J. Pharm. Pharmacol. 1981, 33: 715–719
Received February 10, 1981

0022–3573/81/110715–05 $02.50/0

Noradrenergic component in the vasoconstriction induced by 5-hydroxytryptamine in goat cerebral arteries

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5-Hydroxytryptamine (5-HT) elicited dose-dependent increases in tension in the middle cerebral artery of the goat, which were significantly antagonized by lysergic acid diethylamide (LSD, 10^{-8} M) and methysergide (10^{-7} M). In the presence of phentolamine (10^{-6} M), the dose-response curve to 5-HT was shifted to the right, the pA_2 value for this antagonism was 6·52. Pretreatment of goats with reserpine (0·02 mg kg^{-1} day^{-1} for three days) or removal of both superior cervical ganglia 15 days before the experiment brought about a significant decrease in the vasoconstriction induced by doses of 5-HT higher than 10^{-7} M. The remaining contraction produced by 5-HT in arterial segments from reserpinized or gangliectomized goats was further reduced in the presence of LSD. In addition, high concentrations of 5-HT induced tritium release from goat pial arteries preloaded with (−)-[^{3}H]noradrenaline, 2×10^{-7} M) which was significantly decreased in vessels from gangliectomized or reserpinized goats. These results in goat cerebral arteries indicate that in the contraction evoked by 5-HT there are two components. The first appears with low concentrations (up to 10^{-7} M) in which 5-HT acts directly on 5-HT receptors. The second occurs at high doses (>10^{-7} M) in which 5-HT also acts indirectly on α-adrenoceptors by release of noradrenaline from noradrenergic nerve endings.

5-Hydroxytryptamine (5-HT) has been identified in vascular tissue including cerebral blood vessels (Berkowitz et al 1975; Jarrot et al 1975; Moskowitz et al 1978; Reinhard et al 1979) and possibly is implicated in the maintenance of vascular tone. In addition, 5-HT is a potent vasoconstrictor agent of brain arteries that appears to play an importnt role in the vasospasm occurring in these vessels after subarachnoid haemorrhage (Allen et al 1974; Lobato et al 1980). In vitro studies have shown that this vasoconstriction is mediated by tryptamine receptors since it is blocked by the 5-HT antagonists methysergide and lysergic acid diethylamide (LSD) (Nielsen & Owman 1971; Allen et al 1974; Urquilla et al 1975; Edvinsson et al 1978; Marín et al 1979a). Besides its action on tryptamine receptors, 5-HT has the ability to release noradrenaline from several tissues (Innes 1962; Fillion et al 1971; Pluchino 1972; Fozard & Mwaluko 1976; Marín et al 1979b). Furthermore, its direct administration to cerebral circulation of the goat produces a marked reduction of cerebral blood flow which is partly inhibited by reserpine or phentolamine (Lluch et al 1976). To extend these in vivo observations we have investigated the existence of a direct action of 5-HT on tryptamine receptors and its ability to release noradrenaline from the adrenergic nerve endings in goat middle cerebral artery. The contribution of both effects to the overall contraction induced by 5-HT was also examined. To achieve this, experiments were designed to specifically antagonize 5-HT receptors or to alter the potential for sympathetic activity in cerebral vessels.

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MATERIAL AND METHODS

Female goats (30–45 kg) were killed by injecting intravenously (i.v.) 30 ml of saturated solution of potassium chloride. The brain was removed and the middle cerebral arteries were dissected free and cut into cylindrical segments 4 mm in length. Each segment was prepared for isometric recording in an organ bath according to Edvinsson et al (1974). The bath contained 6 ml of Krebs-Henseleit solution (KHS) continuously bubbled with 95% O_2 and 5% CO_2 to give a pH of 7·3–7·4. Two stainless steel pins, 150 μl diameter, were introduced through the lumen of the segment. One pin was fixed to the bath wall while the other was connected to a strain gauge for isometric tension recording, the latter pin being in parallel position with the former and movable, thus permitting the application of resting tension in a plane perpendicular 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.

The composition of the KHS 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. Disodium salt of ethylene-diamine tetraacetic acid (Na_2EDTA, 3×10^{-5} M) was added to prevent oxidation of unstable substances. A resting tension of 1 g was applied to the tissue and readjusted every 15 min during 60 to 90 min of equilibration before cumulative dose-response curves for the agonist were made. Bath fluid was changed every 30 min.

Drugs were dissolved in 0·9% (w/v) NaCl containing 0·01% (w/v) ascorbic acid. When phentolamine, LSD and methysergide 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. Control and experimental responses were obtained from separate vascular preparations.

In 8 goats anaesthetized with 2% sodium thiopentone administered intravenously, both superior cervical ganglia were exposed, isolated and removed under sterile conditions. These animals were killed 15 days postoperatively and tissues prepared as in the unoperated animals.

In 6 goats, reserpine was administered intravenously in a dose of 0·02 mg kg^{-1} day^{-1} for 3 days before they were killed.

Efflux of radioactivity. The arteries of the circle of Willis and their branches were removed and placed in a Petri dish, which contained ice-cold KHS. In this medium the blood was removed from arteries which were pooled. Each pool of arteries (40–50 mg) was placed in a cylindrical nylon net and after a 15 min equilibration at 37 °C, the tissues were exposed to (−)[^{3}H]noradrenaline ([^{3}H]NA, 2×10^{-7} M, specific activity 9·1 Ci $mmol^{-1}$, Radiochemical Centre, Amersham, England) for 30 min and thoroughly washed with fresh KHS, at 10 min intervals during 100 min. To estimate the spontaneous tritium release, the arteries were successively immersed in 5 vials containing 2 ml of fresh KHS for 3 min. The drug-evoked release was analysed by transfering the tissue to another 4 vials, each one containing 2 ml of KHS with 10^{-5} or 10^{-4} M 5-HT; finally the arteries were again exposed to fresh KHS in another 5 vials in order to recover the basal level of tritium efflux. Total radioactivity present in the media was analysed by adding 0·5 ml of each sample to 10 ml of Bray's solution (Bray 1960) and it was measured in a Nuclear Chicago liquid scintillation counter, model Isocap 300. The pre-drug efflux (at 112 min) was given the value 1 and expressed as counts min^{-1} mg^{-1} of wet tissue. At the end of the experiment the arteries were blotted, weighed, homogenized in 1 ml of 0·4 M perchloric acid, centrifuged for 15 min at 2000 *g*, and an aliquot (0·1 ml) of the supernatant was measured as previously described for the media. The results were expressed as counts min^{-1} mg^{-1}.

Drugs used were: phentolamine methanesulfonate (Ciba), reserpine (Ciba), 5-hydroxytryptamine creatinine sulphate (Sigma), lysergic acid diethylamide and methysergide bimaleate (Sandoz).

The pA_2 value for phentolamine against 5-HT was determined from the Kb (apparent dissociation constant), since the negative logarithm of Kb is considered to be equivalent to pA_2. The Kb was calculated by the quotient of the concentration of antagonist and dose-ratio-1 (Gaddum 1957; Arunlakshana & Schild 1959). Statistical analysis for unpaired preparations was done by means of Student's *t*-test; a probability value of less than 5% was considered significant.

RESULTS

5-HT induced dose-dependent contractile responses in the middle cerebral artery of the goat which were significantly ($P < 0.025$) reduced in the presence of 10^{-8} M LSD at all doses used (Fig. 1A). The contractile effects of 5-HT were equally reduced by 10^{-7} M methysergide (results not shown). Phentolamine (10^{-6} M), an α-adrenoceptor blocking agent, shifted the dose-response curve to the right (Fig. 1B) and the pA_2 value for this antagonism was 6·52.

Fig. 2 shows the dose-response curve for 5-HT determined in vascular segments from goats treated with reserpine (A) and goats in which both cervical ganglia had been removed 15 days before the experiment (B). The magnitude of the contractile response in both cases was significantly reduced ($P < 0.05$) compared with control when doses of

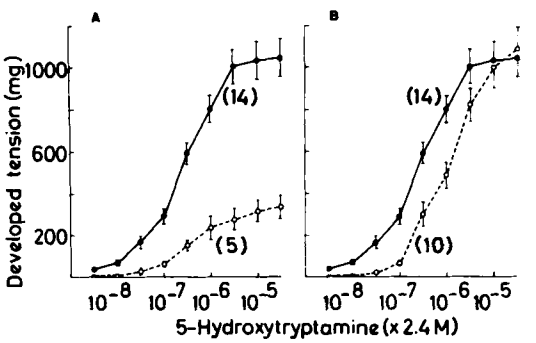

FIG. 1. Effect of 10^{-8} M LSD (A) and 10^{-6} M phentolamine (B) on the dose-response curve to 5-HT. Figures in parentheses indicate the number of arterial segments used. Each point represents the mean ± s.e.m. ●, control; ○, drug.

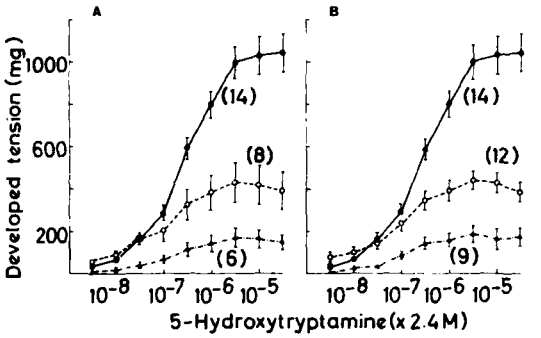

FIG. 2. Effect of pretreatment with reserpine (A) ●, control; ○, reserpine; ▲, reserpine + LSD; and removal of both superior cervical ganglia (B), ●, control; ○, denervated; ▲, denervated + LSD, on the dose-response curve to 5-HT in the absence or in the presence of 10^{-8} M LSD.

5-HT higher than 10^{-7} M were used. However, in the arterial segments from both ganglionectomized and reserpinized goats a significant amount of contraction in response to 5-HT was still evident. To investigate the possibility that this remaining contraction implied activation of tryptaminergic receptors, dose-response curves to 5-HT in segments from ganglionectomized or reserpinized animals were carried out in the presence of LSD. Fig. 2 shows that 10^{-8} M LSD reduced the 5-HT-induced contraction to a level similar to that observed in segments treated only with LSD.

The spontaneous tritium efflux from goat pial arteries preloaded with [^{3}H]NA showed a rapid initial fall which then became slower and levelled off after 90 min of washout. 5-HT (10^{-5} or 10^{-4} M) increased the radioactivity release from these arteries. The peak of this release was reached in the first 6 min of incubation (Fig. 3). The peak of release evoked by 10^{-4} M 5-HT was approximately double than that obtained by 10^{-5} M 5-HT. After denervation or pretreatment with reserpine, the efflux of radioactivity induced by 10^{-4} M 5-HT was considerably diminished ($P < 0.001$). In addition, the basal level of tritium efflux (i.e. pre-drug efflux) of vessels from control animals was 280 ± 40 counts min^{-1} mg^{-1} and diminished significantly ($P < 0.05$) in vessels from ganglionectomized (190 ± 20 counts min^{-1} mg^{-1}) and reserpinized (155 ± 15 counts min^{-1} mg^{-1}) animals. The amount of tritium retained by these tissues was measured at the end of the experiment. There was a significant difference between the radioactivity present in the arteries of normal goats (9·650 ± 840 counts min^{-1} mg) and that present in denervated (3·040 ± 710 counts min^{-1} mg^{-1}) ($P < 0.0001$) and reserpinized (1·820 ± 140 counts min^{-1} mg^{-1}) ($P < 0.001$) goats.

DISCUSSION

The present experiments show that 5-HT induces potent vasoconstrictor responses in goat middle cerebral arteries which can be potently inhibited by LSD and methysergide thus suggesting that these effects are mediated by direct interaction with 5-HT receptors. These results agree with those obtained by others in brain vessels of various animal species (Toda & Fujita 1973; Allen et al 1974; Urquilla et al 1975; Edvinsson et al 1978; Marín et al 1979a). If an indirect sympathomimetic action contributes to the vasoconstriction induced by 5-HT, it would be expected that denervation or reserpine pretreatment would result in a decreased response to 5-HT since both these procedures reduce the noradrenaline content of cerebral vessels to undetectable levels (Urquilla et al 1974; Conde et al 1978; Marín et al 1980) and inhibit the cerebral vasoconstriction induced by tyramine or sympathetic nerve stimulation (Lluch et al 1975; Alborch et al 1977; Conde et al 1978). This was the case in the present experiments in as much as contraction was significantly reduced in arterial segments from reserpinized or ganglionectomized animals for doses of 5-HT higher than 10^{-7} M. These results suggest that part of the cerebral vasoconstriction induced by this amine is due to the noradrenaline released by 5-HT from perivascular adrenergic nerve endings. This noradrenaline would in turn activate α-adrenoceptors as can be inferred

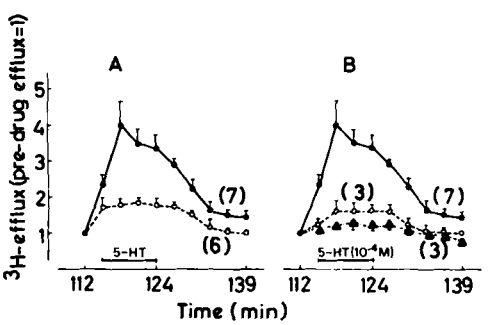

FIG. 3. Tritium efflux induced by (A) 5-HT, 10^{-5}, (○) or 10^{-4} M (●) in cerebral arteries (B) from control (●), reserpinized (▲) and ganglionectomized (○) goats. Tissues were previously labelled with [^{3}H]NA (2×10^{-7} M) and thoroughly washed during a 100 min before the initiation of sample collection. Vials containing 2 ml of bathing solution were collected every 3 min. Each point represents the tritium efflux during a period of 3 min. The pre-drug (basal level) was taken as 1 in all the treatments and the release evoked by 5-HT was calculated with respect to this value. Number of experiments are shown in parentheses. Vertical bars represents s.e.m. Horizontal lines indicate the time of exposure to 5-HT.

from the reduced contractily observed after phentolamine treatment. Similar results have also been described in the dog saphenous vein by Humphrey (1978), although the indirect sympathomimetic effect of 5-HT occurred from about 10^{-6} M. This small discrepancy might be due to differences both in tissues and in animal species used in each case.

The pA_2 value for the antagonism 5-HT-phentolamine was 6·52, i.e. a little greater than that obtained in rabbit aorta (6·21; Apperley et al 1976) and in dog saphenous vein (6·11; Humphrey 1978).

The ability of 5-HT to release noradrenaline from perivascular adrenergic nerve endings was directly demonstrated in arteries preincubated with [^{3}N]NA. In these vessels 5-HT induced tritium release which was markedly reduced in reserpinized and ganglionectomized goats (Fig. 3). Furthermore, both reserpine and denervation reduced the basal level of radioactivity (pre-drug efflux) and the amount of tritium retained by these tissues at the end of the experiment. These results strongly support the view that contraction of cerebral arteries in response to high doses of 5-HT is mediated, to a great extent, through an adrenergic mechanism. The ability of 5-HT to induce adrenergic neurotransmitter release has also been observed in several tissues (Innes 1962; Fillion et al 1971; Fozard & Mwaluko 1976; Marín et al 1979b).

The mechanism involved in noradrenaline secretion induced by 5-HT has been demonstrated to be a Ca^{2+}-dependent process on the rabbit heart, which implies a depolarization of adrenergic nerve endings (Fozard & Mwaluko 1976). Recently, Marín & Sánchez (1980) observed in goat cerebral arteries that the noradrenaline release by 5-HT is a partially Ca^{2+}-dependent process, whereas for tyramine and potassium chloride is Ca^{2+}-independent and Ca^{2+}-dependent, respectively. These results suggest that 5-HT evokes release from these arteries by tyramine-like (stoichiometric displacement mediated via uptake of 5-HT by the noradrenergic $Uptake_1$), and potassium-like mechanisms (by depolarization of perivascular adrenergic endings).

Recently, Marín et al (1979a) have reported that an adrenergic component is of minor importance in the contractile response to 5-HT of the cerebral arteries of the cat, since the increase of tension developed by 5-HT was only slightly reduced by phentolamine or reserpine. This might be due either to a poor ability of 5-HT to displace noradrenaline from the cerebral arteries of the cat or to the predominance of the direct activation of 5-HT receptors over the indirect adrenergic component. The reasons for this difference have not been thoroughly examined but it probably involves unequal density of adrenergic innervation in the cerebral arteries of the two animal species as inferred from their respective noradrenaline content and DBH activity. Both parameters in arteries of the goat are double the value of those in the cat (Marín et al 1980).

In conclusion, the fact that the tritium release by 5-HT occurred at high doses of this amine and the contraction induced by 5-HT was reduced in vessels from ganglionectomized and reserpinized animals seems to indicate that in this contraction there are two components. The first occurring with low concentrations of 5-HT (up to 10^{-7} M) in which 5-HT acts directly on 5-HT receptors, the second at high doses ($>10^{-7}$ M) in which 5-HT releases noradrenaline from adrenergic nerve endings.

Acknowledgements

This work was supported in part by Ministerio de Sanidad and Comisión Asesora de Ivestigación Científica y Técnica.

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