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THE BIPHASIC RESPONSE OF AN ISOLATED ARTERY TO TYRAMINE

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A simple isolated nerve-blood vessel preparation made from the central ear artery of the rabbit has been described by de la Lande & Rand (1965). The authors also described the effect of nerve stimulation and of some drugs on this preparation. Directly acting sympathomimetic amines injected into the artery induced a vasoconstriction which is monophasic in nature. However, it has been observed that the indirectly acting sympathomimetic amine, tyramine, produced a biphasic vasoconstriction of the artery (Farmer, 1966). In this paper the response of the artery to tyramine and its modification by cocaine, sympathetic denervation, reserpine and phentolamine is described.

METHODS

The central artery of the rabbit ear was prepared for perfusion as described by de la Lande & Rand (1965). The rabbit was anaesthetized with pentobarbitone (30 mg/kg I.V.) and the central artery cannulated at the base of the ear. The central artery, vein and adhering tissue was removed from the rabbit and perfused with McEwen's solution at 37° C delivered at a rate of 4 to 6 ml./min from a constant output pump (Watson–Marlow). The preparation was arranged for periarterial nerve stimulation and immersed in McEwen's solution at 37° C. Drugs or procedures which initiate changes in the calibre of the vessel cause changes in the perfusion pressure which are registered with the use of a Devices pressure transducer and a Devices polygraph recorder. Periarterial nerve stimulation was delivered by bipolar platinum electrodes from a Palmer electronic square wave stimulator. The pulses were of 1 msec duration, supramaximal voltage, and were given for 5 sec at frequencies of 1–20 pulses/sec. Noradrenaline and tyramine were injected into the perfusion fluid close to the artery in a dose volume of 0.02 ml. The solutions contained 10^{-6} ascorbic acid as an antioxidant. Phentolamine and cocaine were added to the perfusion fluid at the stated concentration. Reserpine was dissolved in 20% ascorbic acid in distilled water and injected intraperitoneally 24 hr before the artery was removed from the rabbit. The ear artery was denervated 7–10 days before use, in the following way: rabbits were anaesthetized with halothane in nitrous oxide and oxygen (3:1 v/v) and the cervical sympathetic ganglion and a portion of the pre- and post-ganglionic fibres were removed.

Drugs. The following drugs were used: tyramine hydrochloride, (−)-noradrenaline acid tartrate, cocaine hydrochloride, reserpine and phentolamine methane sulphonate. Stated doses and concentrations are in terms of these compounds.

RESULTS

A comparison of the effect of periarterial nerve stimulation, noradrenaline and tyramine on the isolated artery

The responses of the artery to periarterial nerve stimulation 1, 2, 5, 10 and 20 c/s, noradrenaline 0.2, 2.0, 20.0, 200 ng and 2 μg and tyramine 2.0, 20.0, 200 μg and 2 mg

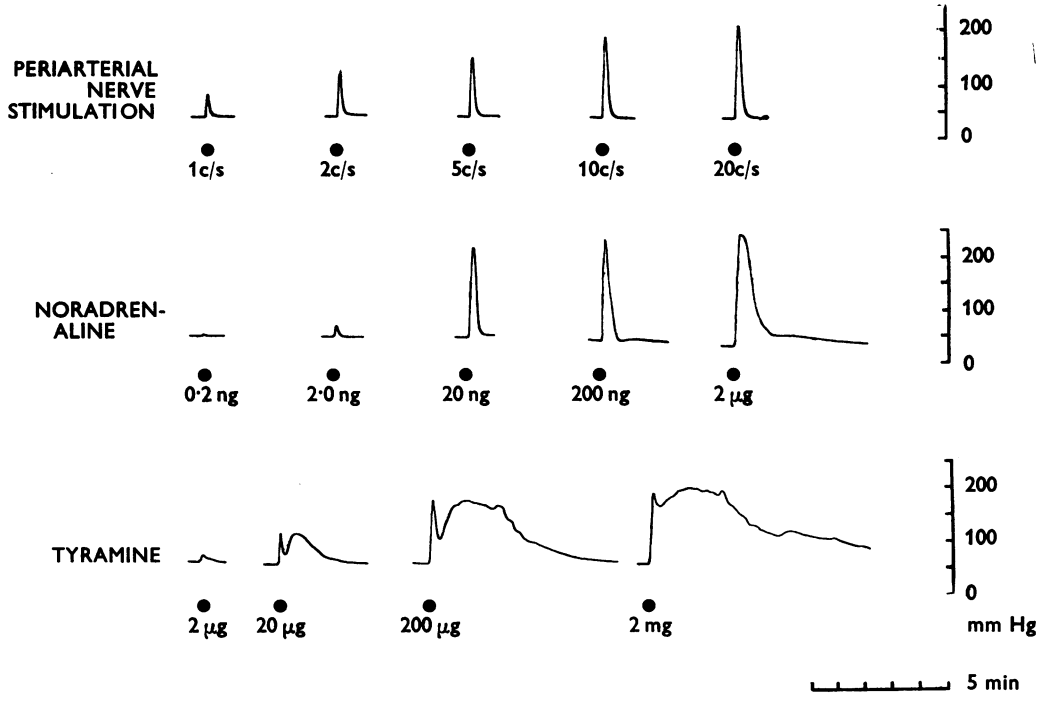

Fig. 1. The response of isolated central artery of the rabbit ear to periarterial nerve stimulation (1, 2, 5, 10 and 20 c/s; pulse width 1 msec, 5 sec duration, supramaximal voltage) and graded doses of noradrenaline and tyramine.

are shown in Fig. 1. Periarterial nerve stimulation and noradrenaline produced a monophasic response while tyramine produced a biphasic response. The first phase of the response to tyramine was short lived and similar to that observed for noradrenaline and was followed by a second phase of similar height to the first but of longer duration.

Modification of the response to periarterial nerve stimulation, noradrenaline and tyramine by cocaine, reserpine and sympathetic denervation

The responses of artery preparations to periarterial nerve stimulation, noradrenaline and tyramine were examined in 12 normal arteries, six perfused with 10 μg/ml. cocaine, 11 denervated arteries and 13 arteries taken from rabbits pretreated with reserpine. The modification of the responses of the artery by these procedures is shown in Figs. 2 and 3. It was observed that when the arteries were perfused with 10 μg/ml. cocaine nerve stimulation was either slightly reduced or potentiated. However, when the responses at each frequency for all the preparations were averaged it was found that responses at 1, 2 and 5 c/s were not significantly affected, while those at 10 c/s and 20 c/s were slightly reduced (Figs. 2 and 3D). The responses at all frequencies of stimulation were more prolonged in the presence of cocaine. Pretreatment with reserpine abolished the response of the artery to stimulation at 1 and 2 c/s and markedly reduced the responses at 5, 10 and 20 c/s (Fig. 3D). No response to periarterial nerve stimulation was observed in

denervated preparations at 1, 2 and 5 c/s; some very small responses were, however, observed at 10 and 20 c/s in a few preparations.

An increase in the sensitivity to small doses of noradrenaline but not to larger doses was observed in preparations made from rabbits pretreated with reserpine. Cocaine 10 μg/ml. tended to reduce the sensitivity of the artery to noradrenaline while the response of denervated arteries was essentially the same as the responses observed in the control preparations (Figs. 2 and 3).

The primary phase of the response to tyramine was not markedly altered by perfusion with cocaine 10 μg/ml. or pretreatment with reserpine or sympathetic denervation (Figs. 2 and 3A, B and C). In general it was observed that the primary phase was affected

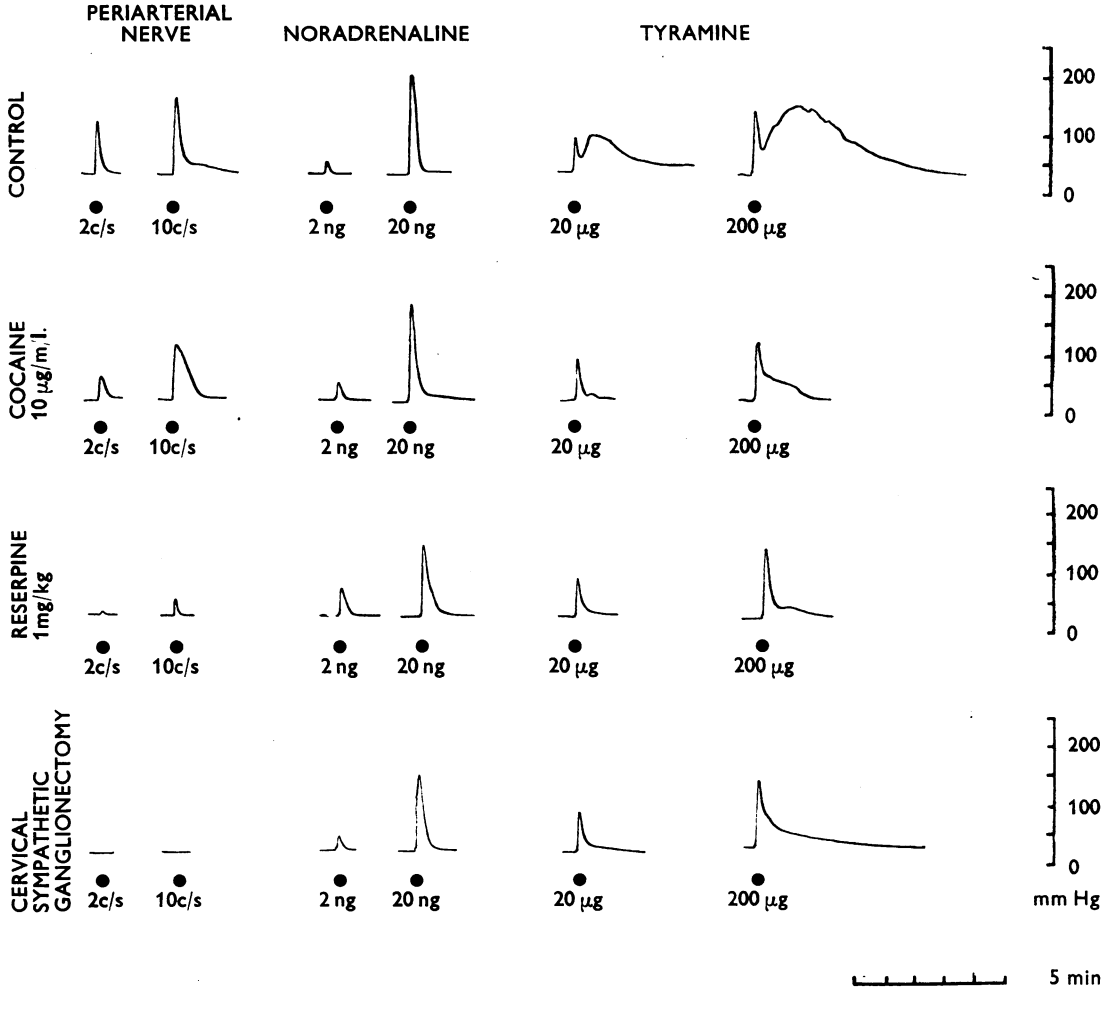

Fig. 2. The modification of the response of the isolated central artery of the rabbit ear to periarterial nerve stimulation, noradrenaline and tyramine by cocaine, pretreatment with reserpine and cervical sympathetic ganglionectomy.

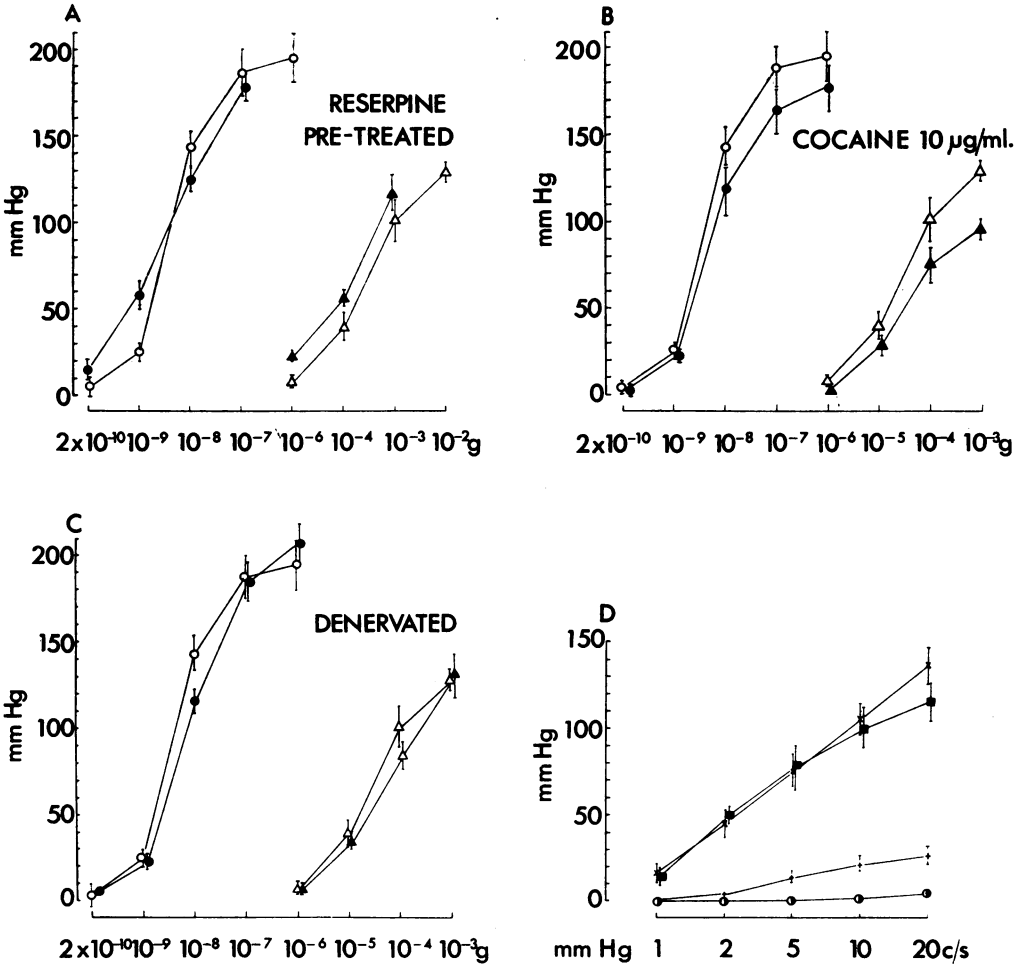

Fig. 3. Dose effect curves for noradrenaline (○) and primary phase of tyramine (△). Open symbols refer to responses obtained in normal arteries, closed symbols to responses obtained in treated preparations (A, B and C). Panel D shows the effect of these treatments on the responses to periarterial nerve stimulation, control (×) in presence of cocaine 10 µg/ml. (■); reserpine pretreated (+); denervated (◑). All results are means ± S.E. Further details see text.

in the same direction as were the responses to noradrenaline. All the treatments, however, reduced or abolished the second phase of the response to tyramine (Fig. 2). Cocaine 10 µg/ml. virtually abolished the second phase of the response to tyramine at 20 µg and markedly reduced that at 200 µg. Similarly, pretreatment with reserpine or sympathetic denervation abolished the second phase of the response to tyramine at low doses (20 µg). The responses obtained were similar in duration to those obtained with equi-active doses of noradrenaline. However, 200 µg tyramine produced a monophasic response in reserpinized or denervated preparations but the time for recovery of the response was slightly longer than was observed for noradrenaline in the same preparations.

The effect of perfusion with noradrenaline on response of the artery to tyramine

The responses of normal, reserpine pretreated and denervated arteries to periarterial nerve stimulation 5 c/s, noradrenaline 2 and 20 ng and tyramine 20 and 200 μg were determined before and after perfusion with a 0.1 μg/ml. noradrenaline for 20 min. In control preparations the infusion of noradrenaline reduced slightly the response to periarterial nerve stimulation; the responses to noradrenaline were not affected while the second phase of the response to tyramine was slightly enhanced. It was observed that the infusion of noradrenaline completely restored the second phase of the responses to tyramine in reserpine pretreated preparations (Fig. 4) but not in those which had been

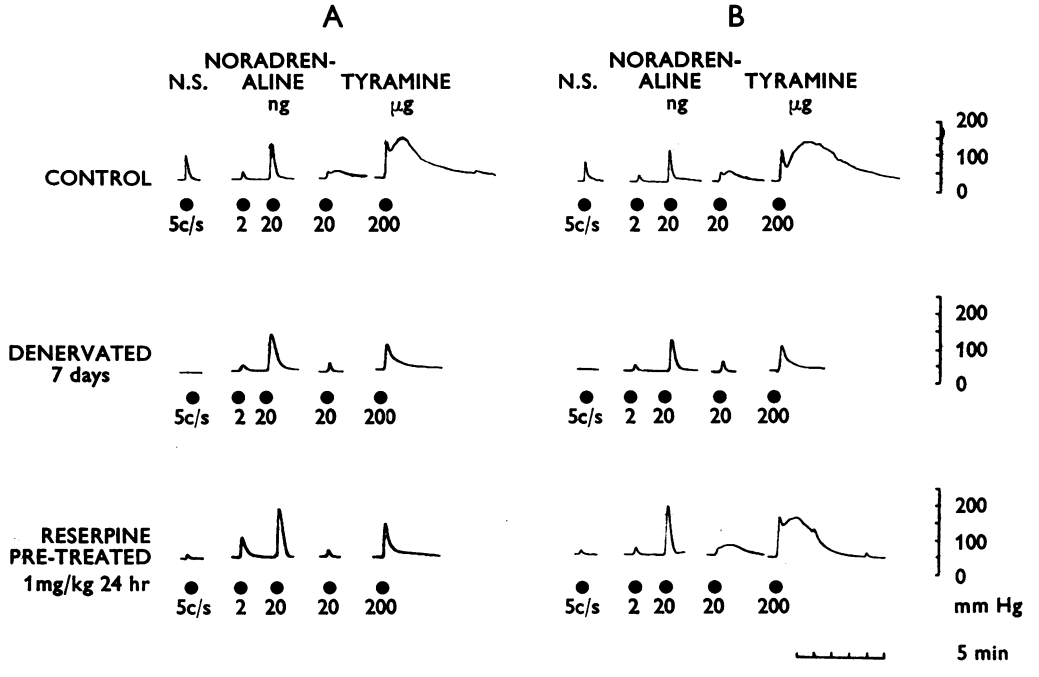

Fig. 4. The effect of perfusion of the isolated artery with 10^{-7} g/ml. noradrenaline for 20 min (between A and B) on the responses to periarterial nerve stimulation, noradrenaline and tyramine. Upper tracing: control preparation; middle tracing: artery denervated seven days previously; lower tracing: rabbit pretreated with 1 mg/kg reserpine. Note restoration of second phase of response to tyramine by noradrenaline in reserpine-treated but not the denervated preparation.

denervated. The restoration was not permanent, and the magnitude of the second phase rapidly diminished when successive injections of tyramine were made. The infusion of noradrenaline did not restore the response to periarterial nerve stimulation at 5 c/s in reserpine pretreated preparations. The enhanced sensitivity of the artery to injected noradrenaline in reserpine pretreated preparations was reduced to normal by the perfusion.

The effect of α-receptor blockade on the response of the artery to tyramine and noradrenaline

Dose effect curves for noradrenaline and tyramine were determined in eight arterial preparations before and after perfusion with phentolamine 0.1, 0.5 and 2.5 μg/ml. A period of 30 min elapsed between the start of each dose effect curve and the perfusion with each concentration of phentolamine. The results are shown in Fig. 5. Perfusion with 0.1 μg/ml. phentolamine shifted the dose effect curves for noradrenaline and tyramine to the right. The effect of phentolamine was selective in that the shifts in dose

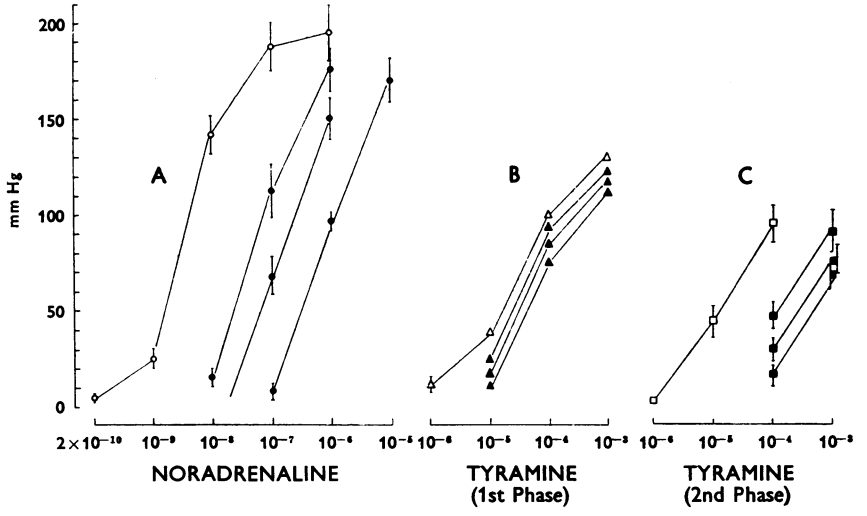

Fig. 5. The effect of phentolamine 0.1, 0.5 and 2.5 μg/ml. on the response of the isolated central artery of rabbit ear to (A) noradrenaline (○); (B) primary phase of response to tyramine (△) and (C) secondary phase of response to tyramine (□). Open symbols are responses before and closed symbols responses after addition of phentolamine. Responses are means ± S.E. Further details see text.

effect curves for these substances were not equal. Thus the curve for noradrenaline and second phase of the response to tyramine were shifted to the right by an almost equal amount but the primary phase of the response to tyramine was much less affected. The further shifts in the dose effect curve for noradrenaline when the concentration of phentolamine was raised to 0.5 and 2.5 μg/ml. were equal. This increase in concentration of phentolamine produced further, but small, shifts in the dose effect curve for tyramine (second phase) and an even smaller shift of the dose effect curve for the primary phase of the response to tyramine.

It was found that phentolamine at very high concentration—for example, 25 μg/ml.—would reduce the primary phase of the response to tyramine by more than 50%, but this concentration of phentolamine also reduced the response of the artery to histamine and 5-hydroxytryptamine. Chlorpheniramine (1 μg/ml.) and lysergic acid diethylamide (100

μg/ml.) produced full blockade of submaximal responses of the artery to histamine and 5-hydroxytryptamine respectively without producing any effect on the primary phase of the response to tyramine.

DISCUSSION

Tyramine exerts a sympathomimetic action on a wide variety of tissues by effecting a release of noradrenaline from storage sites within post-ganglionic adrenergic nerve endings. The evidence for such an action has been well documented by numerous investigators. The second phase of the response to tyramine on the isolated central ear artery of the rabbit is undoubtedly an indirect sympathomimetic action of tyramine. Procedures which reduce the noradrenaline content of tissues, such as prior treatment of the animal with reserpine (Bertler, Carlsson & Rosengren, 1956) or sympathetic denervation (Von Euler & Purkhold, 1951), are known to reduce or abolish the sympathomimetic actions of tyramine (Burn, 1932 ; Carlsson, Rosengren, Bertler & Nilsson, 1957). The second phase of the response to tyramine on the isolated artery is similarly abolished by such procedures. Evidence that this action of tyramine is principally on an intraneuronal store of noradrenaline is provided by the complete restoration of the response by infusion of noradrenalne in arteries taken from rabbits pretreated with reserpine, but not those to which the sympathetic nerve fibres had been cut. Burn & Rand (1960) observed that, in the cat pretreated with reserpine, the sympathomimetic action of tyramine on several tissues could be restored by infusion of noradrenaline only if the sympathetic nerve supply were present. These authors also observed that noradrenaline was more effective in restoring responses of tissues to tyramine than to sympathetic nerve stimulation. In the present experiments it was observed that noradrenaline fully restored the response to tyramine but failed to restore the response to periarterial nerve stimulation in arteries taken from rabbits pretreated with reserpine. Cocaine abolishes the vasopressor actions of tyramine (Tainter & Chang, 1927). This interaction has since been shown to be competitive in nature and that cocaine antagonizes the uptake of sympathomimetic amines into tissue stores and so prevents tyramine from releasing noradrenaline (Lockett & Eakins, 1960 ; Trendelenburg, 1961 ; Farmer & Petch, 1963). On the ear artery the antagonism of the second phase of the response to tyramine is probably competitive, since at a constant concentration of cocaine there was less reduction in the response to large than to small doses of tyramine.

The primary phase of the response to tyramine and the response to noradrenaline was not reduced to the same extent by the α-adrenergic receptor blocking agent, phentolamine. If two substances act on the same receptor it can be expected that the substances would produce the same PA_x (Schild, 1947) with a competitive antagonist. This is a consequence of the mass law and is independent of the intrinsic activity (Ariëns, 1954) or efficacy (Stephenson, 1956) of the agonist (Arunlakshana & Schild, 1959). From these considerations, the primary phase of the response to tyramine should not be due to stimulation of α-adrenergic receptors. On the other hand Mujić & van Rossum (1965) have observed that, of a series of α-receptor stimulants related to oxymetazoline, the more potent members were more easily antagonized by α-receptor blockade than the less active ones. These authors stressed the need for reinvestigation of the principles of receptor theory. The present findings on isolated artery are in agreement with the results of these workers.

If the primary phase of the response to tyramine is due to α-receptor stimulation, then the drug is probably a partial agonist as indicated by the shape of the dose-response curves in Fig. 5B. Also, in other experiments in reserpinized arteries it was found that submaximal responses to noradrenaline and tyramine were not additive, the presence of tyramine reducing the response to noradrenaline.

It is concluded that the biphasic response to tyramine consists of a primary phase which is probably caused by direct α-stimulation with tyramine acting as a partial agonist and a secondary phase due to release of catecholamines from stores within the sympathetic neurone.

SUMMARY

1. The responses of the isolated perfused central artery of the rabbit ear to tyramine, noradrenaline and periarterial nerve stimulation have been described.

2. Tyramine causes a biphasic response of the artery. The first phase is due to a direct stimulant action of tyramine on the smooth muscle, probably mediated through α-adrenergic receptors, but analysis of dose-response curves in the presence of an α-blocking drug indicated that a different receptor may be involved.

3. The second phase is indirect and results from the release of catecholamines from intraneuronal stores. This part of the response is reduced or abolished by cocaine, denervation or reserpine. In reserpine-treated but not denervated preparations the second phase of the response to tyramine may be totally restored by perfusion with noradrenaline.

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