THE UNIVERSITY OF ALBERTA

STIMULATION OF PERIPHERAL VASCULAR BETA-ADRENERGIC RECEPTORS BY NEURONALLY RELEASED NORADRENALINE

by

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ABSTRACT

The role of noradrenaline as a neurotransmitter in the sympathetic nervous system has been well documented. In the peripheral vascular bed noradrenaline released by stimulation of sympathetic nerves causes vasoconstriction and this response is mediated through alpha adrenergic receptors. Stimulation of beta adrenergic receptors produces vasodilatation. It has been suggested that neurotransmittors released from sympathetic nerve terminals did not act on vascular beta adrenergic receptors, although these receptor sites were accessible to blood borne noradrenaline.

The investigations described herein were designed to study the effects of neuronally released noradrenaline on peripheral vascular beta adrenergic receptors. Using the whole hind limb vascular bed of dogs, noradrenaline and tyramine were infused intra-arterially during alpha receptor blockade with phentolamine. Phentolamine reduced the vasoconstrictor response to tyramine, which causes the release of noradrenaline from sympathetic nerve terminals, but the response was not converted to a vasodilalation as was the case with noradrenaline during alpha receptor blockade.

These experiments were repeated with skin and paw flow occluded. It was found that prolonged (15 minute) infusions of tyramine produced a significant vasodilatation in the isolated muscle vascular bed of the dog hind limb. Further experiments were conducted to investigate the response to prolonged (15 minutes) reflex stimulation of the sympathetic nerves by occluding the carotid arteries bilaterally.

Evidence was obtained that endogenous or neuronally released noradrenaline did exert a significant action on peripheral vascular beta receptors and that these receptors are innervated by the sympathetic nervous system. Furthermore, the results suggest that neuronally released and blood borne noradrenaline are acting on similar receptor populations. The magnitude of the beta receptor mediated response depends on the amount of transmitter accumulating at the receptor sites, and this is dependent on the period of stimulation.

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GENERAL INTRODUCTION

One of the pioneers of the hypothesis of chemical transmission in the sympathetic nervous system, T.R. Elliot (1905)[1] compared the effects of adrenaline on a number of organs in a number of species and noted adrenaline had similar effects to sympathetic nerve stimulation. Others, such as Barger and Dale (1910)[2] observed that the action of noradrenaline mimicked stimulation of sympathetic nerves more closely than did the action of adrenaline.

Cannon and Rosenbluth (1933)[3] noting differences in the response of adrenaline and that substance released on stimulation of sympathetic nerves, suggested the chemical should be called sympathin to distinguish it from adrenaline. However, believing the transmittor to be adrenaline, they postulated that adrenaline produced excititory effects by combining with another substance from the organ being stimulated to form sympathin E. Inhibitory effects were caused by Sympathin I formed from adrenaline and another substance from the organ being inhibited by sympathetic nerve stimulation. Bacq (1934)[4] suggested that "sympathin E" was noradrenaline.

In 1946, the work of Von Euler[5] showed the presence of a substance in extracts of heart, spleen and sympathetic nerves which had chemical and biochemical properties which resembled noradrenaline but were distinct from those of adrenaline. Since this work of Von Euler, noradrenaline has been widely accepted as the adrenergic chemical transmitter in the sympathetic nervous system.

In 1948, Ahlquist[6] proposed the concept of alpha and beta adrenergic receptors to explain the mediation of excitation and inhibition of various effector cells as determined by their response to a series of sympathomimetic amines. The alpha receptor is associated

with most excitatory functions: vasoconstriction, contraction of the uterus, nicitating membrane, ureter; and dilatation of the pupil . . . and one inhibitory function--intestinal relaxation. Vasodilation, inhibition of bronchial and uterine musculature; and one excititory function (myocardial stimulation) are functions associated with the beta adrenergic receptor. Beta adrenergic receptors have been further classified as beta-1 receptors and beta-2 receptors as a result of observations which suggest significant difference between myocardial beta adrenergic receptors and vascular beta adrenergic receptors. Beta receptors in the myocardium have been classified as beta-1 receptors whereas those in the vascular smooth muscle have been classified as beta-2 receptors (7), (8), (9), (10).

This thesis deals with the question can sympathetic nerve endings produce stimulation of vascular beta adrenergic receptors and if so,what physiological role does this play in producing active vasodilatation in the peripheral circulation ?

It has been demonstrated in the human forearm[11] and in the perfused hind skeletal vascular bed of the dog leg[12] that the constrictor effect of adrenaline can be reduced or abolished by blockade of alpha adrenergic receptors with phentolamine. As well, after alpha blockade with phentolamine, an intra-arterial dose of noradrenaline produces vasodilatation, which is due to stimulation of beta-adrenergic receptors since the response is abolished by beta blockade with propranolol. Glover and Hutchison[13] (1965) showed that the vasoconstrictor response to circulating noradrenaline was potentiated in the forearm by the pharmacological blockade of beta receptors. Brick, Hutchison and Roddie[14] (1966) demonstrated that after blockade of alpha receptors with phentolamine, infusions

of noradrenaline produced vasodilatation. The vasodilatation response was abolished after the infusion of propranolol. These experiments and others (Brick et al, 1967)[11] indicated that the action of noradrenaline on the forearm vessels is normally a summation of a predominant vasoconstrictor or alpha receptor action and a weaker dilator or beta receptor action.

A method of reflexly stimulating sympathetic nerves in humans was described by Greenfield[15] et al (1963). The method involved exposing the lower half of the body to subatmospheric pressure. This caused an adrenergically mediated vasoconstriction in the forearm since it was abolished by intra-arterial bretylium tosylate (an adrenergic neurone blocking agent) but not by intra-arterial atropine[16]. Using such a stimulus, Brick, Hutchison and Roddie[11] (1967) were unable to demonstrate a vasodilator response after alpha adrenergic blockade or a potentiation of the vasoconstrictor response by beta adrenergic blockade. It was suggested therefore, that reflex activity of the sympathetic nerves to forearm vessels does not result in stimulation of beta adrenergic receptors (Brick, Hutchison and Roddie; 1967, 1968)[11, 14].

A study by Glick, Epstein, Wechsler and Braunwald (1967)[12] investigated the physiological differences between the effects of neuronally released and blood borne noradrenaline on beta adrenergic receptors in the arterial bed of the dog. The responses of control dogs to carotid sinus hypotension and intra-arterially administered noradrenaline was compared to animals subjected to alpha receptor blockade. In the control dogs, carotid occlusion and intra-arterial noradrenaline caused an increase in hind limb perfusion pressure,

whereas in dogs previously treated with phenoxybenzamine, carotid sinus hypotension still produced reflex vasoconstriction, whereas intra-arterially injected noradrenaline produced vasodilation.

The results of this study are in agreement with Brick et al (1967, 1968)[11,14] since no potentiation of the reflex constrictor response was observed after beta receptor blockade. Thus this suggested that while intra-arterial noradrenaline stimulates both alpha and beta adrenergic receptors, neuronally released noradrenaline does not exert a significant effect on beta adrenergic receptors in the arterial bed.

Use of drugs which cause the release of endogenous transmitter from nerve terminals is another way of investigating the response to nerve stimulation. Much of the knowledge of the effect of endogenous noradrenaline released from sympathetic nerve terminals comes from the experimental work with a drug called tyramine. Intravenous infusion of tyramine increases the amount of noradrenaline in the venous outflow of several organs "in vivo" and in the effluent of isolated perfused organs[17]. It has been suggested[18] that tyramine causes the displacement of noradrenaline from sympathetic nerve endings. Thus tyramine was said to exert only an indirect action, and the hemodynamic effects of tyramine were attributed to the release of stored noradrenaline.

There is some disagreement with the hypothesis. In a study by J.N. Cohn (1964)[19] the cardiovascular effects of tyramine, ephedrine and noradrenaline were compared in man. He observed that tyramine had weak vasoconstrictor activity as did ephedrine. The pressure effects of these drugs was largely due to their ability to increase the rate and strength of myocardial contraction.

Cohn, in suggesting tyramine and ephedrine have local

vasoconstrictor actions, similar to noradrenaline, pointed out that larger doses of tyramine did not produce the dose dependent vasoconstriction noted with noradrenaline, but instead produced vasodilatation. He suggested this was a direct response to tyramine after depletion of noradrenaline stores in the nerve endings despite replenishing noradrenaline infusions.

Cohn suggests several possibilities to explain the action of tyramine.

(1) that tyramine caused the release of adrenaline and dopamine as well as noradrenaline from sympathetic neurons which could cause forearm vasodilatation.

(2) that tyramine might exert a direct action on sympathetic nerves.

(3) that tyramine could have both direct and indirect actions.

Frewin and Whelan (1967)[20] studied intra-arterial infusions of noradrenaline and tyramine before and alfter alpha and beta adrenergic blockade in an attempt to establish the mechanism of action of tyramine on the peripheral blood vessels in man. They found that tyramine was without effect on a sympathetically denervated forearm in the doses used, implying its effect was mediated solely by the release of transmitter from nerve endings. Furthermore in a normal forearm, a dose dependent fall in peripheral resistance was observed to increasing intra-arterial doses of tyramine. After alpha receptor blockade, noradrenaline caused vasodilatation whereas tyramine was without effect. These infusions were of ten minutes duration.

More prolonged tyramine infusions (15 minutes duration) were carried out during phentolamine infusions. In these experiments

a vasodilatation response was observed eight minutes (an average) after the tyramine infusion was begun. If a beta receptor blocking agent (propranolol) was infused after a vasodilatation response had been recorded, tyramine then caused a fall in flow. Propranolol caused no potentiation of the constrictor action of tyramine on forearm vessels during five minute infusions, but when the infusions were continued for 15 minutes potentiation of the constrictor response was observed in four out of six experiments. In analysis of their data, Frewin and Whelan concluded that tyramine produced vasoconstrictor and vasodilator responses which were delayed in onset compared to noradrenaline since tyramine must act by releasing noradrenaline from nerve endings.

It was felt worthwhile to investigate further the response of the peripheral vascular bed to intra-arterial infusions of tyramine. Brick et al (1967)[11] and Glick et al (1967)[12] had failed to demonstrate a vasodilator response to reflex sympathetic nerve stimulation, after alpha receptor blockade, or any augumentation of the reflex constrictor response by propranolol. The work of Frewin et al[20] indicates that the time course of stimulation may be a determining factor, and if the stimulation period was too short no vasodilatation response would be observed. Glick et al (1967)[12] reflexely stimulated sympathetic nerves for only 60 seconds; no reversal of the effect of noradrenaline released from nerve stores was observed (noradrenaline still caused vasoconstriction after alpha adrenergic receptor blockade). Brick et al (1967) reflexely stimulated sympathetic nerves for up to 10 minutes, however, because general body vasodilatation caused by spillover of the large doses of phentolamine (1600μg/Kg/minute I.A) they were unable

to expose subjects to lower body suction below -25mm Hg. Thus Glick et al[12] may not have stimulated long enough, whereas the stimulus applied by Brick et al may not have been sufficient by severe to cause the release of the necessary amount of transmitter to manifest a beta response. If it could be demonstrated that prolonged infusion of tyramine (15 minute duration) caused the release of neurotransmittor from nerve endings which, after alpha receptor block, was capable of producing a beta receptor mediated vasodilatation, then it is reasonable to assume that noradrenaline released as a result of sympathetic nerve stimulation could also produce a dilatation after alpha receptor blockade providing the stimulus is of sufficient strength and duration. This would be classed as active reflex dilatation.

Passive dilatation refers to the relaxation of vascular smooth muscle which occurs when tonic secretion of a constrictor substance is interrupted and implies as a prerequisiste the existence of neurogenic tone. Active dilation refers to vascular relaxation caused by the direct action of some substance, following exogenous administration or endogenous release, on vascular smooth muscle.

Even from the times of Claude Bernard[21], who first demonstrated passive dilatation in the rabbit ear and active dilatation in the submaxillary gland upon stimulation of the chorda tympani nerve, the controversy has raged as to the existence of a vasodilator as well as a vasoconstrictor center. Early workers hypothesized only a vasoconstrictor centre and vasodilatation resulted from inhibition of vasoconstrictor activity.

Since then workers have reported demonstrations of active

reflex dilatation in various preparations. There has been a great deal of work done on compounds such as vasopressin, oxytocin, angiotensin and bradykinin which have powerful vasoconstrictor or vasodilator activity depending on the compound. The possibility that bradykinin may be important in vasoregulation is suggested by the studies of Hilton and Lewis[22,76] who showed kallikrein could be obtained from the venous effluent and the secretion of the salivary gland after stimulation of the chorda tympani. However, while kallikrein is found in many glandular tissues, in the blood and in the urine, the physiological role of the kinins as vasoregulators is not fully established.

In a recent review, Schachter (1969)[23] suggested that specific physiological roles cannot as yet be ascribed to these substances with confidence. There has been evidence to suggest that the kallikrein-kinin system may regulate functional hyperemia in glands; and that the system is activated at birth and mediates the vascular changes occuring during the transition from fetal to neonatal circulation.

Studies by Dale (1906)[24], Euler et al (1937)[25], Burn (1932)[26], Bulbring and Burn (1936)[27], Folkow and Uvnas (1948)[28] and others have demonstrated a cholinergic vasodilator system with extensive central representation in cats and dogs. Excitation of the system results in active dilatation of skeletal muscle vasculature. Since peripheral resistance is localized in the arterioles, the increase in blood flow is thought to be due to arteriolar dilatation (Uvnas 1966)[29]. Vasodilator activity in these nerves can be abolished by atropine. Burn and Rand (1958)[30] proposed that nor-

adrenaline release is brought about through the intermediary action of acetylcholine. In experiments in which post ganglionic fibres running to the perfused rabbit ear were stimulated, vasoconstriction due to the release of noradrenaline was greatly potentiated by the addition of physostigmine to the perfusion fluid. An article summarizing the evidence for this hypothesis has been recently published by Burn (1971)[31]. There is no evidence at present which links this "sympathetic cholinergic" system to the cholinergic vasodilator system discussed earlier.

Lindgren and Unvas (1954)[32] produced a vasodilatation in skeletal muscle by stimulation of the depressor area which was not abolished with atropine. This evidence, plus that of Frumin, Ngai and Wong (1953)[33] who used an adrenergic blocking compound to abolish the constrictor response to ventral root stimulation, was taken to indicate that this vasodilatation was the result of a reduction in sympathetic tone mediated through the baroreceptors and was therfore passive in origin. Frumin et al[33] confirmed the existence of sympathetic adrenergic constrictor, sympathetic cholinergic and dorsal-root dilator fibres. Furthermore, he suggested the only mechanism for eliciting dilatation through medullary reflexes was inhibiton of sympathetic constrictor discharge.

In spite of evidence which suggests "active reflex dilatation" is actually only "passive dilation", another candidate for the neurohumoral mediation of active reflex dilatation is histamine. Beck et al (1961)[34] demonstrated that reflex dilatation was not affected by low doses of atropine sufficient to block cholinergic fibres. Since ganglion blocking agents blocked both reflex dilatation and reflex

constriction this showed both passive and active reflex systems were ganglionated.

The beta blocking agent dichloroisoproterenol(DCI) does not block reflex dilatation in doses which abolish an equivalent dilatation induced by the intra-arterial injection of isoproterenol. The results with DCI suggest active reflex dilatation is not simply a phenomenon of adrenaline reversal resulting from a diminished neurogenic release of noradrenaline, nor can it be ascribed to the release of a substance with pure beta stimulating activity. Beck et al (1961)[34] has suggested in experiments with hemicholinium which abolished sympathetic tone and reflex dilatation which was restored after treatment with choline (which had been shown to antagonize hemicholinum) that acetylcholine may be implicated in sympathetic transmission and active reflex dilatation. These experiments demonstrated that reflex dilatation induced by baroreceptor stimulation is not entirely passive in origin. The fact that reflex dilatation exceeds the dilatation resulting from loss of vasoconstrictor tone after acute sympathetic denervation, and that reflex dilatation was shown to be present after vasoconstrictor tone was abolished pharmacologically was taken to suggest that at least part of reflex dilatation was active in nature and involved the release of a vasodilator transmitter from sympathetic nerve ending.

In a paper published in 1965, Beck (1965)[35] presented evidence of the blocking effect of antihistamineson reflex dilatation. A wide variety of antihistaminergic compounds were tested and it was demonstrated that a reduction in reflex dilatation was brought about in some cases through a central action (presumably by depressing the

excitability of vasomotor neurones in the medulla). In cross circulation experiments he showed that antihistamines effect a reduction in reflex activity in the recipient limb without reducing perfusion pressure, demonstrating a second component of reflex blocking activity in the periphery.

It seems reasonably certain that antihistamines block the peripheral effects of vasodilation. What is not clear as yet, is whether antihistamines block the neurogenic release of histamines at the vascular level, or whether the blockade is a result of pharmacological properties shared by antihistamine blocking the passive component of reflex dilatation through a central action. Beck[35] suggests that antihistamines block passive dilatation shown to be due to a reduction in sympathetic vasoconstrictor tone centrally, and active dilatation by an effect at the periphery. Either this implies histamine is an inhibitory transmittor in the CNS or blockade of passive dilatation is due to some other pharmacological effect of antihistamines.

It is clear that histamine is present in nervous tissue and is most concentrated in post ganglionic sympathetic fibres Brody (1966)[36]. Enzymes responsible for its synthesis and degradation also appear to be present in sympathetic nerves. Exogenous histamine produces a response very similar to reflex dilatation and the response is antagonized by pharmacological agents that antagonize histamine. Brody (1966)[36] demonstrated the liberation of isotopically labelled histamine into venous blood during reflex dilatation. He concluded that when baroreceptors are excited by increased pressure, the reflex vasodilator response in skeletal muscles is produced by the inhibition

of noradrenaline secretion and simultaneous neurogenic liberation of the vasodilator histamine.

There is a good deal of evidence to support histamine mediation of active reflex dilatation. However, the fact still remains that not all anti-histamines were capable of blocking reflex dilatation and similarly several histamine releasing drugs tested by Beck (1965)[35] caused a fall in perfusion pressure, and no reduction in reflex dilatation; or a parallel decrease in reflex dilatation and perfusion pressure. There has been no satisfactory answer to the question of why histaminergic dilatation is not readily evoked upon stimulation of lumbar sympathetic chains. Brody's experiments (1966)[36] provided the first direct evidence for the role of histamine as a neurotransmitter of active reflex dilatation. Brody stated that the vasodilation response was produced by noradrenaline inhibition and histamine release. However, Brody was not able to demonstrate any decrease in noradrenaline in venous blood collected during reflex vasodilatation. Glick, Wechsler, and Epstein (1968)[37] perfused the hind limb of dogs at a constant flow rate. The role of histamine as a mediator of active reflex vasodilatation was studied in animals in which basal sympathetic tone in the arterial bed of skeletal muscle was minimized by:

(a) alpha adrenergic blockade with phenoxybenzamine

(b) depletion of endogenous noradrenaline stores by pretreatment with reserpine.

Both techniques essentially abolished reflex vasodilatation. It was concluded that since vascular beds in which reflex vasodilatation was abolished retained a sensitivity to intra-arterial acetylcholine that

the sympathetic cholinergic system does not participate in reflex vasodilatation. Glick et al[37] concluded decreasing levels of noradrenaline at the neuroeffector junction,rather than secretion of histamine or acetylcholine,is the cause of reflex vasodilatation.

Furthermore, it was suggested that the blocking effect of antihistamines (in particular treplennamine) on reflex vasodilatation as observed by Beck (1965)[35] was due to a cocaine like interferance with the neural uptake mechanism. That is, if a means of reducing noradrenaline concentration at its site of action is reduced, reflex vasodilatation is concurrently reduced. The study of Brody (1966)[36] which demonstrated radioactive histamine, and a metabolite (N-methyl-histamine) in the venous effluent of an isolated perfused muscle during reflex vasodilatation was accounted for by the suggestion that large priming doses of radioactive histamine diffused nonspecifically into many areas of the tissue water. Thus reflex dilatation could wash out increased amounts of radioactivity by a redistribution of blood flow. This argument is further strengthened by the fact that a large amount of radioactivity appeared as N-methyl-histamine which had been shown to be widely distributed in the tissue water.

Levin et al (1968)[38] noted adrenaline was a potent dilator in several species of monkeys. It was suggested that adrenaline released from the adrenal medulla into the circulation may participate in active reflex vasodilatation. However, in comparing the reflex dilatation obtained in intact monkeys to adrenalectomized monkeys, the responses were not significantly different. The fact that humorally released adrenaline does not participate in reflex dilatation, does not exclude endogenous transmittor from stimulating receptor sites.

Levin et al[38] noted that a dose of propranolol which abolished a comparable dilatation induced by intra-arterial isoproterenol did not affect reflex vasodilatation. It may be possible that reflex vasodilatation induced by transmitter stimulation of beta receptors may not be amenable to blockade by circulating propranolol. One point of agreement between the studies of Beck (1965)[35], Glick et al (1968)[37] and Levin et al (1968)[38] is that the cholinergic vasodilator fibres do not participate in the pressoreceptor induced reflex vasodilatation.

Folkow et al (1961)[39] suggested vasodilator fibres are distributed to bigger precapillary resistance vessels only. Few if any dilator fibres run to the venous side of the circulation. In a study in which Zimmerman (1963)[40] compared the effects of various sympathomimetic amines upon venous and total vascular resistance in the foreleg of the dog, it was observed the pressor amines had a differential ability to constrict veins in the foreleg. Different degrees of venoconstriction were produced for similar changes in total resistance. The percentage contribution of the increase in venous resistance to the increase in total resistance was greater as the dosage increased. The results suggest the venous segment constricts proportionately more at higher doses than at lower ones - whereas the resistance vessels are relatively more sensitive to lower doses of amines. Kaiser et al (1964)[41] noted that while isoproterenol consistently diminished systemic vascular resistance it also consistently produced venoconstriction. He showed that the venoconstriction very likely resulted from stimulation of beta receptors since it was completely blocked with

nethalide. Phenylephrine constricted the arterial bed, but its effects on the capacitance vessels was similar to isoproterenol. The effects of phenylephrine and noradrenaline on arteriolar and venous beds were blocked with phenoxybenzamine. It was concluded that the effects of phenylephrine and noradrenaline were mediated through alpha adrenergic receptors. These experiments suggest that the systemic venous bed contains alpha and beta adrenergic receptors both of which mediate venoconstriction.

Clement et al (1969)[42] investigated the influence of various pharmacological agents such as noradrenaline, adrenaline, and isoproterenol on isolated vein preparations. It was observed that isoproterenol in small doses produced a venodilatation, whereas in higher doses a venoconstriction was provoked. This response could be abolished with phentolamine. In other preparations if venoconstriction was previously induced by noradrenaline, isoproterenol induced relaxation which could be abolished by previous exposure to propanolol. Thus this suggests that beta adrenergic stimulation produces vasodilatation in veins.

Abboud and Eckstein (1966)[43] demonstrated that sympathetic nerve stimulation causes constriction of the large artery segment of the perfused foreleg of dogs and results in diversion of blood away from the paw to more proximal parts of the foreleg. The effects of noradrenaline differ from those of nerve stimulation. Noradrenaline causes constriction of small vessels in paw predominently. Constriction of both small vessels and venous segments in the paw exceeds constriction of corresponding segments in muscle. Previously it had been reported by Abboud, Eckstein and Zimmerman (1965)[44].

that stimulation of beta receptors with isoproterenol causes much more dilatation in small vessels than in veins in the foreleg and that small vessels of both the paw and muscle dilate equally.

Further studies by Zimmerman (1966)[45] in which the entire left hind limb of dogs was perfused through the femoral artery at a constant flow showed very large resistance changes in the paw as compared to those in the muscle in response to sympathetic nerve stimulation. They found that vasoconstriction in the muscle elicited by sympathetic stimulation was accounted for by small vessel constriction in both arteries and veins. Constrictor responses in the muscle were of much lesser magnitude than that obtained in the paw. After blockade of adrenergic fibres by bretylium tosylate, sympathetic stimulation resulted in vasodilatation in small vessels in paw and muscle. Veins were not dilated during sympathetic stimulation after bretylium; slight residual constriction of the venous segment in the paw remained. This agrees with the results of Folkow et al (1961)[39] who suggested that sympathetic vasodilator system does not innervate the venous circulation.

In the study by Zimmerman (1966)[45] sympathetic stimulation elicited a prominent fall in paw artery perfusion pressure indicating vasodilatation in the paw after adrenergic neurone blockade with bretylium. This dilatation was resistant to atropine blockade in doses which abolished the effect of intra-arterial methacholine. No explanation for the atropine resistance dilatation was available.

In a following study, Zimmerman (1968)[46] observed in the dog hind-limb:

(1) a rapidly developing cholinergic component easily

blocked by atropine.

(2) a slowly developing long lasting component not affected by atropine localized in the cutaneous portion of the bed.

(3) a response histaminergic in nature occurring mainly in skeletal vessels.

The long lasting component was not blocked with anithistamines or antiserotonin, but was abolished after skinning the limb suggesting exclusively cutaneous vasodilatation. A chemical transmitter for this cutaneous vasodilatation has not been identified with certainty but it had been shown not to be acetylcholine, histamine, serotonin or bradykinin. The presence of this dilator system distributed to the cutaneous vessels was confirmed by Brody and Shaffer (1970)[47]. It also indicated that vasodilatation in response to carotid sinus nerve stimulation occurred in the cutaneous as well as muscular components of the limb, but offered no new evidence as to a possible mediator for sustained vasodilatation in the paw. It is not likely that adrenergic mechanisms play a role in this third dilator system since the vasodilator response was obtained 10-30 minutes after adrenergic neurone blockade with bretylium tosylate of guanethidine hydrochloride.

The role of beta adrenergic mechanisms in causing reflex vasodilatation has been all but eliminated. Any effect of beta blockade on reflex vasodilatation has been attributed to a nonspecific vascular depressant or ganglion blocking action. Levin[38] noted that while propranolol abolished a vasodilatation induced by isoproterenol,

it did not affect reflex vasodilatation. It is unlikely that reflex vasodilatation induced by stimulation of beta receptors is not amenable to blockade by circulating beta blocker -- if beta adrenergic receptors were, in fact, involved in this response. Li and Bentley[81] (1970) showed that in cats the direct dilator response to adrenaline which is a beta response in the hind limbs is not reduced by alpha antagonists at a time when reflex responses are abolished. Further more this direct beta dilator response was augmented after azapetine which reduced the pressor response to adrenaline by 50%.[81] These workers[81] concluded that the efferent arm of the reflex vasodilator mechanism does not appear to involve either histamine, muscarinic receptors, or beta adrenergic receptors.

This thesis is concerned with the sympathetic control of peripheral vascular smooth muscle and deals mainly with the response of beta adrenergic receptors in the peripheral vascular system to endogeneously released noradrenaline, i.e., noradrenaline released from sympathetic stores in the nerve endings by means of a "releasing drug" (tyramine) or by reflex stimulation of sympathetic nerves (bilateral carotid occlusion). Included also are evaluations of the alpha adrenergic receptor blocking properties of thymoxamine, dibozine and phentolamine. These drugs were assessed in preliminary experiments to determine which produced the most effective blockade with minimal other effects (such as a direct vasodilatory effect) as evidenced by the change in peripheral resistance in response to intra-arterial noradrenaline, and tyramine.

As well, one section of this thesis deals with the cardiovascular effects of theophylline during infusions of noradrenaline,

and bilateral carotid occlusion, and after alpha and beta receptor blockade. These experiments were conducted to investigate the "beta potentiating" effects of theophylline on peripheral beta receptors.

The thesis deals with the question can sympathetic neurotransmitter released from sympathetic nerve endings produce stimulation of vascular beta adrenergic receptors and, if so, what physiological role does this play in producing active vasodilatation in the peripheral circulation?

SECTION I

The Response of the Whole Hind Limb Vascular Bed of Dog to Intra-arterial Infusions of Noradrenaline and Tyramine.

INTRODUCTION

Frewin and Whelan[20] observed in the human forearm that the action of tyramine is dependent on the presence of sympathetic nerves and that tyramine stimulates both alpha and beta adrenergic receptors. They observed that the alpha receptor action was predominant, the beta effect being seen only after blockade of the alpha receptors with phentolamine. Experiments similar to those of Frewin and Whelan[20] who investigated the mechanism of action of tyramine on the blood vessels of the forearm in man are repeated in the dog.

MATERIALS AND METHODS

Experiments were conducted on five mongrel dogs (average weight of 15 kgms). The dogs were anaesthetized with I.V.pentabarbital sodium (nembutal ^{R} 30mgm/kgm) and maintained on thiopental (pentothal ^{R}) 10mgm (0.5cc)/15 minutes, for the duration of the experiment.

Immediately after induction the trachea was intubated and a catheter was placed in the right cephalic vein of the foreleg for the administration of anaesthetics. The left brachial artery was cannulated to monitor systemic arterial pressure. Arterial pressure was measured with a Statham model P23db pressure transducer and mean arterial pressure was calculated from these recordings (diastolic plus one third Pulse Pressure).

A flow probe was placed around the left iliac artery which had been exposed through an incision just below and paralleling the inguinal ligament. The femoral artery was exposed and freed from tissue where it passed below the inguinal ligament. Proximally the left external iliac artery was dissected free of tissue for a distance of four to five centimeters above the femoral profundus branch up under the inguinal ligament. The position of this branch relative to the iliac artery and the position of the flow transducer illustrated in Figure 1. It should be noted that in this experiment paw flow was not occluded, nor were the carotid arteries exposed or the vagus nerves divided as depicted in Figure 1. The flow probe (A-5000 series transducer, Ward Associates) was connected to a blood flow meter (Doppler flowmeter - Model 1503, Ward Associates) and this output and systemic arterial pressure were recorded simultaneously on a Beckman Dynograph. The flowmeter was calibrated at the end of the

Figure 1:

Experimental preparation.

Diagram of the experimental set-up

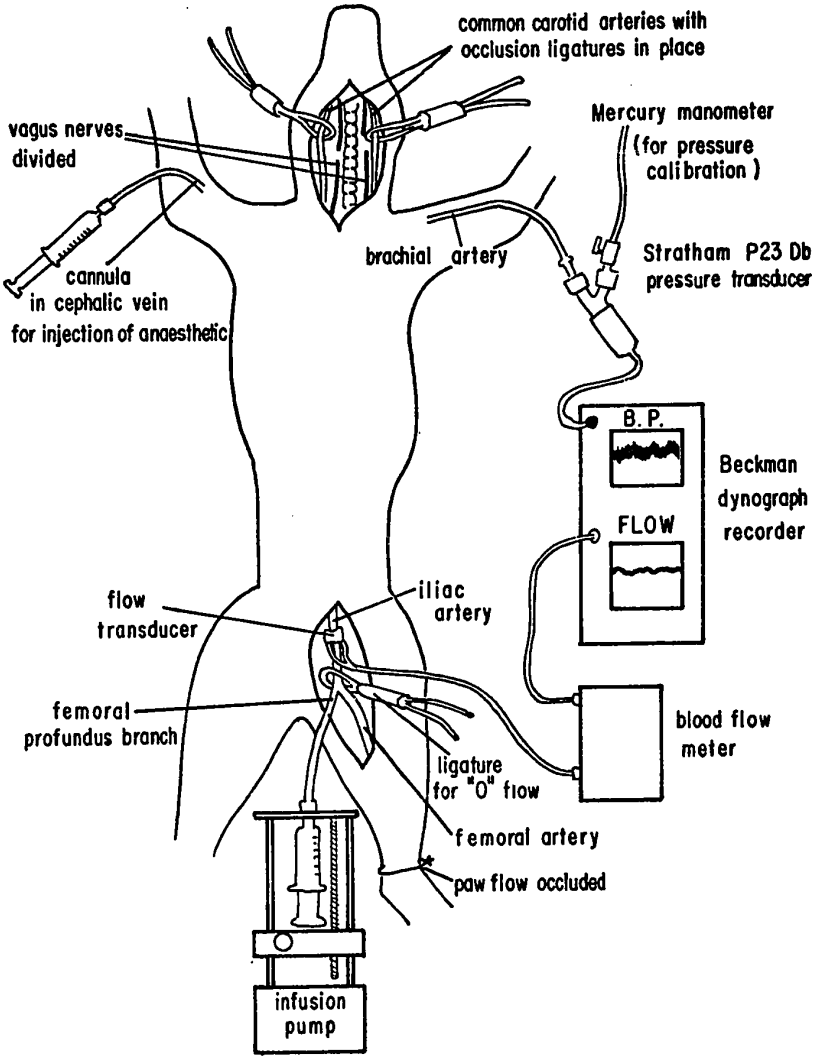


Figure 1

experiment by timed collections from the cut artery distal to the flow probe. Calculation from the recordings of pressure and flow according to the formulae shown below were made:

$$PVR = \frac{\text{Mean Blood Pressure}}{\text{Flow}}$$ (PVR=Peripheral Vascular Resistance.)

$$PVC = \frac{\text{Flow}}{\text{Mean Blood Pressure}}$$ (PVC=Peripheral Vascular Conductance.)

and these values of resistance and conductance were plotted against time.

A catheter was inserted in the femoral profundus branch (see Figure 1) for the infusion of drugs. The catheter was placed so that the tip was not protruding into the lumen of the main artery. (Figure 2). This procedure plus the fact that the infusion rate of 1ml/minute was maintained throughout the experiment resulted in no interference with the flow recording in the main branch. A ligature was passed under the vessel just proximal to the femoral profundus branch. The ligature, when tightened, occluded blood flow in the left iliac artery such that zero flow could be established.

Noradrenaline 0.3 and 1.0µg/Kg/minute were infused for five minutes each; tyramine 10 and 30µg/Kg/minute for 15 minutes each. The drugs were dissolved in ascorbic acid saline (0.9% NaCl, 0.03% ascorbic acid) so that the minute dose was contained in one milliliter. Ascorbic Acid saline alone was infused during control periods.

Figure 2:

Placement of the catheter for drug infusion.

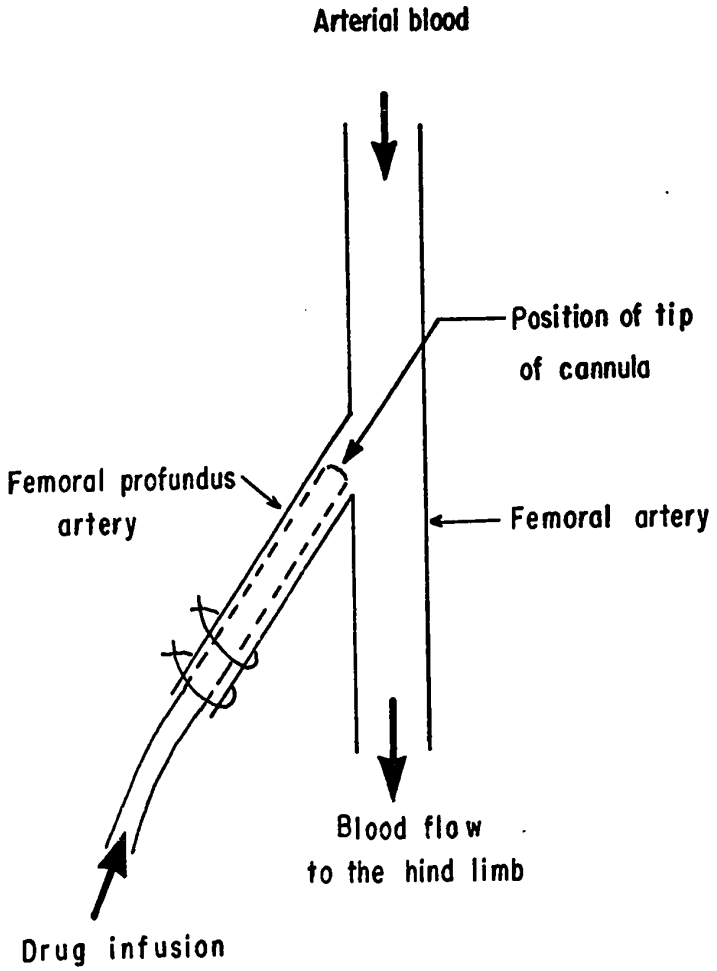

Arterial blood
Position of tip
of cannula
Femoral profundus
artery
Femoral artery
Blood flow
to the hind limb
Drug infusion

Figure 2

RESULTS

The results of one experiment are shown in Figure 3. Ascorbic acid saline alone was infused for 30 minutes before the drug infusions to allow flow to reach a steady level. Intra-arterial noradrenaline (0.3µg/Kg/minute) was infused for five minutes. After flow had stabilized (usually in six to 10 minutes; or until three minutes of level flow had been recorded) noradrenaline (1.0µg/Kg/minute) was infused for five minutes. Noradrenaline produced a dose dependent decrease in vascular conductance. The noradrenaline infusions were followed by intra-arterial tyramine in 10 and 30 µg/Kg/minute for 15 minutes each. Again a dose dependent decrease in vascular resistance was observed. Intra-arterial phentolamine (8µg/Kg/minute) was infused for 20 minutes prior to and during repetition of the noradrenaline and tyramine infusions. Noradrenaline (0.3 and 1.0µg/Kg/minute) infused during phentolamine (8µg/Kg/minute) produced a marked vasodilatation, that is, an increase in vascular conductance. The response to tyramine (10µg/Kg/minute) during phentolamine (8µg/Kg/minute) was abolished, whereas tyramine (30µg/Kg/minute) produced vasodilatation. Phentolamine was increased to 32µg/Kg/minute and infused 20 minutes prior to, and during, repetition of the noradrenaline and tyramine doses. Noradrenaline again produced increases in vascular conductance at both doses. In this run, blood flow did not return to control levels in which case at least three minutes of steady flow was recorded before starting an infusion of noradrenaline or tyramine. The response to tyramine (10µg/Kg/minute) was abolished during phentolamine (32µg/Kg/minute), whereas, tyramine (30µg/Kg/minute) caused a vasoconstriction, that is, a decrease in vascular conductance.

Figure 3:

Effect of phentolamine on the response to intra-arterial noradrenaline and tyramine in the whole hind limb preparation.

Peripheral vascular conductance -o-o-o

Noradrenaline 0.3µg/Kg/minute - NA 0.3

Noradrenaline 1.0µg/Kg/minute - NA 1.0

Tyramine 10µg/Kg/minute - TYR 10

Tyramine 30µg/Kg/minute - TYR 30

Phentolamine 8µg/Kg/minute - Phen 8

Phentolamine 32µg/Kg/minute - Phen 32

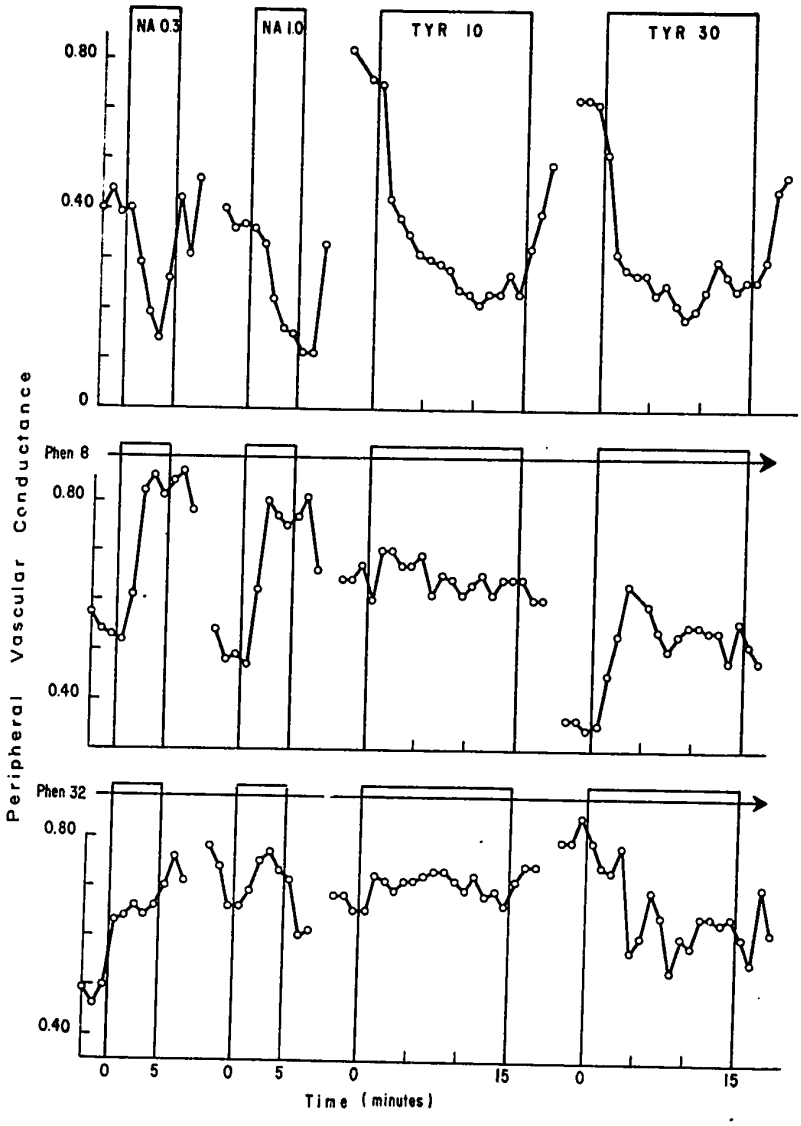

NA 0.3
NA 1.0
TYR 10
TYR 30
0.80
0.40
0
Phen 8
0.80
0.40
Phen 32
0.80
0.40
Peripheral Vascular Conductance
0
5
0
5
0
15
0
15
Time (minutes)

Figure 3

In Figure 4 the average resistance (the reciprocal of conductance) during the fourth and fifth minutes of each infusion has been expressed as a percentage of the average resistance over the last three minutes prior to the infusion. Noradrenaline (0.3 and 1.0μg/Kg/minute) caused dose dependent increases in peripheral vascular resistance (% Ch = 61±15; $p<0.05$ and % Ch = 196±33; $p<0.01$ respectively). During phentolamine (8μg/Kg/minute) noradrenaline (0.3μg/Kg/minute) produced a decrease in vascular resistance (% Ch = -14±5, $p<0.10$). The constrictor response to noradrenaline (1.0μg/Kg/minute) was abolished (% Ch = -9±8) but vascular resistance was not significantly decreased. During phentolamine 32μg/Kg/minute noradrenaline 0.3 and 1.0μg/Kg/minute produced a significant decrease in vascular resistance (% Ch = -21±5, $p<0.01$ for NA 0.3μg and % Ch = -28±7, $p<0.02$ for NA 1.0μg). These responses constituted a reversal of the constrictor effects of noradrenaline.

Tyramine (10μg/Kg/minute) produced a significant change in resistance (% Ch = 84±15, $p<0.01$) as shown in Figure 4. A similar response was observed with tyramine 30μg/Kg/minute (% Ch in resistance = 72±14, $p<0.01$). Phentolamine (8μg/Kg/minute) abolished the response to tyramine 10 and 30μg/Kg/minute (% Ch in resistance = -3±4 and -7±9% respectively), but a significant reversal was not observed at either dose level. As with the smaller dose of phentolamine, Figure 4 (2) the larger dose (32μg/Kg/minute) Figure 4 (3) abolished the constrictor response to tyramine but again no significant reversal was observed even though the response was reversed in four out of five experiments

Figure 4:

The average response in five experiments to noradrenaline and tyramine before (1) and during phentolamine 8μg/Kg/minute ; (2) and 32μg/Kg/minute; (3) expressed as a percentage change in vascular resistance.

1A NA 0.3μg/Kg/minute - ●

1A NA 1.0μg/Kg/minute - ■

1A TYR 10μg/Kg/minute - O

1A TYR 30μg/Kg/minute - □

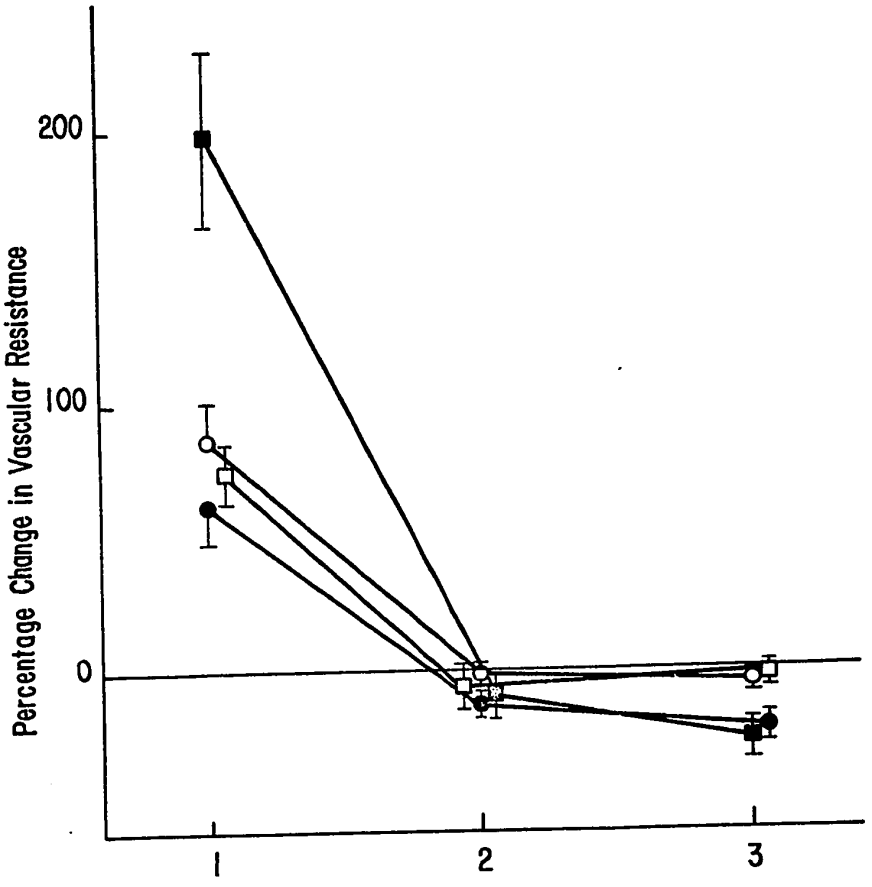


Figure 4

at the five minute point of the infusion in both cases. Tyramine was continued for 15 minutes. The changes in resistance expressed as a percentage change are shown in Table I at five and 15 minutes. The increase in resistance observed with tyramine at five minutes (Figure 4) was sustained for the duration of the infusion (% Ch = 93±21, $p<0.05$; Table I). Tyramine 30μg/Kg/minute also produced a significant change in resistance at 15 minutes (% Ch = 111±28, $p<0.05$; Table I). During phentolamine 8μg/Kg/minute and 32μg/Kg/minute when tyramine 10 and 30μg/Kg/minute were continued for 15 minutes; the constrictor response which was abolished and/or reversed at five minutes showed little change at the 15 minute period (note in Table I).

Table 1: Comparison of the peripheral vascular resistance response to Tyramine (10μg/Kg/minute and 30μg/Kg/minute) at five minutes and 15 minutes after the start of the infusion, average of five experiments. Peripheral vascular resistance has been expressed as a % change from the control and is followed by the standard error of the mean.

* $p<0.05$

** $p<0.01$

Drug infused during → ↓	Control (ascorbic acid saline)			Phentolamine 8μg/Kg/minute			Phentolamine 32μg/Kg/minute		
	5 minutes	15 minutes	Difference between 5 and 15 minutes	5 minutes	15 minutes	Difference between 5 and 15 minutes	5 minutes	15 minutes	Difference between 5 and 15 minutes
TYR 10μg/Kg/minute	84±15 **	93±21 *	9±18	-3±4	-5±5	-2±3	-4±5	-4±8	0±4
TYR 30μg/Kg/minute	72±14 **	111±28 *	39±23	-7±9	0±16	7±13	-3±6	0±7	3±3

DISCUSSION

Intra-arterial noradrenaline produced vasoconstriction in peripheral vascular smooth muscle by an action on alpha adrenergic receptors.[48] The effects have been well documented previously. After alpha adrenergic receptor blockade noradrenaline produced vasodilatation which was abolished after propranolol and therefore is due to stimulation of beta receptors. These results agree with previous work by (Brick et al, 1966;[11] and Glick et al, 1962[12]).

Tyramine produced a vasoconstriction in the hind limb of the dog. Frewin and Whelan (1967)[20] have suggested in man that the effect of tyramine is mediated solely by the release of neuro-transmittor from sympathetic nerve endings. They concluded that because tyramine exerts its effect indirectly as compared to an intra-arterial infusion of noradrenaline, the onset of the maximum response to noradrenaline develops more quickly than that to tyramine. In the present experiments very little, if any, delay was observed in the onset of the response to tyramine infusions. The onset of the response to tyramine and noradrenaline after beginning the infusions was almost immediate. In fact it can be noted in the results that the percentage changes in resistance are greater at five minutes for tyramine than for the smaller dose of noradrenaline. However, since noradrenaline infusions were not continued beyond five minutes, it is not possible to determine if noradrenaline reached a maximum effect at the end of five minutes.

Frewin et al[20] maintained that since tyramine acts indirectly by releasing noradrenaline whereas intra-arterial noradrenaline exerts its action directly on the smooth muscle wall of the blood vessel, the response to tyramine and its dilator effects

would be of lesser magnitude. The fact that tyramine may take longer to exert a maximum effect may be due to the relative amount of transmitter released by tyramine as compared to intra-arterial infusion of noradrenaline. The results of the present experiments suggest a difference between dog and man, i.e. that tyramine may exert some direct vasoconstrictor action on vascular smooth muscle. Either that or it can be said that there is very little time delay between tyramine causing the release of noradrenaline from nerve stores and the vasoconstrictor response to released noradrenaline.

After alpha adrenergic blockade with phentolamine noradrenaline caused a vasodilatation. Phentolamine abolished the constrictor effect of tyramine but a significant vasodilatation was not observed even when the infusions were continued for 15 minutes. (Note Figure 4). Possible explanations for this might be:

(1) that tyramine does not stimulate beta adrenergic receptors.

(2) that phentolamine was not producing adequate blockade of alpha receptors.

(3) that transmitter released by tyramine stimulated beta receptors (in skeletal muscle blood vessels) but the response was being minimized by a high skin blood flow.

It has been shown that skeletal muscle blood vessels contain both alpha and beta adrenergic receptors whereas the skin contains only alpha receptors.[49] Thus a vasodilatation in skeletal muscle produced in response to tyramine in the present experiments after phentolamine may have been minimized by a residual constriction in

the skin and paw mediated through partially unblocked alpha adrenergic receptors.

SECTION II

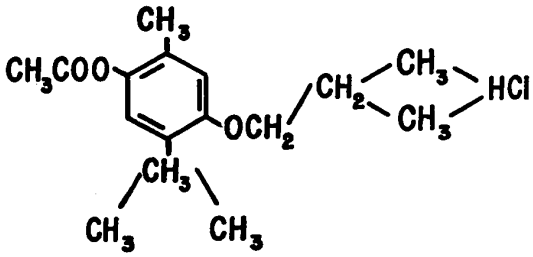
Assessment of the Specific Alpha Adrenergic Blocking Affects of Thymoxamine and Dibozane on the Isolated Muscle Vascular Bed of Dog Hind Limb.

The results of the preceeding study suggested the possibility that the alpha adrenergic blocker phentolamine was not producing an adequate blockade of peripheral alpha adrenergic receptors. Intra-arterial noradrenaline produced vasoconstriction and after alpha adrenergic receptor blockade, vasodilatation. However, while intra-arterial tyramine produced vasoconstriction, after adrenergic receptor blockade with phentolamine, the constrictor response was abolished but a significant dilator response was not observed. Thus, this led to the possibility that the alpha blockade was inadequate and that the vasodilatation to intra-arterial tyramine was being masked by a residual alpha adrenergic response. In this section, two other alpha adrenergic blocking agents were assessed with respect to their ability to block the alpha adrenergic constrictor response of vascular smooth muscle in the dog hind limb. In the first experiments, the ability of intra-arterial thymoxamine to block the response to intra-arterial noradrenaline and tyramine was studied. The structural formulae of thymoxamine and dibozane are given in Figure 5, as well as that of noradrenaline for comparison purposes. In the second groups of experiments, dibozane (1, 4-[bis 1, 4 benzo dioxan-2yl methyl] piperazine)[57] a member of a group of compounds, the benzodioxans, was employed. The alpha adrenergic blocking ability of this compound was assessed with regard to specificity to block the effect of neuronally-released noradrenaline.

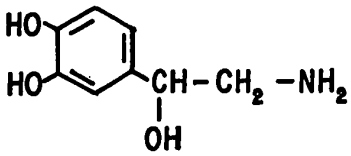
Figure 5:

Comparison of the structural formulae of thymoxamine and dibozane with that of noradrenaline.

THYMOXAMINE



NORADRENALINE



DIBOZANE

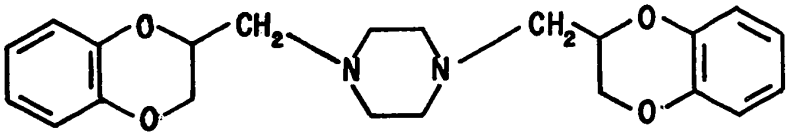


Figure 5

METHODS

Experiments were performed on three mongrel dogs - two employing thymoxamine and one, dibozane. The animals were anaesthetized with sodium pentobarbital (Nembutal ^{R}) (30mgm/Kg/minute) and maintained on pentothal (10mgm/15 minutes) for the duration of the experiments. If necessary, the animals were respired with a Harvard respirometer.

A cannula (O.D. 0.27cm) was placed in the cephalic vein for the administration of anaesthetic. The brachial artery of the left foreleg was cannulated and the catheter was connected to a Statham P23db pressure transducer and the output recorded on a Beckman Type R Dynograph on heat-sensitive paper. Systemic arterial pressure was recorded and mean systemic arterial pressure was calculated from the recordings (diastolic plus one-third Pulse Pressure). A flow transducer (A-5000 series, Ward Assocates) was placed around the iliac artery (see Figure 1). The flow probe was connected to a blood flowmeter (Doppler Flowmeter, Model 503, Ward Associates) and mean flow was recorded on a Beckman Dynograph. As in Section I, the vascular resistance and conductance were calculated from blood flow and mean arterial pressure. A cannula was placed in the femoral profundus branch for the infusion of drugs (see Figure 2). All drugs were made up in ascorbic acid saline (0.9% NaCl, 0.03% ascorbic acid) which was infused during control runs as well as serving as a vehicle for drug administration. Drug infusions were expressed in μg/Kg/minute and the infusion pump was calibrated to deliver one ml/minute. In the present experiment, beta receptor activity localized in the muscle and not the skin was investigated. Paw flow was occluded

(since paw flow is mainly cutaneous) and major cutaneous branches to the skin of the leg were ligated. Therefore, the changes occurring in vascular resistance and conductance were changes occurring in the muscle vascular bed.

Similar procedures were employed in the experiments with thymoxamine and dibozane with the addition that in the dibozane experiment, the carotid arteries were exposed through a mid-line incision in the neck. Ties were placed around the arteries so that they could be occluded when necessary. At the same time, the vagus nerves were ligated and divided. The complete experimental preparation is illustrated in Figure 1.

RESULTS

A. Thymoxamine

The partial results of one experiment are illustrated in Figure 6. Referring to Figure 6, noradrenaline (0.3 µg/Kg/minute) produced an increase in resistance with a concurrent decrease in conductance. This was followed by a 15 minute infusion of tyramine (30µg/Kg/minute) (Figure 6B). Tyramine also produced a decrease in vascular conductance. Thymoxamine (160µg/Kg/minute) was infused 20 minutes prior and continued during repetitive doses of noradrenaline (0.3µg/Kg/minute) and tyramine (30µg/Kg/minute) (Figure 6A, middle and Figure 6B, middle, respectively). This was followed by a 20 minute infusion of thymoxamine (640µg/Kg/minute) which preceeded a further repetition of the above doses. Thymoxamine (640µg/Kg/minute) was continued during these doses. Thymoxamine (640µg/Kg/minute) reversed the constrictor response to noradrenaline (0.3µg/Kg/minute) which now produced an increase in conductance and a decrease in resistance. Tyramine (30µg/Kg/minute) still produced a vasoconstriction during the larger dose of thymoxamine.

In Figure 7, the effects of thymoxamine on the vascular responses to noradrenaline and tyramine are expressed as a percentage change in conductance (average of two experiments). Intra-arterial noradrenaline (0.3µg/Kg/minute) produced a vasoconstriction (% change in conductance = -45%), while noradrenaline (1.0µg/Kg/minute) produced a larger vasoconstriction (% change in conductance = -70%). During thymoxamine (160µg/Kg/minute), noradrenaline still produced a decrease in conductance although the response was reduced

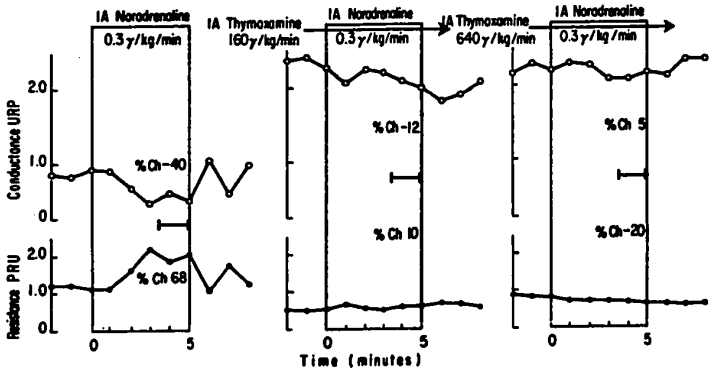
Figure 6:

The response of intra-arterial noradrenaline (0.3μg/Kg/minute) and intra-arterial tyramine 30μg/Kg/minute on hind limb blood flow expressed as changes in vascular resistance and conductance before thymoxamine, after thymoxamine 160μg/Kg/minute, and thymoxamine 640μg/Kg/minute.

Vascular conductance -o-o-o-

Vascular resistance -●-●-●-

A


IA Norodrenaline
0.3γ/kg/min
IA Thymoxamine
160γ/kg/min
IA Norodrenaline
0.3γ/kg/min
IA Thymoxamine
640γ/kg/min
IA Norodrenaline
0.3γ/kg/min
Conductance URP
Resistance PRU
%Ch-40
% Ch 68
%Ch-12
% Ch 10
%Ch 5
%Ch-20
Time (minutes)

B

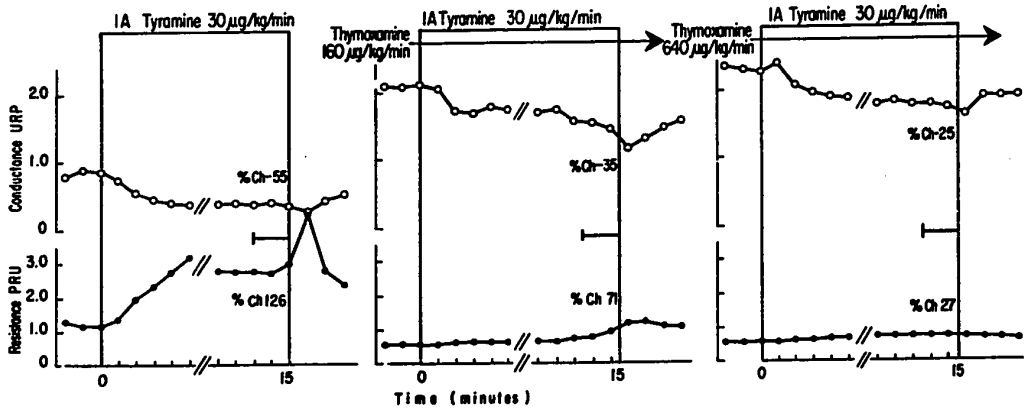

IA Tyramine 30µg/kg/min
IA Tyramine 30 µg/kg/min
Thymoxamine
160µg/kg/min
IA Tyramine 30 µg/kg/min
Thymoxamine
640µg/kg/min
Conductance URP
Resistance PRU
%Ch-55
% Ch126
%Ch-35
% Ch 71
%Ch-25
% Ch 27
Time (minutes)

Figure 6

Figure 7:

The response to noradrenaline (0.3 and 1.0μg/Kg/minute) and tyramine (10 and 30μg/Kg/minute) expressed as a percentage change in vascular conductance from control levels before and during treatment with thymoxamine (160 and 640μg/Kg/minute) ($\bar{X}$ of two experiments).

Noradrenaline 0.3μg/Kg/minute o

Noradreanline 1.0μg/Kg/minute ●

Tyramine 10μg/Kg/minute ▫

Tyramine 30μg/Kg/minute ■

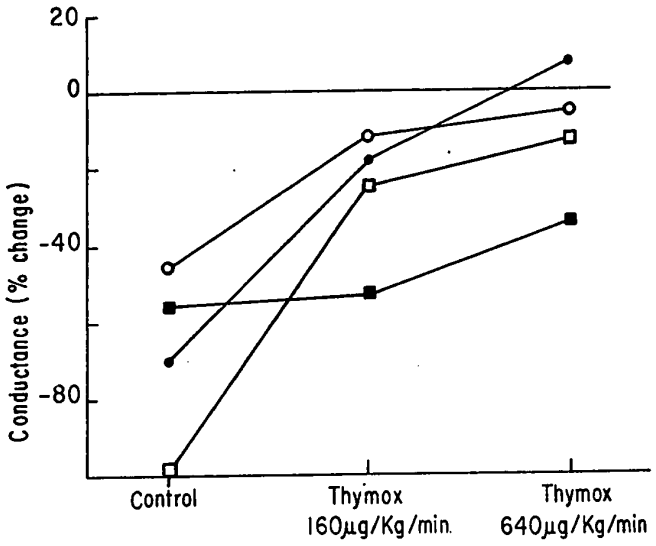

20
0
-40
-80
Conductance (% change)
Control
Thymox
160µg/Kg/min
Thymox
640µg/Kg/min

Figure 7

(% ch for Na, 0.3µg = -12%; for Na, 1.0µg = -18%). During thymoxamine (640µg/Kg/minute), noradrenaline (0.4µg/Kg/minute) produced a decrease in conductance (% ch = -5%), while the response to noradrenaline (1.0µg/Kg/minute) was a small increase in conductance (% ch = 8%).

Before treatment with thymoxamine, tyramine (10µg/Kg/minute) produced a decrease in conductance (% ch = -98%). Similarly, tyramine (30µg/Kg/minute) also decreased conductance (% ch = -55%). During thymoxamine (160µg/Kg/minute), the response to tyramine (10µg/Kg/minute) was reduced (% ch = -25%), but the response to tyramine (30µg/Kg/minute) was relatively unaffected (% ch in conductance = -52%). When thymoxamine was increased to 640µg/Kg/minute, tyramine (10 and 30µg/Kg/minute) still decreased vascular conductance (% ch = -12% and -33%, respectively). In order to evaluate the direct effect of intra-arterial thymoxamine, the vascular conductance during each control period prior to each infusion of noradrenaline and tyramine has been averaged for the three situations before thymoxamine treatment, during thymoxamine (160µg/Kg/minute) and during thymoxamine (640 µg/Kg/minute). The values for the two thymoxamine treatments were then expressed as a percentage of the value before thymoxamine and are plotted as closed circles in Figure 8. It can be seen from the Figure that thymoxamine produced a marked increase in vascular conductance (% ch = 150%).

Figure 8:

The effect of thymoxamine on vascular conductance (expressed as a percentage change from control levels). The open circles represent the individual values obtained from the control periods before each infusion of noradrenaline and tyramine. The vascular conductance for the control period before each drug infusion during treatment with thymoxamine 160µg/Kg/minute and 640µg/Kg/minute has been expressed as a percentage change from the vascular conductance in the control period before that drug infusion before treatment with thymoxamine. The closed circles are the averages of these four values.

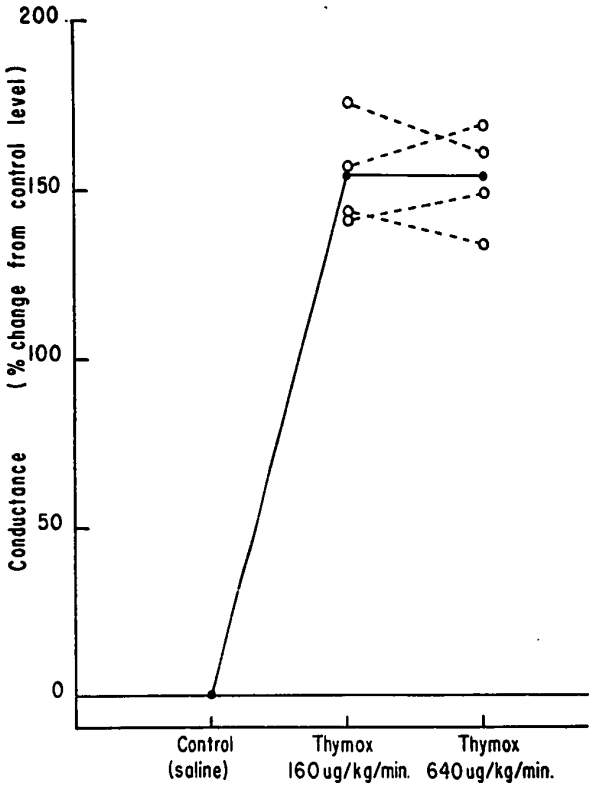

200
150
100
50
0
Conductance (% change from control level)
Control (saline)
Thymox 160 ug/kg/min.
Thymox 640 ug/kg/min.

Figure 8

B. Dibozane Results

The experiment is shown in Figure 9. In Figure 9a, left, noradrenaline was infused for five minutes. A vasoconstriction was produced as indicated by an increase in resistance and a decrease in conductance. After flow stabilized the carotid arteries were occluded for approximately 15 minutes producing a reflex vasoconstriction in the hind limb (Figure 9a). Again after flow had stabilized, tyramine (10μg/Kg/minute) was infused for five minutes (Figure 9a, right) and produced a vasoconstriction. A five minute loading dose of dibozane (0.6μg/Kg/minute) preceeded an infusion of dibozane (10μg/Kg/minute). The control runs were repeated during dibozane (Figure 8b) with the exception that noradrenaline was increased to 0.32μg/Kg/minute. After dibozane, noradrenaline produced a decrease in vascular resistance (Figure 9b, left) whereas, tyramine and bilateral carotid occlusion produced a vasoconstriction (Figure 9b, middle and right). The experiment was repeated with dibozane (20μg/Kg/minute) (Figure 9c). Noradrenaline (0.32 and 0.64μg/Kg/minute) produced a vasoconstriction.

In Figure 10, the responses to noradrenaline during dibozane infusions are expressed as a percentage change from control levels of resistance (closed circles) and conductance (open circles) for one experiment. Noradrenaline (0.16μg/Kg/minute) produced an increase in resistance (% ch = 120%). During dibozane (10μg/Kg/minute), the response to noradrenaline was reversed (% ch in resistance = -37%). Similarly, smaller reversals were observed for noradrenaline (0.32 and 0.64μg/Kg/minute) during dibozane (20μg/Kg/minute) (% ch in resistance = -18 and -13%,

Figure 9:

The peripheral vascular resistance and conductance responses in a typical experiment to intra-arterial noradrenaline (0.16, 0.32 and 0.64μg/Kg/minute), tyramine (10μg/Kg/minute) and bilateral carotid occlusion before and during infusions of dibozane (10 and 20μg/Kg/minute).

Noradrenaline 0.16μg/Kg/minute - NA 0.16μg

Noradrenaline 0.32μg/Kg/minute - NA 0.32μg

Noradrenaline 0.64μg/Kg/minute - Na 0.64μg

Tyramine 10μg/Kg/minute - Tyr 10μg

Bilateral Carotid Occlusion - BCO

Dibozane 10μg/Kg/minute - DIB 10μg

Dibozane 20μg/Kg/minute - DIB 20μg

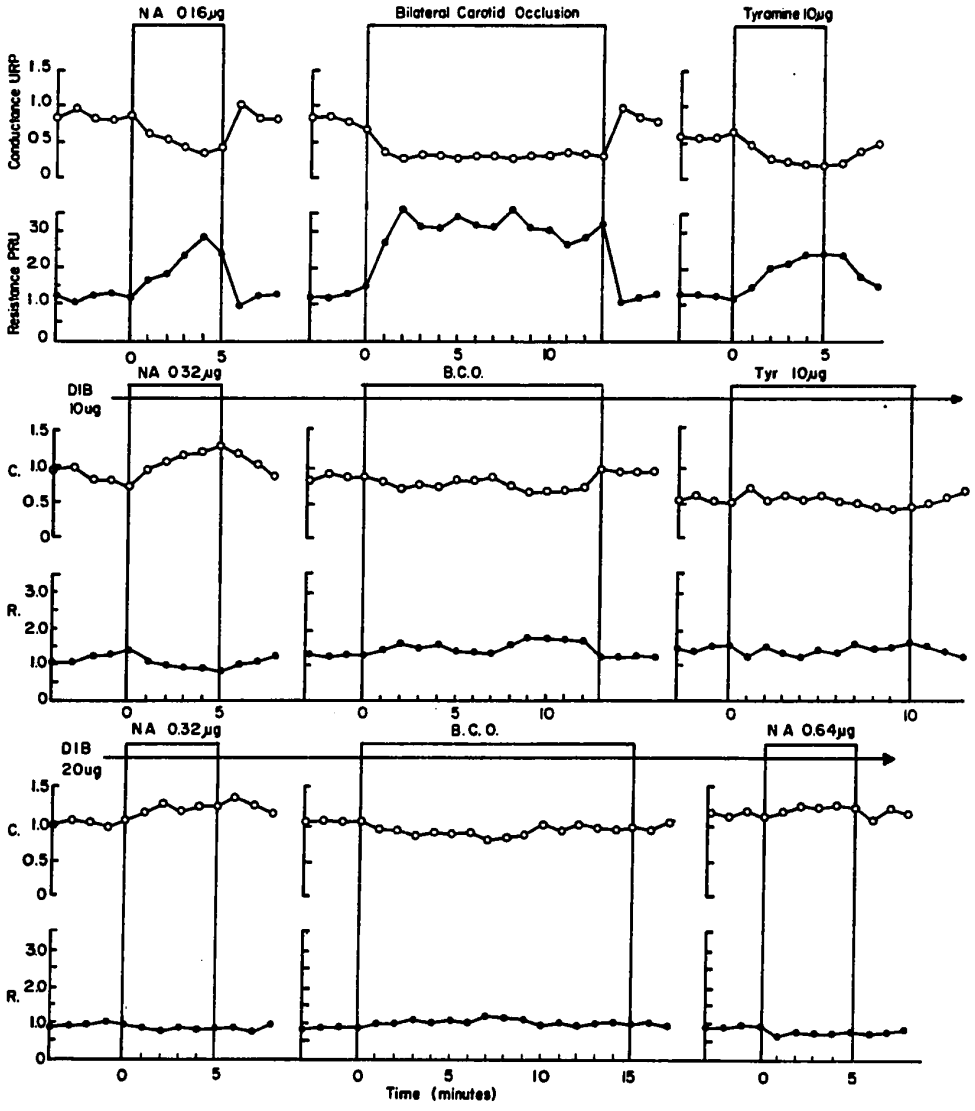

A
B
C
NA 0.16µg
Bilateral Carotid Occlusion
Tyramine 10µg
Conductance URP
Resistance PRU
NA 0.32µg
B.C.O.
Tyr 10µg
DIB 10ug
C.
R.
NA 0.32µg
B.C.O.
NA 0.64µg
DIB 20ug
Time (minutes)

Figure 9

Figures 10 and 11:

The response to noradrenaline, tyramine and BCO expressed as percentage change from control levels for one experiment. Doses are in µg/Kg/minute.

% change in vascular resistance - ●

% change in vascular conductance - o

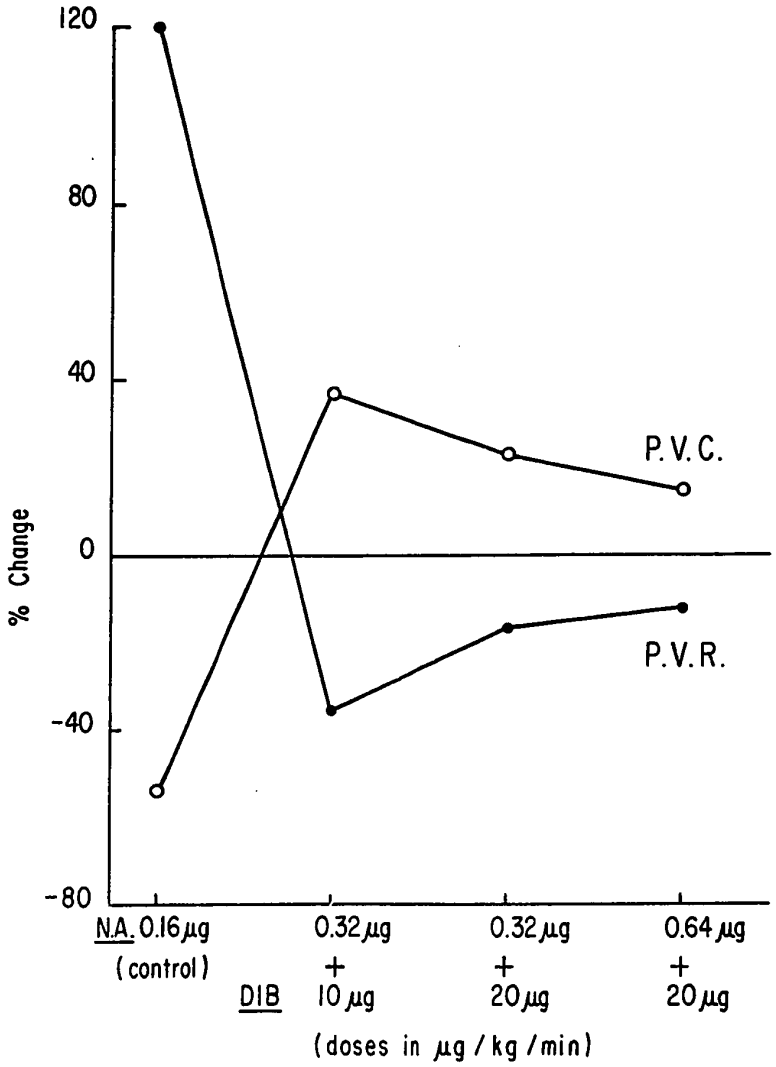

120
80
40
0
-40
-80
% Change
P.V.C.
P.V.R.
N.A. 0.16 µg
(control)
0.32 µg
+
DIB 10 µg
0.32 µg
+
20 µg
0.64 µg
+
20 µg
(doses in µg / kg / min)

Figure 10

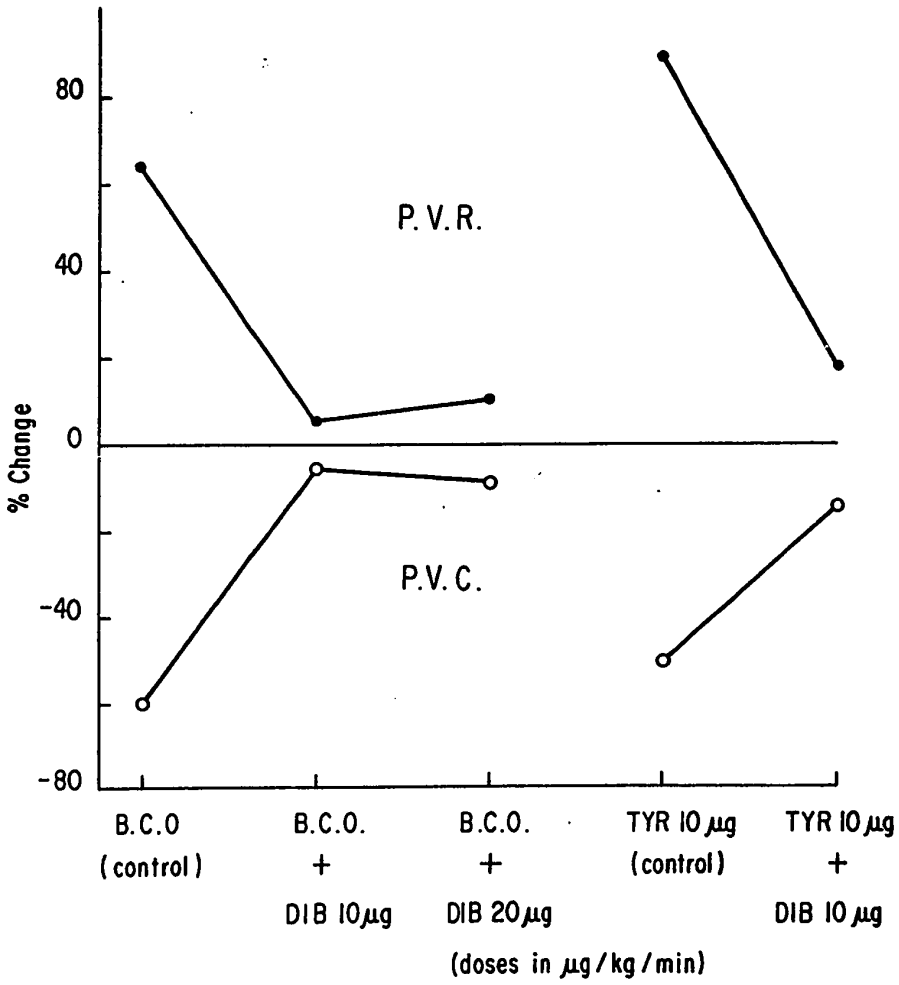

80
40
0
-40
-80
% Change
P.V.R.
P.V.C.
B.C.O (control)
B.C.O. + DIB 10µg
B.C.O. + DIB 20µg
TYR 10µg (control)
TYR 10µg + DIB 10µg
(doses in µg/kg/min)

Figure 11

respectively). Bilateral carotid occlusion produced an increase in resistance (% ch = 65%) (Figure 11). This response was reduced after dibozane (10μg/Kg/minute) (% ch in resistance = 6%). A similar response was produced after dibozane (20μg/Kg/minute) (% ch = 11%). Tyramine (10μg/Kg/minute) produced an increase in resistance (% ch = 89%), but vascular resistance was not reversed after dibozane (10μg/Kg/minute); that is, tyramine still produced a vasoconstriction.

DISCUSSION

Birmingham and Szolcsanyi (1965)[50], using isolated tissue, suggested thymoxamine was a competitive antagonist of adrenergic alpha receptors on vas deferens and vascular smooth muscle and a histamine antagonist on guinea pig ileum. They noted thymoxamine had a relative specificity for alpha adrenergic receptors, but the effect was completely reversible. In experiments by Birmingham et al. (1967)[51] on unconscious cats, thymoxamine reduced the pressor response to intra-venous noradrenaline. The proposed mode of action was on vascular alpha receptors. Turner et al. (1969)[52] applied thymoxamine as an ointment to the feet of patients with severe ischaemic disease to the lower limbs. The results suggested thymoxamine reflected a reduction in alpha adrenergic receptor mediated vasoconstriction. In the present experiments, thymoxamine was ineffective in producing noradrenaline or tyramine "reversal". This suggests thymoxamine is at best a relatively weak alpha receptor blocker. Birmingham et al. (1969)[53] suggested the action of thymoxamine on human arteries indicated simple competitive antagonsim, but in the present experiments, thymoxamine was ineffective in abolishing or reversing the response to noradrenaline even at the highest doses.

Ramsay (1969)[54] found that intra-venous thymoxamine produced a definite cutaneous vasodilatation in patients with vasopastic conditions with no large vessel occlusion, but was ineffective in patients with occlusive vascular disease. Thus, the drug exerts its action directly on vascular smooth muscle. The ability of thymoxamine

to increase blood flow to the foot was explained by Meyers (1968)[55] as an appreciable dilatation of skin vessels, but little dilatation in the muscle circulation. In the present experiments the direct vasodilatory effect was very evident. Since an attempt was made to isolate the muscle circulation by eliminating skin and paw flow, this effect is assumed to be occurring principally in a muscle vascular bed. Kane (1970)[56] found thymoxamine to be a cutaneous dilator of brief duration of action in the hand and foot. The present results and those of Kane[56] indicate thymoxamine causes dilatation in both the skin and muscle. Its usefulness as a drug therefore seems to derive from its own direct vasodilatory effects since its effectiveness as a specific alpha blocker is questionable.

Ahlquist and Levy (1959)[58] demonstrated the alpha adrenergic blocking specificity of dibozane on canine ileum when the intestinal inhibitory effects of phenylephrine were blocked with dibozane and those of isoproterenol by DCI. But the effects of adrenaline were not blocked unless both alpha and beta receptor blockers were present. In the present experiments, the response to intra-arterial noradrenaline was reversed, but the response to tyramine and bilateral carotid occlusion (reflex stimulation of the sympathetic nerves) was only reduced and never completely abolished or reversed. These results are in agreement with those of Rapela and Green (1961)[59] who observed dibozane only reduced the response to lumbar sympathetic nerve stimulation. They also noted that larger doses of dibozane reversed the response to noradrenaline although not as effectively as that to adrenaline. This suggests that dibozane is more effective in producing alpha receptor blockade to circulating noradrenaline than

to neurotransmitter-released from nerve stores. In the experiments with phentolamine, the response to tyramine was reversed in four out of five experiments (although the response was not significant). Thus, phentolamine appears to be more effective than dibozane in blocking the alpha adrenergic effects of endogeneously released noradrenaline, whereas dibozane is equally as effective as phentolamine in reversing the effects of blood-borne noradrenaline. Rapela and Green (1961)[59] suggested dibozane produced a non-competitive blockade whereas Goodman and Gilman[60] note in "The Pharmacological Basis of Therapeutics" that dibozane blockade is competitive and relatively transient. Increasing noradrenaline concentration during an infusion of dibozane indicated the blockade could be overcome. Thus a competitive blockade is possible.

SECTION III

The Effects of Theophylline on Adrenergic Responses in the Dog

In the preceeding experiments involving intra-arterial infusions of phentolamine, noradrenaline and tyramine, a small but consistent (although statistically insignificant) beta-response was obtained in the hind limb vascular bed to tyramine after alpha-receptor blockade with phentolamine. If a small beta receptor component was present it might be made more obvious if the response to beta receptor stimulation was potentiated.

It has been suggested by Robinson, Butcher and Sutherland (1967)[61] that the beta-adrenergic receptor may be related to the adenyl cyclase cyclic AMP system. Cyclic AMP is a nucleotide which plays a unique role in the action of many hormones. Its level in body tissues may be increased or decreased by hormonal action; the effect varies depending on the tissue. Evidence indicates that adenyl cyclase is localized in the cell membrane, therefore hormone action of cyclic AMP levels and membrane phenomena may be related.[62] Tissue levels of cyclic AMP as well as being influenced by hormones, can be affected by nicotinic acid, imidazole and the methyl-xanthines (eg. theophylline) acting on its synthesis and degradation. The only known enzymatic reaction resulting in the formation of cyclic AMP is that catalysed by adenyl cyclase which converts adenosine triphosphate to cyclic AMP and inorganic phosphate in the presence of Mg^{2+} and Mn^{2+} ions.[63] Adenyl cyclase is activated by sodium fluoride, and inhibited by zinc and acetyl-choline. The only known route of cyclic AMP catabolism is hydrolysis of the cyclic nucleotide to 5' AMP catalysed by specific enzymes (one or more phosphodiesterases). Phosphodiesterase which breaks down cyclic AMP is activated by imidazole and inhibited by theophylline.

Evidence that beta adrenergic receptors are closely associated with (if not an integral component of) the adenyl cyclase system is considerable. Murad et al (1963)[64] reported that adrenaline stimulates the synthesis of cyclic 3'5' AMP in heart muscle by direct action on adenyl cyclase and that this stimulating effect is prevented by beta adrenergic receptor blockade. The same workers (1963)[64] investigated the relative potencies of a series of catecholamines in stimulating adenyl cyclase and they were found to be similar to their potencies as inotropic agents *in vivo* . The effects were blocked by dichloroisoproterenol (DCI). Furthermore it was found that the rise of cyclic AMP concentration was very rapid following an injection of adrenaline and the rise preceded the inotropic effect.[65] Other evidence has been provided by studies using theophylline, a potent inhibitor of phosphodiesterase. Potentiation of the inotropic effect of noradrenaline by theophylline was demonstrated by Rall and West (1963)[65] and confirmed by Epstein et al 1970.[66] Both the positive inotropic effects and increase in cyclic AMP levels resulting from catecholamine administration could be prevented by beta-receptor blockade.

From the foregoing it would appear that potentiation of beta adrenergic stimulation in vascular smooth muscle might be accomplished by theophylline. If potentiation occurred this would mean:

(1) first of all, that adenyl cyclase was related to the beta receptor and therefore that potentiation of the beta adrenergic response might occur as a result of theophylline inhibition of the enzyme responsible

for the destruction of 3'5' cyclic AMP. Retarding the destruction of cyclic AMP in the area of the receptor site would increase the observed beta effect by increasing the concentration of cyclic AMP.

(2) secondly, that a relationship could exist between the cardiac beta receptor and cyclic AMP, as reported above, and the peripheral-beta adrenergic receptor.

However, there is little direct evidence to support this hypothesis in smooth muscle. Wikenfeld and Levy (1969)[67] determined the effect of theophylline on the inhibitory response to isoproterenol and phenylephrine in the spontaneously contracting rabbit ileum. The log dose curves of isoproterenol were shifted to the left in the presence of theophylline, while the dose response curves for the inhibitory effect of phenylephrine were not altered by similar treatment with theophylline.

They concluded that 3'5' AMP mediates the beta response in the rabbit ileum, but that cyclic 3'5' AMP is not involved in the inhibitory response due to alpha receptor activation. Morel (1969)[68] compared the effects of oxytocin and noradrenaline on active sodium transport and osmotic water flow across epithelial cells of the skin of amphibians and demonstrated potentiation of beta receptor effects by theophylline. In this preparation addition of cyclic AMP or noradrenaline resulted in increased osmotic water flow and sodium transport. The effect of threshold doses of cyclic AMP and noradrenaline were potentiated by theophylline since the effects produced by both compounds was greater than the sum produced by each alone in the doses used. The effects produced

by noradrenaline were completely suppressed by beta receptor blocking agents such as propranolol, but not by alpha receptor blocking agents such as phentolamine. Somlyo et at (1970)[69] recently reported that cyclic AMP and theophylline hyperpolarize smooth muscle of rabbit main pulmonary artery in low, but not high concentrations of potassium. The dependence of this effect on low external potassium is similar to that observed with isoproterenol. It was also observed that the hyperpolarizing effect of isoproternol was potentiated with prior treatment with theophylline. These findings are compatible with the assumption that potassium dependent beta adrenergic hyperpolarization is mediated by cyclic adenosine monophosphate.

There are those who contend that a relationship exists between adenyl cyclase and the adrenergic alpha receptor. Turtle (1967)[70] found the effect of alpha receptor activation to be opposite to those of cyclic AMP on insulin release. This would imply that sympathetic vasoconstriction could be mediated by a decrease in the level of cyclic AMP in vascular smooth muscle. Sutherland (1968)[71] suggested that effects of alpha receptor activation may be related to a decrease in the intracellular level of cyclic AMP.

Bartelstone (1967)[72] showed that an intravenous infusion of theophylline potentiated the response to carotid occlusion and to the injection of noradrenaline in dogs during M.V.O. (main vessel occlusion). He also observed that cyclic 3'5' AMP potentiated the contractions of rat aortic strip to noradrenaline. Also Bartelstone reported several instances in which small concentrations of theoph-

ylline under certain conditons increased the contraction of smooth muscle in response to catecholamine. The results of Bartelstone et al[72] are interpreted to indicate that enhancement of vascular smooth muscle contraction by inhibition of phosphodiesterase is mediated by sympathetic alpha receptors. Smith and Ireson (1970)[73] obtained results which indicated isoproterenol and noradrenaline were having an inhibitory effect on electrically induced contraction of ileum by an action on sympathetic alpha receptors. These results ([72,73]) suggest sympathetic vasoconstriction is mediated by an increase rather than a decrease in the intracellular level of cyclic AMP as was suggested by Sutherland[71] previously.

The first experiments in this section deal with the effect of intra-arterially infused theophylline on the response of the perfused vascular bed of the dog hind limb to intra-arterial isoproterenol. The effect of theophylline on the response to intravenous noradrenaline and carotid occlusion was investigated in the second group of experiments.

METHODS

A. Effect of Theophylline on the Vascular Response to Intra-arterial Isoproterenol.

Experiments were performed on five mongrel dogs (weights 13-16Kgms). The animals were anaesthetized with pentobarbital sodium (nembutal ^{R}) (30mgm/Kg) and respired if required by means of a Harvard respirometer. One or both of the hind limbs was perfused as follows. The dorsal aorta was cannulated through a midline abdominal incision as shown in Figure 12. The cannula was pushed well up into the aorta and tight ligatures were placed around the aorta to eliminate anastomotic blood flow to the hind limb. Another incision paralleling the inguinal ligament was made to expose the external iliac-femoral artery and a catheter was inserted downstream in this artery. The catheter in the dorsal aorta was connected by means of a stopcock to tygon tubing (2.5mm I.D.) through which blood from the aorta was pumped with a Harvard Peristaltic pump through a thermostatically controlled water bath maintained at 38°C and back into the femoral artery to perfuse the limb. Perfusion pressure was recorded from a three-way connector just prior to where the blood entered the perfused limb. Perfusion pressure was measured with a Statham P23db pressure transducer and recorded on a Beckman dynograph on heat sensitive paper. Systemic arterial pressure was measured from the brachial artery as described in Section I. Perfusion pressure was adjusted according to the systemic arterial pressure. The transducers were calibrated with a mercury manometer at the beginning of the experiment. Drugs were infused through the femoral profundus branch of the femoral artery

Hind limb perfusion apparatus

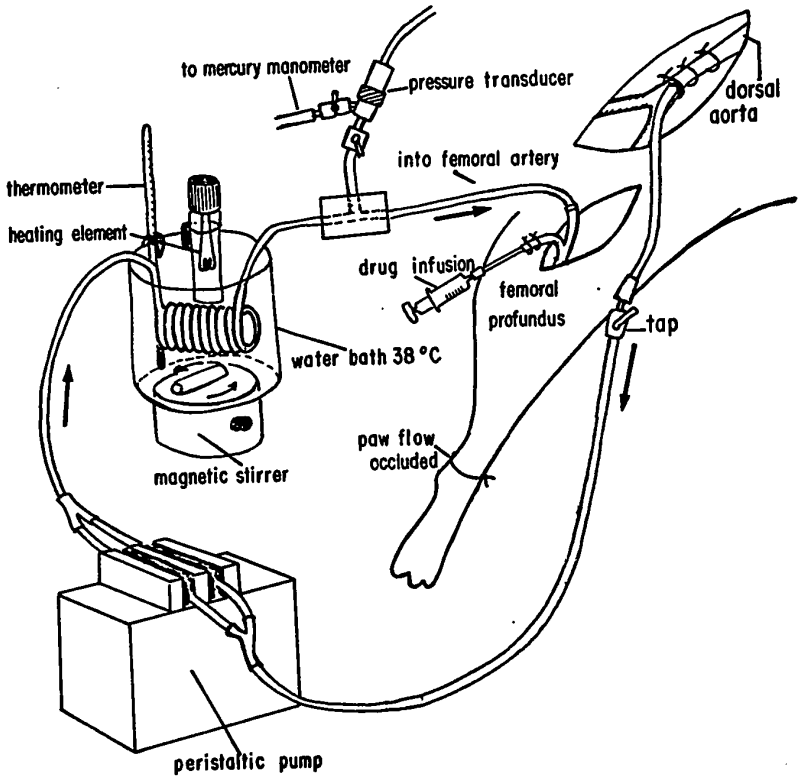


Figure 12

Figure 12:

Prepartion and Apparatus for Hind Limb Perfusion. Blood from the aorta was pumped through a peristaltic pump and back into the hind limb (s) as indicated by the direction arrows.

(see Figure 12). Paw circulation was occluded by means of a tight wire ligature placed at the distal end of the femur. The animal was heparinized with an intravenous injection (5mg/Kg) prior to the start of limb perfusion.

When both the hind limbs were perfused, blood from the aorta flowed through a "Y" connector just distal to the tap. A separate length of tygon tubing went through the peristaltic pump and the water bath; and perfusion pressure was monitored with a duplicate set-up shown in Figure 12. Ascorbic acid saline (0.9% NaCl and 0.03% ascorbic acid)was infused continously in the control side. On the experimental side, ascorbic acid saline was infused during control runs, this was followed by theophylline made up in ascorbic acid saline. Isoproterenolwas made up in theophylline as required.

RESULTS

Figure 13 illustrates the changes in perfusion pressure in one experiment in response to isoproterenol. Three experiments employing the doses shown in Figure 13 were carried out. In the first row isoproterenol ($10^{-4}\mu g$/Kg/minute) was infused for five minutes and a slight drop in perfusion pressure was recorded suggesting a vasodilatory effect. Dose dependent falls in perfusion pressure were observed for subsequent infusions of isoproterenol (10^{-3} and $10^{-2}\mu g$/Kg/minute). Each infusion lasted for five minutes. Theophylline (10µg/Kg/minute) was then infused for ten minutes and the doses of isoproterenol were repeated while the theophylline infusion was continued at this rate. Dose dependent decreases in perfusion pressure in response to IPN were again observed. The last minute of the infusion of isoproterenol was compared to the last minute of pre-run control levels. The difference is expressed as a percentage change from control levels. In Figure 13 theophylline appears to potentiate the response to isoproterenol, however, this effect was not reproducible. The average response (n=3) to isoproterenol ($10^{-4}\mu g$/Kg/minute) was a slight decrease in perfusion pressure (% ch = -3). Larger doses of isoproterenol produced larger dose dependent decreases in perfusion pressure (IPN $10^{-3}\mu g$/Kg/minute, % ch = -16; and IPN $10^{-2}\mu g$/Kg/minute, % ch = -29). The responses to isoproterenol during theophylline (10µg/Kg/minute) was isoproterenol $10^{-4}\mu g$/Kg/minute caused essentially no change (% ch = -1.4); IPN $10^{-3}\mu g$/Kg/minute, % ch = -8, and IPN $10^{-2}\mu g$/Kg/minute (% ch = -22). Thus an average of the reduction in perfusion pressure to the three doses of IPN was less during

Figure 13:

Response of the perfused dog hind limb to isoproterenol (10^{-4}, 10^{-3} x $10^{-2}\mu g$/Kg/minute) before and during infusion of theophylline (10μg/Kg/minute) in one experiment.

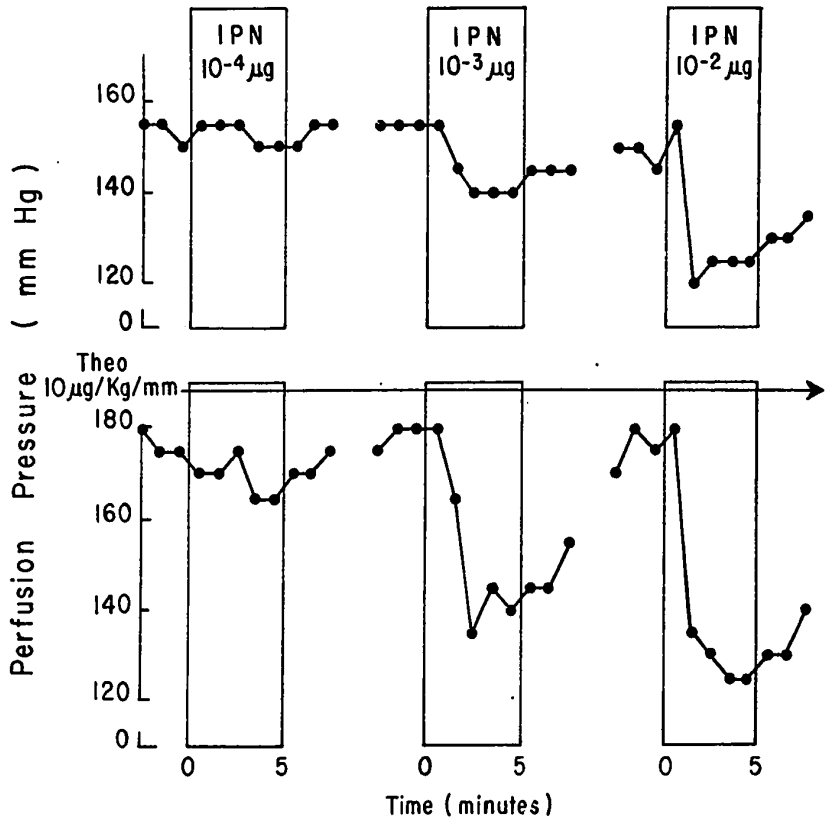

IPN
10-4 µg
IPN
10-3 µg
IPN
10-2 µg
160
140
120
0
(mm Hg)
Theo
10µg/Kg/mm
Perfusion Pressure
180
160
140
120
0
0
5
0
5
0
5
Time (minutes)

Figure 13

theophylline treatment than before each treatment.

In a further experiment (the partial results of which are shown in Figure 14) the effect of increasing doses of theophylline on the response to isoproterenol 10^{-3} and $10^{-2}\mu g$/Kg/minute were observed. The results are shown as a change in perfusion pressure from control levels. In Figure 14 (1), isoproterenol $10^{-2}\mu g$/Kg/minute caused a decrease in perfusion pressure (% ch = -29). Isoproterenol ($10^{-2}\mu g$/Kg/minute) repeated during theophylline (50µg/Kg/minute) (2) then caused a smaller decrease in perfusion pressure (% ch = -27). Increasing theophylline to 100µg/Kg/minute (Figure 14, (3)) reduced the response still further. (% ch = -5). When theophylline was increased to 250µg/Kg/minute (Figure 14 (4)) the fall in perfusion pressure increased slightly from Figure 14 (3), (% ch = -11). Then theophylline was discontinued and isoproterenol was infused for five minutes. Isoproterenol produced a still greater fall in perfusion pressure (% ch = -21) but this was still smaller than the control response. In another experiment theophylline was increased to 500µg/Kg/minute. The effects of intra-arterial theophylline on perfusion pressure were compared to a control limb in which ascorbic acid saline was infused. Theophylline caused a fall in perfusion pressure (% ch = -20) whereas the control side was unaffected.

These effects were investigated further by giving selected doses of theophylline and isoproterenol as injections. In Figure 15, a set of typical responses is illustrated. At time (1) theophylline (1mgm/Kg) and isoproterenol (0.5µg/Kg) were injected simultaneously into the right and left perfused limbs respectively. Isoproterenol produced a fall in perfusion pressure (% ch = -25).

Figure 14:

The response to isoproterenol ($10^{-2}\mu g$/Kg/minute) during increasing doses of theophylline (50, 100, 250µg/Kg/minute) and after theophylline was discontinued.

(1) Isoproterenol $10^{-2}\mu g$/Kg/minute (control).

(2) IPN + theophylline 50µg/Kg/minute.

(3) IPN + theophylline 100µg/Kg/minute.

(4) IPN + theophylline 250µg/Kg/minute.

(5) IPN (theophylline discontinued, saline infused).

The control is shown on both figures.

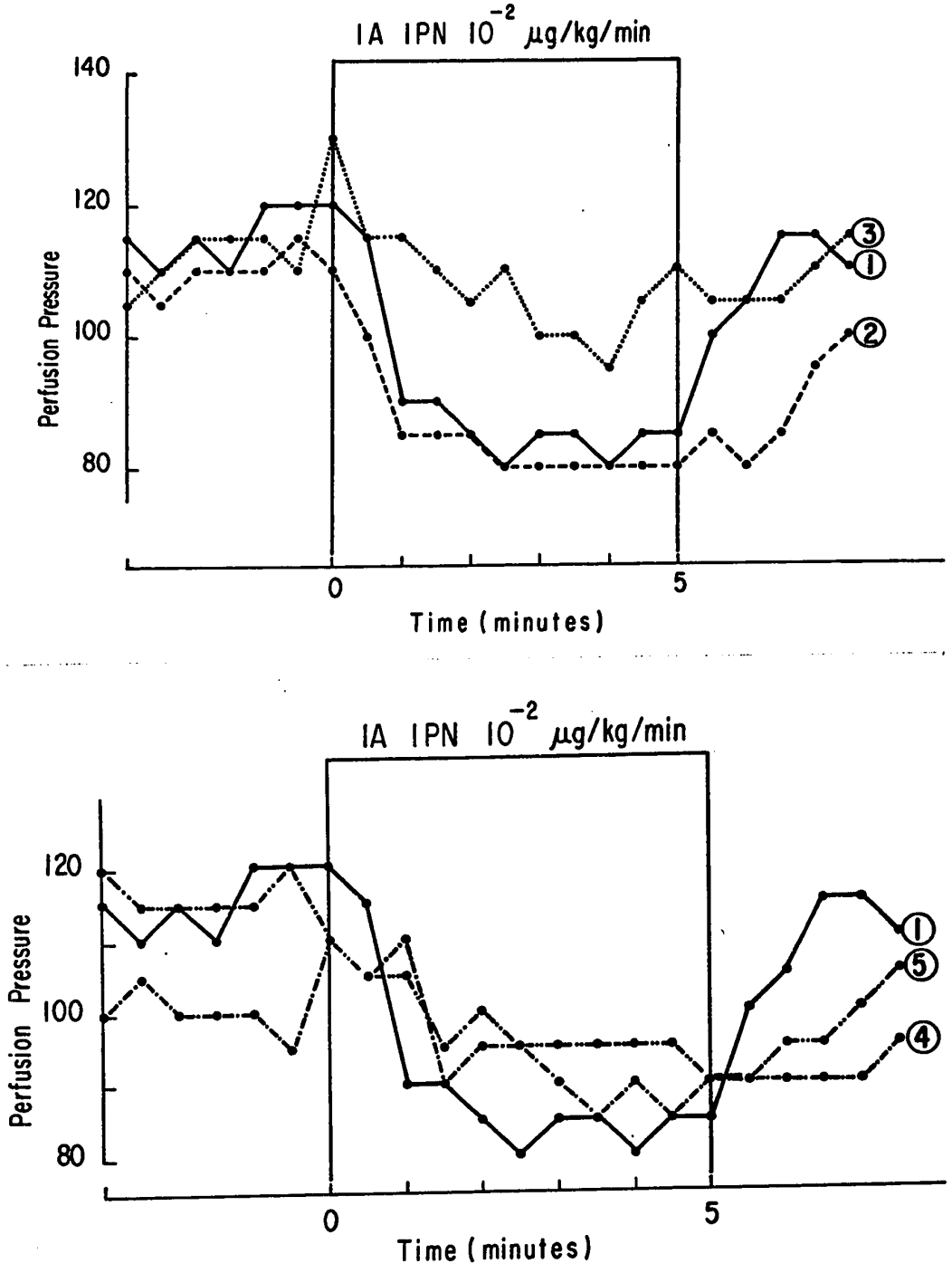

IA IPN 10^{-2} µg/kg/min
140
120
100
80
Perfusion Pressure
0
5
Time (minutes)
③
①
②
IA IPN 10^{-2} µg/kg/min
120
100
80
Perfusion Pressure
0
5
Time (minutes)
①
⑤
④

Figure 14

Figure 15:

The response obtained to selected injections of theophylline and isoproterenol injected simultaneously into the perfused left and right hind limbs in one experiment.

(1) theophylline (1mg/Kgm) to the right $limb^{R}$
isoproterenol (0.5µg/Kg) to the left $limb^{L}$

(2) theophylline (1mg/Kgm) + IPN (0.5µg/Kg) to ^{L}
isoproterenol (1µg/Kg) to the ^{R}

Left perfused limb -●-●-

Right perfused limb -o-o-

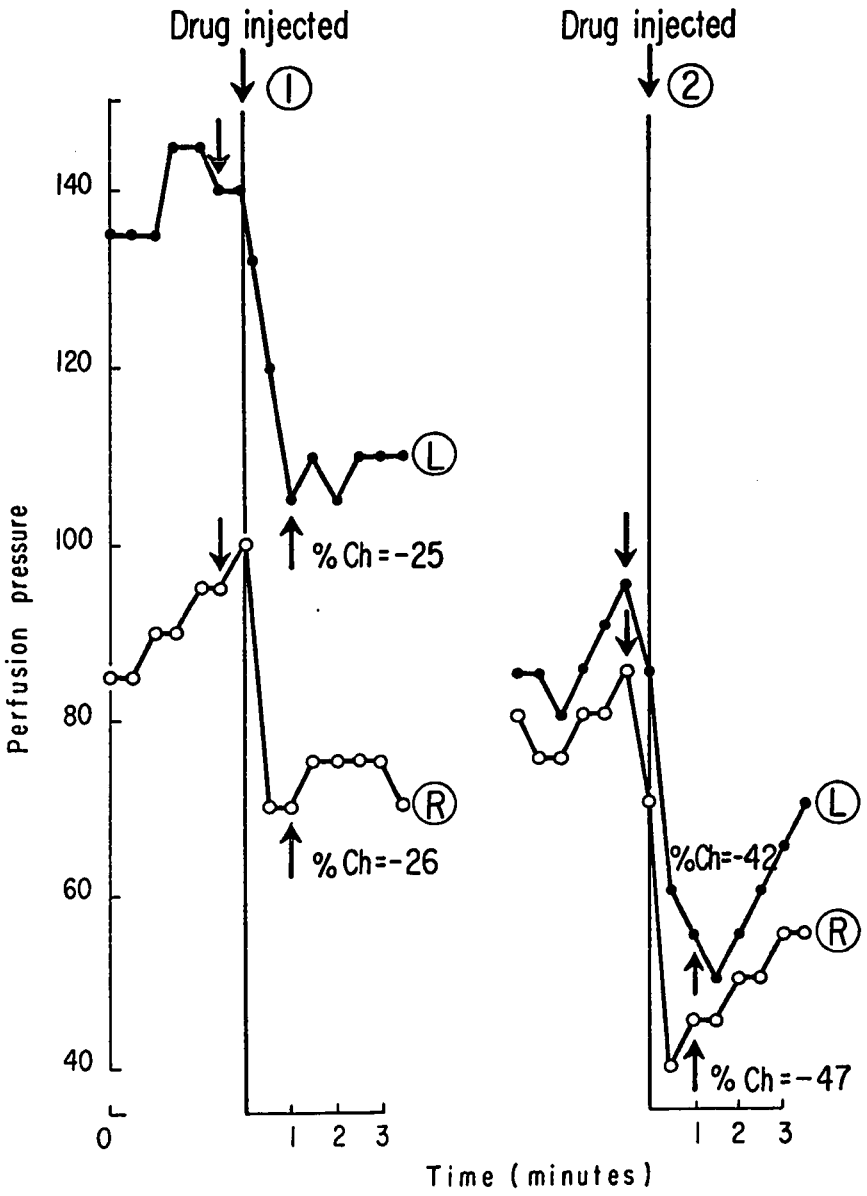

Drug injected
①
Drug injected
②
140
120
100
80
60
40
Perfusion pressure
L
%Ch=-25
R
%Ch=-26
%Ch=-42
L
R
%Ch=-47
0
1 2 3
1 2 3
Time (minutes)

Figure 15

Theophylline produced a fall in perfusion pressure of equal magnitude (% ch = -26) (the percentage change from one minute before injection was compared to one minute after injection). At time (2) in Figure 15, the fall in perfusion pressure (% ch = -47) in response to isoproterenol (0.5μg/Kg) and theophylline (1mgm/Kg) injected intra-arterially into the left limb was only slightly greater than the response to an injection of isoproterenol (1μg/Kg) given to the right perfused limb (% ch = -42).

METHODS

B. The Effect of Theophylline on the Response to Intravenous Noradrenaline and Bilateral Carotid Occlusion.

Experiments were performed on five mongrel dogs weighing 14-18kgms. The dogs were anaesthetized as described in Section I. A catheter was placed in the cephalic vein in the right foreleg. This catheter was connected to a three-way stopcock which was "Luer-locked" to a syringe mounted in the infusion pump. All drugs were given in this catheter. During a continuous infusion, intravenous injections were administered by switching the three-way stopcock to an injection syringe (for a 1 ml injection) and then back to the infusion pump. For all purposes the infusion was continuous and the injected dose was infused in the blood stream by the pump at a rate of 1 ml/minute. All drugs were made up in ascorbic acid saline (0.9% NaCl, 0.03% ascorbic acid) which was infused alone during control runs.

A catheter was placed in the brachial artery, as shown in Figure 1 for measurement of systemic arterial pressure. A flow probe was mounted on the external-iliac-artery to record flow to the left hind limb (also shown in Figure 1). Paw flow was occluded by a tight ligature placed at the distal end of the femur and cutaneous circulation of the leg was minimized by ligating major cutaneous vessels.

The carotid arteries and vagus nerves were exposed through a midline incision in the neck. Ties were placed around the arteries so that they could be intermittently occluded. The vagus nerves were ligated and divided. If necessary the animal was respired

artifically after division of the vagi in the neck. Heart rate was monitered with a cardiotachometer. Heart rate, systemic arterial pressure, and left hind limb blood flow were recorded with a Beckman Dynograph on heat sensitive paper. Peripheral vascular resistance and conductance were calculated from mean arterial pressure and limb blood flow as described in Section I.

RESULTS

The stimuli of intravenous noradrenaline (2μg) injection and carotid occlusion were applied before, after and during increasing levels of theophylline (10, 20, 40, 100μg/Kg/minute). The response to I.V. noradrenaline (2μg) is shown in Figure 16 (far left). Mean blood pressure was increased, heart rate was decreased and conductance was increased and its reciprocal resistance was decreased. When pressure, flow and heart rate had returned to control level, the left carotid artery was occluded for sixty seconds. Figure 17 (far left) shows the average response obtained to left carotid occlusion. Mean blood pressure was markedly increased, heart rate was increased and a peripheral vasoconstriction resulted, indicated by the fall in conductance and increase in resistance. Left carotid occlusion was followed by noradrenaline (2μg) which was in turn followed by left carotid occlusion (LCO) again. Each run consisted of two injections and two periods of occlusion. These were repeated during infusions of theophylline 10, 20, 40, 100μg/Kg/minute and after theophylline was discontinued. Figures 16 and 17 show the responses to noradrenaline and left carotid occlusion during theophylline and after theophylline was discontinued (average of 10 experimental trials, [two each in five dogs] except for heart rate which is based on eight trials [two each in four dogs]). Noradrenaline (2μg) alone (Figure 16) produced an increase in mean blood pressure (% ch = 6, $p<0.001$). The average of two minutes of control was compared to the response of one minute after injection. Heart rate was consistently decreased (% ch = 2%, $p<0.01$) by noradrenaline whereas resistance and

Figure 16:

The average response to intravenous noradrenaline (2μg) of mean arterial pressure, heart rate, and peripheral resistance and conductance (n = 5 except for heart rate for which n = 4), before and during theophylline 10, 20, 40, 100μg/Kg/minute and after theophylline was discontinued.

I.V. Noradrenaline 2μg	▼
Mean BP (mm/Hg)	□
Heart rate (beats/minute)	■
Conductance (URP)	o
Resistance (PRU)	●

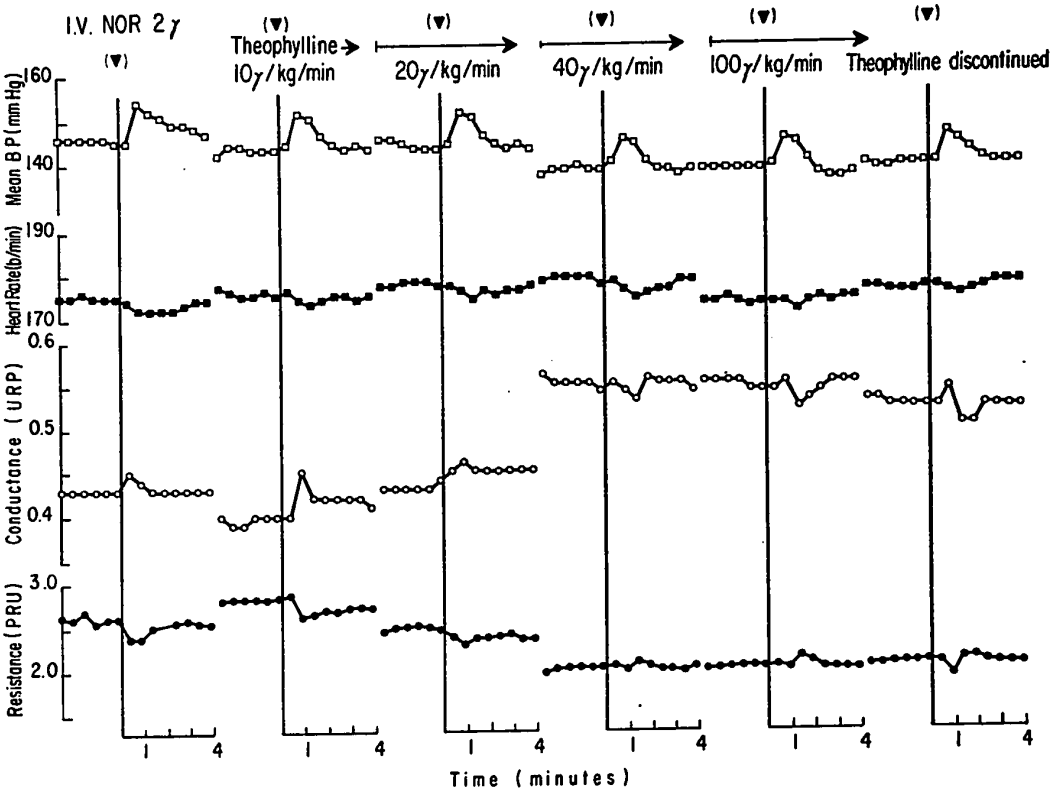

I.V. NOR 2γ
(▼)
(▼)
Theophylline →
10γ/kg/min
(▼)
20γ/kg/min
(▼)
40γ/kg/min
(▼)
100γ/kg/min
(▼)
Theophylline discontinued
Mean BP (mmHg)
160
140
Heart Rate (b/min)
190
170
Conductance (URP)
0.6
0.5
0.4
Resistance (PRU)
3.0
2.0
1
4
Time (minutes)

Figure 16

Figure 17:

The average responses during left carotid occlusion of mean arterial pressure, heart rate, and peripheral resistance and conductance before and during treatment with theophylline 10, 20, 40, 100µg/Kg/minute. The left carotid artery was occluded for 60 seconds.

Left carotid occlusion	LCO
Control (ascorbic acid saline infused)	A
Theophylline 10 g/Kg/minute	B
Theophylline 20 g/Kg/minute	C
Theophylline 40 g/Kg/minute	D
Theophylline 100 g/Kg/minute	E
Theophylline discontinued (saline infused)	F
Mean blood pressure (mm/Hg)	▫
Heart rate (beats/minute)	■
Vascular conductance	o
Vascular resistance	●

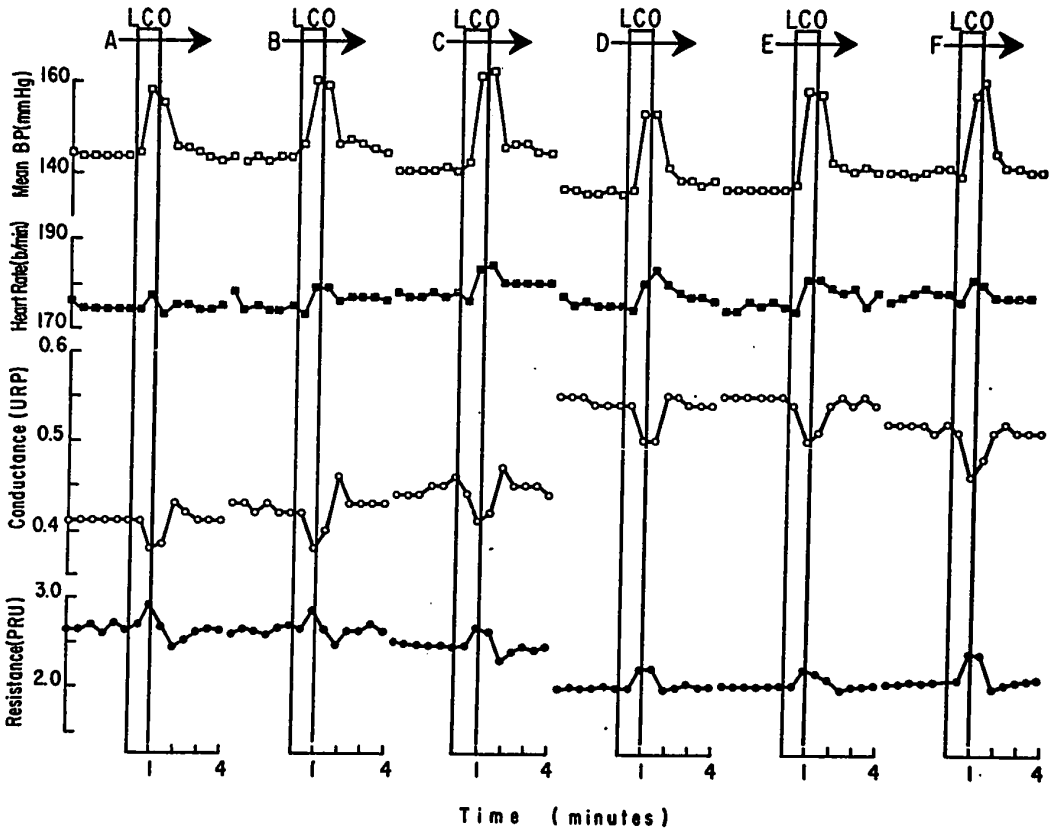

LCO
A
B
C
D
E
F
Mean BP(mmHg)
160
140
Heart Rate(b/min)
190
170
Conductance (URP)
0.6
0.5
0.4
Resistance(PRU)
3.0
2.0
1
4
Time (minutes)

Figure 17

conductance were essentially unchanged. During theophylline (10μg/Kg/minute) the pressure response (% ch = 6%) was not different from the control. The heart rate response did not differ significantly from the control response. (% ch = 2.8±1, $p<0.10$). While a significant increase in conductance (% ch = 12.5%, $p<0.01$) and a decrease in resistance (% ch = 5, $p<0.05$ occurred, these values were not significantly different from the control ($p<0.05$). Noradrenaline (2μg) injected during theophylline 20μg/Kg/minute increased mean blood pressure (% ch = 6%), did not change heart rate, produced an increase in resistance (% ch = 7%) and a decrease in conductance. Comparing these responses to those of the control, no significant differences were observed. Noradrenaline (2μg) during theophylline (40μg/Kg/minute) produced approximately the same change in pressure (% ch = 5%) as the control, similarly heart rate and conductance did not significantly differ from the control. Noradrenaline given during theophylline 100μg/Kg/minute produced a change in heart rate (% ch = 5%) which was significantly different from the control ($p<0.05$). Mean arterial pressure and peripheral blood flow were not significantly altered from the control after theophylline was discontinued, the response of noradrenaline remained as previously. Mean blood pressure was increased (% ch = 6%, same as control) whereas peripheral flow did not change. Heart rate was unchanged, and this response was different from the control value (% ch = 2.3±0.8, $p<0.06$).

In Figure 17A the left carotid artery was occluded for 60 second during an infusion of ascorbic acid saline. Left carotid occlusion produced a rise in mean blood pressue (% ch = 11%,

$p<0.001$). Heart rate was unchanged while resistance increased and conductance decreased, but the changes were not significant. During theophylline 10μg/Kg/minute (Figure 17B) mean blood pressure was increased, heart rate increased while conductance was decreased by left carotid occlusion. When theophylline was increased to 20μg/Kg/minute (Figure 17C) mean blood pressure was increased by left carotid occlusion (% ch = 16%). This was significantly different from the control (% ch = 6, $p<0.01$). Heart rate increased slightly (% ch = 3.4), conductance decreased and resistance increased, but these parameters did not vary from control values. Figure 17D shows the response to left carotid occlusion during theophylline 40μg/Kg/minute. Mean blood pressure was increased (% ch = 13), heart rate increased (% ch = 4.6), while conductance decreased (% ch = -7), and resistance increased (% ch = 10). None of these responses differed significantly from the control responses. In Figure 17E, mean blood pressure increased (% ch = 14.5) during theophylline 100μg/Kg/minute, heart rate increased (% ch = 31%), resistance increased % ch = 8% and conductance was decreased (% ch = -9). No significant differences were observed between these and control response. Similar responses were observed after theophylline was discontinued, as before when the responses were compared to the control no changes were observed.

Summarizing the responses to noradrenaline and left carotid occlusion during theophylline compared to control responses, theophylline (20μg/Kg/minute) potentiated the pressure response to left carotid occlusion (absol. diff = 5.8±1.7, $p<0.01$) but this effect was not observed again for other doses of theophylline.

The pressure response to noradrenaline was not potentiated in any situation. Theophylline (100µg/Kg/minute) prevented the decrease in heart rate in response to noradrenaline (absol. diff = 2.6±1.1, $p<0.05$) and this effect persisted after theophylline was discontinued (absol. diff = 2.3±0.8, $p<0.05$). In all cases the response to heart rate was quite small (max. change five beats/minute) while resting heart rate averaged between 150-200 beats/minute. Therefore, the maximum percentage change observed would be of the order of five percent. This means that the changes that were produced, although small, were very consistent. Theophylline had no effect on the increase in heart rate produced during left carotid occlusion.

Theophylline did not potentiate the response to intravenous noradrenaline in the peripheral circulation although in the several instances at the high doses of theophylline the increase in conductance observed in the control response was converted to a decrease in conductance after theophylline. The effects of left carotid occlusion on peripheral blood flow were not potentiated by theophylline. Left carotid occlusion consistently produced an increase in resistance with a decrease in conductance.

The effect of theophylline directly on vascular conductance is shown in Figure 18. The percentage change from a control level (average of 10 trials) prior to noradrenaline is shown before theophylline (A) during theophylline (B, C, D, E) after theophylline was discontinued (F). Theophylline (10µg/Kg/minute) decreased conductance, but the effect is not significant. Theophylline 20µg/Kg produced little change in conductance (% ch = 2) from control levels. Theophylline 40µg/Kg/minute increased vascular conductance significantly

Figure 18:

The effect of theophylline on hind limb blood flow expressed as a percentage change from control levels of vascular conductance.

A. Before theophylline (ascorbic acid saline infused).
B. Theophylline 10μg/Kg/minute.
C. Theophylline 20μg/Kg/minute.
D. Theophylline 40μg/Kg/minute.
E. Theophylline 100μg/Kg/minute
F. Theophylline discontinued (ascorbic acid saline infused).

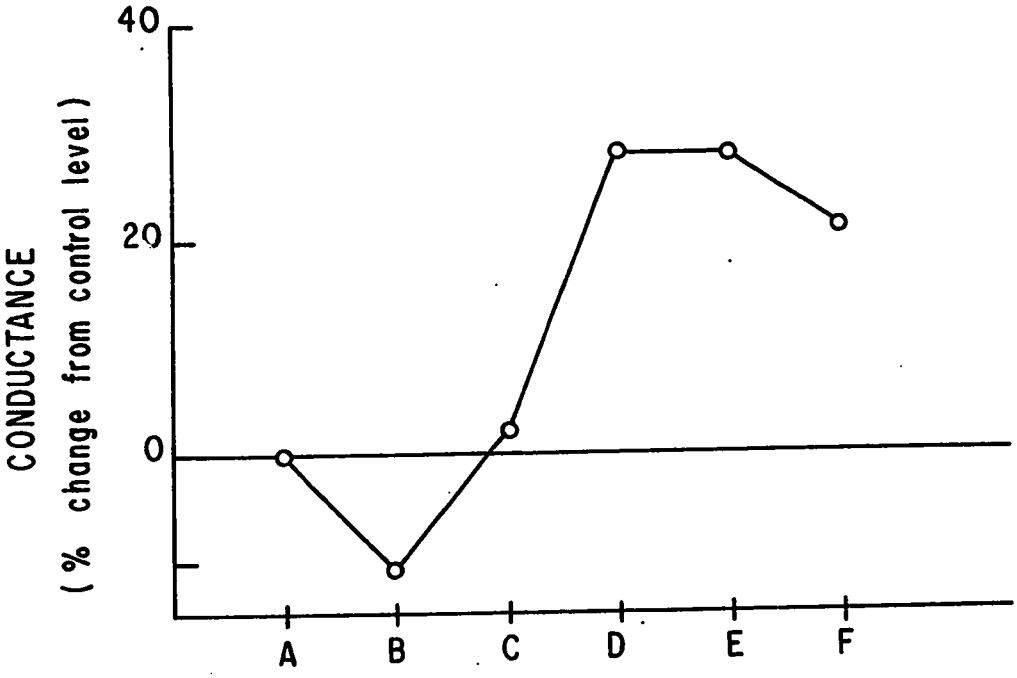


Figure 18

(% ch = 28, $p<0.001$). This effect was observed for theophylline (100μg/Kg/minute) also, (% ch = 28, $p<0.001$). The effect of theophylline on conductance persisted after theophylline was discontinued (% ch = 21, $p<0.01$).

DISCUSSION

If a relationship does exist between the beta receptor and adenyl cyclase, then theophylline (a potent inhibitor of phosphodiesterase) would potentiate the peripheral beta affect of isoproterenol by retarding the breakdown of cyclic AMP at the receptor site. Murad (1967)[64] obtained evidence that isoproterenol, adrenaline and noradrenaline produced a concentration dependent increase in the activity of adenyl cyclase. Bartelstone[72] suggested that theophylline will only produce significant effects *in vivo* in the presence of an agent which activates adenyl cyclase and this would suggest that an agent which did not activate adenyl cyclase would not be affected by theophylline. Since in the present experiments no potentiation by theophylline of the fall in perfusion pressure produced by intra-arterial isoproterenol was observed, it seems unlikely that the proposed relationship between the beta receptor and cyclic AMP is supported in the peripheral circulation. In fact it was demonstrated that an intra-arterial injection of theophylline produced a fall in perfusion pressure of the same magnitude as an equipotent injection of isoproterenol. A combination of the two given to one perfused limb produced approximately the same fall in perfusion pressure as twice the dose of isoproterenol (Figure 15). It seems unlikely that any potentiation was produced since the effects of the two drugs was purely additive. This evidence does not detract from other evidence relating cyclic AMP to other metabolic responses to catecholamines, such as hepatic and muscle glycogenolysis, lipolysis in adipose tissue, inotropic cardiac effects or intestinal and uterine motility supposedly

mediated by beta-adrenergic receptors. Recent work by Lucchesi and Hodgeman (1971)[10] suggested a structural difference between beta adrenergic receptors in the myocardium and coronary bed, and those in the peripheral circulation. This could imply that the peripheral beta receptor is not related to adenyl cyclase and that no potentiation of isoproterenol by theophylline would be expected. Furthermore, recent work by Shanfeld et al (1969)[74] has shown that the increase in phosphorylase A activity induced by noradrenaline could be blocked or markedly inhibited by IMA (N-isopropylmethoxamine) without significantly depressing the inotropic response. Furthermore IMA prevented the rise in cyclic AMP after noradrenaline injection without significantly impairing the increase in cardiac contractility. Thus the hypothesis that cyclic AMP mediates the inotropic response to catecholamines was not supported. In the present experiments theophylline had the reverse effect of what was expected. In Figure 14, increasing doses of theophylline decreased the fall in perfusion pressure in response to a standard dose ($10^{-2}\mu g$) of isoproterenol. More experiments would have to be conducted, but this paradoxical observation may be related to the finding that methyxanthines as in other tissues may have more than one effect on smooth muscle.

Bartelstone (1967)[72] found that cyclic AMP potentiated the contraction of rat aortic strip to noradrenaline. He also observed that in certain situations theophylline increased the contractions of smooth muscle in response to catecholamines. Smith and Ireson (1970)[73] proposed a relationship between the sympathetic alpha receptor and cyclic AMP to explain the inhibitory effects of

isoproterenol and noradrenaline on electriclly induced contractions of ileum. In the second group of the present experiments intravenous noradrenaline and left carotid occlusion were stimuli applied to further investigate this proposed relationship between the adrenergic alpha receptor and the adenyl cyclase system. Both noradrenaline (2μg) and left carotid occlusion produced minimal responses on the heart rate, blood pressure and hind limb blood flow. Thus if theophylline potentiated any of these parameters, the response could be readily observed. The results tended to vary, that is, theophylline potentiated the response to noradrenaline, or left carotid occlusion at one dose and not at another. For example, theophylline (20μg/Kg/minute) enhanced the pressure response to left carotid occlusion but the effect was not reproducible at higher doses of theophylline. Similarly theophylline (100μg/Kg/minute) enhanced the heart rate response to intravenous noradrenaline but only at this dose level. Theophylline did not potentiate the response to noradrenaline or left carotid occlusion in the peripherial circulation. It seem unlikely that theophylline produced any peripheral effects which could be interpreted as potentiating either alpha adrenergic (characteristically a vasoconstriction) or beta effects (vasodilatation). The fact that theophylline did potentiate the pressure response to left carotid occlusion, and the heart rate response to noradrenaline (both central effects) may lend support to evidence that a relationship exists between the myocardial beta receptor and the adenyl cyclase system. Theophylline produced a marked dilatation in the peripheral circulation at high doses as

shown in Figure 18. This was preceeded by an initial vasoconstriction and has been ascribed to stimulation of the medullary vasomotor center.[75] At therapeutic doses the peripheral vasodilator action predominates. This effect appeared to be the result of a direct action on vascular smooth muscle tone and did not seem to be related to adrenergically mediated responses to catecholamines. It is interesting to observe that infusions of small dosages of theophylline appeared to potentiate the effects of intravenous noradrenaline (a dilatation) in the peripheral circulation but this effect was obscured by the vasodilatory effect of higher doses of theophylline and the response converted to a constriction. Theophylline did not have significant effects on the constrictor response to noradrenaline. This is contrasted to the peripheral affects of theophylline on left carotid occlusion. In this case higher doses of theophylline appeared to increase the reflex vasoconstriction produced by left carotid occlusion. However, this was not a significant effect. Theophylline seems to have a somewhat variable effect on the peripheral circulation. Generally, theophylline was not of value in unmasking, or potentiating small beta stimulation in the peripheral circulation, nor did any enhancement of the alpha adrenergically mediated vasoconstrictor response to noradrenaline or carotid occlusion occur during theophylline infusion. These effects may be more clearly defined on isolated vessel preparations. The results of these experiments suggest that theophylline does produce some enhancement of the central response to noradrenaline. This could provide support for the evidence that the myocardial beta receptor is related to the adenyl cyclase system. These responses may have

been more clearly defined had the experiments been repeated during beta adrenergic receptor blockade.

If propranolol abolished the theophylline enhanced effect on heart rate and pressure of noradrenaline then it could be said with certainty that these were beta effects.

SECTION IV

Response of the Isolated Muscle Vascular Bed of Hind Limb of Dog to Intra-Arterial Noradrenaline and Tyramine

INTRODUCTION

It has been shown in Section I that intra-arterial tyramine which caused the release of noradrenaline from sympathetic nerve endings produced a vasoconstriction. After alpha receptor blockade with phentolamine, the response to tyramine was abolished, and in some cases reversed, but this response was not significant. Section II, investigated the possibility that other alpha receptor blockers might produce a more effective blockade of alpha adrenergic receptors, and Section III described an attempt to potentiate the adrenergic beta response. The possibility had been discussed that the cutaneous response which was exclusively "alpha" had been masking the beta adrenergic response to tyramine (in the muscle vascular bed) after alpha adrenergic receptor blockade. Therefore, in these experiments major cutaneous branches to the skin were ligated and paw flow was occluded to isolate the limb muscle circulation. In the first experiments various doses of noradrenaline and tyramine were used, as well as two doses of phentolamine. For the present study the optimum doses were chosen from Section I. In order that the responses might be subjected to more worthwhile statistical evaluation the number of experiments was increased from five in Section I to 10.

METHODS

Experiments were conducted on 10 mongrel dogs (average weight 17.7kgm). Figure 1 shows the experimental apparatus and the procedure is also described in Section I. As before, all drugs were made up in ascorbic acid saline (0.9% NaCl, and 0.03% ascorbic acid) which was infused continously from the beginning of the experiment during control runs, and served as a vehicle for drug administration during the experimental runs.

RESULTS

Figure 19 shows the results of a typical experiment. Noradrenaline 0.3μg/Kg/minute was infused intra-arterially for five minutes. The results are expressed as changes in conductance which were calculated from mean arterial pressure and hind limb flow. Noradrenaline produced a decrease in vascular conductance. This was followed,after flow stabilized, by an infusion of tyramine (10μg/Kg/minute) which was continued for 15 minutes. Tyramine produced a similar effect on vascular conductance as noradrenaline. Following tyramine an intra-arterial infusion of phentolamine 32μg/Kg/minute was given for 20 minutes before and during repetition of the noradrenaline and tyramine infusions. Where noradrenaline was repeated during phentolamine (middle Figure 19), an increase in vascular conductance was observed. The response had been converted from a vasoconstriction to a vasodilatation after alpha adrenergic receptor blockade. Tyramine (10μg/Kg/minute) infused during phentolamine (32μg/Kg/minute) also produced a dilatation although the response was not as great as "noradrenaline reversal". This was followed by a 20 minute infusion of phentolamine 32μg/Kg/minute and propranolol (5μg/Kg/minute). Propranolol essentially abolished the vasodilatation response to noradrenaline and tyramine observed after alpha receptor blockade indicating the vasodilatation was mediated via beta adrenergic receptors.

Figure 20 shows the percentage change in peripheral vascular resistance and conductance for noradrenaline (n=10) at five minutes before alpha receptor blockade with phentolamine (1),

Figure 19:

The results of a typical experiment. Top row: intra-arterial noradrenaline (0.3μg/Kg/minute) and tyramine (10μg/Kg/minute); middle row: noradrenaline and tyramine were repeated during phentolamine (32μg/Kg/minute); bottom row: noradrenaline and tyramine were repeated during phentolamine (32μg/Kg/minute) and propranolol (5μg/Kg/minute).

Noradrenaline 0.3μg/Kg/minute - NA 0.3μg
Tyramine 10μg/Kg/minute - TYR 10μg
Phentolamine 32μg/Kg/minute - PHEN 32μg
Propranolol 5μg/Kg/minute - PROP 5μg

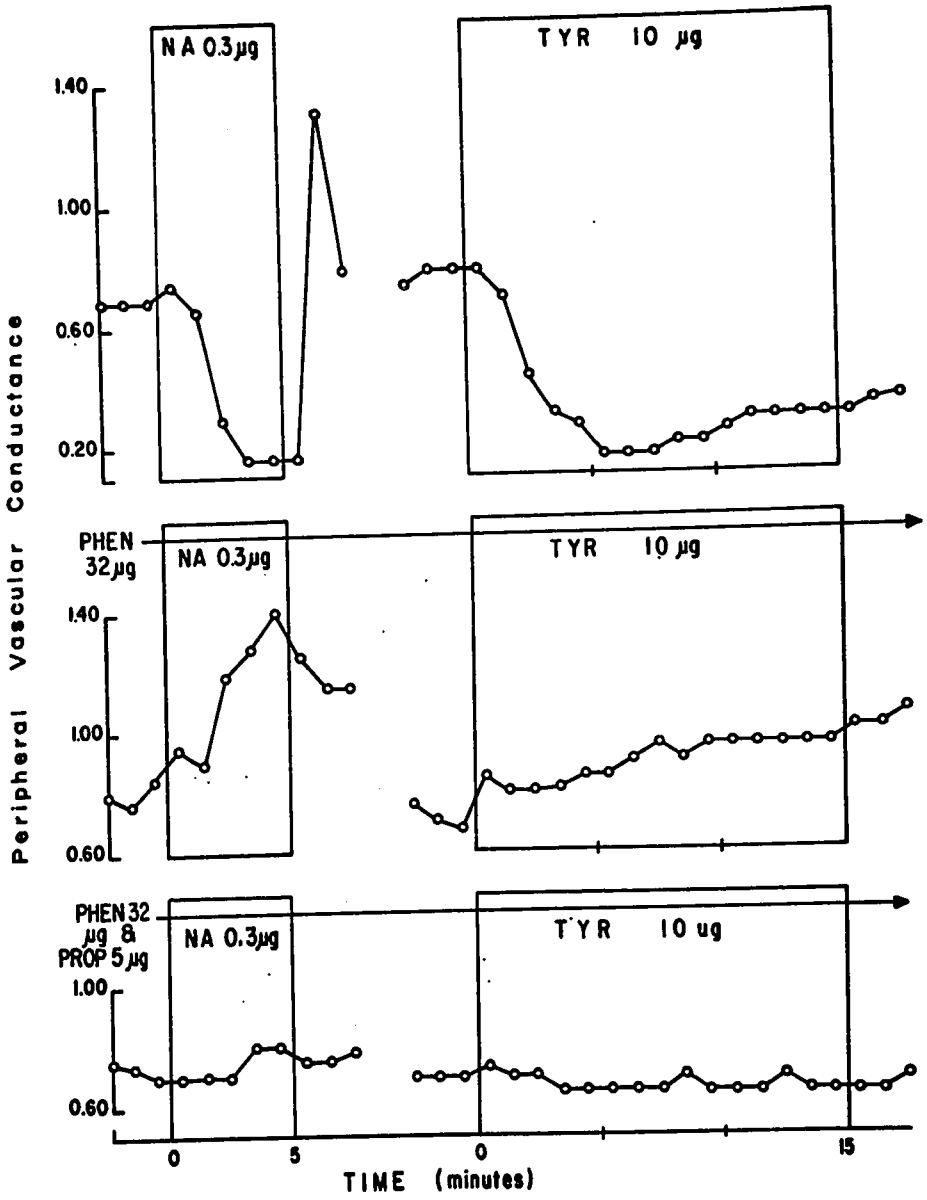

NA 0.3µg
TYR 10 µg
1.40
1.00
0.60
0.20
Peripheral Vascular Conductance
PHEN 32µg
NA 0.3µg
TYR 10 µg
1.40
1.00
0.60
PHEN 32 µg & PROP 5µg
NA 0.3µg
TYR 10 ug
1.00
0.60
0
5
0
15
TIME (minutes)

Figure 19

Figure 20:

The average results (n=10) expressed as a percentage change in resistance (closed circles) and conductance (open circles) for noradrenaline (0.3µg/Kg/minute) after five minutes before phentolamine (1), during phentolamine (32µg/Kg/minute) and during phentolamine (32µg/Kg/minute) and propranol (5µg/Kg/minute). Similar results are shown for tyramine (10µg/Kg/minute) before phentolamine at five minutes (4) and at the end of a 15 minute infusion (5); during phentolamine at five (6) and 15 minutes (7); and during phentolamine and propranol at five minutes (8) and 15 minutes (9). (same doses of phentolamine and propranolol).

I - Standard error of the mean

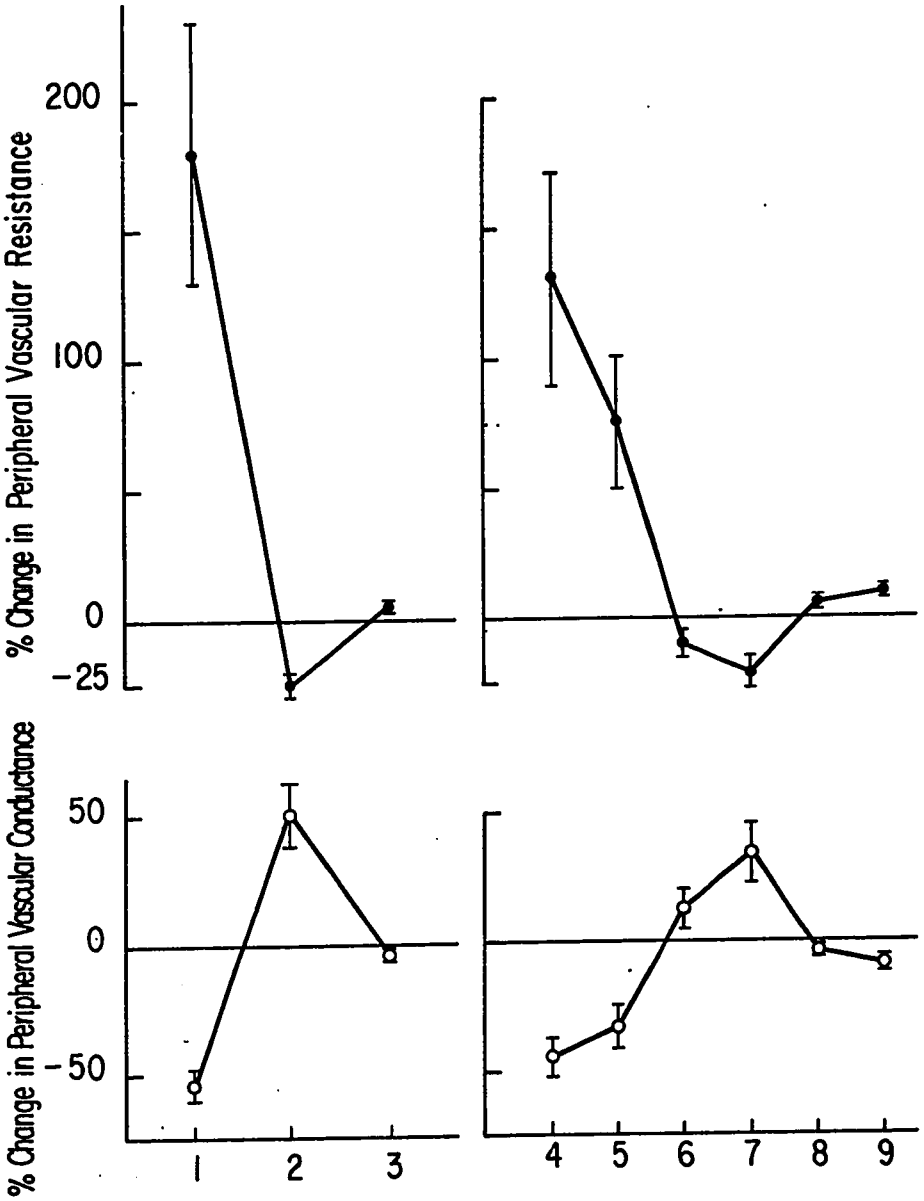

% Change in Peripheral Vascular Resistance
200
100
0
-25
% Change in Peripheral Vascular Conductance
50
0
-50
1
2
3
4
5
6
7
8
9

Figure 20

during alpha receptor blockade (2) and after both alpha receptor blockade and beta receptor blockade (3). Similar values are shown for tyramine before alpha receptor blockade at five minutes (4) and at the end of a 15 minutes infusion (5). Points (6) and (7) represent the percentage changes in resistance and conductance during alpha adrenergic receptor blockade at five minutes and 15 minutes respectively. The percentage changes for resistance (top) and conductance (bottom) during alpha and beta adrenergic receptor blockade at five minutes and 15 minutes are represented by (8) and (9) respectively.

Before alpha adrenergic receptor blockade intra-arterial noradrenaline (1) produced an increase in peripheral vascular resistance (% ch = 180±50, $p<0.01$). After alpha receptor blockade noradrenaline (2) produced a fall in resistance (% ch = -25±3, $p<0.001$). This vasodilator response (2) was converted back to a vasoconstriction (3) after beta receptor blockade with propranolol (% ch in resistance = 5±1, $p<0.05$).

Intra-arterial tyramine (4) caused an increase in resistance at five minutes (% ch = 131±42, $p<0.02$). When the infusion was concluded after 15 minutes (5) resistance had decreased (% ch = 75±24, $p<0.02$). There was a significant difference ($p<0.05$) between the response at 15 minutes compared to the five minute point of the infusion indicating the tyramine response was tending to fall off towards the end of the infusion. During phentolamine 32μg/Kg/minute, tyramine 10μg/Kg/minute produced a decrease in resistance (6) (% ch = -9±5 ($p<0.20$)) after five minutes. At the

end of 15 minutes the resistance (7) had decreased further (% ch = -21±6, $p<0.01$). There was a significant difference between the response at five and 15 minutes. ($p<0.05$). During alpha receptor blockade the vasodilatation response increased over a period of 15 minutes whereas before phentolamine the constrictor response fell off towards the end of a 15 minute infusion.

After beta receptor blockade the response was converted back to a vasoconstriction. After five minutes the percentage change in resistance was 4.8±2%, and after 15 minutes the percentage change in resistance = 9±2% ($p<0.01$). The difference in responses at five and 15 minutes was not significant.

DISCUSSION

This study confirms the results of Frewin and Whelan[20] (1967) in which it was observed that prolonged (15-minute) infusions of tyramine produced a vasodilatation after alpha adrenergic receptor blockade. This response was converted to a vasoconstrictor response if propranolol was infused after the vasodilator response had been recorded. However, Frewin et al[20] observed that the vascular responses to tyramine were delayed in onset since tyramine must act by releasing noradrenaline from sympathetic nerve endings. In the present experiments, it was observed that the constrictor response to tyramine developed as rapidly as the response to intra-arterial noradrenaline, and then tended to fall off towards the end of 15 minutes. This response was reversed after alpha adrenergic receptor blockade, that is, the vasodilator response to tyramine increased from five to 15 minutes. At first, it was thought that this might be due to a depletion of noradrenaline stores in nerve endings. However, the vasodilator response to tyramine after alpha receptor blockade was not significant at five minutes, but reached a significant level by the end of 15 minutes. Therefore it may be possible that the vasoconstrictor response before phentolamine decreased from five to 15 minutes as a result of increased beta receptor stimulation. This hypothesis is further strengthened since the vasodilator response was converted to a vasoconstriction after beta adrenergic blockade with propranolol. Brick et al[11] (1967) had shown previously that the constrictor response to noradrenaline in the human forearm was a summation of alpha and beta adrenergic

responses.

Glick et al[12] suggested neuronally released noradrenaline did not exert a significant beta effect in the perfused limb vascular bed. However, if tyramine causes the release of noradrenaline from sympathetic nerves, and it has been shown in this study that a significant beta effect is produced after phentolamine, then it must be concluded that endogenous noradrenaline is capable of exerting a significant effect on vascular beta receptors.

It must be noted that Glick et al[12] attempted to stimulate beta receptors reflexly by means of bilateral carotid occlusion, and that this stimulus may not have caused the release of sufficient transmitter to produce significant vascular effects. Glick et al occluded the carotid arteries for 45 to 60 seconds while in the present study the vasodilatation response to tyramine had only reached significance by the end of 15 minutes. The period of time that Glick et al stimulated would not be sufficient to observe the dilator response which was observed in the present study. Since this response was abolished with propranolol it was in all probability a beta adrenergic mediated response. Therefore, if it was possible for endogenous noradrenaline released by tyramine to produce significant stimulation of beta receptors in the peripheral circulation, it is also probable that noradrenaline released by reflex stimulation of the sympathetic nerves, if the stimulus is sufficient, should cause vasodilatation after alpha receptor blockade.

SECTION V

The Response of the Isolated Muscle Vascular Bed of Dog Hind Limb to Reflex Stimulation of the Sympathetic Nerves

INTRODUCTION

It has been shown in the previous section (IV) that tyramine released noradrenaline from sympathetic nerve stores in sufficient quantity to produce significant beta adrenergic receptor stimulation after alpha adrenergic blockade with phentolamine. The response developed only after prolonged (15 minute) infusions of tyramine and was abolished with propranolol. These results indicated that endogenous noradrenaline did exert significant beta adrenergic effects after alpha receptor blockade. The following study is an attempt to produce a similar beta adrenergic response by reflex stimulation of the sympathetic nerves (by means of bilateral carotid occlusion) during alpha adrenergic receptor blockade with phentolamine.

METHODS

Ten mongrel dogs (weight range 15-27kgms) were anaesthetized with pentobarbitol sodium (nembutal^{R}) 30mgm/Kgm. The animal was intubated and respired artifically if required. Figure I depicts the experimental set up. The procedure is also described in Section I with the exception that the carotid arteries and vagus nerves were exposed through a midline incision in the neck. Ligatures that could be temporarily tightened and then released were placed around the arteries while the vagus nerves were divided between ligatures.

Flow was recorded from the left external iliac artery. At the conclusion of the experiment, a cannula was inserted into the femoral artery and pushed upwards to lie just distal to the flow transducer where the cannula was secured in place. The flowmeter was calibrated as described earlier in Section I. From the pressure and flow recordings obtained vascular resistance and conductance were calculated at 30 second intervals throughout the experiment.

RESULTS

Ascorbic acid saline (0.9% NaCl and 0.03% ascorbic acid) was infused for approximately 30 minutes prior to control runs to allow the hind limb flow to reach a steady state. Intra-arterial tyramine 10μg/Kg/minute was infused for 15 minutes. Figure 21 shows the results expressed as changes in vascular conductance of a typical experiment. Tyramine (top row left) caused a decrease in vascular conductance. The response tended to fall of towards the end of the infusion. After tyramine was discontinued, ascorbic acid saline was infused and the carotid arteries were occluded for 15 minutes. Vascular conductance decreased (Figure 21 top row right) and the response was sustained for the duration of the stimulus. Phentolamine 32μg/Kg/minute was infused for 20 minutes prior to repeating tyramine and bilateral carotid occlusion during phentolamine infusions. Tyramine caused an increase in conductance which reached a maximum by 15 minutes, and bilateral carotid occlusion after an initial biplasic response caused an increase in vascular peripheral conductance in this experiment. When tyramine and B.C.O. were repeated during phentolamine (32μg/Kg/minute) and propranolol (5μg/Kg/minute) which had been infused for 20 minutes previously, tyramine caused essentially no change in vascular conductance, (Figure 21, bottom left) whereas B.C.O. converted to a vasoconstriction (bottom right).

The average percentage changes in vascular resistance and conductance at five and 15 minutes for tyramine (Figure 22, left) and bilateral carotid occlusion (right) before, during alpha receptor

Figure 21:

Shows the vascular conductance response in one experiment to intra-arterial tyramine and bilateral carotid occlusion (BCO) before phentolamine (top panel), during phentolamine (middle panel) and during phentolamine and propranolol (bottom panel).

Tyramine 10µg/Kg/minute	- TYR 10µg
Bilateral Carotid Occlusion	- BCO
Phentolamine 32µg/Kg/minute	- Phen 32µg
Propranolol 5µg/Kg/minute	- Prop 5µg

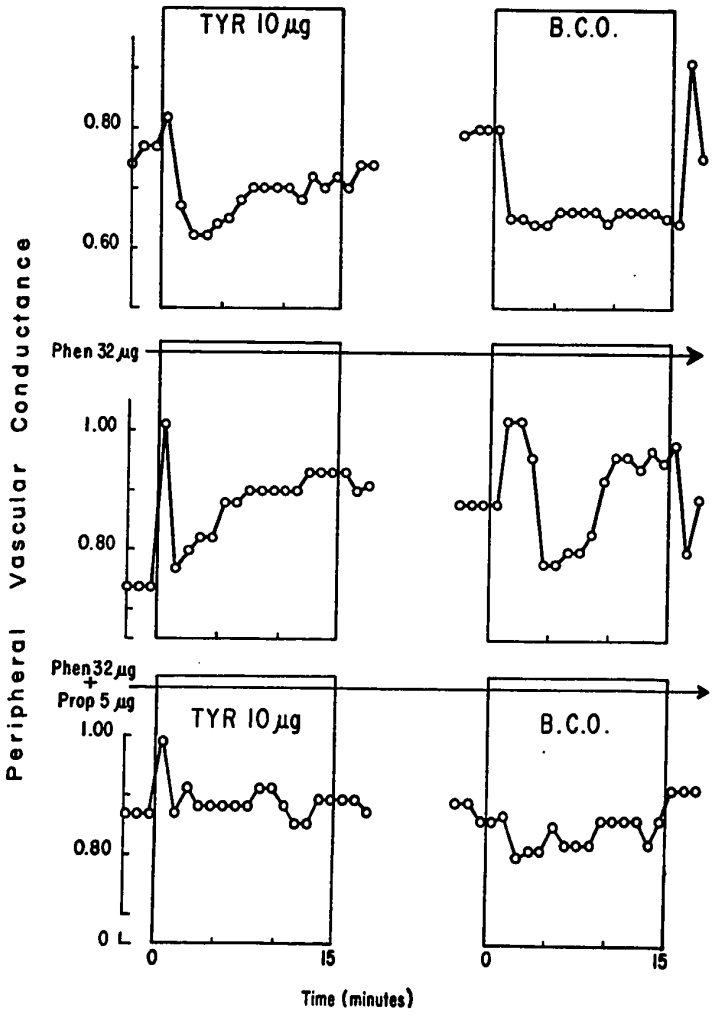

TYR 10 µg
B.C.O.
0.80
0.60
Phen 32 µg
1.00
0.80
Phen 32 µg
+
Prop 5 µg
TYR 10 µg
B.C.O.
1.00
0.80
0
0
15
0
15
Peripheral Vascular Conductance
Time (minutes)

Figure 21

blockade, and after alpha and beta adrenergic receptor blockade are shown in Figure 22. Tyramine (10µg/Kg/minute) produced a marked vasoconstriction at five minutes. (1) (% Ch in resistance 116±19, $p<0.001$). This effect on resistance was sustained at 15 minutes (2) (% Ch = 125±27, $p<0.01$). When tyramine 10µg/Kg/minute was infused during phentolamine 32µg/Kg/minute (3 and 4 left, Figure 22) vascular resistance was decreased (% Ch =-5±4, $p<0.30$) at five minutes, (but not significantly reversed). However, after 15 minutes the response to tyramine was "reversed" (% Ch = -11±5, $p<0.05$). After beta adrenergic receptor blockade with propranolol 5µg/Kg/minute in addition to alpha receptor blockade, the vasodilatation observed during alpha blockade alone was converted to a vasoconstriction. At five minutes the change in resistance was small (% Ch = 8±4, $p<0.10$) but at 15 minutes (% Ch = 10±3, $p<0.01$), a significant vasoconstriction was observed.

Referring to Figure 22 (right), bilateral carotid occlusion produced an increase in vascular resistance at five minutes (1) (% Ch = 32±11, $p<0.02$) but the response tapered off after 15 minutes (21) (% Ch in resistance = 24±14, $p<0.20$). When the carotid arteries were occluded during phentolamine 32µg/Kg/minute, the vasoconstrictor response was reduced, but not reversed as with tyramine. The percentage changes in resistance at five minutes (3) was 5±8 and at 15 minutes (4) 5±6. After beta and alpha receptor blockade, vascular resistance was increased at five minutes (5) (% Ch - 24±6, $p<0.01$) and fifteen minutes (6) (% Ch = 24±7, $p<0.01$) during bilateral carotid occlusion. Although a significant reversal of the response to prolonged carotid

Figure 22:

Shows the average results (n=10) for tyramine (left) and bilateral carotid occlusion (right) expressed as a percentage change in vascular resistance (o) and conductance (o) from control levels. Points were plotted at the five and 15 minutes points of each run.

	TYRAMINE left	BCO right
1.	TYR 10µg/Kg/minute (five minutes)	BCO (five minutes)
2.	TYR 10µg/Kg/minute (15 minutes)	BCO (15 minutes)
3.	TYR 10µg/Kg/minute + Phentolamine 32µg/Kg/minute (five minutes)	BCO + Phentolamine (five minutes)
4.	TYR 10µg/Kg/minute + Phentolamine 32µg/Kg/minute (15 minutes)	BCO + Phentolamine (15 minutes)
5.	TYR + Phen + Propranolol (5µg/Kg/minute) (five minutes)	BCO + Phen + Propranolol (five minutes)
6.	TYR + Phen + Propranolol (5µg/Kg/minute) (15 minutes)	BCO + Phen + Propranolol (15 minutes)

I - Standard Error of Mean

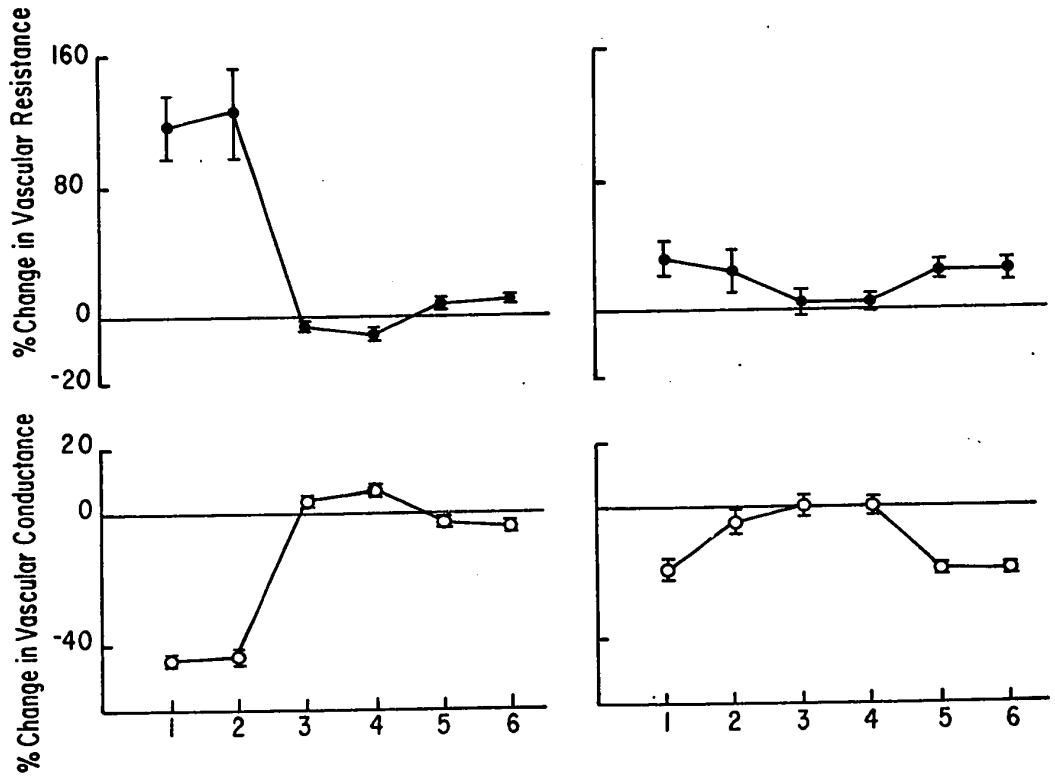

% Change in Vascular Resistance
160
80
0
-20
% Change in Vascular Conductance
20
0
-40
1
2
3
4
5
6

Figure 22

occlusion could not be demonstrated as with tyramine, in comparing the percentages changes in conductance (the inverse of resistance) at five minutes during phentolamine 32μg/Kg/minute and after propranolol 5μg/Kg/minute (points 3 and 5 in Figure 22 right), a significant difference was observed (% Ch = -27±7, $p<0.01$). A similar comparison was made at 15 minutes (4 and 6 in Figure 22 right) and a similar decrease in conductance was noted (% Ch = - 30±6, $p<0.001$). Thus these results suggest that propranolol potentiated the residual vasoconstriction produced during bilateral carotid occlusion after alpha adrenergic receptor blockade, thus suggesting a beta adrenergic receptor component to the response to bilateral carotid occlusion.

DISCUSSION

Reflex stimulation of the sympathetic nerves (bilateral carotid occlusion) after alpha adrenergic receptor blockade resulted in a reduction in the constrictor response to bilateral carotid occlusion, but a reversal of a vasoconstriction did not occur unlike the response to intra-arterial tyramine. Propranolol (a beta receptor blocking agent) infused with phentolamine converted the vasodilatation after tyramine back to a vasoconstriction. After propranolol, bilateral carotid occlusion produced a significant vasoconstriction. Thus propranolol has the effect of potentiating the residual vasoconstrictor response to carotid occlusion after alpha receptor blockade. This suggests that the reduction in resistance after phentolamine was due partly to a reduction in constrictor tone, partly to stimulation of peripheral beta receptors. These results support the hypothesis that neuronally released noradrenaline does act on peripheral beta receptors. This is further supported by the observation that in situations involving prolonged stimulation of vascular receptors prior to blockade, the vasoconstrictor response to bilateral carotid occlusion or tyramine began to fall off by the end of a 15 minute period. Furthermore it is suggested that the constrictor response to carotid occlusion decreased with time as a result of increased activation of vascular beta receptors. That is to say that only at the end of a prolonged period was there enough transmittor accumulated at the receptor site to manifest an observable beta response. Comparison of the graphs in Figure 22 finds them

objectively similar, differing only in the magnitude of the responses obtained which further suggests a difference in the relative amount of transmittor released with tyramine as compared to bilateral carotid occlusion. Intra-arterial tyramine was relatively effective in producing a response limited to the vessels of the hind limb concerned, however occlusion of the carotid arteries had more non-specific effects including an increase in the rate and strength of contraction of the heart, and perhaps the release of catecholamines from the adrenal medulla. The results obtained in the present experiments do not rule out the possibility that the response obtained during bilateral carotid occlusion were due in part to blood borne catecholamines released from the adrenal medualla. Grant et al[78] and Unväs[79], in a quantitative study of catecholamine release from the adrenal medualla on sympathetic vasodilator activation reported that such activation always led to the discharge of adrenaline. However, they suggested that the amount discharged was too low to produce any vascular reaction in normally innervated vessels. In addition, Levin and Beck (1967)[80] compared the blocking effects of phenoxybenzamine on neurogenically versus injected noradrenaline and suggested that if a mixed alpha-beta adrenergic amine such as adrenaline were released during stimulation of sympathetic nerves supplying extremities the alpha adrenergic blocking drug would produce a greater reduction than the noradrenaline induced response. In the present study phentolamine reversed the vasoconstrictor response to tyramine but only reduced the response to bilateral carotid occlusion. If adrenaline had been involved in this response, then

according to Levin et al[80] the reduction in constrictor activity during phentolamine should have been at least equal to the reduced vasoconstrictor activity observed with tyramine during phentolamine.

To completely eliminate adrenaline secretion as a possibility, these experiments could have been conducted after bilateral adrenalectomy or by imposing a delay circuit as was done by Glick et al[12]. However, because of the long period of stimulation (15 minutes) employed, the large reservoir of blood required made this technique impractical.

Glick et al[12] concluded that injected and neuronally released noradrenaline did not act on identical receptor populations. The results of the present study do suggest however, that neuronally released and intra-arterial noradrenaline do act on similar receptor populations. The difference in magnitude of the response is a function of the quantity of neurotransmittor reaching the receptor sites and appears to be time-dependent.

SUMMARY AND CONCLUSIONS

CONCLUSIONS

Noradrenaline has been accepted as the neurotransmitter in the sympathetic nervous system[2,4,5]. Stimulation of sympathetic nerves innervating blood vessels results in a vasoconstriction[6]. This vasoconstriction is supposedly mediated through adrenergic alpha receptors. Stimulation of beta adrenergic receptors[11,12] produces vasodilatation. Several authors have suggested that neurotransmitter released from sympathetic nerve terminals does not act on vascular beta adrenergic receptors, although beta adrenergic receptors were accessible to blood borne noradrenaline[12,13].

The investigations described herein were designed to study the effects of neuronally released noradrenaline on beta adrenergic receptors. In Section I, using the whole hind limb vascular bed of dogs, noradrenaline and tyramine were infused intra-arterially during alpha adrenergic receptor blockade. Although tyramine, which causes the release of noradrenaline from sympathetic nerve terminals, reduced the vasoconstrictor response after alpha receptor blockade, the response was not converted to a vasodilatation as was the case with noradrenaline during alpha receptor blockade. In Section II, two other alpha blocking drugs were used to assess the effectiveness of the alpha receptor blockade, but they were found to be no better than the first alpha receptor blocker (Phentolamine). Another series of experiments, in Section III, employed the use of theophylline to investigate a proposed relationship between the peripheral vascular beta adrenergic receptors and the adenyl cyclase-cyclic 3'5'

monophosphate system in an attempt to potentiate a small beta receptor component observed in the earlier experiments with tyramine. No potentiation of the responses to isoproterenol, noradrenaline or carotid occlusion could be demonstrated and the evidence which suggests a relationship between the peripheral vascular beta receptor and the adenyl cyclase system was not supported.

The earlier experiments suggested the possibility that a vascular beta receptor response after alpha receptor blockade was being masked by an unblocked alpha receptor response localized in the skin and paw. Thus the experiments with noradrenaline and tyramine were repeated in Section IV with paw and skin flow occluded. It was found that with prolonged infusions (15 minutes), intra-arterial tyramine produced a significant vasodilatation in the isolated muscle vascular bed of the dog hind limb. This indicated that endogenous or neuronally released noradrenaline did exert a significant action on peripheral vascular beta receptors.

In Section V, the response of the isolated muscle vascular bed of the dog hind limb to prolonged reflex stimulation (bilateral carotid occlusion) of the sympathetic nerves during alpha receptor blockade and during combined alpha and beta adrenergic receptor blockade was investigated. Bilateral carotid occlusion produced a vasoconstriction before alpha adrenergic receptor blockade. During alpha receptor blockade the vasoconstrictor response to carotid occlusion was reduced but not converted to a vasodilatation -- unlike the response to intra-arterial tyramine. During combined alpha and

beta adrenergic receptor blockade the response was converted back to a significant constriction. These results suggested that propranolol (the beta receptor blocker) potentiated the vasoconstrictor response to carotid occlusion during alpha receptor blockade. This suggests that neurotransmittor released by sympathetic nerve stimulation does act on peripheral vascular beta adrenergic receptors and that peripheral vascular beta receptors are innervated by the sympathetic nervous system. In addition the results suggest neuronally released and blood borne noradrenaline are acting on similar receptor populations and that a difference in the magnitude of response between neuronally released and blood borne noradrenaline depends on the amount of transmittor reaching the receptor sites and this is dependent on the period of stimulation of the sympathetic nerves.

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"I Am The Master Of My Fate, The Captain Of My Soul"

William Ernest Henley.