Short communications

Effect of tyramine on human umbilical artery *in vitro*

O. D. GULATI and V. V. KELKAR
Department of Pharmacology, Medical College, Baroda, India

Fifteen of twenty-one perfused human umbilical artery preparations constricted in response to tyramine. These responses were unaffected by atropine, mepyramine or bromolysergic acid diethylamide but were blocked by phentolamine or dihydroergotamine. Cocaine augmented the constriction responses to tyramine or noradrenaline but not those to adrenaline, acetylcholine or 5-hydroxytryptamine. Infusion of noradrenaline restored the responses of preparations which had become tachyphylactic to tyramine.

In 1964, Fischer & Kopin reported that noradrenaline taken up by chronically denervated rat salivary gland could not be depleted by reserpine. Tyramine released a portion of the noradrenaline, and cocaine (but not reserpine) interfered with the entry of noradrenaline into the storage site (Kopin, 1964). Responsiveness of the chronically denervated nictitating membrane of the cat to tyramine is partly restored by noradrenaline (Trendelenburg & Pfeffer, 1964). This was taken to indicate a noradrenaline binding site which differs from the receptors of the effector organ and from the stores in the nerve endings.

In view of these reports, it was of interest to examine the action of tyramine on the non-innervated vascular smooth muscle of human umbilical artery *in vitro*.

Methods.—Human umbilical artery preparations were set up and perfused as described by Gokhale, Gulati, Kelkar & Kelkar (1966). The perfusion fluid (composition, g/l.: NaCl, 9·00; KCl, 0·42; $CaCl_2$, 0·24; $NaHCO_3$, 0·2; and dextrose 1·0), aerated with 5% CO_2 in O_2, was allowed to flow from a Mariotte bottle through a cannula in the foetal end of the vessel.

Drugs: Acetylcholine chloride, (±)-noradrenaline hydrochloride, 5-hydroxytryptamine creatinine sulphate (5-HT), tyramine hydrochloride, dihydroergotamine methanesulphate (DHE), phentolamine methanesulphonate, mepyramine maleate, cocaine hydrochloride and atropine sulphate were used; the doses refer to the base. Adrenaline base and bromolysergic acid diethylamide (BOL) were prepared in 0·9% sodium chloride immediately before use. All drugs, diluted in 0·9% sodium chloride solution, were injected in a fixed volume of 0·3 ml through the rubber tubing close to the cannula. The interval between successive injections was 7 to 10 minutes.

Results.—Of a total of twenty-one preparations exposed to tyramine (80–400 μg) fifteen gave constrictions lasting for 1–5 min, three gave dilations lasting for 1–3 min and three did not respond. Six of the fifteen vessels contracted by tyramine became tachyphylactic to the amine sooner or later (Fig. 1, A); the others responded regularly and the drug effect was proportional to the dose. The magnitude of the constriction also varied considerably. The highest dose given reduced the outflow only by 20–25% of the control in eight experiments, but by nearly 100% in the other seven experiments.

All preparations used in this study showed constriction in response to adrenaline or noradrenaline. The threshold doses ranged between 0·01–0·05 μg and 0·024–0·064 μg respectively. Four of six preparations which constricted with tyramine responded similarly to 5-HT (threshold dose range, 0·04–0·08 μg); two did not respond even to doses as high as 0·5 μg. As judged from the threshold doses in individual experiments, sensitivity to tyramine was unrelated to sensitivity to catecholamines or to 5-HT. These agents elicited responses even in preparations which became tachyphylactic to tyramine.

Effect of antagonists

Contractions of the vessels in response to tyramine or noradrenaline were unaltered by atropine (6·3 μg, three experiments), mepyramine (132 μg, four experiments), or BOL (15 μg, three experiments); these doses of antagonists blocked the contraction in response to acetylcholine (24–72 μg), histamine (0·36–18 μg) and 5-HT (0·023–0·064 μg) respectively. Phentolamine (300 μg, two experiments) or DHE (23 μg, two experiments) promptly abolished constrictions in response to tyramine and noradrenaline.

Effect of cocaine

In a total of sixteen experiments cocaine (350 μg) did not alter the outflow from the vessels. It augmented by 20% or more constriction to noradrenaline, tyramine and 5-HT in nine of the eleven, four of the six and three of the eight preparations respectively. Cocaine had no effect on responses to adrenaline or acetylcholine. In two experiments phentolamine (300 μg) abolished the cocaine augmented responses to noradrenaline or tyramine.

Effect of noradrenaline infusion

In five experiments in which tyramine produced a reproducible contraction of the vessels, noradrenaline (0·83 μg) was slowly infused over a period of 8–12 minutes. The action of tyramine was augmented by 10–15% of the control response to this drug in three experiments, whilst in others it remained unaltered.

In three experiments in which the vessels became tachyphylactic to tyramine (Fig. 1, A), noradrenaline completely restored sensitivity to tyramine (Fig. 1, B); this phenomenon was reproducible. However, administration of cocaine (350 μg) before the infusion of noradrenaline did not affect the capacity of the latter to restore response to tyramine (Fig. 1, C); on the other hand tachyphylaxis was now minimal (Fig. 1, C).

Discussion.—Cocaine induced supersensitivity of the umbilical artery to noradrenaline but not to adrenaline. This suggests that in giving rise to supersensitivity cocaine does not act directly on the α-adrenoceptor of the arterial smooth muscle.

Supersensitivity of an innervated tissue to noradrenaline induced by cocaine is due to block of uptake of noradrenaline at sympathetic nerve endings (Trendelenburg,

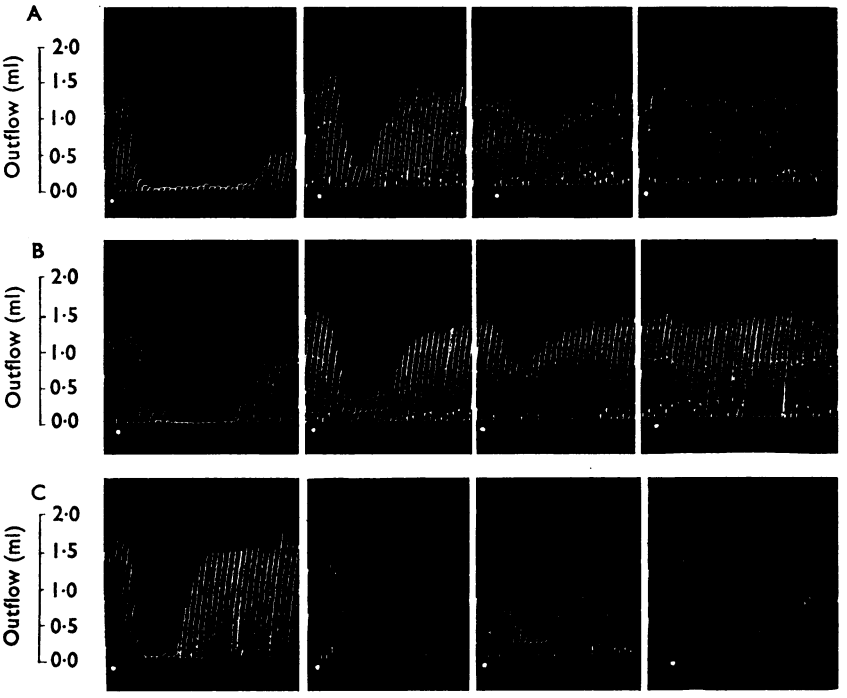

FIG. 1. Perfused human umbilical artery. Records of outflow per 10 seconds. Responses to tyramine (240 μg) injected at intervals of 8 min (at white dots) showing the development of tachyphylaxis (A). Noradrenaline (0·83 μg) was slowly infused over 10 min before (B), restoring the response but not preventing further tachyphylaxis. Cocaine (350 μg) over a period of 5 min and then noradrenaline (0·83 μg) were given before C. Cocaine had no effect on the restorative action of noradrenaline, but after cocaine, tachyphylaxis to tyramine was less marked.

1966), resulting in increased effective concentration of the amine at the receptor site. This 'deviation concept' could also explain the augmenting effect of cocaine on the non-innervated umbilical artery, if the latter is assumed to have a significant, cocaine sensitive mechanism which takes up and binds noradrenaline but not adrenaline. A cocaine sensitive, extraneuronal site which binds noradrenaline is present in the denervated rat salivary gland (Kopin, 1964).

Our experiments with antagonists exclude the possibility that the action of tyramine is on the tryptamine, acetylcholine or histamine receptors of the artery. With respect to tryptamine receptors, it is pertinent that there were preparations responding to tyramine but not to 5-HT. On the other hand, α-adrenoceptors do seem to be involved in the action of tyramine.

Potentiation of responses to tyramine by infusion of noradrenaline following the development of tachyphylaxis would suggest that tyramine then acted by releasing noradrenaline from the sites of binding. Inherent in this view is the assumption that the indirect action would occur only if there is a sufficient number of noradrenaline binding sites and a sufficient amount of noradrenaline is bound at these sites. The failure in some experiments of an infusion of noradrenaline to augment the action of tyramine and the weaker and less consistent action of tyramine than that of the directly acting constrictors of the vessels are also easily explicable on this assumption.

The action of tyramine is not dependent upon the infusion of noradrenaline and the absence of tachyphylaxis in some experiments could be due to a direct action of tyramine on α-adrenoceptors. That tyramine can act directly (through α-adrenoceptors or otherwise) is well documented for non-innervated (Takenaka, 1963; Somlyo, Woo & Somlyo, 1965) as well as other tissues (Luduena, 1963; Furchgott, Kirpekar, Rieker & Schwab, 1963; Hudgins & Fleming, 1966; Bevan & Verity, 1967; Arthur & Fleming, 1968).

Whilst tyramine reacts with the extraneuronal sites (which bind noradrenaline) to cause a partial release of noradrenaline, it is in all probability also taken up and bound at these sites (Fischer, Kopin & Axelrod, 1965). Thus cocaine, which is a competitive antagonist of tyramine (Muscholl, 1966), would reduce the binding of tyramine just as it reduces the binding of noradrenaline. Under these circumstances the direct action of tyramine may become evident or the weak antagonist action of tyramine may become augmented. This would explain the augmentation by cocaine of the action of tyramine seen in our experiments. The augmented direct action could also be the basis of our observations that cocaine could not abolish the restoration of action of tyramine by the infusion of noradrenaline in preparations which had become tachyphylactic to tyramine, and that following cocaine tachyphylaxis was not seen.

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