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THE MECHANISM OF ACTION OF TYRAMINE ON THE BLOOD VESSELS OF THE FOREARM IN MAN

BY

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In 1926 Tainter showed that tyramine acted as a circulatory stimulant in rabbits, cats and dogs. The stimulation involved the heart and blood vessels and occurred independently of the vasomotor centres, sympathetic ganglia and the adrenal glands. The pressor response that resulted from the infusion of tyramine resulted partly from the constriction of the peripheral vessels and partly from a direct action on cardiac muscle. The vasoconstriction that occurred in the cat was attributed to stimulation of the sympathetic nerve endings and in the dog to direct stimulation of vascular smooth muscle. Further experimentation using a variety of isolated organs suggested that direct smooth muscle stimulation was the predominant action of tyramine.

In 1958 Burn & Rand found that tyramine lost its pressor action in a cat which had been pretreated with reserpine and no longer caused contraction of the nictitating membrane or the spleen. The action of tyramine could be restored in such an animal by infusion of noradrenaline into the blood stream. These observations suggested that tyramine acted by releasing noradrenaline or adrenaline from stores in the artery wall.

Farmer (1966) studied the central artery of the rabbit ear using the technique described by de la Lande and Rand (1965) and reported that tyramine had both direct and indirect constrictor actions. The indirect action was abolished by sympathectomy and reserpine pretreatment, while the direct action remained unaffected by these procedures.

In human subjects Cohn (1965) showed that tyramine infused into the brachial artery usually caused constriction of forearm vessels, but with large doses (more than 80 μg/min) vasodilatation sometimes occurred. In explanation of these responses Cohn offered a number of possibilities: (*a*) that tyramine causes release of adrenaline and dopamine as well as noradrenaline from storage sites in the adrenergic neurones, and these could cause forearm vasodilatation; (*b*) that tyramine might exert a direct sympathomimetic action without the intervention of noradrenaline and (*c*) that tyramine might have both direct and indirect actions.

In the present study the effects of local intra-arterial infusions of noradrenaline and tyramine on the forearm blood vessels have been studied before and during blockade of the alpha- and beta-adrenaline receptors in an endeavour to establish the mechanism of action of tryamine on the peripheral blood vessels in man.

METHODS

The subjects for the experiments were normal volunteer medical students and one patient (W. W.) who had undergone bilateral cervical sympathectomy 6 yr previously for mild Raynaud's phenomenon in the hands. The vessels of the forearm of this patient responded in a normal fashion to drugs which act directly on vascular smooth muscle (Parks, Sandison, Skinner & Whelan, 1961 ; Parks, Skinner & Whelan, 1961) but did not respond to sympathetic stimuli such as application of ice to the face, deep inspiration or mental arithmetic (Cooper, Fewings, Hodge & Whelan, 1963 ; Blair, Glover, Greenfield & Roddie, 1959).

The experiments were carried out at laboratory temperatures ranging from 23° to 26° C, the subjects lying recumbent on a couch for at least 30 min before observations were made, during which time recording apparatus was applied and the infusion needle inserted.

Forearm blood flow was measured by venous occlusion plethysmography, using water-filled plethysmographs maintained at a temperature of 34°–35° C (Greenfield, 1954), three or four records of flow being obtained each minute.

Intra-arterial infusions were given into the brachial artery at the elbow of one side through a 22 or 23 gauge needle, connected by a length of polyethylene tubing to a mechanically driven syringe which delivered 2 ml. of solution per min. Saline (0.9% w/v) was infused during control periods and was also used as a vehicle for the drugs. The doses of the drugs were such that they did not produce systemic effects, making it possible to use the opposite uninfused limb as a control.

Percentage changes in forearm flow produced by tyramine and noradrenaline were determined from the averaged flow values during the 2 min before the drug infusion and the last 2 min of the infusion period by which time the responses to the drugs had become stable. Allowance was made for spontaneous variations in flow unrelated to drug action by assuming that in the absence of the drug infusion the infused and the control sides would have maintained the same relationship to each other as in the pre-infusion period (Duff, 1952).

The drugs used were tyramine hydrochloride (Koch-Light Laboratories Ltd.), phentolamine methanesulphonate (Regitine, Ciba), propranolol hydrochloride (Inderal, I.C.I.) and noradrenaline bitartrate monohydrate (Levophed, Winthrop). The doses of noradrenaline are expressed as weights of the base and those of the other drugs as weights of their salts. Ascorbic acid (1 : 50,000) was added to the noradrenaline solutions.

RESULTS

The constrictor action of tyramine on the vessels of the forearm of one subject is illustrated in Fig. 1. Doses of 12.5, 25 and 50 μg/min infused into the brachial artery of one side for 5 min caused a fall in blood flow which increased in magnitude with increasing doses. There was no effect on the vessels of the opposite control forearm.

This action of tyramine was absent in the sympathetically denervated forearm of the patient (W. W.) even when very large doses were used (Fig. 2). It was also abolished by alpha-receptor blockade. Fig. 3 shows the changes in forearm blood flow in one subject during intra-arterial infusion of tyramine (25 μg/min) and noradrenaline (0.4 μg/min), in another subject before (left of figure) and during blockade of alpha-receptors for adrenaline with phentolamine (50 μg/min, right of figure). Before alpha-receptor blockade the tyramine and noradrenaline infusions produced approximately equal degrees of reduction of forearm flow. During administration of phentolamine, tyramine was without effect whereas a vasodilatation was caused by noradrenaline. Similar results were obtained in two other subjects, using 5 and 10 min infusions of the drugs. In two further subjects, however, an increase in forearm blood flow occurred after 10 min tyramine infusions had ceased and while the phentolamine infusion was continued (Fig. 4).

In view of the delayed dilator responses seen in these two experiments, more prolonged infusions of 15 min duration were carried out in four subjects during phentolamine infusion. A vasodilatation was seen after the onset of the tyramine infusion, taking 6, 6, 9 and 12 min respectively to develop. In two of the subjects the beta-receptor blocking agent propranolol was administered after the above response had been recorded,

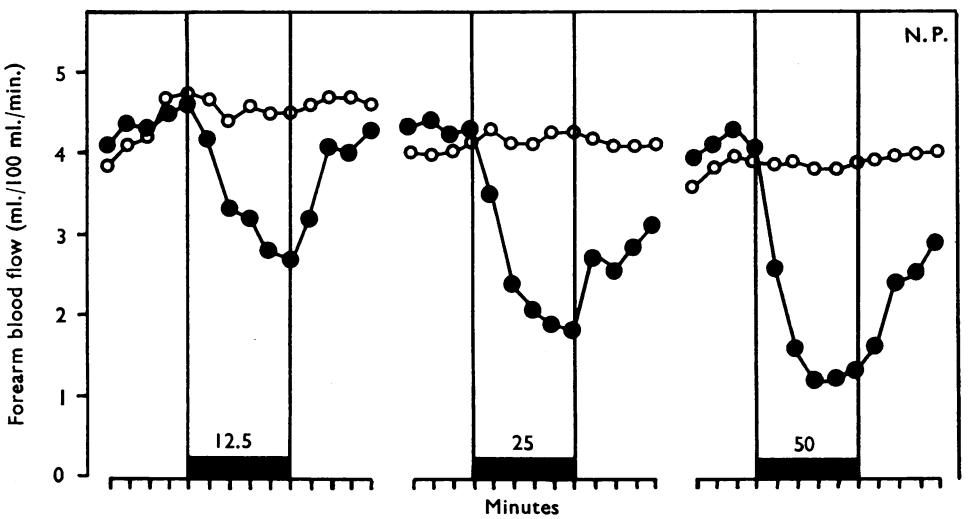

Fig. 1. Effect on the blood flow through the forearm of three doses of tyramine infused into the brachial artery in a normal subject. ●, Infused side ; ○, control side. The 5 min periods of drug infusion are indicated by the black rectangles, above which are shown the doses of tyramine in μg/min.

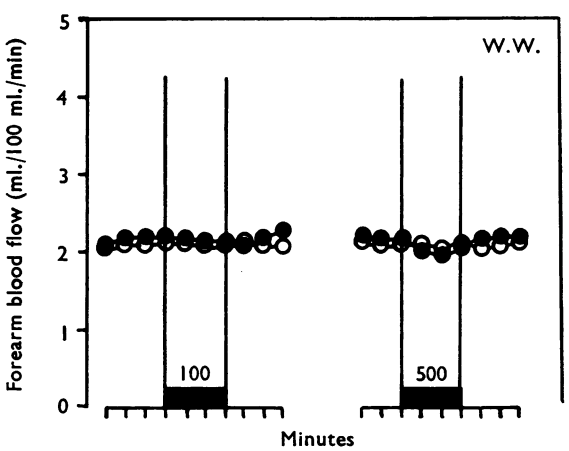

Fig. 2. Effect on the forearm blood flow of intra-arterial infusions of tyramine (100 and 500 μg/min) in a surgically sympathectomized limb. ●, Infused side ; ○, control side. The periods of drug infusion are indicated by the black rectangles.

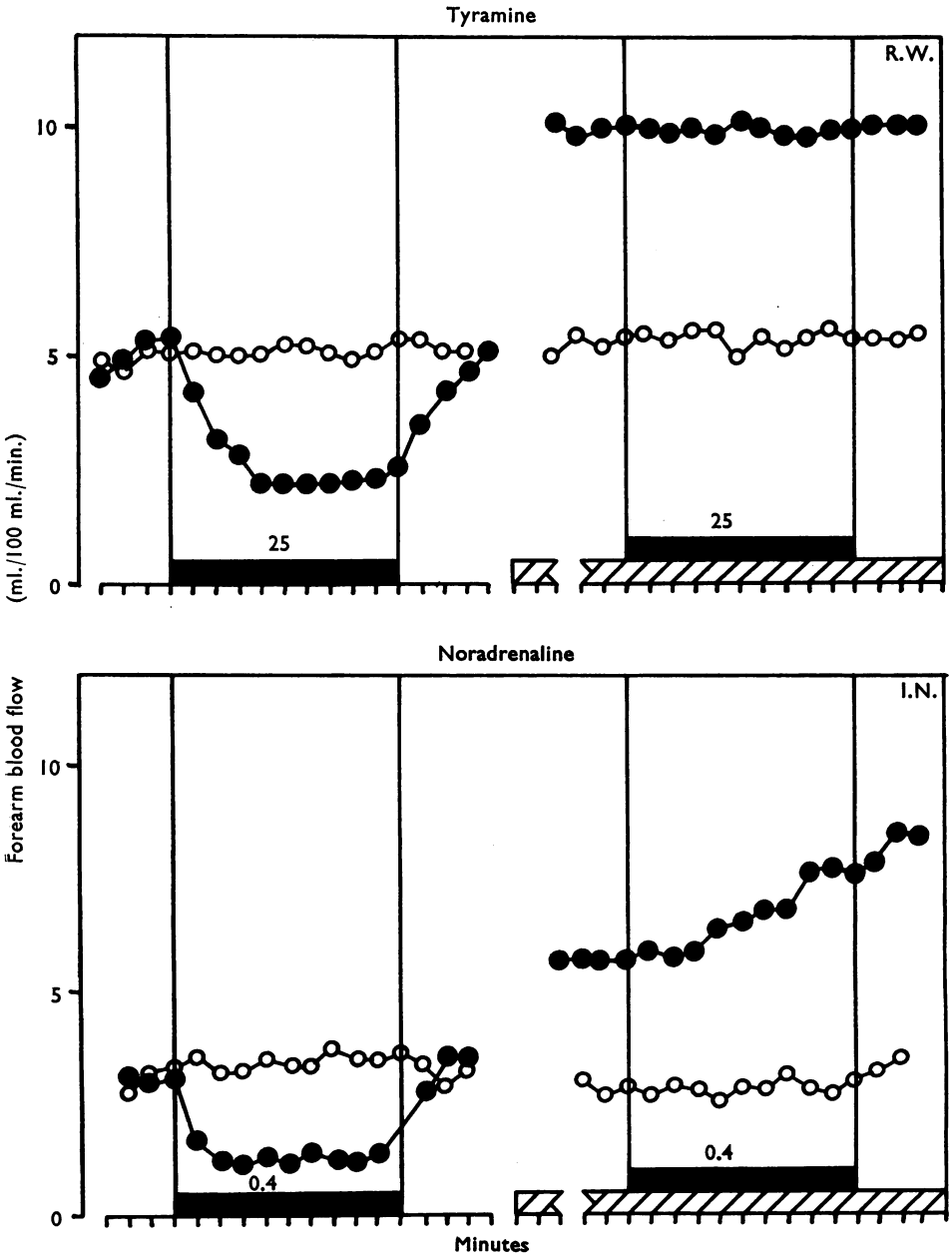

Fig. 3. Effect on the forearm blood flow of intra-arterial infusions of tyramine (upper frame) and noradrenaline (lower frame) before (left) and during (right) intra-arterial infusion of phentolamine (50 μg/min). ●, Infused side ; ○, control side. The infusions of tyramine (25 μg/min) and of noradrenaline (0.4 μg/min) are indicated by the black rectangles and the infusions of phentolamine by the hatched rectangles, the interruptions in which represent time intervals of 12 min (R.W.) and 23 min (I.N.).

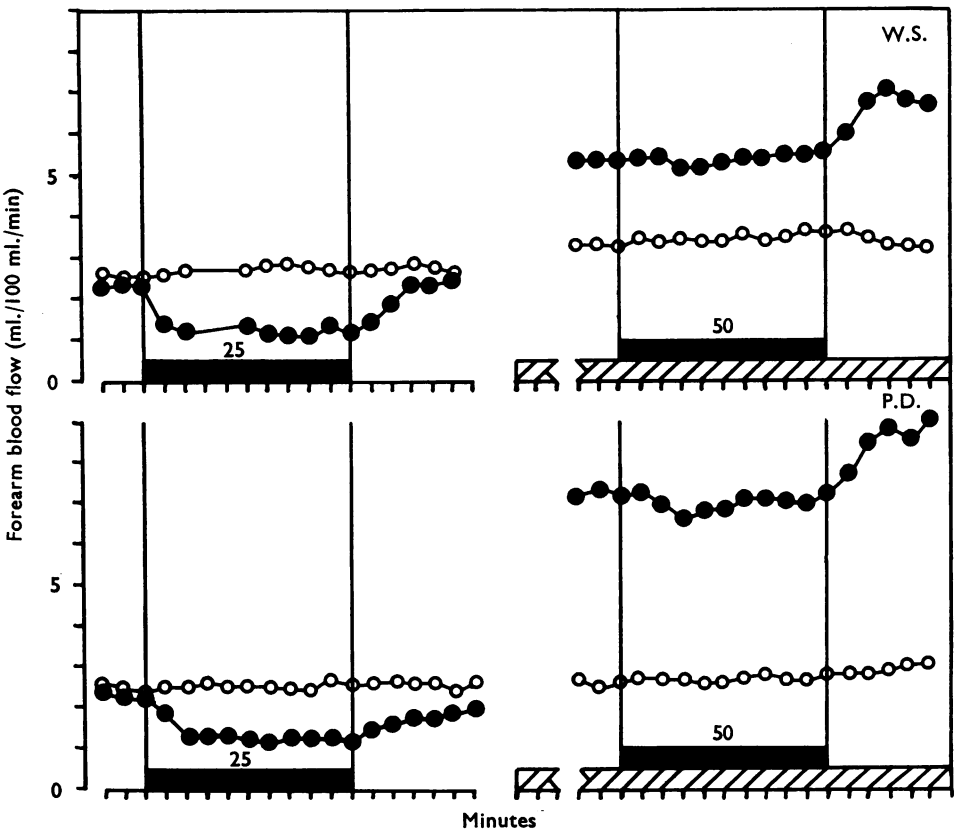

Fig. 4. Effect on the forearm blood flow in two subjects of intra-arterial infusions of tyramine (black rectangles) before (left) and during (right) intra-arterial infusion of phentolamine (50 μg/min, hatched rectangles). ●, Infused side ; ○, control side. The figures above the black rectangles indicate the doses of tyramine in μg/min. The interruptions in the hatched rectangles represent time intervals of 17 min (W.S.) and 12 min (P.D.).

and tyramine now caused a fall in flow. The response obtained with tyramine on one of these subjects is illustrated in Fig. 5 and is compared with the similar pattern of response seen with noradrenaline.

In the experiments illustrated in Fig. 4, the same dose of drug was given before and during the phentolamine infusion. In all subsequent experiments, however, an attempt was made to compensate for the increase in blood flow produced by phentolamine by appropriately increasing the doses of tyramine which were given during the phentolamine blockade, so that comparable concentrations of the drug arrived at the vessels of the forearm before and during the phentolamine infusions (Frewin & Whelan, 1968).

The effect of propranolol alone on the response of the forearm vessels to tyramine was studied in ten experiments. When the responses were observed over periods of 5 min (four experiments), no potentiation of the constrictor action of tyramine was observed

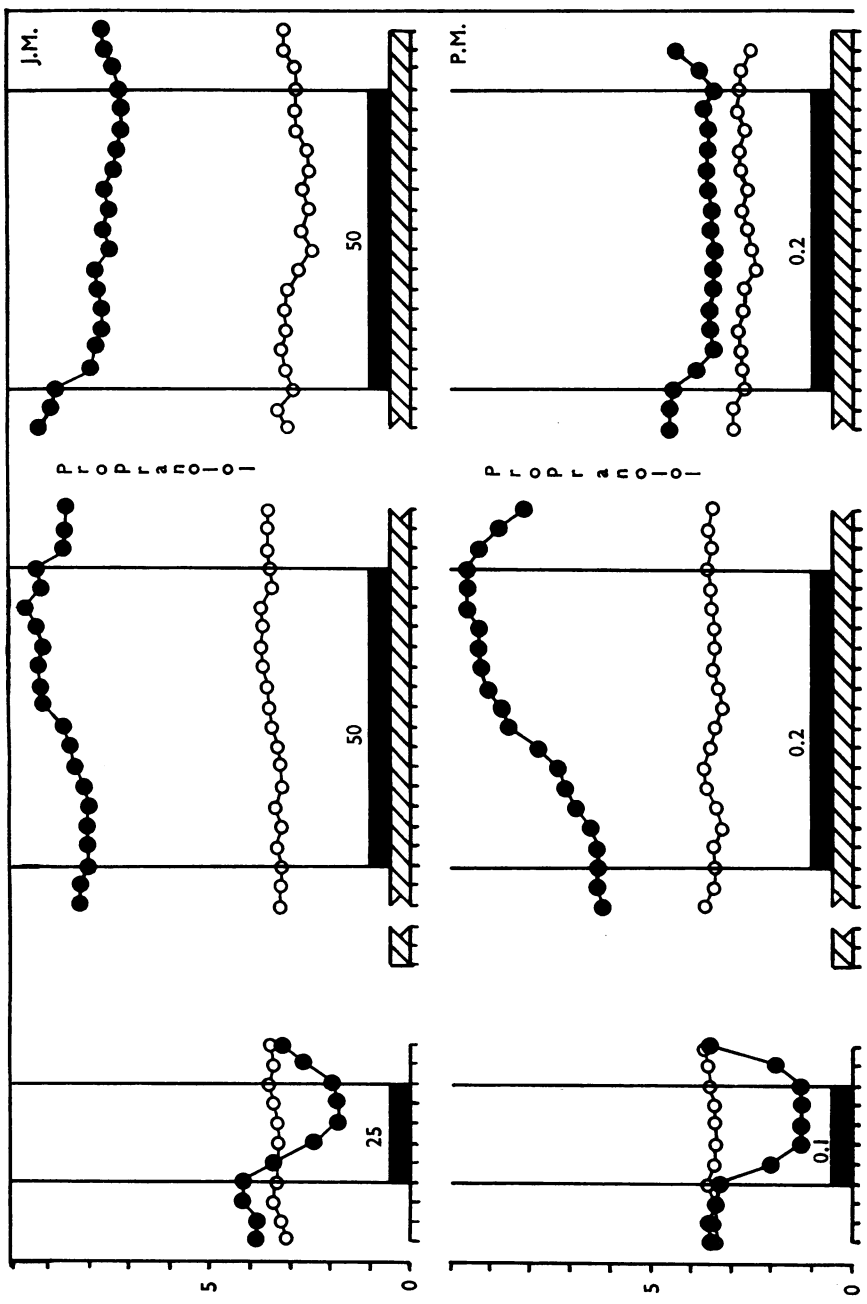

Fig. 5. Effect on the forearm blood flow of intra-arterial infusions of tyramine (upper frames) and of noradrenaline (lower frames) before (left) and during intra-arterial administration of phentolamine (50 μg/min, middle) and during phentolamine administration after intra-arterial propranolol (100 μg/min for 8 min, right). The infusions of tyramine and noradrenaline are represented by the black rectangles and the figures above each indicate the doses in μg/min. The interruptions in the hatched areas represent time intervals of 10 min and 32 min (J.M.) and 21 min and 23 min (P.M.).

after beta-blockade. With 15 min infusions, however, in four out of six experiments a potentiation of 6, 12, 9 and 6% respectively was obtained, while in two there was no increase in the constrictor response. The pooled data from all these experiments are shown in Fig. 6.

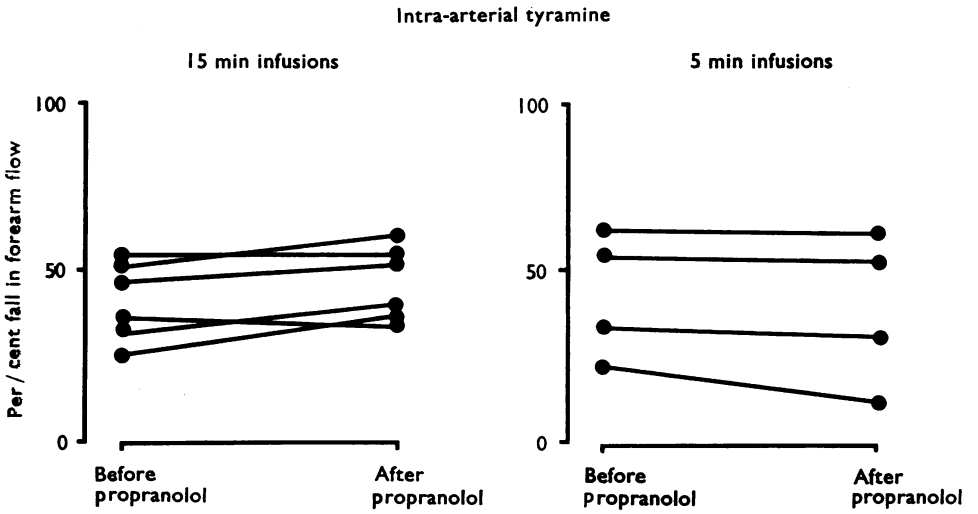

Fig. 6. Fall in forearm blood flow during the last 2 min of intra-arterial infusion of tyramine expressed as the percentage fall from the pre-infusion level before and after intra-arterial administration of propranolol (100 μg/min for 8 min). The left hand frame includes data from six 15 min infusions of tyramine each in a different subject and the right hand frame from four 5 min infusions each on a different subject.

Time course of the responses

The length of the polyethylene connexion between the infusion syringe and the needle in the artery was constant in all experiments (35 cm), and the time taken for the drug solution to reach the needle at an infusion rate of 2 ml./min was 6–9 sec. The time of onset of the infusion of drug into the vessels could thus be determined within a second or two.

The onset of the response of the vessels was taken to be that time at which the first flow measurement after the beginning of the infusion was either greater or less than the control level of flow. When doses of the two drugs producing similar constrictor effects were compared, the responses to noradrenaline were more rapid in onset than those to tyramine.

Fig. 7a shows the pooled data from fifteen infusions of tyramine and eleven infusions of noradrenaline on sixteen subjects, in doses which had approximately equal constrictor effects on the forearm vessels, the time of onset of the constrictor effect being plotted against the maximum fall in flow attained during the infusion period, expressed as a percentage fall from the pre-infusion level of flow. For a given degree of vasoconstrictor response, noradrenaline had a more rapid onset of effect than did tyramine (means:

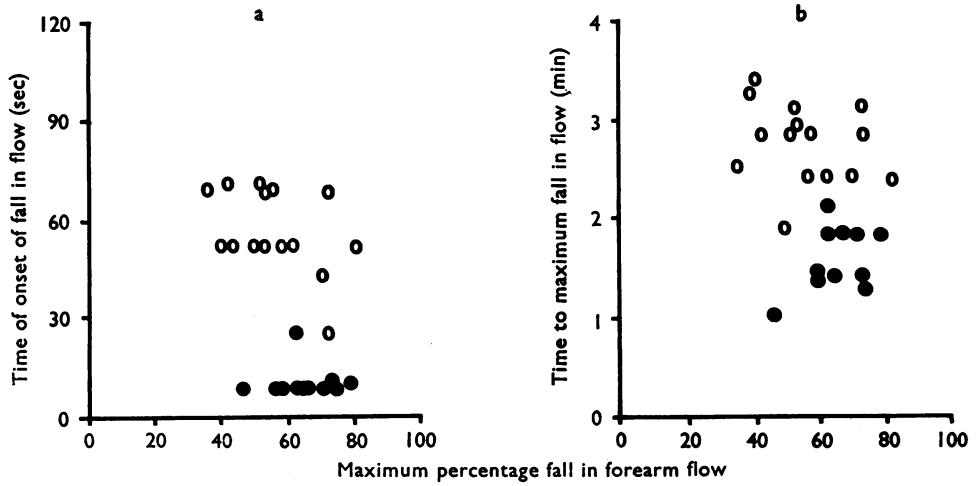

Fig. 7. Time of onset of the reduction in blood flow (left) and the time taken for maximum flow reduction to develop (right) with infusions of tyramine (○) and of noradrenaline (●) plotted against the maximum fall in blood flow attained during the infusions, expressed as per cent fall from the pre-infusion level of flow corrected for any spontaneous changes in blood flow by reference to the opposite control side.

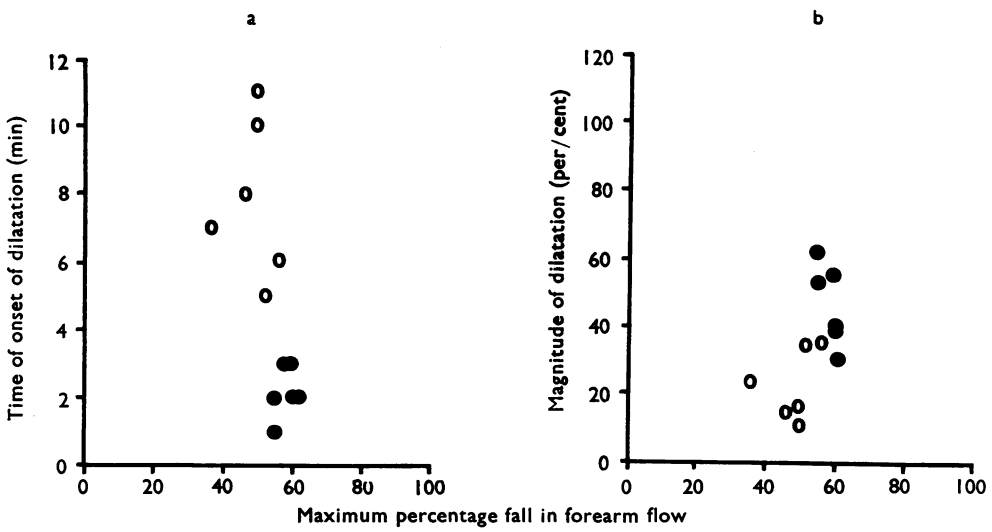

Fig. 8. Time of onset (left) and magnitude (right) of the increase in forearm flow above the control level produced by infusions of tyramine (○) and noradrenaline (●) following alpha-receptor blockade by phentolamine, plotted against the maximum fall in blood flow attained during infusions of the drugs before phentolamine administration.

noradrenaline 10 sec ; tyramine 55 sec). The time taken for the maximum constrictor effect to be attained was also much shorter with noradrenaline (mean: 1 min 47 sec) than with tyramine (2 min 59 sec) (Fig. 7b).

A similar time relationship was seen between the onset of the dilator actions of the two drugs when given during administration of phentolamine (Fig. 8a). The time of onset of the dilator response to noradrenaline was 2 min 10 sec, while that to tryamine was 7 min 50 sec (means of six infusions in six subjects with each drug). With doses of noradrenaline and tyramine which had comparable vasoconstrictor actions (Fig. 8b and Fig. 5) the magnitude of the dilator response was less with tyramine.

DISCUSSION

The fact that tyramine is without effect on the sympathetically denervated forearm vessels indicates that in the doses used the drug has no significant direct action on the vessels and implies that its effect is mediated solely by the release of a substance from the nerve endings.

The abolition of the constrictor action of tyramine by alpha-receptor blockade with phentolamine and the unmasking of a dilatation which is caused by beta-receptor stimulation indicates that the released substance has properties similar to those of adrenaline and noradrenaline. The beta-receptor stimulating action of adrenaline on forearm vessels in the presence of alpha-receptor blockade was demonstrated by de la Lande & Whelan (1959) and Allwood & Ginsburg (1961). That of both adrenaline and noradrenaline was inferred by Lowe & Robinson (1964) from the enhancement of the constrictor response to these drugs after beta-receptor blockade with pronethalol. Further evidence of the beta-stimulating action of noradrenaline was provided by Glover & Hutchison (1965) from the potentiation of the effect of noradrenaline by the beta-receptor antagonist propranolol and also supported by the observation by Brick, Hutchison & Roddie (1967) of a dilatation of the forearm vessels with noradrenaline in the presence of phentolamine. The latter authors used large doses of both noradrenaline and of phentolamine. The results of the present experiments confirm their findings and extend them by showing that a beta-receptor stimulating action of noradrenaline can be elicited with small doses of the two drugs.

When beta-receptor blockade was introduced following alpha-receptor blockade, the dilator responses to both noradrenaline and tyramine were abolished, and a small vasoconstriction occurred (Fig. 5). The mechanism of this residual constrictor response is not clear. A similar effect has been observed in the action of ephedrine on forearm vessels (Frewin & Whelan, 1968). It is possible that the administration of propranolol after phentolamine may have had the effect of antagonizing the alpha-blocking activity of the latter and the infused noradrenaline and the transmitter released by tyramine were then able to act on receptors (Prichard & Ross, 1966). Another explanation might be that the phentolamine had not been fully effective in blocking the alpha-receptors and the partial constrictor action of noradrenaline and of tyramine became apparent when their dilator effects were blocked.

The vasoconstrictor and the vasodilator actions of tyramine differed from those of noradrenaline in that there was a greater delay between the beginning of the infusions

and the onset of the responses of the vessels. Such a difference in time course between the effect of a catecholamine released by tyramine from nerve endings and that of catecholamines injected intra-arterially may be accounted for in a number of ways.

It has been shown that the sympathetic nerve endings in man lie between the adventitia and the media of the vessels (Falck & Rorsman, 1963; Waterson, 1967). Tyramine introduced into the arterial blood stream would need to penetrate through the intima and the media before reaching the nerve stores, which would account for the delayed onset of effect compared to infused noradrenaline.

Once released by tyramine, the transmitter substance would, in addition to being subject to degradation by monoamine oxidase, be also readily accessible for re-uptake by the store. Catecholamine introduced intra-arterially, on the other hand, would reach the smooth muscle receptors more rapidly and uptake by the nerve stores would occur only after it had exerted its effect on the muscle (de la Lande, Frewin & Waterson, 1967). These factors could account for the beta-stimulating action of tyramine being less readily demonstrable with infusions which lasted less than 5 min.

When a dose of tyramine was given which matched in its constrictor effect a given dose of noradrenaline (and hence might be presumed to achieve a similar quantity of transmitter at the smooth muscle), the dilator effect of this dose, adjusted for increased flow after alpha-receptor blockade, was less marked than that of noradrenaline (Fig. 5). There are a number of possible explanations for this difference. The location of the beta-receptors in relation to the lumen of the vessel might be of relevance. If these lay closer to the intimal surface of the smooth muscle coat than to the adventitia they would be more readily accessible to infused noradrenaline arriving in the blood stream than to the substance released from the nerve plexus between the adventitia and the muscle coats.

Another factor capable of influencing the time course and magnitude of the dilatation induced by tyramine compared with that of noradrenaline is a blocking action of phentolamine on the transport of tyramine into the storage sites. Phentolamine has been shown to have a weak inhibitory effect on noradrenaline uptake by these sites (Iversen, 1967) and it is likely that it has the same effect on tyramine, which probably has a similar uptake mechanism. Such inhibition would reduce the rate and amount of transmitter released after phentolamine treatment, while the amount of infused noradrenaline accumulating at the receptor sites would be increased.

If the substance released from the sympathetic nerve endings by tyramine can be presumed to be the natural transmitter, the demonstration that it has both alpha and beta stimulating properties implies that it could be noradrenaline, adrenaline or a similar sympathomimetic substance. This conclusion is at variance with that of Brick, Hutchison & Roddie (1966) who compared the responses of the phentolamine-treated forearm vessels to noradrenaline with the reflex activity of the sympathetic nerves to the vessels induced by exposure of the legs and lower trunk to negative pressure. Noradrenaline caused an increase in flow but reflex nerve activity did not, implying that the transmitter was not noradrenaline. The present observation of a weaker and slower beta-stimulating effect of transmitter released by tyramine when compared with infused noradrenaline may offer an explanation for these findings. The reflex activity induced by the application of negative pressure may have been transient and the transmitter released in the presence of phentolamine may have been insufficient in amount to affect the beta-receptors or to

reach them if these are situated at a more distant site from the point of release than the alpha-receptors.

The failure of Brick *et al.* (1966) to demonstrate a potentiation by propranolol of the constrictor effect of nerve activity might be accounted for in the same way, because in the present investigation no potentiation was observed with infusions of tyramine which lasted less than 5 min, and with 15 min infusions it was present in only four of six subjects and was small in degree.

The relative distribution and densities of alpha and beta receptors in the smooth muscle of blood vessels are not known but the results of the present investigation suggest that such relationships might influence vascular responses to locally released and to circulating catecholamines.

SUMMARY

1. The constrictor action of tyramine on the blood vessels of the human forearm is dependent on the presence of the sympathetic nerves.

2. The constrictor substance liberated from these nerve endings by tyramine has both alpha-receptor and beta-receptor stimulating properties.

3. The alpha-receptor action of tyramine is its predominant effect, whereas the beta-receptor activity, which is modest, is only seen after blockade of the alpha-receptors with phentolamine.

4. Comparison of the times of onset of the constrictor and dilator responses, using doses of tyramine and noradrenaline which produced constrictor effects of comparable magnitude, demonstrated that responses to tyramine always appeared later than those to noradrenaline and its dilator effects were of lesser magnitude.

5. It is suggested that the differences between the times of onset of the actions of tyramine and noradrenaline might be the result of the fact that infused noradrenaline exerts its action directly on the vascular smooth muscle coat, whereas tyramine is required to penetrate to the nerve plexus and release transmitter which may be subject to re-uptake and degradation before reaching its site of action. The actions of the two substances might also be influenced by the relative distributions of the alpha and beta receptors in the vascular smooth muscle.

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REFERENCES

ALLWOOD, M. J. & GINSBURG, J. (1961). The effect of phenoxybenzamine (Dibenyline) on the vascular responses to sympathomimetic amines in the forearm. *J. Physiol., Lond.*, **158**, 219–228.

BLAIR, D. A., GLOVER, W. E., GREENFIELD, A. D. M. & RODDIE, I. C. (1959). Excitation of cholinergic vasodilator nerves to human skeletal muscles during emotional stress. *J. Physiol., Lond.*, **148**, 633–647.

BRICK, I., HUTCHISON, K. J. & RODDIE, I. C. (1966). A comparison of the effects of circulating noradrenaline and vasoconstrictor nerve stimulation on forearm blood vessels. *J. Physiol., Lond.*, **189**, 27–28*P*.

BRICK, I., HUTCHISON, K. J. & RODDIE, I. C. (1967). The vasodilator properties of noradrenaline in the human forearm. *Br. J. Pharmac. Chemother.*, **30**, 561–567.

BURN, J. H. & RAND, M. J. (1958). The action of sympathomimetic amines in animals treated with reserpine. *J. Physiol., Lond.*, **144**, 314–336.

COHN, J. N. (1965). Comparative cardiovascular effects of tyramine, ephedrine and norepinephrine in man. *Circulation Res.*, **16**, 174–182.

COOPER, C. J., FEWINGS, J. D., HODGE, R. L. & WHELAN, R. F. (1963). Effects of bretylium and guanethidine on human hand and forearm vessels and on their sensitivity to noradrenaline. *Br. J. Pharmac. Chemother.*, **21**, 165–173.

DE LA LANDE, I. S. & WHELAN, R. F. (1959). The effect of antagonists on the response of the forearm blood vessels to adrenaline. *J. Physiol., Lond.*, **148**, 548–553.

DE LA LANDE, I. S. & RAND, M. J. (1965). A simple isolated nerve-blood vessel preparation. *Aust. J. exp. Biol. med. Sci.*, **43**, 639–656.

DE LA LANDE, I. S., FREWIN, D. & WATERSON, J. G. (1967). The influence of sympathetic innervation on vascular sensitivity to noradrenaline. *Br. J. Pharmac. Chemother.*, **31**, 82–93.

DUFF, R. S. (1952). Effect of sympathectomy on the response to adrenaline of the blood vessels of the skin in man. *J. Physiol., Lond.*, **117**, 415–430.

FALCK, B. & RORSMAN, H. (1963). Observation on the adrenergic innervation of the skin. *Experientia*, **19**, 205–206.

FARMER, J. B. (1966). The biphasic response of an isolated artery to tyramine. *Br. J. Pharmac. Chemother.*, **28**, 340–347.

FREWIN, D. B. & WHELAN, R. F. (1968). The action of ephedrine on forearm blood vessels in man. *Aust. J. exp. Biol. med. Sci.*, in the Press.

GLOVER, W. E. & HUTCHISON, K. J. (1965). The effect of a beta-receptor antagonist (propranolol) on the cardiovascular response to intravenous infusions of noradrenaline in man. *J. Physiol., Lond.*, **177**, 59–60*P*.

GREENFIELD, A. D. M. (1954). A simple water-filled plethysmograph for the hand and forearm with temperature control. *J. Physiol., Lond.*, **123**, 62*P*.

IVERSEN, L. I. (1967). *The uptake and storage of noradrenaline in sympathetic nerves*, p. 156. London: Cambridge University Press.

LOWE, R. D. & ROBINSON, B. F. (1964). Effect of differential adrenergic blockade on response of forearm blood flow to infused catecholamines. *Clin. Sci.*, **26**, 81–87.

PARKS, V. J., SANDISON, A. G., SKINNER, S. L. & WHELAN, R. F. (1961). Sympathomimetic drugs in orthostatic hypotension. *Lancet*, **1**, 1133–1136.

PARKS, V. J., SKINNER, S. L. & WHELAN, R. F. (1961). Mechanisms in the return of vascular tone following sympathectomy in man. *Circulation Res.*, **9**, 1026–1034.

PRICHARD, B. N. C. & ROSS, E. J. (1966). Use of propranolol in conjunction with alpha-receptor blocking drugs in phaeochromocytoma. *Am. J. Cardiol.*, **18**, 394–398.

TAINTER, M. L. (1926). The actions of tyramine on the circulation and smooth muscle. *J. Pharmac.*, **30**, 163–184.

WATERSON, J. G. (1967). Fluorescent structures in the rabbit dental pulp. *Aust. J. exp. Biol. med. Sci.*, **45**, 309–311.