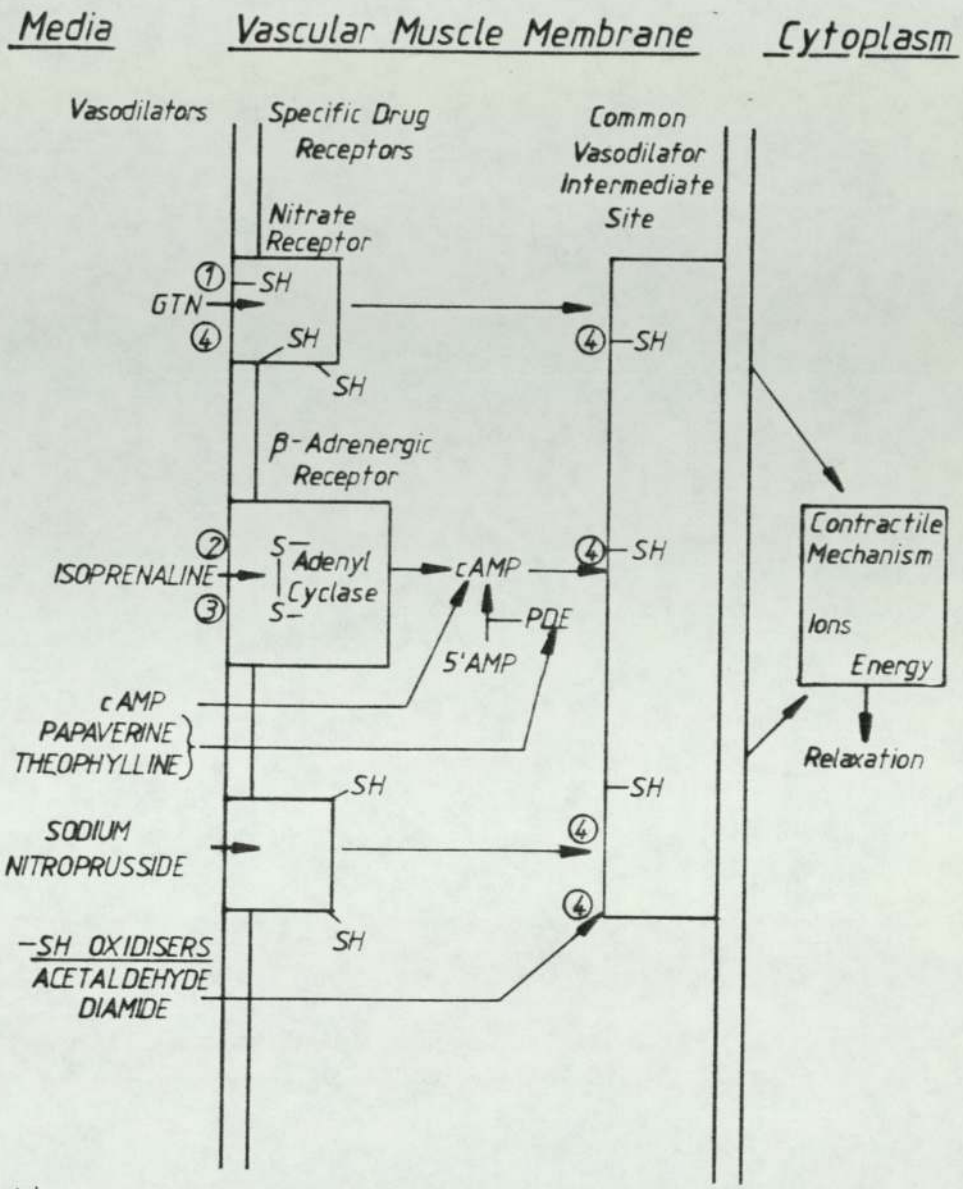
is an aryl-sulphoxide derivative and not a nitrate. The agents which exhibited no selectivity of action were VER, DIAZ and HYD. VER has its vascular muscle relaxant action by inhibiting $Ca^{2+}$ fluxes across cell membranes (Henry, 1980) and a similar, though less well defined, action has been suggested for DIAZ (Wohl, *et al.*, 1968; Janis & Triggle, 1973). Similarly McLean (1978) has proposed a $Ca^{2+}$ entry blocking action for HYD. However this action of HYD has been contested by Chevillard and co workers (1980) and Trapani *et al.* (1980) who have suggested it acts by interacting with vasoactive endogenous purines. It is possible that the classification of vasodilators by their actions on the end organ responses of the rat portal vein may be a reflection in gross differences in mechanism of action. If the underlying mechanisms of action of these drugs does produce these diverse spectra of action it would appear that drugs acting directly on $Ca^{2+}$ handling produce a uniform reduction in various parameters of reactivity which probably reflects the very high dependence of the rat portal vein on extracellular $Ca^{2+}$ as a source of activator $Ca^{2+}$ (Johansson *et al.*, 1967).

A putative explanation of the mechanism of action of GTN and NP was put forward by Needleman and co-workers (1973) on the basis of a model of tissue response to nitrates (Fig.68). Needleman (1972) showed that the intact GTN molecule was necessary for vasodilatation and explained this by a hypothetical GTN-'nitrate receptor' interaction. It was also suggested that the GTN-'nitrate receptor'

interaction was the source of GTN tolerance displayed by vascular muscle both *in vivo* and *in vitro*, the tolerance being due in some way to receptor desensitization. The action of NP on a 'nitrate receptor' is in much greater doubt as there is no known desensitization effect exhibited by NP. By comparing the profile of action on NP and GTN with procedures likely to cause hyperpolarization of rat vascular muscle it has been suggested (and supported by separate observations by other workers; Kreye, 1980; Kreye, *et al.* 1977 & 1981) that the common basic mechanusm of these two agents is membrane hyperpolarization.

More recent work (Kreye, 1980) has suggested that the common mechanistic link between GTN and NP (and another 'nitrate' vasodilator isosorbide dinitrate) is induced by alteration of the membrane $Cl^-$ handling. Whatever the underlying mechanism of action is it would appear, both from published data and results presented here, that GTN and NP probably share a common mode of action. The demonstrated similarity in the actions of TOLM to those of GTN and NP may indicate a similar mechanism of action for this agent which may warrent further investigation.

The relative actions of the vasodilator drugs on rat portal veins aortae were used to assess arterio- or veno-selectivity. As the haemodynamic consequences and therapeutic use of any vasodilator are to some extent determined by arterio- or veno-selectivity *in vivo* a comparison of results obtained on the rat preparations with




Schematic representation of interaction between vasodilators and tissue componants bringing about vasodilatation. Abreviations: --SH sulphhydryl Grouping; PDE phosphodiesterase enzyme; 1. Blocked by GTN tolerance; 2. Blocked by isoprenaline tolerance; 3. Blocked by propranolol; 4. Blocked by ethacrynic acid.

FIG. 68. Putative mechanism of action of the 'nitrate' vasodilators proposed by Needlemen (1973)

published data for human vessels was made.

As discussed previously, the responses of the rat portal vein were not inhibited to a uniform extent by the addition of all the vasodilators, for example NP selectively inhibited spontaneous activity while having little or no action of KCl induced responses. Bearing this in mind it can be seen that each parameter of portal vein responsiveness should be considered in selecting a measure of this vessels sensitivity to relaxation by vasodilators.

When the results obtained in this study were compared with thos publishe dfor human vessels by Collier and co-workers (1978) and Robinson *et al.* (1979) it was found that the action of vasodilators on the spontaneous activity of the portal vein most closely resembled the action of these agents on human hand veins. If the rat portal vein were to be included in an initial screening method for noval vasodilators, as has been suggested by Mackenzie and Parratt (1977), then this study would suggest that spontaneous activity would be the most useful parameter to measure for predictive purposes.

One problem encountered in the rat/human comparison is the question of how representative human hand veins are of human venous muscle in general. When the results obtained by Collier *et al.* (1978) and Robinson *et al.* (1979) for the action of vasodilators on hand veins were compared with what is known of the general haemodynamic properties of these

agents (Opie, 1980; Brown, 1980; Cohn & Franciosa, 1977; Parratt, 1975) then there is seen to be general accord in the findings. Thus although human hand veins are not an ideal system to test vasodilator agents they provide a reasonably accurate model of the general behaviour of venous muscle.

When these comparitive studies were extended to encompass the comparison of arterio- and veno-dilator potency then there was a marked reduction in the degree of agreement between the results obtained in this study and those published by Collier (1978) and Robinson (1979). From the results presented here it would appear that the rat aorta was a poor model for vasodilator action in the human forearm arteriolar bed. The rat aorta appeared to be considerably more sensitive to NP than were the *in vivo* preparations of Collier *et al.* (1978) and Robinson *et al.* (1979) who calculated that in human forearm arteriolar beds VER was 16 times more potent than NP in inducing relaxation whereas in this study, in the rat aorta, NP was 56 times more potent than VER.

Similarly when arterio-venous comparisons were made the results obtained in this study were at considerable variance with those of Collier *et al.* (1978) and Robinson *et al.* (1979). Thus on the whole, as discussed previously, that rat aorta and portal vein make a poor model for predicting Human vasodilator activity as reflected by the responses of human hand veins and forearm arteriolar beds. However, it

should be born in mind that the experimental measurement of vasodilator activity in human preparations may not accurately reflect vasodilator action on the entire human circulation, and thus that the testing of agents in healthy human subjects may not produce similar effects to those seen in patients. For example, Collier *et al.* (1978) showed NP to be 6 times more potent in relaxing human hand veins than human forarm arterioles while Opie (1980) suggested that NP to be the drug of choice in acute severe low-output left-sided heart failure because he saw it as having a balanced effect; equally dilating both arterioles and veins. The advantages of testing vasodilators in human tissues or subjects are obvious in that no species or gross metabolic differences between experimental and clinical situations should occur, however isolated animal tissues have the great benefits of low cost and ease of use and thus have considerable value for obtaining an initial 'first-order' estimate of vasodilator activity.

## 2. Vascular Muscle Reactivity and Vasodilators.

Vascular muscle tone is the main determinant of distribution of blood throughout the body and thus the consequences of altered vascular muscle reactivity can have profound effects on the prevailing haemodynamic situation. Vasodilators are drugs which can relax vascular muscle and as such can alter blood distribution. The first step in understanding the mode of action of directly acting vasodilators is to elucidate how they interact with the physiological processes which control vascular muscle tone as it is these mechanisms which must be influenced to bring about relaxation.

In vascular muscle it is generally accepted that the contractile proteins are stimulated to increase tension by a rise in $[Ca^{2+}]_i$ and consequently any agent or procedure which inhibits or reduces $[Ca^{2+}]_i$ will reduce vascular muscle tone (Endo, *et al.*, 1977; Gordon, 1978). There are several methods by which vasodilators could alter $[Ca^{2+}]_i$; i) inhibition of $[Ca^{2+}]$ ; ii) stimulation of $Ca^{2+}$ removal from the cell; iii) stimulation of calcium sequestration by intracellular stores or organelles; iv) inhibition of the processes linking raised $[Ca^{2+}]_i$ to the machinery of muscular contraction. A directly acting vasodilator could interact with any of the mechanisms which link $Ca^{2+}$ with activation of vascular muscle (excitation-contraction coupling), as outlined above.

Factors other than direct interference with intracellular $Ca^{2+}$ handling could alter vascular muscle reactivity via an indirect action on excitation-contraction coupling, thus an agent or procedure need not alter $[Ca^{2+}]_i$ directly but could have a 'knock on' effect by altering some factor which is linked to the control of $[Ca^{2+}]_i$. One factor which has long been known to alter vascular muscle reactivity is the ionic mileu of the smooth muscle cell. Tobian and co-workers (1959) have shown that changes in $[H^+]$ could alter vascular muscle reactivity to NA stimulation. Siegel *et al.* (1976 a & b) have shown, in dog carotid artery, that changes in $[H^+]$ cause changes in vascular muscle membrane potential, alkalosis causing depolarization and contraction and acidosis causing hyperpolarization and relaxation. Somlyo and co-workers (1968, 1976) have suggested that changes in membrane potential induced by alteration of $[H^+]$ could be due to changes in the membrane properties or in the local concentrations of ions around the membrane. Siegel *et al.* (1978) have shown that $K^+$-binding in dog carotid artery increases with increased $[H^+]$ over the pH range of 4 to 6.8 and then falls as pH becomes more basic. Furthermore Siegel has suggested (Siegel, *et al.*, 1978) that these effects may be explained by competitive binding between $K^+$ and $Ca^{2+}$ for the same sites in connective tissue. At basic pH, $Ca^{2+}$-binding is increased and as $Ca^{2+}$ has a higher affinity for the sites, $K^+$ is displaced. At pH 7.4 to acidity $K^+$ is bound to the connective tissue, when pH rises then $K^+$ is released and $Ca^{2+}$ is bound. If this is indeed the case then during alkalosis the extracellular $[K^+]$ may

increase in the vicinity of the vascular muscle cells sufficient to cause a depolarization and contraction, during acidosis the $[K^+]$ will be reduced causing a hyperpolarization and relaxation, as originally observed by Tobian and co-workers (1959). It is possible that a directly acting vasodilator could in some way alter the binding of either ion on the connective tissue, this could alter membrane potential indirectly and subsequently alter vascular muscle reactivity as outlined above.

A more direct interaction between vascular muscle cell potential and reactivity has been demonstrated by Siegel and Schnieder (1981) for alteration of $[K^+]_o$ in the dog carotid artery; Konold and co-workers (1968a & b) had shown that alteration of $[K^+]_o$ over a physiological range (2 - 6 mM) caused an alteration of tone in bovine facial artery and Nguyen-Duong *et al.* (1977) suggested that the changes in tone observed during alteration of $[K^+]_o$ are due to altered membrane potential. Siegel and Schnieder (1981) demonstrated that a relationship does exist between membrane polarization,

$[K^+]_o$ and tension in the dog carotid artery. They noted that the lowest tension was found at levels of $[K^+]_o$ which produced the greatest hyperpolarization and that both increasing and decreasing the $[K^+]_o$ from this level caused a depolarization and contraction. Links between vascular muscle reactivity and the concentration of $Mg^{2+}$ and $Cl^-$ ions, membrane polarization and ion binding have been suggested and are discussed in the introduction (Sections

4iii & iv).

Results presented in this study have shown that independent alteration of the concentrations of $Ca^{2+}$; $Mg^{2+}$; $Cl^{-}$; $H^{+}$ and $K^{+}$ can alter the reactivity of vascular muscle. The actions of altered $[H^{+}]$ and $[K^{+}]$ have been discussed above. As expected lowering the $[Ca^{2+}]_o$ caused an inhibition of responses of the portal vein. Johansson *et al.* (1967); Sigurdsson and co-workers (1975) and Ebeigbe (1982) have all shown a similar dependence upon $[Ca^{2+}]_o$ to maintain contractions and spontaneous activity in the rat portal vein, although under certain circumstances a superficially bound $Ca^{2+}$ store may be important (Ebeigbe, 1982). The sensitivity of this tissue to the $Ca^{2+}$ entry blocker, VER, is probably due to VER's ability to inhibit rises in activator $Ca^{2+}$ due to $Ca^{2+}$ influx.

The mechanisms by which $Mg^{2+}$ has its diverse actions in the portal vein are discussed in detail above (Results Sect.3.). The two major hypotheses to explain the actions of $Mg^{2+}$ are; i) that it directly competes with $Ca^{2+}$ for binding sites in membrane stores (Altura & Altura, 1978a) and ii) that increased $[Mg^{2+}]_o$ inhibites smooth muscle by causing a membrane hyperpolarization (Sigurdsson & Uvelius, 1977). Theoretically therefore, vasodilatation could be produced by agents which mimicks or interfers with the binding of $Mg^{2+}$

From the results obtained in this study with $Mg^{2+}$ and

$Ca^{2+}$ and from the published data discussed above it is obvious that ionic mileu can profoundly alter vascular reactivity and that the interaction of drugs with ions is a possible mechanism of vasodilator action. In this study it was not possible to measure membrane potential or ion fluxes directly, however it was thought worthwhile to attempt to investigate by less direct methods, the actions of directly acting vasodilators in the light of the possible mechanisms outlined above. During the initial assessment of vasodilator efficacy and action carried out on the portal vein, it was found that a classification of vasodilators by their spectrum of action was possible. The basis of the classification being the ability to selectively inhibit spontaneous activity. An attempt was made to find a link between the underlying mechanisms of action of the vasodilators tested and their spectrum of action on the end organ responses of vascular muscle.

The agent found in this study to be the most selective inhibitor of spontaneous activity in the portal vein was NP. Hausler and Thorens (1976) have shown that NP caused a dose dependent hyperpolarization of rabbit main pulmonary artery associated with muscular relaxation. It was therefore considered worthwhile to investigate other procedures known to cause hyperpolarization in vascular muscle in attempt to gain circumstancial evidence for any link that might exist between membrane hyperpolarization and selective suppression of spontaneous activity in the portal vein. The results obtained in these experiments have been discussed previously

(Results & Discussion Section 11.), they showed that acidosis (Siegel *et al.*, 1976a & b), reduction of $[K^+]_o$ (Siegel & Schnieder, 1981) and increased $[Mg^{2+}]_o$ (Sigurdsson & Uvelius, 1977) (all procedures shown to hyperpolarize vascular muscle) caused a selective inhibition of spontaneous activity in the rat portal vein.

Kreye (1980) has suggested that NP could bring about a hyperpolarization in at least four different ways; i) by activating an electrogenic $Na^+$ pump, ii) by increasing the $K^+$ permeability of the cell membrane, or iii & iv) by reducing membrane permeability to $Na^+$ or $Cl^-$. Kreye *et al.* (1977 & 1981) have shown that in rabbit aorta $Cl^-$ may be implicated in the mechanism of action of both NP and GTN. In this study it was observed that in rat aorta reduction of $Cl^-$ inhibited the action of NP while no such interaction was noted in the portal vein. The results presented here and discussed more fully above (Section 12., Results and Discussion.) do not rule out the possibility that hyperpolarization is the mechanism of selective suppression of spontaneous activity in the rat portal vein but they do suggest that any drug induced hyperpolarization observed in this study was not dependent upon $[Cl^-]$.

During the investigation of hyperpolarizing events and vascular muscle activity the actions of STRY were investigated since Hausler and Thorens (1977) had reported a STRY induced hyperpolarization of vascular muscle. When the actions of STRY, as well as those of TEA and 4-AP, were

investigated on the portal vein and aorta it was found that STRY had inhibitory actions on both induced phasic and tonic responses of the aorta and also inhibited the phasic activity of the portal vein in high concentrations. At lower concentrations STRY stimulated tha spontaneous activity of the portal vein in a manner similar to TEA and 4-AP. These results are discussed at length elsewhere (Section 13, Results & Discussion) but it may be that the inhibitory actions of STRY are due to hyperpolarization while its stimulant actions are dependent upon a completely different mechanism.

The interest of all the observations discussed above, and elsewhere, is that they show how readily drugs can have actions upon vascular muscle reactivity by interacting with the processes which control excitation-contraction coupling. If, as is widely accepted, $Ca^{2+}$ acts in vascular muscle to determine the contractile state it can be seen from the above results that the activator $Ca^{2+}$ can be modified in a number of ways. Also, although directly acting vasodilators do not all have immediate effects on $Ca^{2+}$, as for example do agents selectively blocking $Ca^{2+}$ entry, it should be born in mind that any drugs acting on vascular muscle may ultimately alter activator $Ca^{2+}$ and consequently the contractile state, via numerous alternative pathways.

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