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Analysis of the contractile effect of 5-hydroxytryptamine on the isolated posterior communicating artery of the cat

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5-Hydroxytryptamine (5-HT) induced dose-dependent increases in tension on the isolated posterior communicating artery (PCA) of the cat were significantly antagonized by lysergic acid diethylamide (LSD, 6×10^{-9} M). In the presence of phentolamine (10^{-6} M) the contraction induced by the two lowest doses of 5-HT was significantly reduced. Pretreatment of the animals with reserpine (3 mg kg^{-1}, i.p., total dose) did not modify the dose-response curve to 5-HT except for the lowest dose. Removal of both superior cervical sympathetic ganglia 15 days before the experiment brought about a significant increase in the vasoconstriction induced by 5-HT at all the doses compared with the control. Cocaine (10^{-6} M) induced a significant shift to the left of the dose-response curve to 5-HT but the maximum response was the same as in the control. The augmented response to 5-HT after denervation was partially antagonized by LSD (6×10^{-9} M) but not by phentolamine (10^{-6} M). These results show that the vasoconstriction elicited by 5-HT in the PCA of the cat is mainly due to direct stimulation of tryptaminergic receptors. The participation of an indirect adrenergic component in the contractile effects of 5-HT seems to be negligible.

The existence of specific receptors for 5-hydroxytryptamine (5-HT) in the cerebral blood vessels has been demonstrated *in vivo* as well as *in vitro* (Nielsen & Owman 1971; Allen et al 1974a; Urquilla et al 1975; Edvinsson & Hardebo 1976; Lluch et al 1976; Edvinsson et al 1978). In addition to the direct activation of these tryptaminergic receptors there is evidence that 5-HT might have an indirect adrenergic mechanism in its action on cerebral vessels. Thus, the reduction of cerebral blood flow elicited by 5-HT in unanaesthetized goats may be partially blocked by the previous administration of the α-adrenoceptor antagonist phentolamine or by pretreatment of the animals with reserpine (Lluch et al 1976). On the other hand, the cerebral vasospasm induced in the dog by intracisternal administration of 5-HT may be reversed by phenoxybenzamine (Allen et al 1974b).

The aim of the present work was to study whether 5-HT showed an indirect sympathomimetic mechanism in its contractile effect on the isolated posterior communicating artery of the cat. To achieve this, experiments were designed to specifically antagonize 5-HT and α-adrenoceptors or to alter the sympathetic activity present in cerebral vessels.

MATERIALS AND METHODS

The drugs used were: 5-hydroxytryptamine creatinine sulphate (Sigma), (−)-noradrenaline bitartrate (Sigma), cocaine hydrochloride (Abelló), reserpine (Ciba), phentolamine methanesulphonate (Ciba), lysergic acid diethylamide (Sandoz), sodium pentobarbitone (Abbott), and atropine sulphate (Miró).

Cats of either sex, 1·5–4 kg, were anaesthetized with sodium pentobarbitone (35 mg kg^{-1} i.p.) and killed by bleeding. The brain was carefully removed and the posterior communicating cerebral arteries dissected into cylindrical segments 4 mm in length. Each arterial cylinder was set up for isometric recording in an organ bath according to Nielsen & Owman (1971). Briefly, this consists in passing two fine stainless steel pins through the lumen of the vascular segment. One pin is fixed to the organ bath wall while the other is connected to a strain gauge for isometric recording. The latter pin is parallel to the former and movable, allowing the application of resting tension at right angles to the long axis of the vascular cylinder. The recording system included a Universal Transducing Cell UC3, a Statham Micro-Scale Accessory UL5 and a Beckman Type RS recorder. A resting tension of 0·5 g was applied to the tissue and readjusted every 15 min during a 90–120 min equilibration period before cumulative dose-response curves for the different agonist were made.

The organ bath which contained 3 ml of Krebs-Henseleit solution at 37 °C was continuously bubbled with 5% CO_2 in oxygen which gave a pH

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of 7·3 to 7·4. The composition of the Krebs-Henseleit solution was (mM): NaCl, 115; KCl, 4·6; $CaCl_2$, 2·5; KH_2PO_4, 1·2; $MgSO_4 \cdot 7H_2O$, 1·2; $NaHCO_3$, 25; glucose, 11·1. Ethylenediaminetetraacetic acid (EDTA, 3×10^{-5}M) was added to prevent oxidation of unstable substances. Drugs were dissolved in physiological saline solution containing 0·01 % (w/v) ascorbic acid. When cocaine, phentolamine and lysergic acid diethylamide (LSD) were used, they were added to the bath 10 min before 5-HT and allowed to remain in contact with the tissue throughout the determination of the dose-response relationship. The dose-response curves were made in a cumulative manner and control and experimental responses were obtained from separate vascular preparations.

Reserpine (3 mg kg^{-1} i.p., total dose) was administered as follows: 2 and 1 mg kg^{-1}, 48 and 24 h respectively, before the experiment.

Cats previously injected with atropine (0·5 mg kg^{-1} i.p.) were anaesthetized with sodium pentobarbitone (35 mg kg^{-1} i.p.) and, under aseptic conditions, both superior cervical sympathetic ganglia were removed. All the experiments performed with arterial segments obtained from these animals, were carried out 14 or 16 days after surgery at a time when the adrenergic nerve endings would be expected to have degenerated (Edvinsson et al 1975; Lee et al 1976).

Statistical analysis was by means of Student's *t*-test; a probability value of less than 5% was considered significant (Snedecor 1956).

RESULTS

5-HT elicited dose-dependent contractile responses in the posterior communicating artery of the cat which were significantly reduced ($P < 0{\cdot}025$ or less) in the presence of 6×10^{-9}M LSD at all the doses used (Fig. 1).

Phentolamine (10^{-6}M) blocked significantly the vasoconstriction induced by 5-HT only at the two first doses (Fig. 2A). This concentration of phentolamine was able to produce a significant 46-fold shift of the dose-response curve to noradrenaline to the right (results not shown). On the other hand, pretreatment of the animals with reserpine decreased contractile response to 5-HT only at the lowest dose (Fig. 2B).

When cocaine 10^{-6}M was added to the bath, the dose-response relationship was shifted to the left but the maximum response was not significantly different from the control (Fig. 3A). A potentiation was also found at all the doses tested ($P < 0{\cdot}05$ or less) in the dose-response curves to 5-HT performed in the arterial segments from animals in which both superior cervical sympathetic ganglia had been removed 15 days before the experiment (Fig. 3B).

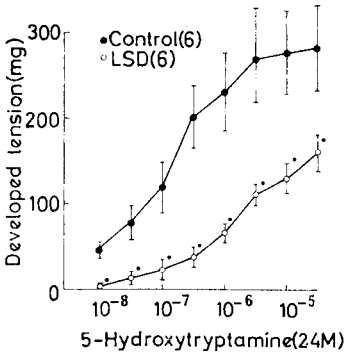

FIG. 1. Effect of 6×10^{-9} M LSD on the dose-response curve to 5-HT. Figures in parentheses indicate the number of arterial segments used. Each point represents the mean ± s.e.m. Dots denote statistically significant differences.

To elucidate whether the potentiation of the 5-HT contraction obtained in denervated vascular segments implied activation of α-adrenoceptors or tryptaminergic receptors, dose-response curves to 5-HT in denervated segments were carried out in the presence of phentolamine or LSD. Fig. 4 shows that phentolamine 10^{-6}M did not affect the response of 5-HT whereas LSD 6×10^{-9}M reduced significantly the contractile effects of 5-HT in denervated segments.

DISCUSSION

The release of noradrenaline has been involved in the mechanism of the contractile action of 5-HT on the

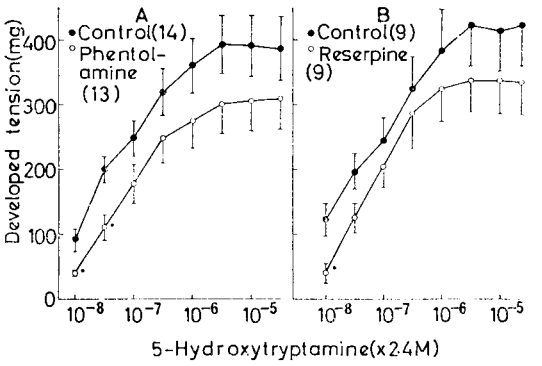

FIG. 2. A: Effect of 10^{-6} M phentolamine on the dose-response curve to 5-HT. B: Effect of the pretreatment with reserpine on the dose-response curve to 5-HT. Figures in parentheses indicate the number of arterial segments used. Each point represents the mean ± s.e.m. Dots denote statistically significant differences.

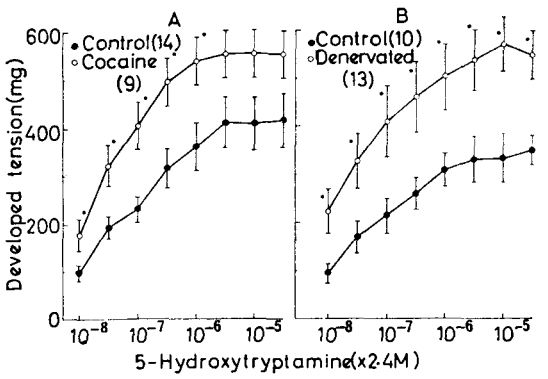

FIG. 3. A: Effect of 10^{-6} M cocaine on the dose-response curves to 5-HT. B: Effect of the removal of both superior cervical sympathetic ganglia on the dose-response to 5-HT. Figures in parentheses indicate the number of arterial segments used. Each point represents the mean ± s.e.m. Dots denote statistically significant differences.

nictitating membrane and splenic strips of the cat (Pluchino 1972), on dog and rabbit hearts (Fillion et al 1971; Fozard & Mwaluko 1976), and vascular smooth muscle (McGrath 1977). Indirect evidence of these effects is also seen in experiments with unanaesthetized goats showing that the decrease in cerebral blood flow due to 5-HT is partially abolished by phentolamine or reserpinization (Lluch et al 1976). The results reported here suggest that an adrenergic component is of minor importance in the contractile response to 5-HT of the posterior communicating artery of the cat, since the increases in tension developed by 5-HT were only slightly reduced by phentolamine—at a dose that clearly antagonized the effect of noradrenaline—and by pretreating the animals with reserpine.

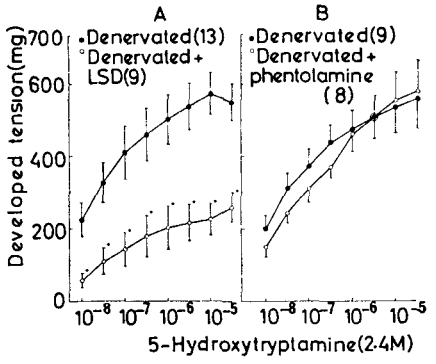

FIG. 4. A: Effect of 6×10^{-9} M LSD on the dose-response curve to 5-HT performed in cerebral arteries from denervated animals. B: Effect of 10^{-6} M phentolamine on the dose-response curve to 5-HT performed in cerebral arteries from denervated animals. Figures in parentheses indicate the number of arterial segments used. Each point represents the mean ± s.e.m. Dots denote statistically significant differences.

If an indirect sympathomimetic action contributes to the vasoconstriction induced by 5-HT, it would be expected that denervation would result in a decreased contractile response to 5-HT. However, in the present study 5-HT produced augmented responses in the denervated arteries. We, therefore, conclude that it does not produce contraction via an indirect sympathetic mechanism under the present experimental conditions. The supersensitivity to 5-HT obtained in denervated segments does not seem to involve α-adrenoceptors, since in the presence of phentolamine the response was unaffected; it more than likely implicates activation of tryptaminergic receptors due to the finding that LSD was able to decrease this response. Both cocaine and denervation made the vessels supersensitive to the contractile effects of 5-HT, which is consistent with findings in other preparations (Wakade et al 1970). We cannot exclude the possibility that this potentiated response might also result from the abolition of the neuronal uptake of 5-HT after denervation or cocaine treatment.

The virtual absence of an adrenergic component in the contractile effect of 5-HT might be due either to a very poor ability of 5-HT to displace noradrenaline from its synaptic stores or to the predominance the direct activation of 5-hydroxytryptaminergic receptors over the indirect adrenergic component, taking into account the poor response that noradrenaline can elicit in cerebral blood vessels compared with 5-HT (Nielsen & Owman 1971; Toda & Fujita 1973; Urquilla et al 1975).

The present results differ from those obtained in unanaesthetized goats, where an important indirect adrenergic component seems to be acting in the marked reduction of cerebral blood flow induced by 5-HT (Lluch et al 1976). The reasons for this difference have not been examined but it probably involves differences in the density of adrenergic innervation in the cerebral arteries of the two species as inferred from their respective noradrenaline content (1·5 $\mu g\ g^{-1}$ in the cat vs 2·10 $\mu g\ g^{-1}$ in the goat, Edvinsson et al 1972; Urquilla et al 1974). It should also be borne in mind that in the experiments using unanaesthetized animals multiple factors are implicated in the response of cerebral vessels leading to conditions far removed from the situation present in isolated cerebral arteries.

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