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Effects of 5-HT receptor agonists on depolarization-induced [^{3}H]-noradrenaline release in rabbit hippocampus and human neocortex

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1 The present study attempted to determine whether noradrenaline (NA) release in rabbit hippocampus and human neocortex is modulated by presynaptic 5-hydroxytryptamine (5-HT) receptors.

2 Slices of rabbit hippocampus and human neocortex, loaded with [^{3}H]-noradrenaline ([^{3}H]-NA) were superfused and the effects of 5-hydroxytryptamine (5-HT) receptor ligands on electrically evoked [^{3}H]-NA release were investigated.

3 In rabbit hippocampus, 5-HT, 5-carboxamidotryptamine (5-CT; 32 μM) and 2-CH_3-5-HT (32 μM) increased [^{3}H]-NA release elicited with 360 pulses/3 Hz. Facilitation of transmitter release was not influenced by the 5-HT_3 receptor antagonist, tropisetron but was prevented by the α_2-adrenoceptor antagonist, rauwolscine. When autoinhibition was avoided by stimulating the tissue with 4 pulses/100 Hz (pseudo-one pulse-(POP) stimulation), 2-CH_3-5-HT decreased evoked transmitter release, whereas 5-HT and 5-CT had no effect. Inhibition caused by 2-CH_3-5-HT was not affected by tropisetron but counteracted by the α_2-adrenoceptor ligands, clonidine and rauwolscine. Inhibition caused by clonidine was diminished in the presence of 5-CT or 2-CH_3-5-HT.

4 In human neocortex, [^{3}H]-NA release elicited with 360 pulses/3 Hz was increased by 10 μM 5-HT and 32 μM 5-CT, whereas 2-CH_3-5-HT was ineffective. [^{3}H]-NA release evoked with a modified POP stimulation (2 bursts of 4 pulses/100 Hz, 3.5 min apart) was not affected by 2-CH_3-5-HT or 5-CT.

5 The present results indicate that 5-HT, 2-CH_3-5-HT and 5-CT can act on presynaptic α_2-autoreceptors as partial agonists (2-CH_3-5-HT; in rabbit hippocampal tissue) or antagonists (5-HT and 5-CT; in tissue of rabbit hippocampus and human neocortex). Furthermore the existence of autoinhibition dictates whether these drugs cause facilitation of release, inhibition or have no effect.

Keywords: Noradrenaline release; presynaptic 5-hydroxytryptamine receptors; presynaptic α_2-autoreceptors; 2-CH_3-5-hydroxytryptamine; 5-carboxamidotryptamine; human neocortex; rabbit hippocampus

Introduction

The existence of regulatory 5-hydroxytryptamine (5-HT) receptors located on postganglionic sympathetic nerve terminals has been demonstrated for various tissues of different species. In rat vena cava (Molderings *et al.*, 1987) and human saphenous vein (Molderings *et al.*, 1990) depolarization-induced noradrenaline (NA) release is inhibited by activation of 5-HT_{1B} and 5-HT_{1D} receptors, respectively. In pig coronary artery (Molderings *et al.*, 1989) the inhibitory presynaptic 5-HT receptor seems to belong to the 5-HT_4 receptor class. In contrast, for the central nervous system, it is not clear whether 5-HT receptors modulate NA release. For example, electrically-evoked [^{3}H]-NA release from slices of rat neocortex was not affected by 5-HT and 5-HT receptor ligands (Taube *et al.*, 1977), whereas in rabbit hippocampus 5-HT, blockers of 5-HT re-uptake, the 5-HT_1 receptor agonist 5-CT (Pedigo *et al.*, 1981), and the 5-HT_3 receptor agonist, 2-CH_3-5-HT (Richardson & Engel, 1986) caused an increase in transmitter release (Feuerstein & Hertting, 1986). Moreover, Blandina *et al.* (1991) demonstrated that K^+-evoked release of endogenous NA in rat hypothalamic slices was inhibited by activation of 5-HT_3 receptors. The reasons for these discrepancies are unknown. They might result from (i) the use of non-selective receptor ligands affecting more than one presynaptic receptor (ii) differences in the biophase concentration of NA so that a given partial agonist at α_2-adrenoceptors could either facilitate or inhibit release, or (iii) a functional interaction between various presynaptic receptors (*e.g.* Limberger *et al.*, 1988; Allgaier *et al.*, 1989; 1991a; Molderings & Göthert, 1990).

The present investigation continues the previous work of Feuerstein & Hertting (1986) who investigated whether noradrenergic nerve terminals of rabbit hippocampus are equipped with 5-HT receptors modulating depolarization-induced noradrenaline release. To this end, slices derived from either rabbit hippocampus or human neocortex were labelled with [^{3}H]-NA and the effects of 5-HT receptor ligands on electrically-evoked [^{3}H]-NA release were measured by choosing experimental conditions in which autoinhibition by released NA was avoided.

Preliminary accounts of the present results were given at the Joint Meeting of the Deutsche Gesellschaft für Pharmakologie und Toxikologie and the Association Francaise des Pharmacologistes (Lille, France, 1993).

Methods

Superfusion experiments

Slices (350 μm) of rabbit hippocampus were prepared according to Allgaier *et al.* (1986). Human neocortex slices (350 μm; 1.5-4 mg wet wt.) were obtained from patients (20-78 years) with brain tumours during surgical access to the subcortical neoplasms as described by Feuerstein *et al* (1990); between 4 and 12 slices were obtained per patient. For incubation and superfusion of the brain slices see Allgaier *et al.* (1991a). In brief: usually 15 slices were incubated a t 37°C in 2 ml of modified Krebs-Ringer bicarbonate buffer (KRBB;

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composition in mM: NaCl 118, KCl 4.8, $CaCl_2$ 1.3, $MgSO_4$ 1.2, $NaHCO_3$ 25, KH_2PO_4 1.2, glucose 10, ascorbic acid 0.57, Na_2 EDTA 0.03; saturated with 5% CO_2 in O_2, pH 7.4) containing a final concentration of 0.1 μM [^{3}H]-NA (42.1 Ci mmol^{-1}). Following ^{3}H-labelling (30 min), slices were rinsed and transferred to 12 superfusion chambers (each containing one slice) where they were situated between two platinum plate electrodes. Superfusion (at a flow rate of 0.6 ml min^{-1}) was followed with KRBB containing 1 μM (+)-oxaprotiline, an inhibitor of noradrenaline re-uptake (Waldmeier *et al.*, 1982). A 'conditioning' stimulation with 18 rectangular pulses of 2 ms duration of 1 Hz was routinely applied 20 min after the beginning of superfusion, since this yielded more reproducible responses to subsequent stimulations. For the 'conditioning' and all subsequent stimulations, voltage drop and current strength were 15 V cm^{-1} and 60 mA (≈1.2 mA mm^{-2} electrode area). Fractions (4 min) of the superfusate were collected, starting after 40 min of superfusion. Electrical field stimulation was carried out after 48 min (S_1) and 80 min (S_2) with 360 pulses/3 Hz or 4 pulses/100 Hz (POP stimulation). Drugs were added 8 or 16 min before S_2. α_2-Adrenoceptor ligands were present throughout superfusion as indicated. Superfusion was terminated after 96 min. S_1 served as the reference stimulus.

In some experiments on human neocortex slices, the usual POP stimulation was replaced by a modified POP stimulation consisting of two bursts of 4 pulses/100 Hz, 3.5 min apart in order to enhance the amount of the evoked tritium overflow. It was established in separate control experiments that with this modified POP stimulation, autoinhibition did not occur (i.e. yohimbine had no facilitatory effect on the evoked overflow; see Results).

Fractional rate of outflow of tritiated compounds per 4 min was determined by expressing the radioactivity in a 4 min-superfusate-fraction as a percentage of the radioactivity in the slice at the beginning of the respective fraction. Stimulation-evoked overflow of tritium from brain slices, which reflects exocytotic release of [^{3}H]-NA (Allgaier *et al.*, 1991a), was calculated as the difference between the total radioactivity in the 12 min superfusion period after the start of stimulation and the basal radioactivity in that period (basal outflow of tritium was assumed to decline linearly). The difference was expressed as a percentage of the radioactivity in the slice at the beginning of the respective stimulation period (S_1%, S_2%). Evaluation of drug effects on evoked tritium overflow was performed by calculating the ratio between the overflow evoked by S_2 and the overflow evoked by S_1 (S_2/S_1). Drug effects on resting outflow of tritium were calculated from the ratio between the fractional rates of tritium outflow in the fraction preceding S_2 (b_2) and the fraction before S_1 (b_1). S_2/S_1 and b_2/b_1 ratios were expressed as a percentage of the corresponding mean S_2/S_1 and b_2/b_1 ratios of appropriate controls.

Statistics

All data are given as arithmetic means ± s.e.mean. After Kruskal-Wallis analysis, the significance of differences between the means of various groups was determined by the Mann-Whitney test.

Drugs

(-)-[Ring-2,5,6-^{3}H]-NA (42.1 Ci mmol^{-1}; NEN, Dreieich, Germany); 2-CH_3-5-HT maleate, 5-CT maleate (Biotrend, Köln, Germany); clonidine HCl (Sigma, Deisenhofen, Germany); 5-HT creatinine sulphate, yohimbine HCl (Merck, Darmstadt, Germany) and rauwolscine HCl (Roth, Karlsruhe, Germany) were used. The following drugs were kindly donated: (+)-oxaprotiline HCl (Ciba-Geigy, Basel, Switzerland), tropisetron (Sandoz, Basel, Switzerland).

Results

Rabbit hippocampus: effects of 5-HT receptor ligands on [^{3}H]-NA release elicited with 360 pulses/3 Hz

Electrical field stimulation of rabbit hippocampal slices with 360 pulses/3 Hz induced a reproducible release of [^{3}H]-NA of approximately 5% of tissue tritium (Table 1). Transmitter release was significantly facilitated by 5-HT, 5-CT and by 2-CH_3-5-HT, each used at 32 μM (Figure 1). The 5-HT_3 receptor antagonist, tropisetron (10 μM) had no effect on the agonist-induced facilitation of release which was, however, prevented in the presence of the α_2-adrenoceptor antagonist, rauwolscine (1 μM) (Figure 1). Rauwolscine dramatically increased [^{3}H]-NA release (Table 1) presumably by counteracting autoinhibition mediated by activation of α_2-adrenoceptors.

None of the compounds had an effect on resting tritium outflow (data not shown) except 5-HT ($b_2/b_1 = 133.3 \pm 2.0$% of control for 32 μM 5-HT, $n = 5$; $P < 0.01$ vs. control).

Table 1 [^{3}H]-noradrenaline ([^{3}H]-NA) release from slices of rabbit hippocampus or human cortex

	Drug throughout (μM)	S_1(%)	S_1(nCi)	n
A *Rabbit hippocampus*				
4 pulses/100 Hz	–	1.07 ± 0.02	1.50 ± 0.03	67
4 pulses/100 Hz	Rauwloscine (1)	1.12 ± 0.02	1.37 ± 0.03	18
4 pulses/100 Hz	Tropisetron (0.01)	1.17 ± 0.05	1.43 ± 0.07	8
4 pulses/100 Hz	Clonidine (0.003)	0.69 ± 0.04**	0.99 ± 0.05	12
4 pulses/100 Hz	5-CT (32)	1.02 ± 0.05	1.42 ± 0.07	11
4 pulses/100 Hz	2-CH_3-5-HT (10)	0.96 ± 0.08	1.39 ± 0.08	12
360 pulses/3 Hz	–	5.13 ± 0.15	7.57 ± 0.22	30
360 pulses/3 Hz	Tropisetron (0.01)	5.02 ± 0.19	7.13 ± 0.41	22
360 pulses/3 Hz	Rauwolscine (1)	29.01 ± 0.60**	48.26 ± 1.41	40
B *Human neocortex*				
4 pulses/100 Hz *(2 consecutive bursts)*		1.05 ± 0.04	0.31 ± 0.02	33
360 pulses/3 Hz		6.09 ± 0.13	1.88 ± 0.04	250

Slices were electrically stimulated with either 360 pulses/3 Hz or 4 pulses/100 Hz except slices of human cortices which were stimulated with a modified POP stimulation consisting of two bursts of 4 pulses/100 Hz, 3.5 min apart. Rauwolscine, clonidine or tropisetron was present throughout as indicated. [^{3}H]-NA release elicited at S_1 is given as a percentage of the radioactivity in the tissue and as nCi. Means ± s.e.mean from n slices are given. Significant differences from respective drug-free control: **$P < 0.01$.

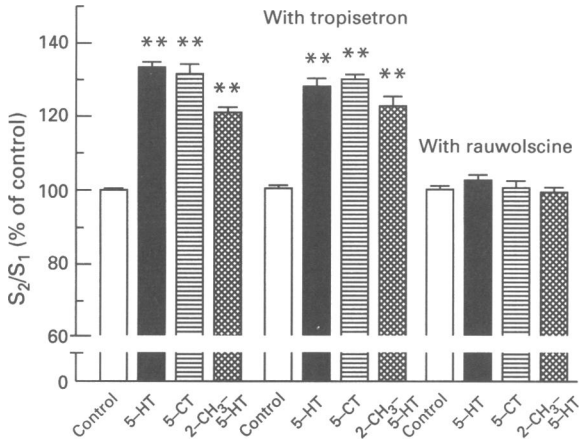

Figure 1 [^{3}H]-noradrenaline ([^{3}H]-NA) release from rabbit hippocampal slices evoked with 360 pulses/3 Hz: effects of 5-hydroxytryptamine (5-HT) receptor agonists in the absence and presence of tropisetron or rauwolscine. Slices were stimulated twice (S_1, S_2). 5-HT (32 μM; solid columns), 5-carboxamidotryptamine (5-CT; 32 μM; columns with horizontal hatching), or 2-methyl-5-hydroxytryptamine (2-CH_3-5-HT; 32 μM; cross-hatched columns) was added 16 min before S_2; tropisetron (0.01 μM) or rauwolscine (1 μM) was present throughout as indicated; open columns, control. S_2/S_1 ratios obtained in the presence of a 5-HT receptor agonist are expressed as a percentage of the mean ratio of respective control S_2/S_1 ratio: 1.01, no antagonist; 0.96, with tropisetron; 1.05, with rauwolscine. Means ± s.e.mean from 4–8 slices. Significant differences from corresponding control: $^{**}P<0.01$.

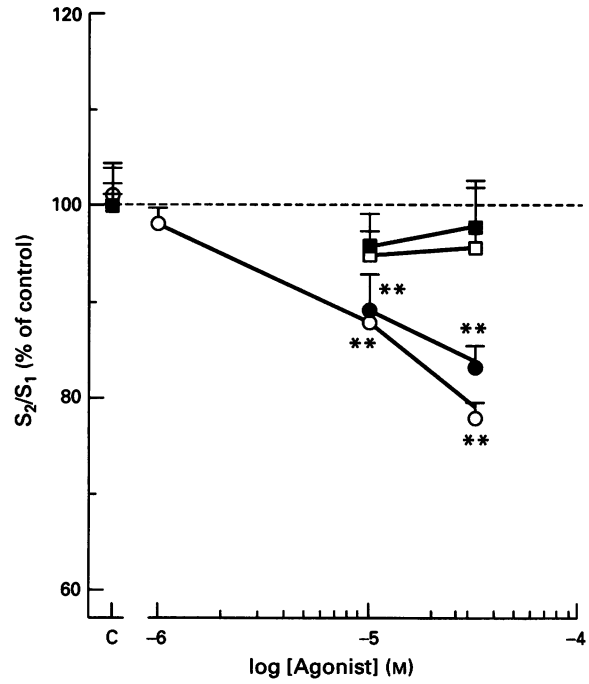

Figure 2 Rabbit hippocampus: effects of 2-methyl-5-hydroxytryptamine (2-CH_3-5-HT) on [^{3}H]-noradrenaline ([^{3}H]-NA) release evoked with 4 pulses/100 Hz. Slices were stimulated twice (S_1, S_2). 2-CH_3-5-HT (○) was added 16 min before S_2. Tropisetron (●) or rauwolscine (0.03 or 1 μM; □ or ■, respectively) was present throughout as indicated. S_2/S_1 ratios obtained in the presence of 2-CH_3-5-HT were expressed as a percentage of the mean ratio of respective control. S_2/S_1 control ratios were 1.11 (no rauwolscine), 1.16 (with 0.03 μM rauwolscine), and 1.14 (with 1 μM rauwolscine). Means ± s.e.mean from 4–11 slices. Significant differences from respective control: $^{**}P<0.01$.

Rabbit hippocampus: effects of 5-HT receptor agonists on [^{3}H]-NA release elicited with 4 pulses/100 Hz

In response to electrical stimulation with 4 pulses/100 Hz, [^{3}H]-NA release from slices of rabbit hippocampus amounted to approximately 1% of tissue tritium (Table 1). 5-HT and the 5-HT receptor agonists, 5-CT and 2-CH_3-5-HT, were added to the superfusion buffer usually 16 min before S_2. 2-CH_3-5-HT significantly decreased evoked [^{3}H]-NA release at 10 and 32 μM (Figure 2); it also inhibited transmitter release by 20.4 ± 3.3% (compared to control) when added only 8 min before S_2 (32 μM 2-CH_3-5-HT, $n=6$; $P<0.01$ vs. control). Tropisetron (10 nM) did not diminish the 2-CH_3-5-HT-induced inhibition of release (Figure 2). 5-HT (10 or 32 μM) and 5-CT (1, 10, or 32 μM) had no effect on evoked transmitter release (data not shown).

The inhibitory effect of 2-CH_3-5-HT was not changed by tropisetron (10 nM) but was abolished by rauwolscine (0.03 or 1 μM) (Figure 2). In accordance with previous results (Allgaier *et al.*, 1991b; 1992) rauwolscine had no effect on [^{3}H]-NA release evoked with 4 pulses/100 Hz (Table 1). Also the α_2-adrenoceptor agonist, clonidine (3 nM), which diminished [^{3}H]-NA release by approximately 40% when given alone (Table 1), counteracted the inhibition caused by 2-CH_3-5-HT (Table 2) when it was present throughout the superfusion.

When 5-CT or 2-CH_3-5-HT was present throughout superfusion and clonidine (10 nM) was added before S_2, the inhibitory effect of clonidine was significantly diminished (Table 3).

Human neocortex: effects of 5-HT receptor agonists on [^{3}H]-NA release elicited with 360 pulses/3 Hz

[^{3}H]-NA release elicited with 360 pulses/3 Hz was significantly increased by 10 μM 5-HT and 32 μM 5-CT (Figure 3), whereas 2-CH_3-5-HT was ineffective at the same concentrations (S_2/S_1 values in % of control were 99.0 ± 7.4 [$n=3$] for 10 μM 2-CH_3-5-HT and 96.6 ± 9.2 [$n=4$] for 32 μM 2-CH_3-5-HT). None of the compounds affected basal outflow of tritium (data not shown).

Table 2 Rabbit hippocampus: influence of clonidine on the 2-methyl-5-hydroxytryptamine (4 pulses/100 Hz)-induced inhibition of [^{3}H]-noradrenaline ([^{3}H]-NA) release evoked with 4 pulses/100 Hz

Drug (μM)	S_2/S_1 (% of control)	n
–	100.0 ± 1.9	6
2-CH_3-5-HT (32)	81.8 ± 2.3**	6
Clonidine (0.003)	100.0 ± 2.4	6
2-CH_3-5-HT/clonidine	97.7 ± 5.1^{NS,*}	6

Hippocampal slices were stimulated twice (S_1, S_2). 2-CH_3-5-HT was added from 16 min before S_2 onwards, clonidine was present throughout superfusion. S_2/S_1 ratios obtained in the presence of 2-CH_3-5-HT are expressed as a percentage of the mean ratio of respective controls. S_2/S_1 control ratios were 1.10 (no clonidine) and 1.00 (with clonidine). Means ± s.e.mean from n slices. $^*P<0.05$, compared with the effect of 2-CH_3-5-HT in the absence of clonidine; $^{**}P<0.01$, compared with clonidine-free control; NS, not significant compared with the clonidine control group.

Human neocortex: effects of 5-HT receptor agonists on [^{3}H]-NA release elicited with 4 pulses/100 Hz

Slices of human neocortex were stimulated with a modified POP stimulation (2 bursts of 4 pulses/100 Hz, 3.5 min apart) to obtain a sufficiently high [^{3}H]-NA release (Table 1). Using the α_2-adrenoceptor antagonist, yohimbine (1 μM) it was established that no autoinhibition occurred. The mean S_2/S_1 value for control and yohimbine was 1.10 ± 0.04 ($n=10$) and 1.11 ± 0.02 ($n=4$), respectively. Neither 2-CH_3-5-HT nor 5-CT significantly affected [^{3}H]-NA release induced by modified

Table 3 Rabbit hippocampus: influence of 5-carboxamidotryptamine (5-CT) or 2-methyl-5-hydroxytryptamine (2-CH_3-5-HT) on the clonidine-induced inhibition of [^{3}H]-noradrenaline ([^{3}H]-NA) release evoked with 4 pluses/100 Hz

Drug (μM)	S_2/S_1 (% of control)	n
–	100.0 ± 1.9	6
Clonidine (0.01)	32.2 ± 1.8	6
5-CT (32)	100.0 ± 1.8	7
5-CT/clonidine	48.3 ± 1.6**	4
2-CH_3-5-HT (10)	100.0 ± 2.2	8
2-CH_3-5-HT/clonidine	39.9 ± 2.3*	4

Hippocampal slices were stimulated twice (S_1, S_2). Clonidine was added from 16 min before S_2 onwards, 5-CT or 2-CH_3-5-HT was present throughout superfusion. S_2/S_1 ratios obtained in the presence of clonidine were expressed as a percentage of the mean ratio of respective controls. S_2/S_1 control ratios were 1.12 (drug-free), 1.19 (with 5-CT), and 1.12 (with 2-CH_3-5-HT). Means ± s.e.mean from *n* slices. *$P < 0.05$, **$P < 0.01$ compared with the clonidine group in the absence of 5-CT or 2-CH_3-5-HT, respectively.

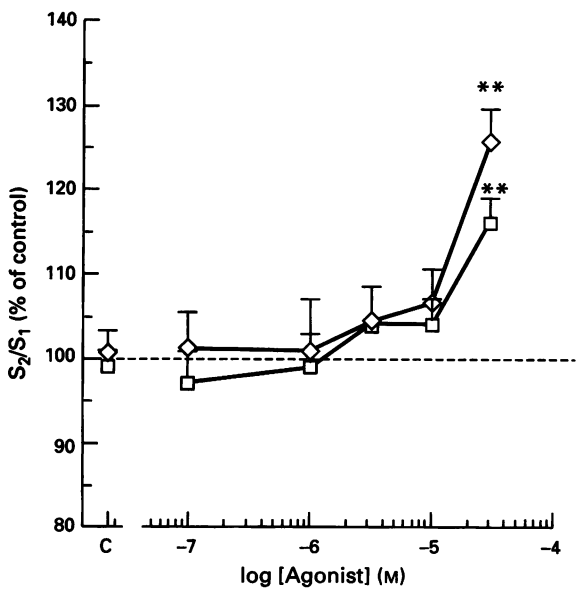

Figure 3 Human neocortex: effects of 5-hydroxytryptamine (5-HT) and 5-carboxamidotryptamine (5-CT) on [^{3}H]-noradrenaline ([^{3}H]-NA) release evoked with 360 pulses/3 Hz. Slices were stimulated twice (S_1, S_2). 5-HT (□) or 5-CT (◇) was added 16 min before S_2. S_2/S_1 ratios obtained in the presence of an agonist were expressed as a percentage of the mean S_2/S_1 ratio of controls (0.97). Means ± s.e.mean from 4–10 slices.

POP stimulation (Figure 4). Yohimbine slightly affected Ca^{2+}-independent tritium outflow under resting conditions, as previously observed (Allgaier *et al.*, 1992).

Human neocortex: dependence of [^{3}H]-NA release on the age of the patients

[^{3}H]-NA release evoked from human neocortex slices with 360 pulses/3 Hz was independent of the age of the patients (Figure 5). In addition, it was not influenced by the sex of the patients: the mean S_1 value from male patients was 6.13 ± 0.14 ($n = 178$), and that from female patients was 6.00 ± 0.28 ($n = 72$).

Discussion

In the present study we investigated whether presynaptic 5-HT receptors modulate depolarization-induced NA release in

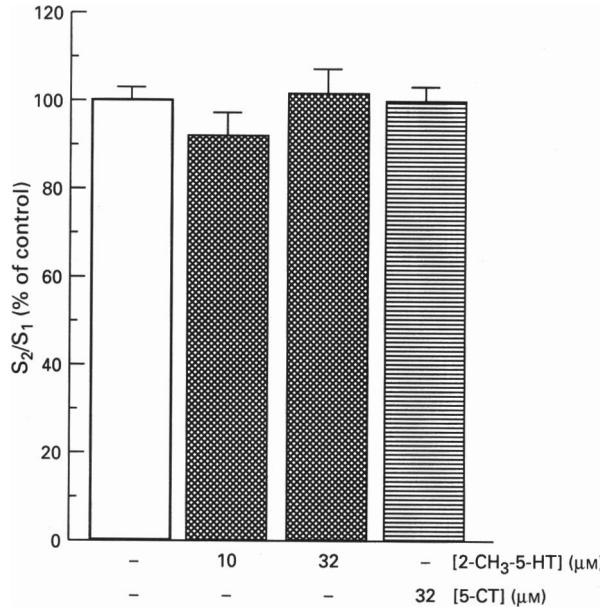

Figure 4 Human neocortex: effects of 5-hydroxytryptamine (5-HT) receptor agonists on [^{3}H]-noradrenaline ([^{3}H]-NA) release evoked with a modified POP stimulation consisting of two bursts of 4 pulses/100 Hz, 3.5 min apart. Cortical slices were stimulated twice (S_1, S_2). 2-Methyl-5-hydroxytryptamine (2-CH_3-5-HT; cross-hatched columns) or 5-carboxamidotryptamine (5-CT; columns with horizontal hatching) was added 16 min before S_2. S_2/S_1 ratios obtained in the presence of an agonist are expressed as a percentage of the mean S_2/S_1 ratio of controls (1.10). Means ± s.e.mean from 4–10 slices.

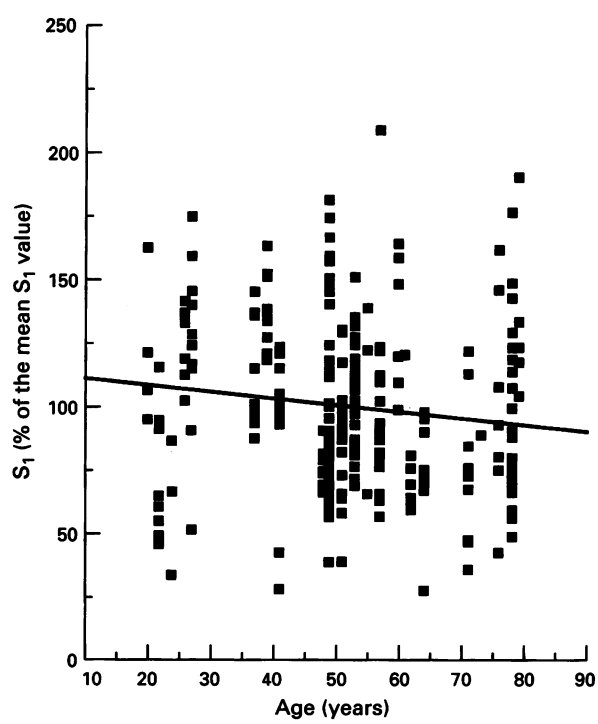

Figure 5 Human neocortex: [^{3}H]-noradrenaline ([^{3}H]-NA) release evoked with 360 pulses/3 Hz independently of the age of patients. S_1 values (in % of the tritium content of the tissue) are expressed as a percentage of the mean S_1 value from a total number of 250 slices of 33 patients (mean S_1 ± s.e.mean was 6.09 ± 0.13). The slope of the regression line was −0.25 ± 0.14, and was not significantly different from zero. Thus, a possible overestimation of the slope of the regression line due to the partial dependence of the single S_1 values within each patient was not critical (see Feuerstein et al., 1992a).

rabbit hippocampus and human neocortex. In a previous study, performed on rabbit hippocampus (Feuerstein & Hertting, 1986) 5-HT, as well as 5-CT, a nanomolar affinity 5-HT_1 receptor agonist without significant affinity for 5-HT_3 receptors (Pedigo *et al.*, 1981; Heuring & Peroutka, 1987), and 2-CH_3-5-HT, a micromolar 5-HT_3 receptor agonist having low affinity for either 5-HT_1 or 5-HT_2 receptors (Richardson & Engel, 1986), enhanced the electrically evoked release of [^{3}H]-NA at μmolar concentrations. The facilitatory effects of these compounds persisted in the presence of the non-selective α-adrenoceptor antagonist, phentolamine. In the study of Feuerstein & Hertting (1986) electrical stimulation was carried out with 360 pulses/3 Hz, a condition under which autoinhibition by released NA develops very rapidly (Valenta *et al.*, 1988). Using the same experimental paradigm we could confirm the capacity of 5-HT, 5-CT, and 2-CH_3-5-HT to enhance electrically evoked noradrenaline release in rabbit hippocampus. However, facilitation of transmitter release was not blocked by the 5-HT_3 receptor antagonist, tropisetron but was prevented by the α_2-adrenoceptor antagonist, rauwolscine. Previous studies have shown that tropisetron and MDL 72222, another 5-HT_3 receptor antagonist, inhibited the facilitatory effects of 5-HT agonists in rabbit hippocampus only at concentrations much higher than those required to block 5-HT_3 receptors of the periphery (Feuerstein & Hertting, 1986). Rauwolscine by itself increased stimulus-evoked transmitter release approximately 6 fold, whereas phentolamine, a partial agonist at the α_2-adrenoceptor (Heepe & Starke, 1985) caused only a 1.5 fold increase (Feuerstein & Hertting, 1986). Considering the prominent enhancement of release caused by rauwolscine it is noteworthy that evoked release in the presence of the α_2-antagonist does not reflect maximum release under these conditions. Compounds such as the potassium channel inhibitor, tetraethylammonium chloride or the protein kinase C activator, 4β-phorbol 12,13-dibutyrate, also enhanced transmitter release in the presence of α_2-adrenoceptor antagonists (Heepe & Starke, 1985; Allgaier *et al.*, 1987).

The present results suggest that 5-HT, 5-CT, and 2-CH_3-5-HT increased NA release by impairing autoinhibition. This hypothesis is supported by data from experiments in which autoinhibition was avoided by stimulating the tissue with 4 pulses/100 Hz (POP stimulation): (i) 5-HT and 5-CT were ineffective, whereas 2-CH_3-5-HT decreased [^{3}H]-NA release; (ii) the inhibition caused by 2-CH_3-5-HT was not antagonized by tropisetron but by rauwolscine and the α_2-adrenoceptor agonist, clonidine; (iii) the inhibition caused by clonidine was diminished by 5-CT or 2-CH_3-5-HT. Since rauwolscine has been shown to be an agonist at the presynaptic 5-HT_{1D} autoreceptor in rabbit hippocampus (Feuerstein *et al.*, 1985) and caudate nucleus (Feuerstein *et al.*, 1992b) it has to be considered that it counteracted the enhancement of NA release via activation of inhibitory 5-HT_{1D} receptors. However, the typical 5-HT_1 receptor agonists, 5-HT and 5-CT, had no effect on NA release in the absence of autoinhibition so that the existence of inhibitory 5-HT_{1D} receptors can be ruled out. Clonidine (3 μM) caused approximately 40% inhibition of [^{3}H]-NA release in rabbit hippocampal slices. As shown in detail previously, clonidine reduced [^{3}H]-NA release evoked with POP stimulation from rabbit hippocampal slices with a EC_{50} value of 57 nM and virtually abolished release at concentrations of 100 nM and more (Allgaier *et al.*, 1991b). Since the effect of 3 nM clonidine was far from being maximal, inhibition caused by 2-CH_3-5-HT should have been additive to that of the α_2-agonist, if mediated by a receptor other than the α_2-adrenoceptor.

Taken together, in rabbit hippocampus, 5-HT, 5-CT, and 2-CH_3-5-HT modulated evoked NA release by acting on presynatic α_2-adrenoceptors as partial agonist (2CH_3-5-HT) or antagonist (5-HT, 5-CT). Furthermore the existence of autoinhibition dictates whether these drugs cause facilitation of release (autoinhibition present), inhibition or had no effect (no autoinhibition).

In human neocortex, [^{3}H]-NA release induced with 360 pulses/3 Hz did not change with the age of the patients. This observation is in contrast to acetylcholine release which decreases with age (Feuerstein *et al.*, 1992a). [^{3}H]-NA release from human neocortex slices was increased by μmolar concentrations of 5-HT and 5-CT, but not by 2-CH_3-5-HT. Autoinhibition of [^{3}H]-NA release during stimulation of human neocortex slices (Feuerstein *et al.*, 1990) was comparable to that measured for rabbit hippocampal slices. However, when autoinhibition was avoided by eliciting transmitter release with a modified POP stimulation, both 5-CT and 2-CH_3-5-HT remained ineffective. The modified POP stimulation consisted of two bursts of 4 pulses at 100 Hz, 3.5 min apart and was used to obtain a sufficiently high [^{3}H]-NA release from human neocortex slices. It has previously been applied also to rabbit brain tissue (Allgaier *et al.*, 1992). The data obtained with human neocortex tissue are in agreement with the antagonistic properties of 5-CT on presynaptic α_2-adrenoceptors in the rabbit hippocampus. They reveal, in addition, species differences between human neocortex and rabbit hippocampus for which distinct α_2-adrenoceptor subtypes might exist, since 2-CH_3-5-HT inhibited NA release only in the rabbit in the absence of autoinhibition. For rabbit brain it was demonstrated that the α_2-adrenoceptor regulating NA release belongs to the α_{2A} subtype (Trendelenburg *et al.*, 1993b), whereas for human kidney tissue it was classified as α_{2C} subtype (Trendelenburg *et al.*, 1993a).

In the literature, there are a number of reports demonstrating an affinity of 5-HT-receptor ligands for adrenoceptors and, conversely, of adrenoceptor ligands for 5-HT receptors. For example, the 5-HT_2 antagonist, ketanserin, binds to α_1-adrenoceptor in human, rat and pig brain (Hoyer *et al.*, 1987), 5-methyl-urapidil shows equal affinity for α_{1A}- and 5-HT_{1A}-receptors in rat cortex membranes (Groβ *et al.*, 1987), and the 5-HT_1-receptor agonist, RU 24969, is a weak antagonist for presynaptic α_2-adrenoceptors in rat cortex (Schlicker *et al.*, 1988).

The present data provide no evidence for the existence of presynaptic 5-HT receptors modulating NA release in rabbit hippocampus and human neocortex. The effects of 5-HT, 2-CH_3-5-HT and 5-CT were most probably due to an interaction with presynaptic α_2-autoreceptors. While this manuscript was in preparation Mongeau *et al.* (1994) reported that 2-CH_3-5-HT enhanced NA release from slices of *rat* hippocampus via 5-HT_3 receptors. However, Schlicker *et al.* (1994) demonstrated that facilitation of NA release from *mouse* cerebral cortex caused by the 5-HT_3 receptor agonist 1-(*m*-chlorophenyl)-biguanide was due to α_2-adrenoceptor blockade. In radioligand binding studies they determined the affinities of 2-CH_3-5-HT and 5-CT for α_2-adrenoceptors which correspond to the concentrations affecting NA release in the present study.

The present paper describes a facilitatory influence of 5-HT on NA release in human neocortex. In addition, there is another presynaptic interaction between the noradrenergic and the 5-hydroxytryptaminergic system in cortical tissue of animals (e.g. Feuerstein *et al.*, 1985) and man (Feurerstein *et al.*, 1993), as endogenous NA inhibits 5-HT release via α_2-heteroreceptors. This regulatory circuit between both neurotransmitter systems may have clinical implications since several CNS disorders may be based on interrelated disturbances of both, the noradrenergic and the 5-hydroxytryptaminergic neurotransmission, e.g. cyclothymia, pain syndromes and anxiety disorders.

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