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A proposed mechanism for the biphasic vasoconstrictor responses to 5-hydroxytryptamine and methysergide in the rabbit ear artery

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Rabbit ear arteries were isolated and perfused at a constant flow rate so that the perfusate flowed into the fluid bathing the adventitial surface of the artery. Submaximal doses of intraluminally applied noradrenaline injected as a bolus into the perfusion fluid produced transient monophasic vascoconstrictor responses. In contrast, similarly administered 5-hydroxytryptamine (5-HT) or methysergide caused prolonged biphasic vascoconstrictor responses. The extraluminal/intraluminal potency ratios for noradrenaline, 5-HT and methysergide were 230, 15 and 6 respectively, which indicates that 5-HT and methysergide are relatively more potent when administered extraluminally than noradrenaline. Cocaine ($3{\cdot}0 \times 10^{-5}$ mol litre^{-1}) markedly increased the potency of extraluminally administered noradrenaline and converted the monophasic responses produced by noradrenaline to biphasic responses. It is concluded that under the experimental conditions used 5-HT and methysergide produced biphasic responses by an action on the medial smooth muscle firstly *via* the intraluminal surface and secondly an additional direct action *via* the adventitial surface. Noradrenaline's extraluminal potency is low because of its neuronal uptake and hence the responses are normally monophasic.

5-Hydroxytryptamine (5-HT) and methysergide produce biphasic vasoconstrictor responses when injected as a bolus into the rabbit isolated perfused ear artery (Fozard, 1973; Apperley, Humphrey & Levy, 1976). In contrast, noradrenaline so administered produces monophasic vasoconstrictor responses (Farmer, 1966). Tyramine also produces biphasic vasoconstrictor responses in the ear artery which are mediated by an initial direct stimulant action followed by a second phase due to noradrenaline release which is diminished or abolished by pretreatment with reserpine (Campbell & Farmer, 1968). However, the biphasic nature of the responses to 5-HT and methysergide is not affected by pretreatment with reserpine (Fozard, 1973; Apperley, Humphrey & Levy, 1974) which suggests that release of noradrenaline is not involved. The purpose of the present experiments was to determine the cause of the second phase of the responses to 5-HT and methysergide.

MATERIALS AND METHODS

Physiological saline. A modified Krebs saline (Krebs & Henseleit, 1932) of the following composition (mmol litre^{-1}) was used; Na^+ 143·4, K^+ 5·9, Mg^{2+} 0·6, Ca^{2+} 1·3, Cl^- 124·5, $H_2PO_4^-$ 1·2, SO_4^{2-} 0·6, HCO_3^- 25·0, glucose 11·1. The mixture was gassed with 5% CO_2 in oxygen at 37°.

Drugs. (−)-Noradrenaline bitartrate, mol wt 337 (Koch-Light), 5-hydroxytryptamine creatinine sulphate, mol wt 405 (Koch-Light), methysergide bimaleate, mol wt 470 (Sandoz), cocaine hydrochloride, mol wt 340 (MacFarlane-Smith Ltd.). Methysergide and 5-HT were dissolved in distilled water and noradrenaline in isotonic saline (0·9% w/v sodium chloride) containing 0·2 mg ml^{-1} ascorbic acid.

Rabbit isolated ear artery

Right and left auricular arteries were removed from New Zealand white rabbits of either sex (2–3 kg) under pentobarbitone anaesthesia (36 mg kg^{-1}, i.v.). The vessels were carefully cleared of all connective tissue and perfused intraluminally at a constant flow rate of 7 ml min^{-1} with modified Krebs solution. The perfusion fluid was allowed to bathe the adventitial surface of the artery before being removed by overflow which maintained a constant volume of perfusate (15 ml) in the bath. Changes in perfusion pressure were recorded by means of a pressure transducer. The method is similar to that of de la Lande & Rand (1965). Arteries were set up in pairs from a single animal and both vessels were perfused

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for 60 min before the experiment commenced. Control responses were obtained for each agonist in both arteries before the effect of cocaine was investigated. One vessel was then used as a control and the other was perfused with cocaine which was added to the physiological saline in the reservoir 30 min before re-determining agonist responses.

Intraluminal administration. Agonists were added to the perfusion fluid immediately before entry into the peristaltic pump which was situated close to the artery. A constant injection volume (100 μl) which produced no injection artifact was used. Consistent responses were obtained with a 5 min interval between doses.

Extraluminal administration. Agonists were administered at a point near the bottom of the organ bath in close proximity to the open end of the artery. The injection volume (100 μl) was kept constant. As the responses were of longer duration by this route a 15 min interval was used between doses.

Comparison of potency of agonists administered intraluminally and extraluminally

Doses of agonist which produced submaximal responses were given either intraluminally or extraluminally. The relative magnitude of the responses was compared by bracketing a single extraluminal dose by two intraluminal doses. The results were analysed as for a (2 and 1) dose assay (Gaddum, 1955) and the extraluminal/intraluminal potency ratio calculated as defined by:

$$\frac{\text{Extraluminal dose required to produce response x mmHg}}{\text{Intraluminal dose required to produce response x mmHg}}$$

Since the responses to intraluminal 5-HT and methysergide were biphasic the larger response was used for calculation. That is, the initial phase was used for 5-HT and the second phase for methysergide with which the initial phase was less discrete, although the difference in magnitude between the initial and second phase of these responses was small.

RESULTS

Intraluminal administration

Fig. 1 a shows typical vasoconstrictor responses to bolus injections of noradrenaline, 5-HT and methysergide. Noradrenaline ($1{\cdot}0 \times 10^{-11}$–$1{\cdot}0 \times 10^{-9}$ mol) produced transient monophasic responses which were dose dependent. However, large doses of noradrenaline which were greater than those necessary for a maximal response gave biphasic responses (Fig. 2 a). In contrast, 5-HT ($1{\cdot}0 \times 10^{-7}$–$2{\cdot}5 \times 10^{-6}$ mol) and methysergide ($1{\cdot}0 \times 10^{-7}$–$1{\cdot}0 \times 10^{-6}$ mol) each produced prolonged biphasic responses over the whole dose-range.

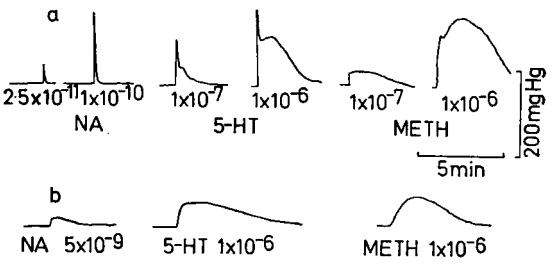

FIG. 1. Perfused rabbit ear artery. Vasoconstrictor responses to bolus injections of a—intraluminal noradrenaline (NA), 5-hydroxytryptamine (5-HT) and methysergide (METH). The doses (mol) are indicated. Note the transient monophasic response with NA. In contrast, 5-HT and methysergide produce prolonged biphasic responses. b. Extraluminal administration. All the agonists are less potent by this route, but this is most marked in the case of NA where much larger doses are necessary than those administered intraluminally (compare doses above in Fig. 1a).

Extraluminal administration

Noradrenaline ($4{\cdot}0 \times 10^{-9}$–$1{\cdot}0 \times 10^{-6}$ mol), 5-HT ($1{\cdot}0 \times 10^{-7}$–$2{\cdot}5 \times 10^{-6}$ mol) and methysergide ($1{\cdot}0 \times 10^{-7}$–$1{\cdot}0 \times 10^{-6}$ mol) produced constrictor responses after bolus injection into the Krebs bathing the adventitial surface of the artery (Fig. 1 b). With all three agonists the rate of onset of the

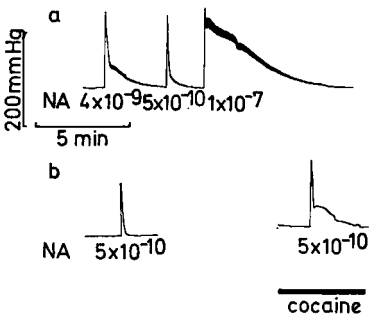

FIG. 2. Perfused rabbit ear artery. Vascoconstrictor responses to bolus injections of intraluminal noradrenaline (NA). a—The characteristic monophasic response is seen at 5×10^{-10} mol, but a biphasic response is seen at 4×10^{-9} mol and $1{\cdot}0 \times 10^{-7}$ mol. The magnitude of the initial phase of the response is the same with all three doses. b—NA (5×10^{-10} mol) in the absence and presence of cocaine (3×10^{-5} mol litre^{-1}). The intraluminal monophasic response was converted to a biphasic response in the presence of cocaine.

responses, which were essentially monophasic, was slower and the potency reduced compared to intraluminal administration.

Comparison of potency of agonists when administered intraluminally and extraluminally

The three agonists were all less potent when administered extraluminally. However, there were marked differences in the extraluminal/intraluminal potency ratios between agonists. That for noradrenaline (Table 1) was high since relatively large doses were necessary to produce vasoconstriction via the extraluminal route. In contrast, methysergide was active at similar doses by either route and the potency ratio was small. With 5-HT the potency ratio was greater than that of methysergide but was much less than that of noradrenaline.

The potency ratio for all three agonists did not change appreciably throughout the course of an experiment and it was therefore possible to determine the effect of cocaine. Cocaine ($3{\cdot}0 \times 10^{-5}$ mol litre^{-1}) reduced the potency ratio for noradrenaline (Table 1). Regression analysis of the dose-response data confirmed that this was almost entirely due to an increased sensitivity of the artery to extraluminally administered noradrenaline. In addition, cocaine altered the nature of the vasoconstrictor responses to intraluminally administered noradrenaline (Fig. 2 b). The monophasic responses to noradrenaline were changed to biphasic responses with small second phases (6 out of 6 arteries). With both 5-HT and methysergide neither the potency ratio nor the nature of the response were significantly altered by cocaine (Table 1).

DISCUSSION

The aim of this study was to investigate the cause of the biphasic vasoconstrictor responses to 5-HT and methysergide in the rabbit isolated perfused ear artery. The prolonged second phase of the response could theoretically arise from either a direct or an indirect mechanism.

An indirect mechanism would involve local release of a vasoconstrictor agent. This was of particular interest in view of the ability of 5-HT to release noradrenaline (Fozard & Mwaluko, 1975) and prostaglandin (Alabaster & Bahkle, 1970). However, release of noradrenaline does not appear to be involved (see Introduction). Furthermore, indomethacin ($3{\cdot}0 \times 10^{-6}$ mol litre^{-1}) did not change the biphasic nature of the response to methysergide (unpublished observation) which suggests that release of prostaglandins is not important. Histamine release can be ruled out since pizotifen, a potent histamine antagonist (Fozard, 1976), only weakly antagonized the second phase of the responses to 5-HT and methysergide (Apperley & others, 1974). The finding that antagonists blocked both phases of the response to 5-HT and methysergide to a similar degree (Apperley & others, 1976) suggested that the second phase more probably resulted from a direct mechanism.

A direct mechanism was further implicated by the finding that intraluminally administered noradrenaline would itself produce a biphasic response providing a dose was used which exceeded that necessary for a maximal response. We were able to show that these high doses of noradrenaline, which produced biphasic responses, produced vasoconstrictor responses when administered extraluminally. The responses were of roughly similar magnitude to the second phase produced by intraluminal administration of that dose. An analysis of the extraluminal/intraluminal potency ratio showed that noradrenaline was relatively much less potent when administered extraluminally.

Table 1. *Comparison of the potency of noradrenaline, 5-HT and methysergide when given intraluminally and extraluminally in the rabbit ear artery.*

	Noradrenaline		5-HT		Methysergide	
Potency ratio	Control	Cocaine (3×10^{-5} mol litre^{-1})	Control	Cocaine (3×10^{-5} mol litre^{-1})	Control	Cocaine (3×10^{-5} mol litre^{-1})
Before	230 (142–374)	196 (115–336)	15·3 (7·4–31·5)	23·4 (12·5–43·7)	6·0 (2·1–17·6)	3·8 (2·1– 6·9)
After	182 (114–291)	34·5* (20·0–59·5)	9·3 (3·8–23·0)	11·0 (5·7–21·0)	7·9 (2·7–23·1)	8·4 (6·4–11·1)

The potency ratio is defined as the ratio of the extraluminal to intraluminal dose of agonist required to produce an equi-active response. This is not an absolute extraluminal/intraluminal potency ratio but reflects the relative potency of the agonists when administered by the two routes described (see methods).

Each value is the mean of 6 determinations (95% confidence limits).

* Significantly different from control ($0{\cdot}02 > P > 0{\cdot}01$).

These observations are consistent with the following scheme. Intraluminally administered noradrenaline injected as a bolus transiently activates receptors in the medial smooth muscle cells lining the inner surface of the artery. After traversing the length of the artery the bolus of noradrenaline passes out into the organ bath and is then much diluted. Some noradrenaline will penetrate the adventitial surface of the artery but will be subjected to inactivation, by uptake into the sympathetic nerves ($Uptake_1$, Iversen, 1971) which form a dense layer at the medial-adventitial border, by uptake into arterial smooth muscle cells ($Uptake_2$, Iversen, 1965) and non-specific binding to connective tissue. Thus, in low doses, little of the intraluminally administered noradrenaline will reach the medial smooth muscle cells via the adventia, consequently the responses to low doses of noradrenaline are monophasic. Only when the intraluminal dose of noradrenaline is high, i.e. in excess of the dose required for a maximal response, will the concentration of noradrenaline in the organ bath be sufficient to ensure that a proportion penetrates from the adventitial surface to the medial smooth muscle cells. In this situation a biphasic response is observed. Cocaine will largely prevent the inactivation of noradrenaline, at least by $Uptake_1$ (Callingham & Burgen, 1966), during its passage from the outer to the inner surface of the artery. Thus, for any given dose, a larger proportion of noradrenaline will reach the medial smooth muscle cells *via* this route. These findings explain why noradrenaline is much less potent when administered extraluminally than intraluminally (de la Lande, Cannell & Waterson, 1966; de la Lande, Frewin & Waterson, 1967) and why the monophasic responses seen with low doses of noradrenaline given intraluminally are converted to biphasic responses in the presence of cocaine.

Methysergide invariably produced biphasic responses when administered intraluminally and its potency was not markedly different by the intraluminal or extraluminal route (i.e. the potency ratio was low). It seems likely that methysergide is resistant to inactivation processes. Thus, the extraluminal/intraluminal potency ratio was not decreased by cocaine and this is in agreement with the finding that methysergide does not compete for amine uptake sites in arterial smooth muscle (Buchan, Lewis & Sugrue, 1974). A high proportion of an intraluminally administered dose of methysergide would therefore be expected to reach the medial smooth muscle cells via the adventitia, thus accounting for the biphasic response. The 4–8 fold greater intraluminal than extraluminal potency is probably a reflection of differences in the degree of dilution by the different routes.

The extraluminal/intraluminal potency ratio for 5-HT was intermediate between those for noradrenaline and methysergide. 5-HT probably undergoes some inactivation during passage across the arterial wall. Thus, it has some affinity for noradrenaline neuronal uptake sites (Thoa, Eccleston & Axelrod, 1969; Iversen, 1974) and a small amount may be removed by $Uptake_2$ or non-specific binding, as suggested for noradrenaline. Nevertheless, a considerable proportion of an intraluminal dose of 5-HT would be expected to penetrate from the adventitia to the medial smooth muscle cells, thus accounting for the biphasic responses.

The extraluminal/intraluminal potency ratio we found for noradrenaline in the presence of cocaine is not as low as that for methysergide. This would suggest that even in the presence of cocaine there is still some loss of extraluminally administered noradrenaline. It might be that $Uptake_1$ was not completely blocked or that $Uptake_2$ was also removing some noradrenaline before it reached its site of action. Alternatively, it is probable that the extraluminal/intraluminal potency ratio for methysergide is not an absolute measure of differences in the degree of dilution by the different routes. Even in the absence of uptake or removal processes it is likely that the extraluminal/intraluminal potency ratio for different agonists would vary because of differences in kinetics of diffusion and receptor activation.

It is possible that an agonist might produce a biphasic response by a direct mechanism which involves activation of two separate contractile mechanisms. A situation such as this has been demonstrated for noradrenaline where a prolonged second phase was seen which was considered to be an equilibrium reaction as a result of penetration of the noradrenaline throughout the smooth muscle of the artery (Bevan & Waterson, 1971). Further work suggested the mechanism for the two phases of the contraction were different (Bevan, Garstka, & others, 1973). This is unlikely to be relevant to our experiments where equilibrium concentrations of agonist are not achieved and where noradrenaline clearly produces a monophasic response of short duration.

It is concluded that the biphasic vasoconstrictor response to 5-HT and methysergide is the result of a direct action of the agonist on the smooth muscle cells, firstly *via* the intraluminal surface and secondly, *via* the extraluminal surface. With nor-

adrenaline the extraluminal potency is low since this agent is effectively removed by the neuronal uptake process and hence the responses are normally monophasic.

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REFERENCES

ALABASTER, V. A. & BAKHLE, Y. S. (1970). *Br. J. Pharmac.*, **40**, 582P.
APPERLEY, E., HUMPHREY, P. P. A. & LEVY, G. P. (1974). *Ibid.*, **52**, 131P–132P.
APPERLEY, E., HUMPHREY, P. P. A. & LEVY, G. P. (1976). *Ibid.*, **58**, 211–221
BEVAN, J. A., GARSTKA, W., SU, C. & SU, M. O. (1973). *Eur. J. Pharmac.*, **22**, 47–53.
BEVAN, J. A. & WATERSON, J. G. (1971). *Circulation Res.*, **28**, 655–661.
BUCHAN, P., LEWIS, A. J. & SUGRUE, M. F. (1974). *Br. J. Pharmac.*, **52**, 132P–133P.
CALLINGHAM, B. A. & BURGEN, A. S. V. (1966). *Mol. Pharmac.*, **2**, 37–42.
CAMPBELL, I. K. & FARMER, J. B. (1968). *J. Pharm. Pharmac.*, **20**, 19–25.
DE LA LANDE, I. S., CANNELL, V. A. & WATERSON, J. G. (1966). *Br. J. Pharmac. Chemother.*, **28**, 255–272.
DE LA LANDE, I. S., FREWIN, D. & WATERSON, J. G. (1967). *Ibid.*, **31**, 82–93.
DE LA LANDE, I. S. & RAND, M. J. (1965). *Aust. J. exp. Biol. med. Sci.*, **43**, 639–656.
FARMER, J. B. (1966). *Br. J. Pharmac. Chemother.*, **28**, 340–347.
FOZARD, J. R. (1973). In: *Background to Migraine.* Editor: Cumings, J. N., London: Heinemann.
FOZARD, J. R. (1976). *Eur. J. Pharmac.*, **36**, 127–139.
FOZARD, J. R. & MWALUKO, G. M. P. (1975). *Br. J. Pharmac.*, **53**, 449P–450P.
GADDUM, J. H. (1955). *Pharmacology*, 4th Edition. Oxford: University Press.
IVERSEN, L. L. (1965). *Br. J. Pharmac.*, **25**, 18–33.
IVERSEN, L. L. (1971). *Ibid.*, **41**, 571–591.
IVERSEN, L. L. (1974). *Biochem. Pharmac.*, **23**, 1927–1935.
KREBS, H. A. & HENSELEIT, K. (1932). *Hoppe-Seyler's Z. physiol. Chem.*, **210**, 33–66.
THOA, N. B., ECCLESTON, D. & AXELROD, J. (1969). *J. Pharmac. exp. Ther.*, **169**, 68–73.