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Receptors Involved in the Modulation of 5-Hydroxytryptamine Release in Bovine Cerebral Arteries

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Abstract—The uptake of [^{3}H]5-hydroxytryptamine (5-HT) in bovine cerebral arteries was reduced by cocaine (1 μM), ouabain (100 μM), pretreatment with 6-hydroxydopamine (6-OHDA) (1·46 mM, 10 min) and metitepine (1 μM). Electrically-stimulated tritium release was decreased by tetrodotoxin (0·8 μM), Ca-free medium, denervation with 6-OHDA (1·46 mM, 10 min), 5-HT (10 μM), noradrenaline (1 μM) and the agonist of α_2-adrenoceptors B-HT 920 (0·1 and 1 μM), enhanced by metitepine (1 μM, antagonists of presynaptic 5-HT_1 receptors) and rauwolscine (1 μM, antagonist at α_2-adrenoceptors, and also of 5-HT_{1D} receptors) and not affected by ketanserin (1 μM, antagonist of 5-HT_2 receptors), methysergide (0·1 μM, antagonist of 5-HT_1 and 5-HT_2 receptors) and phentolamine (1 and 3 μM antagonist of α-adrenoceptors and less potent of 5-HT_1 receptors). The inhibitory action of 10 μM 5-HT was partially reversed by phentolamine (3 μM) and cocaine (1 μM) and completely reversed by both metitepine (1 μM) and rauwolscine (1 μM). Ketanserin (1 μM), methysergide (0·1 μM) or phentolamine (1 μM) had no effect. Rauwolscine (1 μM) antagonized the inhibition induced by both noradrenaline (1 μM) and B-HT 920 (0·1 and 1 μM). 5-HT induced tritium release which was inhibited by cocaine (an antagonist of 5-HT_3 receptors) and denervation with 6-OHDA. These results suggest that 5-HT is mainly accumulated in adrenergic nerve endings, that evoked [^{3}H]5-HT release is modulated by 5-HT_1-like receptors, but the participation of α_2-adrenoceptors cannot be discounted, or more probably both types of receptors have features in common, and evoked [^{3}H]5-HT release elicited by 5-HT may be partially mediated by activation of 5-HT_3 receptors.

Cerebral vessels have adrenergic (Nelson & Rennels 1970; Edvinsson & MacKenzie 1977; Marín et al 1980a, b) and 5-hydroxytryptaminergic (Chan-Palay 1976; Edvinsson et al 1984; Marco et al 1985; Scatton et al 1985; Moreno et al 1991) innervations, originated in the sympathetic superior cervical ganglia and the raphe nuclei of the brain stem, respectively. The existence of the latter innervation has been a matter of controversy and the presence of 5-hydroxytryptamine (5-HT) in these cerebral vessels has been demonstrated to be localized in perivascular adrenergic nerves (Jackowski et al 1988). This amine, in contrast to noradrenaline, produces powerful vasoconstrictor responses in cerebral arteries (Marín et al 1979; Edvinsson et al 1985; Van Nueten et al 1985). Furthermore, 5-HT released from platelets has been implicated in vasospasm following subarachnoid haemorrhage (Allen et al 1976).

The ability of 5-HT to be taken up by adrenergic nerve terminals of different vessels has been previously reported (Verbeuren et al 1983; Kawasaki & Takasaki 1984; Scatton et al 1985; Levitt & Duckles 1986; Barrús et al 1990). The release of this accumulated 5-HT is modulated by presynaptic 5-HT receptors (Feniuk et al 1979; Schlicker & Göthert 1987) and α_2-adrenoceptors (Kawasaki & Takasaki 1986).

The objective of this study was to assess in bovine cerebral arteries if 5-HT is accumulated in adrenergic or 5-HT-ergic nerve endings, and if its release is modulated by presynaptic 5-HT receptors or α_2-adrenoceptors.

Materials and Methods

Experimental design and superfusion studies

Bovine brains were obtained from a slaughterhouse and transported to the laboratory in Krebs–Henseleit solution (KHS) at 4°C. In this medium, branches of middle cerebral arteries were isolated, pooled and carefully cleaned of traces of blood and adherent tissues. They were set up in a nylon net and immersed for 30 min in 10 mL of KHS at 37°C, continuously gassed with 95% O_2–5% CO_2 (stabilization period). Subsequently, they were incubated for 60 min in 1 mL oxygenated KHS at 37°C containing [^{3}H]5-HT (1 μCi mL^{-1} 38 nM, sp. act. 26·7 Ci $mmol^{-1}$). Afterwards, the arteries were transferred into a superfusion chamber with two parallel platinum electrodes, 0·5 cm apart, connected to a stimulator (Cibertec model CS9, modified to supply the adequate current strength) for electrical field stimulation (200 mA, 0·3 ms, 2 Hz). The arteries were superfused at a rate of 2 mL min^{-1} with KHS at 37°C for 100 min (washout period). During this time the basal tritium efflux reached steady-state. Then, the superfusate was collected in vials (10 in total) at 30 s intervals. Two superfusate samples were collected before stimulation, to determine the basal tritium efflux, 2 were collected during and 6 after the stimulation; the latter ones were sufficient for recovering basal levels of tritium secretion. Two electrical stimulation periods of 75 s (S_1 and S_2) were applied to the arteries separated by a 30 min interval. Drugs used to interfere with the stimulated tritium release were added 20 min before S_2.

The effect of 5-HT (10 and 100 μM) and noradrenaline (0·1, 1 and 10 μM) on basal tritium release was also analysed. For this purpose, after washout, either 5-HT or noradrenaline was added to the superfusion medium for 10 min, collecting the superfusate in vials at 2 min intervals from 2 min before (basal tritium efflux) until 4 min after (to recover basal radioactivity release) the addition of drugs.

The action of 1 μM cocaine, 100 μM ouabain and 1 μM metitepine on [^{3}H]5-HT uptake was assessed. The drugs were

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added to the bath 20 min before incubation with [^{3}H]5-HT. The arteries were then washed for 100 min and the radioactivity retained was measured. The effect of denervation with 6-hydroxydopamine (6-OHDA) on this uptake was similarly analysed. To analyse the effect of cocaine on [^{3}H]5-HT release, it was added to the superfusion medium from the beginning of the washout period until the end of the experiment.

The radioactivity present in the superfusate was measured by scintillation counting (Beckman LS 28000) by the addition of Ready Protein (Beckman). To measure radioactivity accumulated in the arteries, the vessels were first blotted, weighed and digested in 1 mL H_2O_2 (30% w/v) at 100°C for 5 h.

The stimulation-evoked tritium efflux was calculated by subtracting the basal tritium release from that elicited by electrical stimulation. In order to eliminate differences among the arteries, the ratios of the net tritium efflux between S_2 and S_1 were calculated. The effects of drugs on the stimulated efflux were referred to by their actions on these ratios. Tritium release induced by noradrenaline or 5-HT was expressed as a percentage of basal release and the radioactivity accumulated in the arteries in d min^{-1} mg^{-1}.

Denervation with 6-OHDA

The method used to produce in-vitro chemical denervation of sympathetic nerves of cerebral arteries was that described by Aprigliano & Hermsmeyer (1976), with the modifications of Levitt & Duckles (1986). Briefly, the arteries were incubated at room temperature (21°C) for 10 min in KHS ($NaHCO_3$ and NaH_2PO_4 were omitted, unbuffered solution) containing 0·02 mM glutathione and 1·46 mM 6-OHDA. The pH of this solution was adjusted to 4·9 with 0·05 M NaOH, and then covered with paraffin oil; control vessels were submitted to the same conditions, except for 6-OHDA. Subsequently, the arteries were immersed in normal KHS.

Solutions, drugs and statistical evaluation

The composition of KHS (mM) was as follows: NaCl 115, $CaCl_2$ 2·5, KCl 4·6, NaH_2PO_4 1·2, $MgSO_47H_2O$ 1·2, $NaHCO_3$ 25, glucose 11·1, Na_2EDTA 0·03 (to prevent the oxidation of unstable substances). To analyse the effect of extracellular Ca^{2+} on tritium secretion caused by electrical stimulation, the arteries were immersed in Ca^{2+}-KHS 20 min before S_2. The composition of the latter solution was equal to KHS, except that $CaCl_2$ was omitted and 1 mM ethyleneglycol-bis (β-aminoethyl-ether) *N,N'*-tetraacetic acid (EGTA) was added.

Statistics

Results are given as mean ± s.e.m. Statistical analysis was by the Student's *t*-test for unpaired experiments; a probability value of $<0·05$ was considered significant.

Drugs

The drugs used were: [^{3}H]5-hydroxytryptamine creatinine sulphate (New England Nuclear, USA), tetrodotoxin, 5-hydroxytryptamine creatinine sulphate, (−)-noradrenaline bitartrate and 6-hydroxydopamine (Sigma, USA), phentolamine methanesulphonate (Geigy, Switzerland), rauwolscine hydrochloride (Carl Roth KG, Germany), cocaine hydrochloride (depósito de estupefacientes, Ministerio de Sanidad y Consumo, Spain), B-HT 920 (Boehringer Ingelheim, Germany), methysergide bimaleate (Sandoz, Switzerland), metitepine maleate (Hoffman-La Roche, Switzerland), ketanserin tartrate (Janssen, Belgium). All drugs were dissolved in distilled water, except noradrenaline and 5-HT, which were made in a saline (0·9% NaCl)-ascorbic acid (0·1% w/v) solution to prevent oxidation. The different concentrations of drugs used were obtained from stock solutions (10 mM) which were kept at −20°C.

Results

Cocaine (1 μM), ouabain (100 μM), pretreatment with 6-OHDA (1·46 mM, 10 min) and metitepine (1 μM) reduced [^{3}H]5-HT uptake in bovine cerebral arteries (Fig. 1a).

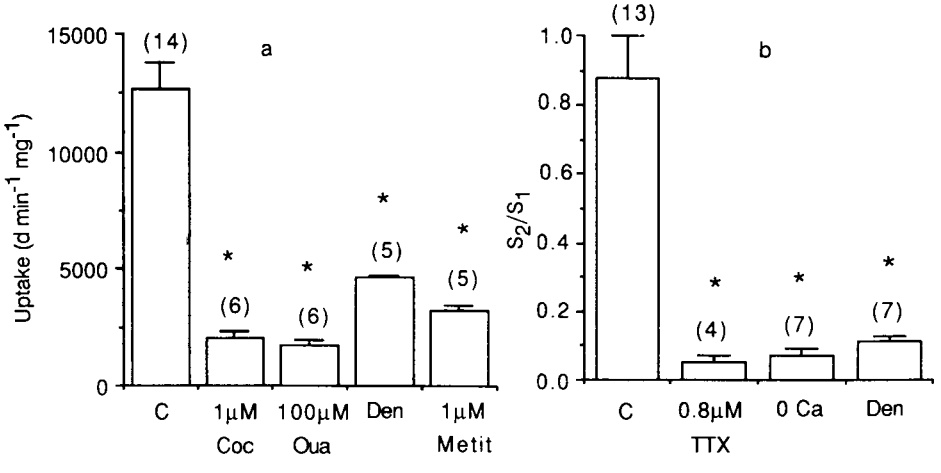

FIG. 1. (a) Effect of cocaine (Coc), ouabain (Oua), denervation (Den) with 6-OHDA and metitepine (Metit) on the uptake of [^{3}H]5-HT in bovine cerebral arteries. The vessels were pretreated with the drugs 20 min before incubation with [^{3}H]5-HT and then washed with KHS for 100 min, and the radioactivity retained measured. (b) Effect of tetrodotoxin (TTX), Ca^{2+}-free medium and denervation with 6-OHDA on electrically (200 mA, 0·3 ms, 2 Hz, for 75 s)-induced tritium secretion from bovine cerebral arteries preincubated with [^{3}H]5-HT. The ratios of tritium release by the stimulation periods S_2 and S_1 separated by a 30 min interval are shown in ordinate. Columns and vertical bars represent means ± s.e.m. Number of experiments is shown in parentheses. C = control, * $P<0·001$.

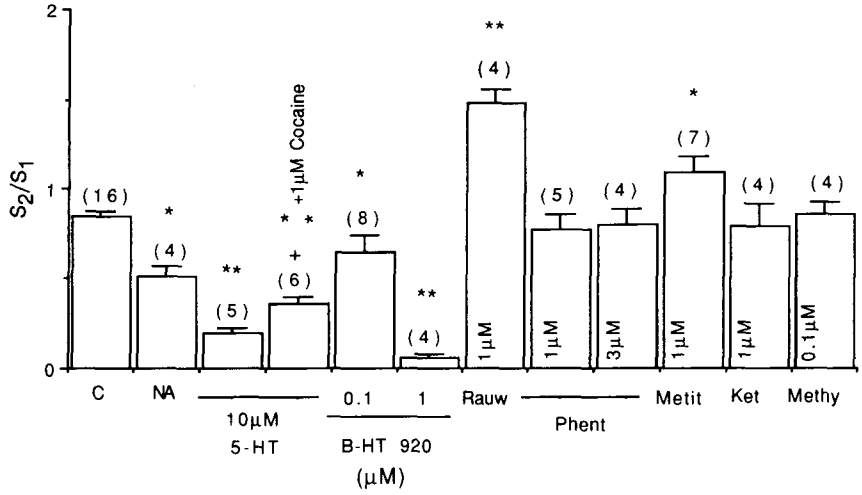

FIG. 2. Effect of noradrenaline (NA), 5-hydroxytryptamine (5-HT, with or without cocaine), B-HT 920, rauwolscine (Rauw), phentolamine (Phent), metitepine (Metit), ketanserin (Ket) and methysergide (Methy) on the electrically-induced tritium release from bovine cerebral arteries preincubated with [^{3}H]5-HT. The ratios of tritium release by the stimulation periods S_2 and S_1 separated by a 30 min interval are shown in ordinate. Columns and vertical bars represent means ± s.e.m. Number of experiments is shown in parentheses. C = control, * $P<0.05$, ** $P<0.005$, with respect to control; $^+$ $P<0.05$ with respect to 10 μM 5-HT.

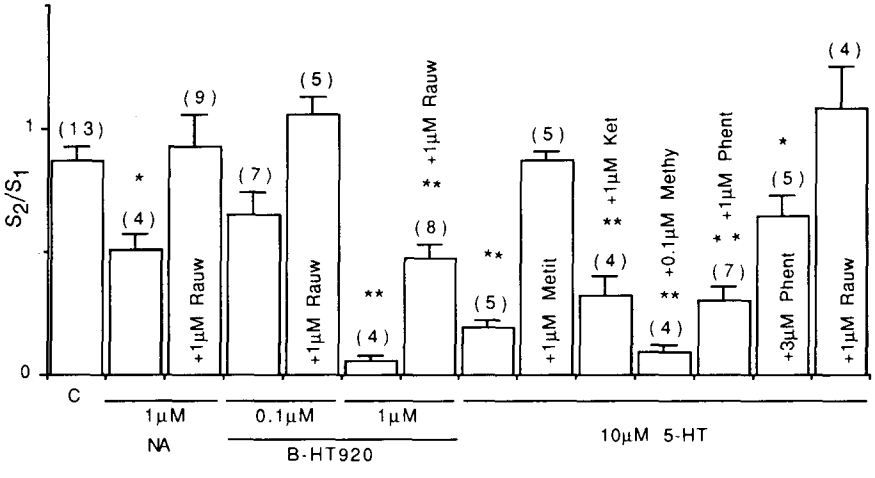

FIG. 3. Antagonism of the inhibitory effects of noradrenaline (NA), and B-HT 920 by rauwolscine (Rauw), and those of 5-hydroxytryptamine (5-HT) by rauwolscine, metitepine (Metit), ketanserin (Ket), methysergide (Methy) or phentolamine (Phent) on the electrically-induced tritium release from bovine cerebral arteries preincubated with [^{3}H]5-HT. The ratios of tritium release by the stimulation periods S_2 and S_1 separated by a 30 min interval are shown in ordinate. Columns and vertical bars represent means ± s.e.m. Number of experiments is shown in parentheses. C = control, * $P<0.05$, ** $P<0.005$.

Electrically-induced tritium release was reduced by tetrodotoxin (0·8 μM), Ca^{2+}-free medium and denervation with 6-OHDA in arteries preincubated with [^{3}H]5-HT (Fig. 1b). 5-HT (10 μM), noradrenaline (1 μM) and B-HT 920 (0·1 and 1 μM) also reduced the evoked tritium release, whereas it was increased by metitepine (1 μM) and rauwolscine (1 μM). Ketanserin (1 μM), methysergide (0·1 μM) and phentolamine (1 and 3 μM) had no effect. The addition of cocaine (1 μM) to the superfusion medium reduces the action of 10 μM 5-HT (Fig. 2). The inhibitory effect induced by both noradrenaline (1 μM) and B-HT 920 (0·1 and 1 μM) was reversed by rauwolscine (1 μM). The action of 10 μM 5-HT was completely antagonized by both metitepine (1 μM) and rauwolscine (1 μM) but only partially by phentolamine (3 μM). Ketanserin (1 μM), methysergide (0·1 μM) and phentolamine (1 μM) failed to alter the effect of 5-HT (Fig. 3).

5-HT (10 and 100 μM) and noradrenaline (0·1–10 μM) evoked concentration-dependent tritium release, which was reduced by chemical denervation with 6-OHDA (Fig. 4).

5-HT (10 μM), noradrenaline (1 μM), metitepine (1 μM) and ketanserin (1 μM) increased the basal tritium release. The increase in the basal release caused by 5-HT was reduced by cocaine (1 μM), but not affected by metitepine or methysergide (Table 1).

Discussion

The results obtained in this study show that [^{3}H]5-HT is mainly stored in noradrenergic nerve endings of bovine cerebral arteries. This is supported by the finding that cocaine, a drug that blocks the uptake of noradrenaline and 5-HT into the nerves (Henseling et al 1976; Paiva et al 1984),

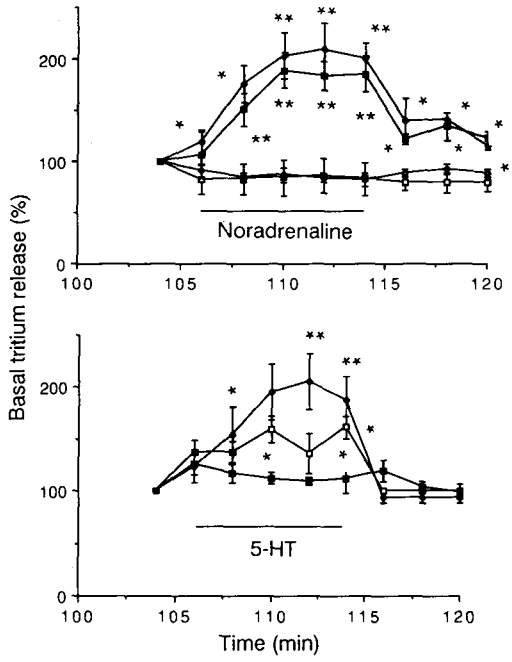

FIG. 4. Time-course of the effects of several concentrations of noradrenaline and 5-hydroxytryptamine (5-HT) on the basal tritium release in control or denervated (with 6-OHDA) bovine cerebral arteries preincubated with [^{3}H]5-HT. Horizontal bars represent the time of superfusion with these drugs. Results (means ± s.e.m.) were expressed in % of basal release (100% = 86 ± 3 d min^{-1} mg^{-1}). Number of experiments is shown in parentheses. $*P < 0.05$, $**P < 0.005$, with respect to tritium release obtained in denervated arteries. Noradrenaline: □ 0·1 (5), ◆ 1 (4), ■ 10 (4) and ◇ 10 μM denervated (4). 5-HT: □ 10 (4), ◆ 100 (4) and ■ 100 μM denervated (4).

Table 1. Effect of different drugs on the ratio b_2/b_1 (i.e. between the basal release before S_2 and S_1) in bovine cerebral arteries preincubated with [^{3}H]5-HT.

Drugs (μM)	[^{3}H]5-HT	No. of experiments
Control	0·88 ± 0·12	13
+ cocaine(1)	0·75 ± 0·04	5
Ketanserin (1)	1·52 ± 0·29**	4
Metitepine (1)	1·65 ± 0·20**	7
Noradrenaline (1)	1·67 ± 0·29**	4
5-HT (10)	1·60 ± 0·31**	5
+ metitepine (1)	1·54 ± 0·36*	5
+ methysergide (0·1)	2·13 ± 0·26**	4
+ cocaine (1)	1·10 ± 0·16†	4

Results are expressed as mean ± s.e.m. $b_1 = 76 \pm 3$ d min^{-1} mg^{-1}. $*P < 0.05$, $**P < 0.005$, †$P < 0.05$ (compared with 5-HT 10 μM).

and 6-OHDA, which specifically destroys noradrenergic nerve endings but has little effect on 5-HT-ergic nerves (Sachs & Jonsson 1972; Levitt & Duckles 1986), greatly reduced [^{3}H]5-HT uptake. Ouabain, an inhibitor of Na-K ATPase (Cantley 1986), also reduced the uptake; the ability of ouabain to block noradrenaline and 5-HT uptake has been reported (Tissari et al 1969; Leitz & Stefano 1970; Barrús et al 1990). The incomplete abolition of this uptake by denervation, may mean that the method used did not produce a complete noradrenergic denervation, or that the amine may be accumulated in other nerve endings, such as in the 5-HT-ergic nerve terminals present in cerebral vessels (Marco et al 1985; Edvinsson et al 1985; Scatton et al 1985) or extraneuronally (endothelium or smooth muscle). However, other reports question the existence of these nerves and their physiological role, having suggested that the 5-HT detected in cerebral vessels is mainly stored in perivascular adrenergic nerves (Saito & Lee 1987; Jackowski et al 1988). In different vessels, chemical denervation with 6-OHDA and treatment with cocaine decreased (Verbeuren et al 1983; Levitt & Duckles 1986; Barrús et al 1990) or did not affect (Fukuda et al 1986) [^{3}H]5-HT uptake. These findings lead to the conclusion that 5-HT is essentially accumulated in perivascular sympathetic nerves from which it is co-released with noradrenaline by nerve stimulation. This is consistent with the finding that the release of [^{3}H]5-HT elicited by 5-HT and mainly by noradrenaline was almost abolished in vessels pretreated with 6-OHDA. The ability of 5-HT to release noradrenaline from adrenergic nerve terminals has been observed in cerebral and peripheral arteries (Marín et al 1981; Van Nueten et al 1985; Houston & Vanhoutte 1986).

The evoked release of ^{3}H is mainly of [^{3}H]5-HT and basal release is of ^{3}H-metabolites (Verbeuren et al 1983). Stimulation-evoked tritium efflux was largely reduced by tetrodotoxin, an inhibitor of propagated nerve impulses (Narahashi et al 1964), indicating that the amine is released by action potentials. This release was also markedly decreased by denervation with 6-OHDA and Ca^{2+}-free medium, suggesting that an exocytotic mechanism mediates the efflux of 5-HT from adrenergic nerve endings. A reduction of stimulated [^{3}H]5-HT release by tetrodotoxin or by denervation (Verbeuren et al 1983; Kawasaki & Takasaki 1984; Barrús et al 1990) and Ca^{2+} omission (Kawasaki & Takasaki 1984; Scatton et al 1985) has been obtained in other vessels.

The modulation of stimulated tritium release by presynaptic α_2-adrenoceptors was investigated. This release was inhibited by both the selective (B-HT 920) and non-selective noradrenaline agonists of these receptors (Hammer et al 1980; Van Meel et al 1982; Docherty 1989; Molderings & Göthert 1990). The α_2-antagonist, rauwolscine (Van Meel et al 1982; Docherty 1989), abolished the release-inhibiting effect of both agonists. These findings suggest the presence of such receptors, modulating 5-HT release from the nerve endings, as described in other vessels (Kawasaki & Takasaki 1986; Barrús et al 1990). Nevertheless, phentolamine, a well known nonselective blocker of α-receptors, had no effect on tritium release, probably due to reduced potency or selectivity in the blockage of the presynaptic receptors involved in [^{3}H]5-HT release. Phentolamine and yohimbine, an antagonist of α_2-receptors, increased the electrically-induced tritium release in dog saphenous vein (Verbeuren et al 1983) and in rat perfused mesenteric bed (Kawasaki & Takasaki 1986) preincubated with [^{3}H]5-HT.

The possible existence of presynaptic 5-HT-receptors modulating the evoked 5-HT release was also studied. The results obtained show that this amine reduced the release; this reduction was blocked by metitepine (an antagonist of 5-HT_1 and 5-HT_2 receptors with presynaptic selectivity for 5-HT_1 (Martin & Sanders-Bush 1982; Schlicker et al 1987; Göthert & Schlicker 1987; Hoyer & Schoeffter 1991)), attenuated by cocaine, and unaffected by ketanserin (a blocker of 5-HT_2 receptors (Saxena et al 1987)) or methyser-

gide (a 5-HT-ergic antagonist with certain postsynaptic selectivity for 5-HT_1 and 5-HT_2 receptors (Cerrito & Raiteri 1979)). These findings suggest the implication of 5-HT_1, but not 5-HT_2, receptors, on the release-inhibiting effect of 5-HT, and that the effect of cocaine may be partially due to blockade of the reuptake of the evoked [^{3}H]5-HT release. In some tissues, such receptors modulate 5-HT release (Cerrito & Raiteri 1979; Monroe & Smith 1985; Schlicker & Göthert 1987), but not in others (Molderings et al 1989). Nevertheless, some results appear to suggest presynaptic 5-HT_1 receptors are not involved: high phentolamine concentrations (3 μM) were needed to partially inhibit the release-inhibiting effect of 5-HT and these concentrations had no effect on the evoked tritium release; rauwolscine antagonized the depressor effect of 5-HT and increased the evoked [^{3}H]5-HT release; and methysergide, in contrast to rauwolscine, modified neither the evoked tritium release nor the inhibitory action of 5-HT. All these findings appear to suggest that the modulation of the tritiated amine release by 5-HT is produced via presynaptic α_2-adrenoceptors, as reported for other preparations (Schlicker et al 1987; Frankhuijzen et al 1988). However, high phentolamine concentrations block 5-HT_1 receptors, i.e. phentolamine is a weak antagonist of these receptors (Bradley et al 1986; Limberger et al 1989) and rauwolscine is, in addition to being an α_2-antagonist, a 5-HT_1 receptor antagonist (mainly 5-HT_{1D} subtype) (Fozard 1987; Hoyer & Schoeffter 1991), and the observations suggest that 5-HT_1-like receptors may be involved in the inhibitory action of 5-HT.

5-HT increased the basal tritium release in intact segments, blocked by cocaine. This indicates that the amine may produce a displacement of the [^{3}H]5-HT stored in adrenergic nerve endings (an effect which might be blocked by cocaine), but also may activate 5-HT_3 receptors. Indeed, 5-HT may produce depolarization of noradrenergic nerves causing noradrenaline release—in our case [^{3}H]5-HT release—an effect mediated by 5-HT_3 receptors, which are blocked by cocaine (Göthert & Schlicker 1987; Hoyer 1990).

Rauwolscine and metitepine enhanced evoked tritium release, which indicates that the 5-HT_1 receptors are functionally active in inhibiting neurotransmitter release. In addition, ketanserin increased the basal tritium release, which may be due to an interference with the neuronal uptake of [^{3}H]5-HT (Monroe & Smith 1985). The increase of basal tritium release induced by metitepine is probably related to its ability to block the amine uptake (Blier et al 1989; Limberger et al 1989). Indeed, the presence of this drug in the incubation medium produced a marked reduction of [^{3}H]5-HT uptake which was similar to that caused by cocaine.

In conclusion, 5-HT is incorporated into adrenergic nerve terminals of bovine cerebral arteries, being co-released with noradrenaline. Evoked 5-HT release appears to be modulated by presynaptic 5-HT_1-like receptors, although with the present results the participation of α_2-adrenoceptors cannot be discounted. It is interesting to note that 5-HT_1 receptors (5-HT_{1A} and 5-HT_{1B}) and α_2-adrenoceptors (mainly those of α_{2B} subtype) have marked similarities with many features in common (Molderings & Göthert 1990; Hartig et al 1990). Recently, our group has reported that the presynaptic receptors present in perivascular nerve endings of these arteries are of the α_{2B} subtype (Arribas et al 1991). The present findings support the contention that 5-HT_1-like receptors and the α_2-adrenoceptors present in these arteries are similar entities and thus are similarly affected by drugs acting on both types of receptors. The [^{3}H]5-HT release induced by 5-HT may be mediated by 5-HT_3 receptors. In addition, the action of metitepine is in part caused by blockage of neurotransmitter uptake.

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References

Allen, G., Gross, C. J., French, L. A., Chou, S. N. (1976) Cerebral arterial spasm. Part 5: in vitro contractile activity of vasoactive agents including human CSF on human basilar and anterior cerebral arteries. J. Neurosurg. 44: 594–600

Aprigliano, O., Hermsmeyer, K. (1976) In vitro denervation of the portal vein and caudal artery of the rat. J. Pharmacol. Exp. Ther. 198: 568–577

Arribas, S., Galván, R., Ferrer, M., Herguido, M. J., Marín, J., Balfagón, G. (1991) Characterization of the subtype of presynaptic α_2-adrenoceptors modulating noradrenaline release in cat and bovine cerebral arteries. J. Pharm. Pharmacol. 43: 855–859

Barrús, M. T., Balfagón, G., Salaices, M., Marín, J. (1990) [^{3}H]5-Hydroxytryptamine uptake and release in cat cerebral arteries. Gen. Pharmacol. 21: 189–194

Blier, N., Ramdine, R., Galzin, A.-H., Langer, S. Z. (1989) Frequency-dependence of serotonin autoreceptor but not α_2-adrenoceptor inhibition of [^{3}H]-serotonin release in rat hypothalamic slices. Naunyn Schmiedebergs Arch. Pharmacol. 339: 60–64

Bradley, P.B., Engel, G., Feniuk, W., Fozard, J.R., Humphrey, P.P.A., Middlemiss, D.N., Mylecharane, E.J., Richardson, B.P., Saxena, P. R. (1986) Proposal for the classification and nomenclature of functional receptors for 5-hydroxytryptamine. Neuropharmacology 25: 536–576

Cantley, L. (1986) Ion transport systems sequences. Trends Neurosci. 9: 1–3

Cerrito, F., Raiteri, M. (1979) Serotonin release is modulated by presynaptic autoreceptors. Eur. J. Pharmacol. 57: 427–430

Chan-Palay, V. (1976) Serotonin axons in the supra- and subependymal plexuses and in the leptomeninges; their roles in local alterations of cerebrospinal fluid and vasomotor activity. Brain Res. 102: 103–130

Docherty, J. R. (1989) The pharmacology of α_1- and α_2-adrenoceptors: evidence for and against a further subdivision. Pharmacol. Ther. 44: 241–284

Edvinsson, L., MacKenzie, E. T. (1977) Amine mechanisms in the cerebral circulation. Pharmacol. Rev. 28: 275–348

Edvinsson, L., MacKenzie, E. T., Scatton, B. (1985) Evidence for a central serotonergic projection to the cerebral resistance vessels: possible functional implications. Prog. Appl. Microcirc. 8: 213–224

Edvinsson, L., Birath, E., Uddman, R., Lee, T. J.-F., Duverger, D., MacKenzie, E. T., Scatton, B. (1984) Indoleaminergic mechanisms in brain vessels; localization, concentrations, uptake and in vitro responses of 5-hydroxytryptamine. Acta Physiol. Scand. 121: 291–299

Feniuk, W., Humphrey, P. P. A., Watts, A. D. (1979) Presynaptic inhibitory action of 5-hydroxytryptamine in dog isolated saphenous veins. Br. J. Pharmacol. 67: 247–254

Fozard, J. R. (1987) 5-HT: the enigma variations. Trends Pharmacol. Sci. 8: 501–506

Frankhuijzen, A. L., Wardeh, G., Hogenboom, F., Mulder, A. H. (1988) α_2-Adrenoceptor mediated inhibition of the release of radiolabelled 5-hydroxytryptamine and noradrenaline from slices of the dorsal raphe region of the rat brain. Naunyn Schmiedebergs Arch. Pharmacol. 337: 255–260

Fukuda, S., Su, C., Lee, T. J.-F. (1986) Mechanisms of extraneuronal serotonin uptake in the rat aorta. J. Pharmacol. Exp. Ther. 239: 264–269

Göthert, M., Schlicker, E. (1987) Classification of serotonin receptors. J. Cardiovasc. Pharmacol. 10 (Suppl. 3): S3–S7

Hammer, R., Kobinger, W., Pichler, L. (1980) Binding of an imidazoline (clonidine and oxazoloazepin (B-HT 993)) and a thiazoloazepin (B-HT 920) to rat brain α-adrenoceptors and relation to cardiovascular effects. Eur. J. Pharmacol. 62: 277–285

Hartig, P., Kao, H.-T., Macchi, M., Adham, N., Zgombick, J., Weinshank, R., Banchek, T. (1990) The molecular biology of serotonin receptors. Neuropsychopharmacology 3: 355–347

Henseling, M., Eckert, F., Trendelenburg, U. (1976) The effect of cocaine in the distribution of labelled noradrenaline in rabbit aortic strips and on efflux of radioactivity from the strips. Naunyn Schmiedebergs Arch. Pharmacol. 292: 231–241

Houston, D. S., Vanhoutte, P. M. (1986) Serotonin and vascular system. Role in health and disease and implications for therapy. Drugs 31: 149–163

Hoyer, D. (1990) Serotonin 5-HT_3, 5-HT_4 and 5-HT-M receptors. Neuropsychopharmacology 3: 371–387

Hoyer, D., Schoeffter, P. (1991) 5-HT receptors: subtypes and second messengers. J. Recept. Res. 11: 197–214

Jackowski, A., Crockard, A., Burnstock, G. (1988) Ultrastructure of serotonin-containing nerve fibres in the middle cerebral artery of the rat and evidence for its localisation within catecholamine-containing nerve fibres by immunoelectron microscopy. Brain Res. 443: 159–165

Kawasaki, H., Takasaki, K. (1984) Vasoconstrictor responses induced by 5-hydroxytryptamine release from vascular adrenergic nerves by periarterial nerve stimulation. J. Pharmacol. Exp. Ther. 229: 816–822

Kawasaki, H., Takasaki, K. (1986) Pharmacological characterization of presynaptic alpha-adrenoceptors in the modulation of the 5-hydroxytryptamine release from vascular adrenergic nerves in the rat. Jpn. J. Pharmacol. 42: 561–570

Leitz, F. H., Stefano, F. J. E. (1970) Effect of ouabain and cisipramine on the uptake and storage of norepinephrine and metaraminol. Eur. J. Pharmacol. 11: 278–285

Levitt, B., Duckles, S. P. (1986) Evidence against serotonin as a vasoconstrictor neurotransmitter in the rabbit basilar artery. J. Pharmacol. Exp. Ther. 238: 880–885

Limberger, N., Fisher, M. R. G., Wichmann, T., Starke, K. (1989) Phentolamine blocks presynaptic serotonin autoreceptors in rabbit and rat brain cortex. Naunyn Schmiedebergs Arch. Pharmacol. 340: 52–61

Marco, E. J., Balfagón, G., Salaices, M., Sánchez-Ferrer, C. F., Marín, J. (1985) Serotonergic innervation of cat cerebral arteries. Brain Res. 338: 137–139

Marín, J., Salaices, M., Marco, E. J., Gómez, B., Lluch, S. (1979) Analysis of the contractile effect of 5-hydroxytryptamine on the isolated posterior communicating artery of the cat. J. Pharm. Pharmacol. 31: 456–459

Marín, J., Salaices, M., Garcia, A. G. (1980a) Dopamine-β-hydroxylase activity and noradrenaline content in pial arteries of cat and goat. Ibid. 32: 64–65

Marín, J., Salaices, M., Rivilla, F., Burgos, J., Marco, E. J. (1980b) Bilateral innervation of the cerebral arteries by the superior cervical ganglion in cats. J. Neurosurg. 53: 88–91

Marín, J., Salaices, M., Gómez, B., Lluch, S. (1981) Noradrenergic component in the vasoconstriction induced by 5-hydroxytryptamine in goat cerebral arteries. J. Pharmacol. Exp. Ther. 33: 715–719

Martin, L. L., Sanders-Bush, E. (1982) Comparison of the pharmacological characteristics of 5-HT_1 and 5-HT_2 binding sites with those of serotonin autoreceptors which modulate serotonin release. Naunyn Schmiedebergs Arch. Pharmacol. 321: 165–170

Molderings, G. J., Göthert, M. (1990) Mutual interaction between presynaptic α_2-adrenoceptors and 5-HT_{1B} receptors on the sympathetic nerve terminals. Ibid. 341: 391–397

Molderings, G. J., Göthert, M., Fink, K., Roth, E., Schlicker, E. (1989) Inhibition of noradrenaline release in the pig coronary artery via a novel serotonin receptor. Eur. J. Pharmacol. 164: 213–222

Monroe, P. J., Smith, D. J. (1985) Demonstration of an autoreceptor modulating the release of [^{3}H]5-hydroxytryptamine from a synaptosomal-rich spinal cord tissue preparation. J. Neurochem. 45: 1886–1894

Moreno, M. J., Conde, M. V., Fraile, M. L., Fernández-Lomana, H., López de Pablo, A. L., Marco, E. (1991) Lesion of the dorsal raphe nucleus induces supersensitivity to serotonin in isolated cat cerebral artery. Brain Res. 538: 324–328

Narahashi, T., Moore, J. W., Scott, W. R. (1964) Tetrodotoxin blockage of sodium conductance increase on lobster giant axons. J. Gen. Physiol. 47: 965–974

Nelson, E., Rennels, M. (1970) Innervation of intracranial arteries. Brain 93: 475–490

Paiva, M. Q., Caramona, M., Osswald, W. (1984) Intra- and extraneuronal metabolism of 5-hydroxytryptamine in the isolated saphenous vein of the dog. Naunyn Schmiedebergs Arch. Pharmacol. 325: 62–68

Sachs, C., Jonsson, G. (1972) Degeneration of central and peripheral noradrenaline neurons produced by 6-hydroxy-dopa. J. Neurochem. 19: 1561–1575

Saito, A., Lee, T. J.-F. (1987) Serotonin as an alternative transmitter in sympathetic nerves of large cerebral arteries of the rabbit. Circ. Res. 60: 220–228

Saxena, P. R., Bolt, G. R., Dhasmana, K. M. (1987) Serotonin agonists and antagonists in experimental hypertension. J. Cardiovasc. Pharmacol. 10 (Suppl. 3): S12–S18

Scatton, B., Duberger, D., L'Heureux, R., Serrano, A., Fage, D., Nowicki, J.-P., MacKenzie, E. T. (1985) Neurochemical studies on the existence, origin and characteristics of the serotonergic innervation of small pial vessels. Brain Res. 345: 219–229

Schlicker, E., Göthert, M. (1987) Presynaptic serotonin receptors on peripheral noradrenergic and central serotoninergic neurons of spontaneously hypertensive and Wistar-Kyoto rats. J. Cardiovasc. Pharmacol. 10 (Suppl. 3): S51–S53

Schlicker, E., Fink, K., Classen, K., Göthert, M. (1987) Facilitation of serotonin (5-HT) in the rat brain cortex by cAMP and probable inhibition of adenylate cyclase in 5-HT nerve terminals release by presynaptic α_2-receptors. Naunyn Schmiedebergs Arch. Pharmacol. 336: 251–256

Tissari, A. H., Schönhöfer, P. S., Bogdanski, D. G., Brodie, B. B. (1969) Mechanism of biogenic amine transport. II. Relationship between sodium and the mechanisms of ouabain blockade of the accumulation of serotonin and norepinephrine by synaptosomes. Mol. Pharmacol. 5: 593–604

Van Meel, J. C. A., Timmermans, P. B. M. W. M., van Zwieten, P. A. (1982) Interaction between calcium antagonists and vascular postsynaptic α-receptors. J. Pharmacol. 13: 317–379

Van Nueten, J. M., Janssens, W. J., Vanhoutte, P. M. (1985) Serotonin and vascular reactivity. Pharmacol. Res. Commun. 17: 585–608

Verbeuren, T. J., Jordaens, F. H., Herman, A. G. (1983) Accumulation and release of [^{3}H]5-hydroxytryptamine in saphenous veins and cerebral arteries of the dog. J. Pharmacol. Exp. Ther. 226: 579–588