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Effect of 5-hydroxytryptamine on [^{3}H]-acetylcholine release from guinea-pig striatal slices

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1 The effect of 5-hydroxytryptamine (5-HT) on spontaneous and electrically-evoked tritium efflux was studied in guinea-pig caudate nucleus slices preloaded with [^{3}H]-choline.

2 5-HT, 10–300 μmol l^{-1}, temporarily increased the spontaneous tritium efflux (as well as the endogenous acetylcholine (ACh) release) and, after 15 min perfusion, inhibited it. The facilitatory effect of 5-HT on spontaneous efflux was increased while the inhibitory effect did not occur in slices taken from dopamine-depleted guinea-pigs.

3 The increase in spontaneous tritium efflux by 5-HT was blocked by methiothepin, methysergide (pA_2 8.7) and by the selective 5-HT_2 antagonist, ritanserin (pA_2 6.7).

4 The inhibition of spontaneous tritium efflux by 5-HT was prevented by methysergide and methiothepin but not by ritanserin and (−)-propranolol.

5 5-HT, 100 μmol l^{-1}, inhibited the electrically-evoked tritium efflux and this effect was unchanged in dopamine-depleted slices.

6 The inhibition of electrically-evoked tritium efflux by 5-HT was blocked by methiothepin and methysergide but not by (−)-propranolol or ritanserin.

7 These results suggest that 5-HT may exert a rapid and transient (excitatory) and a more prolonged (inhibitory) control over striatal cholinergic neurones.

Introduction

There is a great deal of evidence to suggest that 5-hydroxytryptamine (5-HT) exerts an inhibitory effect on the cholinergic system. In fact, in the whole animal, 5-HT or 5-HT-mimetic drugs increase acetylcholine (ACh) content in different brain areas (Envrard *et al.*, 1977; Samanin *et al.*, 1978; Consolo *et al.*, 1980), probably by inhibiting cholinergic neurone activity. Accordingly, 5-HT and 5-HT-releasing drugs reduce the ACh efflux from rat nucleus accumbens slices (De Belleroche & Gardiner, 1982) and from rat hippocampal synaptosomes (Maura & Raiteri, 1986).

However, the views concerning the striatum are conflicting. Some authors have reported that 5-HT reduces the activity of striatal cholinergic neurones (Vizi *et al.*, 1981; Gillet *et al.*, 1985), others have claimed that neurones of the raphe nucleus do not modulate these cholinergic cells (Robinson, 1983) while Butcher *et al.* (1976) demonstrated that dorsal raphe neurones may exert an excitatory effect on striatal cholinergic interneurones. No doubt the influence of 5-HT on the cholinergic system is very complex: for instance it has been found that i.c.v. injection of 5-HT increased ACh outflow from the exposed cortex of freely moving guinea-pigs but inhibited it in methiothepin-pretreated animals (Bianchi *et al.*, 1986).

Starting from these discrepancies, experiments have been performed to re-examine the effect of 5-HT on ACh release from guinea-pig caudate nucleus slices kept at rest or electrically-stimulated.

A preliminary account of this study has been presented (Beani *et al.*, 1985).

Methods

Tritium efflux experiments

Superfusion of guinea-pig caudate nucleus slices preincubated with [^{3}H]-choline was performed as previously described (Beani *et al.*, 1984). The cerebral tissue was sliced with a vibratome-like apparatus and the slices (0.4 μm thick) were incubated at

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37°C for 30 min in 2 ml Krebs solution containing 0.1 μmol l^{-1} [^{3}H]-choline (80 Ci mmol^{-1}). The slices were then rinsed and transferred to superfusion chambers and superfused at 0.5 ml min^{-1} with Krebs solution containing hemicholinium-3 (HC-3) (mmol l^{-1}: NaCl 118.5, KCl 4.8, $CaCl_2$ 2.5, $MgSO_4$ 1.2, $NaHCO_3$ 25, KH_2PO_4 1.2, glucose 11, HC-3 0.01), which was bubbled with 95% O_2 and 5% CO_2.

Tritium efflux at rest The basal release of tritium was determined during a period of 5 min and the effect of the drugs on resting release was evaluated by calculating the ratio between postdrug and predrug release rates. The predrug value was taken at the 55th min and the postdrug value was taken at the 60th min (i.e. 5 min after the drug was added) and at the 80th min (i.e. 25 min after the drug was added).

The effect of 5-HT was studied by adding the amine to the perfusate either for 2 min at 56th min or at the 56th min continuously until the end of the experiment.

Electrically-evoked tritium efflux The slices were electrically stimulated on two occasions for periods of 2 min with rectangular pulses of alternate polarity (5 ms duration, 0.2 Hz, 30 mA cm^{-2}). The stimulation frequency of 0.2 Hz was chosen since at this low frequency, the evoked tritium efflux as a percentage of tritium content remained constant (S_2/S_1 ratio near 1). This was done at the 45th (S_1) and 85th (S_2) min of perfusion. Drug effect on electrically-evoked release was estimated as the ratio (S_2/S_1) of the net extra release (Beani *et al.*, 1984). This was calculated by subtracting resting release from the total electrically-evoked release.

The effect of 5-HT was tested by adding the amine either for 2 min at S_2 or 15 min before S_2 and continuing until perfusion ended. Drugs were added to the superfusion medium 15 min before S_2 to check for their effect on tritium efflux or at the beginning of superfusion to test their influence on 5-HT effects.

In some experiments the basal and electrically-evoked tritium efflux was studied in slices taken from dopamine-depleted guinea-pigs which had been given reserpine 5 mg kg^{-1} i.p. plus α-methyl-*p*-tyrosine 100 mg kg^{-1} i.p. 18 h before the experiment as well as a further 200 mg kg^{-1} i.p. of the latter drug 2 h before the experiment.

Tritium determination

At the end of the experiment the tissue was removed from the chambers, weighed and solubilized with 1 ml of Protosol (New England Nuclear, Boston, MA, U.S.A.). This tissue sample and the 5 min samples of Krebs solution were added to scintillation liquid for tritium determination (beta spectrometer Beckman LS 1800). The tissue tritium at each sampling time was calculated by adding the final tissue tritium of the slices to the whole amount of tritium released by the tissue from that time until the end of the superfusion.

All the results were given as mean ± s.e.mean of tritium efflux expressed as a percentage of tissue tritium (fractional rate) in a given time interval.

Endogenous acetylcholine release

In another set of experiments the endogenous ACh release was studied according to the method previously described (Beani *et al.*, 1978) by perfusing the slices with Krebs solution containing physostigmine sulphate 30 μmol l^{-1} and choline 20 μmol l^{-1}. The ACh content of the samples was bioassayed on tetrodotoxin-pretreated guinea-pig ileum in the presence not only of cyproheptadine and morphine (Beani *et al.*, 1978) but also methysergide (0.02 μmol l^{-1}) to prevent 5-HT-mediated responses of the ileum.

Statistical analysis

The differences between control and treated slices were determined by analysis of variance, Student's *t* test for paired and non paired data and the Wilcoxon two sample test. The apparent pA_2 values of the antagonists, tested at three concentrations, were calculated according to Schild (1947). Linear regression lines and correlation coefficient were calculated to quantify the effect of treatment.

Drugs

The drugs used were: [^{3}H]-choline (80 Ci mmol^{-1}, NEN), Protosol (NEN), 5-hydroxytryptamine creatinine sulphate and methysergide maleate (Sigma), methiothepin maleate (Hoffmann La Roche), (−)-propranolol HCl (ICI), ritanserin (prepared as a hydrochloride, Janssen), hemicholinium-3 (Sigma), tetrodotoxin (Sankyo), paroxetine hydrochloride (Beecham). Freshly prepared solutions were used for each experiment.

Results

Tritium efflux under control conditions

The basal overflow of tritium from guinea-pig caudate nucleus slices kept in Krebs solution containing HC-3 (10 μmol l^{-1}) was 1.18 ± 0.03% of tritium content in 5 min sample (48 expts). In the slices perfused with a solution in which the Ca^{2+}

Table 1 Effect of 5-hydroxytryptamine (5-HT) on tritium efflux from caudate nucleus slices electrically stimulated at 0.2 Hz for 2 min

Experimental conditions	*No. of expts*	*Efflux of tritium in* S_1 *as % of tritium content*	S_2/S_1	% *changes*
Normal guinea-pigs:				
$[Ca^{2+}]$ 2.5 mmol l^{-1}				
Without paroxetine:				
Controls	14	7.9 ± 0.31	0.91 ± 0.01	100
5-HT 100 μmol l^{-1}	14	7.5 ± 0.30	0.72 ± 0.04*	82 ± 4
With paroxetine:				
Controls	23	6.7 ± 0.24	0.81 ± 0.02	100
5-HT 100 μmol l^{-1}	20	6.01 ± 0.37	0.67 ± 0.02*	82 ± 3
$[Ca^{2+}]$ 1.25 mmol l^{-1}				
With paroxetine:				
Controls	15	2.63 ± 0.15†	0.92 ± 0.02	100
5-HT 100 μmol l^{-1}	15	2.90 ± 0.19	0.76 ± 0.02*	80 ± 2
Dopamine-depleted guinea-pigs:				
$[Ca^{2+}]$ 2.5 mmol l^{-1}				
With paroxetine:				
Controls	9	8.02 ± 0.42††	0.79 ± 0.02	100
5-HT 100 μmol l^{-1}	9	8.16 ± 0.35	0.67 ± 0.03*	85 ± 3

5-HT was added 15 min before S_2. Paroxetine was present at 3.2 μmol l^{-1}.
The difference with respect to corresponding controls is statistically significant *$P < 0.01$, according to the Wilcoxon two sample test. The difference with respect to 2.5 mmol l^{-1} $[Ca^{2+}]$ is statistically significant †$P < 0.01$, according to Student's t test for non paired data. The difference with respect to normal guinea-pigs is statistically signifcant ††$P < 0.01$, according to Student's t test for non paired data.

concentration was reduced to half the normal amount or in the presence of tetrodotoxin, 0.5 μmol l^{-1}, the tritium outflow was significantly reduced to 0.97 ± 0.03% with reduced Ca^{2+} concentration (27 expts) and to 1.05 ± 0.05% with tetrodotoxin (20 expts, $P < 0.05$ with respect to basal values in normal Krebs solution). The electrically (0.2 Hz)-evoked tritium efflux (expressed as net extra

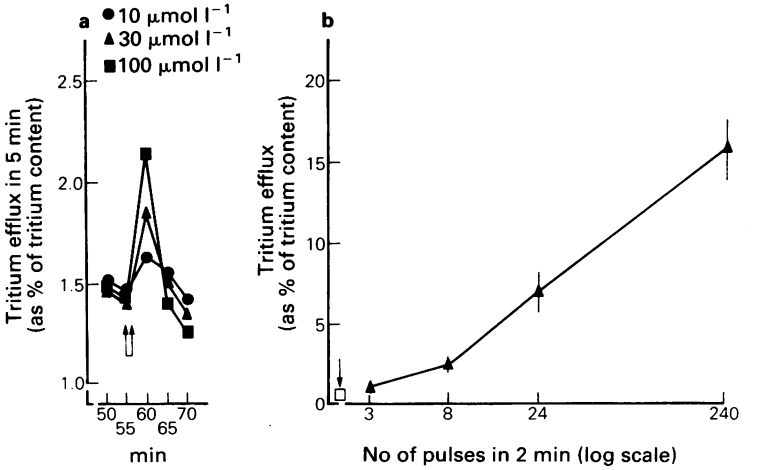

Figure 1 Effect of 5-hydroxytryptamine (5-HT) on tritium efflux from guinea-pig caudate nucleus slices. (a) A typical example of the effect of 5-HT, applied as 2 min pulse (arrows), on tritium efflux of unstimulated slices. (b) Relationship between the number of electrical pulses, applied in a 2 min period and stimulus-induced net extra efflux as % of tritium content. At the arrow, the net extra efflux in 2 min caused by 5-HT (100 μmol l^{-1}) in unstimulated slices. Each point is the mean of at least 5 experiments.

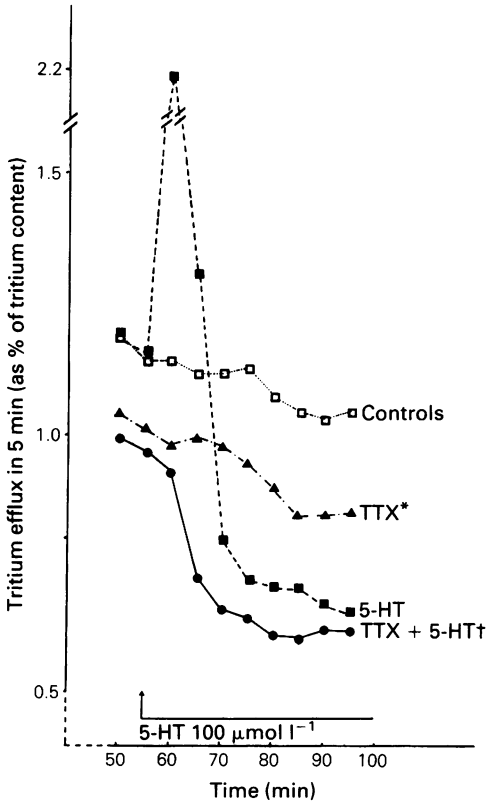

Figure 2 Effect of 5-hydroxytryptamine (5-HT, 100 μmol l^{-1}) (arrow) on tritium efflux from unstimulated caudate nucleus slices. The effect of tetrodotoxin (TTX) 0.5 μmol l^{-1}, added from the beginning of the experiment is also shown. Each point is the mean of at least 10 expts. Error bars, less than 10% of the respective means were omitted. Significantly different from control, *$P < 0.05$, Wilcoxon two sample test; significantly different from TTX alone †$P < 0.05$, Student's t test for non paired data.

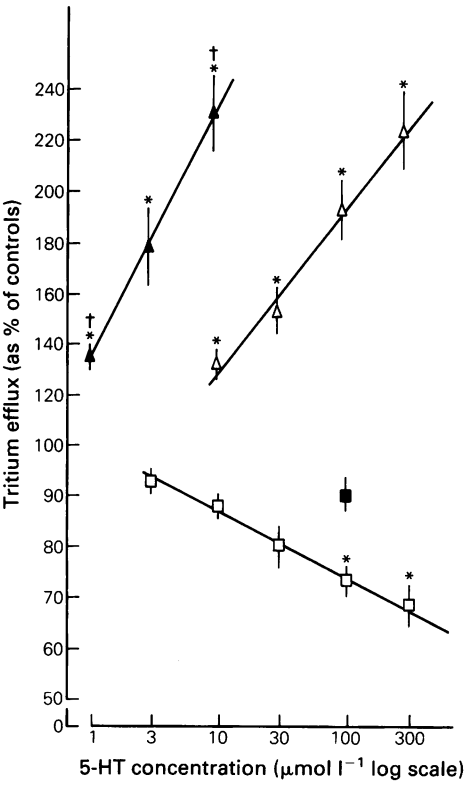

Figure 3 Dual effect of 5-hydroxytryptamine (5-HT) on tritium overflow from guinea-pig caudate nucleus unstimulated slices. Each point, as percentage of predrug value, represents the mean of 10–20 experiments; vertical lines show s.e.mean. The 5-HT-induced increase in tritium efflux from normal (△) [$r = 0.65$, $P < 0.01$] and dopamine-depleted slices (▲) [$r = 0.90$, $P < 01$] was estimated at the 60th min (i.e. 5 min after starting 5-HT perfusion). The 5-HT-induced reduction in tritium efflux from normal (□) [$r = 0.55$, $P < 0.01$] and dopamine-depleted slices (■) was estimated at the 80th min i.e. 25 min after starting 5-HT perfusion. Significantly different from the corresponding control (*$P < 0.05$) according to the Wilcoxon two sample test. Significantly different from normal guinea-pigs (†$P < 0.05$) according to Student's t test for non paired data.

release in 2 min) was 6.7 ± 0.2% of the radioactivity present in the tissue at the start of the stimulation (Table 1). If the Ca^{2+} concentration was halved, the outflow was significantly reduced (Table 1). The electrically-evoked efflux was completely abolished by tetrodotoxin.

Effect of 5-HT on tritium efflux under rest conditions

5-HT, 10–100 μmol l^{-1}, added to the superfusion medium for 2 min temporarily increased spontaneous tritium efflux in a dose-dependent manner (Figure 1a). The increase, expressed as net extra efflux in 2 min, was very small (5-HT 10 μmol l^{-1} 0.41 ± 0.09%, 5-HT 30 μmol l^{-1} 0.63 ± 0.11%, 5-HT 100 μmol l^{-1} 0.84 ± 0.13% of tritium content, 6 expts). In order to quantify the 5-HT-induced increase in tritium outflow, some slices were submitted to a different number of electrical pulses applied over a 2 min period. Figure 1b shows that 5-HT, applied for 2 min, caused a net extra efflux of tritium which was lower than that produced by applying 3 electrical pulses (5-HT 100 μmol l^{-1}: 0.84 ± 0.13; 3 electrical pulses

1.21 ± 0.012, see also Figure 1b). If the amine were present in the Krebs solution until the end of the experiment, the increase in tritium efflux vanished within a few min and after 15 min the tritium outflow was significantly reduced (Figure 2). Both the facilitatory and inhibitory effects were dose-dependent (see Figure 3) and calcium-sensitive. Tetrodotoxin, 0.5 μmol l^{-1} prevented only the facilitation leaving the inhibition of tritium efflux unchanged (Figure 2).

5-HT, 10 μmol l^{-1}, also increased the basal release of endogenous ACh from 0.39 ± 0.05 pmol g^{-1} min^{-1} to 0.54 ± 0.07 pmol g^{-1} min^{-1} (142 ± 2%) after 5 min and to 0.87 ± 0.14 pmol g^{-1} min^{-1} (224 ± 14%) after 10 min (5 expts). The facilitatory effect of 5-HT on endogenous ACh release was more persistent than the effect on tritium efflux overflow.

Effect of 5-HT antagonists on tritium efflux under resting conditions

None of the antagonists tested modified the tritium efflux at rest. Figure 4 shows that methysergide, methiothepin and ritanserin, all at 1 μmol l^{-1}, antagonized the facilitatory effect caused by 5-HT on tritium efflux. In the presence of methysergide and ritanserin, the concentration-effect curves shifted to the right. The pA_2 values were 8.7 and 6.7, respectively. In contrast, (−)-propranolol, up to 1 μmol l^{-1}, was unable to prevent the action of 5-HT on tritium overflow (Figure 4a). Ritanserin, 1 μmol l^{-1}, also prevented the 5-HT-mediated facilitation of endogenous ACh release from unstimulated slices (5 expts).

In order to examine the effect of 5-HT antagonists on 5-HT (100 μmol l^{-1})-induced inhibition, tetrodotoxin, 0.5 μmol l^{-1}, was added from the beginning of the perfusion (see Figure 2). Only methysergide, 3 μmol l^{-1}, and methiothepin, 1 μmol l^{-1}, antagonized the inhibition of resting transmitter overflow induced by 5-HT, whereas (−)-propranolol, 1 μmol l^{-1}, and ritanserin, 3 μmol l^{-1}, were ineffective both alone (Figure 4b) and when added together (data not shown).

Possible involvement of other transmitters in 5-HT effect

Bicuculline, 100 μmol l^{-1}, and naloxone, 1 μmol l^{-1}, did not change the facilitatory or inhibitory action of 5-HT on tritium efflux (Table 2). Similarly, atropine, 0.15 μmol l^{-1}, did not modify the increase in tritium overflow induced by 5-HT, 30 μmol l^{-1}, but it prevented the reduction of the efflux induced by 5-HT 100 μmol l^{-1}. Haloperidol, 0.25 μmol l^{-1}, almost completely antagonized both effects of 5-HT. Since this dopamine antagonist has an important anti-5-HT action (Leysen *et al.*, 1983), some experiments were performed in caudate nucleus slices taken from dopamine-depleted guinea-pigs. As shown in Figure 3, in depleted slices 5-HT displayed a facilitatory

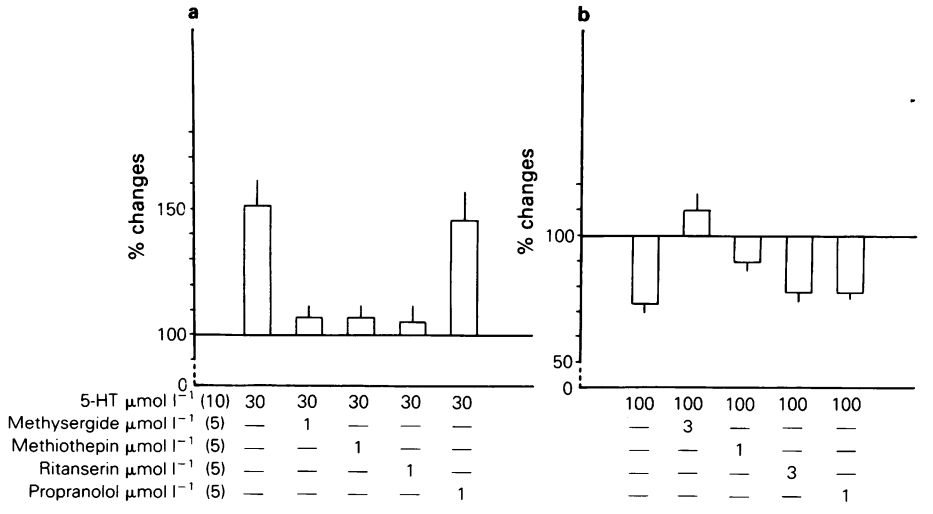

Figure 4 Effect of 5-hydroxytryptamine (5-HT) antagonists on the 5-HT-induced facilitation (a) and inhibition (b) of tritium efflux. The antagonists were added to Krebs solution at the start of perfusion. Two different sets of experiments were performed: with 5-HT 30 μmol l^{-1} to induce facilitation and with 5-HT 100 μmol l^{-1} (in the presence of tetrodotoxin 0.5 μmol l^{-1}) to induce inhibition. The data represent the percentage changes with respect to predrug efflux. Mean values are shown with s.e.mean indicated by vertical lines. Number of experiments in parentheses.

Table 2 Effect of different antagonists on spontaneous tritium efflux induced by 5-hydroxytryptamine (5-HT) from guinea-pig caudate nucleus slices

Experimental conditions	*No. of expts.*	*% of tritium efflux* (basal value = 100) *Increase by 5-HT* ($30\,\mu mol\,l^{-1}$)	*Decrease by 5-HT* ($100\,\mu mol\,l^{-1}$)
No pretreatment	15	149 ± 10*	73 ± 3*
Bicuculline $100\,\mu mol\,l^{-1}$	5	164 ± 7*	70 ± 6.3*
Naloxone $1\,\mu mol\,l^{-1}$	5	159 ± 2.5*	72 ± 2.4*
Atropine $0.15\,\mu mol\,l^{-1}$	5	198 ± 28*†	92 ± 1.7†
Haloperidol $0.25\,\mu mol\,l^{-1}$	5	108 ± 6.3†	90 ± 4.6†

Data represent the % changes with respect to predrug efflux. 5-HT $100\,\mu mol\,l^{-1}$ was tested in the presence of tetrodotoxin $0.5\,\mu mol\,l^{-1}$.
The antagonists were added to the Krebs solution from the beginning of the experiments.
The difference from predrug value is statistically significant $^*P < 0.05$, according to the Wilcoxon two sample test.
The difference from 5-HT alone (no pretreatment group) is statistically significant $\dagger P < 0.05$, Student's t test for non paired data.

effect greater than that observed in normal tissue. The concentration-effect curve was shifted to the left so that the threshold dose needed to cause an appreciable increase was reduced more than ten fold (Figure 3). In contrast, the late inhibition of resting overflow caused by 5-HT, $100\,\mu mol\,l^{-1}$, was prevented (Figure 3).

Effect of 5-HT on tritium efflux induced by electrical pulses

5-HT applied during a 2 min period of stimulation (S_2) did not change the S_2/S_1 ratio (controls: 0.92 ± 0.05; 5-HT $100\,\mu mol\,l^{-1}$ 0.93 ± 0.06, 5 expts). Conversely, when 5-HT was added 15 min before starting the second cycle of stimulation (S_2), the tritium efflux was significantly reduced (Table 1). This inhibitory effect was unchanged in slices perfused with paroxetine or with halved calcium concentration. Methysergide, $1\,\mu mol\,l^{-1}$, and methiothepin, $3\,\mu mol\,l^{-1}$, completely prevented inhibition of stimulated overflow by 5-HT but ritanserin and (−)-propranolol, up to $3\,\mu mol\,l^{-1}$ and $1\,\mu mol\,l^{-1}$ respectively, were ineffective (data not shown).

To ascertain whether the 5-HT-induced inhibition of the electrically-evoked tritium efflux was dopamine-mediated, some experiments were performed in dopamine-depleted slices. The results showed that (i) the net extra-overflow was significantly increased with respect to control slices reaching 8.02 ± 0.42% of tritium content and (ii) 5-HT maintained its inhibitory effect unchanged (Table 1). Methysergide, $1\,\mu mol\,l^{-1}$, and methiothepin, $3\,\mu mol\,l^{-1}$, antagonized this effect of 5-HT.

Discussion

The data in this study demonstrate that 5-HT can both increase and inhibit the tritium efflux from guinea-pig caudate nucleus slices preloaded with [^{3}H]-choline. Similar results were obtained by Kilbinger & Pfeuffer-Friederich (1985) in the guinea-pig myenteric plexus. On the other hand, Gillet *et al.* (1985) reported that 5-HT was ineffective on basal tritium efflux in the rat striatum. Our unpublished data obtained in rat caudate nucleus slices also agree with this last finding. This discrepancy might be due to the animal species used which can display different responsiveness to 5-HT (de Fatima Campos & das Chagas Rodriguez, 1987; Moser *et al.*, 1988). Moreover, it has recently been reported that no spontaneous synaptic activity was observed in rat neostriatal neurones (Galarraga *et al.*, 1987). This is in contrast to previous results obtained by us (Bianchi *et al.*, 1982) showing that in guinea-pig caudate nucleus slices a large part of the resting ACh release is tetrodotoxin-sensitive.

Effect of 5-HT on tritium efflux at rest

Facilitation Most of the experiments were performed by measuring tritium efflux, assuming that the released radioactive material for the most part represented [^{3}H]-ACh. Previous experiments carried out by different authors (Richardson & Szerb, 1974; Wikberg, 1977; Hertting *et al.*, 1980; Lehmann & Scatton, 1982) have, in fact, demonstrated that tritium recovered from superfused slices preloaded with [^{3}H]-choline and in the presence of HC-3 is a faithful tracer of the endogenous transmitter actually

released and makes it possible to estimate the influence of drugs on the release process.

The release of endogenous ACh as well as the efflux of tritium from unstimulated caudate nucleus slices partly represent the release of transmitter from the cholinergic nerve-endings at rest and partly must be ascribed to nerve-mediated activity. In fact, in guinea-pig caudate nucleus slices, tetrodotoxin reduces the endogenous ACh release to about 1/3 and the tritium efflux to about 75% (present results, see also Bianchi *et al.*, 1982; Beani *et al.*, 1984). Since cholinergic interneurones are present in this brain area, one can infer that their firing rate consistently contributes to the so-called spontaneous ACh (or tritium) efflux. Consequently the effect of drugs can depend on changes in the basal release from the nerve endings at rest and/or in the firing rate of the cholinergic interneurones. Tetrodotoxin treatment makes it possible to discriminate between these two components (Lehmann & Scatton, 1982). The facilitation of spontaneous tritium efflux induced by 5-HT vanished in a few minutes, was dose-dependent and was prevented by tetrodotoxin. This pattern of action suggests that 5-HT promotes the repetitive firing of cholinergic cells present in caudate nucleus slices. The short-lived effect may reflect the refractoriness of cholinergic neurones to 5-HT facilitation, although exhaustion of tritium stores cannot be ruled out. In fact change in the specific activity of the released material could occur in response to 5-HT agonists and/or antagonists. Thus the reliability of the response found with the tritium efflux method was verified by measuring the endogenous ACh release. These control experiments, limited to the relatively new aspect of 5-HT action i.e. to the facilitation, confirmed the findings obtained with tritium efflux studies. Obviously a quantitative comparison of the results achieved with the two approaches is unreliable (Beani *et al.*, 1984).

The receptor involved in the increase of tritium efflux by 5-HT seems to be of 5-HT_2 type. In fact, not only methysergide and methiothepin (effective at 5-HT_1 and 5-HT_2 sites) but above all ritanserin, a specific ligand of the 5-HT_2 sites (Janssen, 1985; Bradley *et al.*, 1986), antagonized 5-HT facilitation. In contrast (−)-propranolol which is a ligand of 5-HT_1 sites (Nahorski & Willcocks, 1983), failed to prevent the increase of tritium release by 5-HT.

The experiments performed to ascertain whether 5-HT directly or indirectly increased the spontaneous tritium efflux are in favour of a direct action, although other neurotransmitters or modulators, different from those tested in this study, must be considered.

The antagonism by haloperidol of the dual effect of 5-HT may be related to the anti-5-HT action of butyrophenone derivatives (Leysen *et al.*, 1983). On the other hand, the increase in tritium efflux induced by 5-HT in dopamine-depleted slices, was potentiated as if a tonic inhibitory control by dopamine on the cholinergic interneurones had been removed (Bianchi *et al.*, 1979; Hertting *et al.*, 1980).

Inhibition 5-HT, applied for a long time, decreased tritium efflux at rest in a concentration-dependent manner. The effect was evident even in the presence of tetrodotoxin; therefore its dependence on the preceding facilitation, as well as the involvement of voltage-dependent sodium channels, can be excluded. The inhibition is long lasting (see Figure 2) at variance with the rapid disappearance of 5-HT facilitation found even with low 5-HT doses, unable to cause inhibition.

Attempts to identify the putative transmitters possibly involved in this inhibition demonstrate that bicuculline (a GABA_A receptor antagonist) and naloxone (an opioid receptor antagonist) were ineffective. In contrast, 5-HT, tested in dopamine-depleted slices failed to inhibit tritium efflux at rest. Therefore 5-HT does not seem to exert its inhibitory effect directly but by releasing dopamine which, in turn, inhibits ACh release (Bianchi *et al.*, 1979; Hertting *et al.*, 1980).

Another point that must be considered is the antagonism displayed by atropoine versus 5-HT inhibition. Probably the increase in Ca^{2+} influx induced by atropine (Nordstrom *et al.*, 1981), as reflected by the increased tritium efflux (see Table 2), could have obscured the 5-HT effect. This interpretation agrees with what has been reported by Pedata *et al.* (1983) who demonstrated that, in the presence of atropine, adenosine did not reduce ACh release. This would suggest that heteromodulation of ACh release is effective only when the autoreceptors are operative.

Among the antagonists tested, ritanserin and (−)-propranolol were totally ineffective while methysergide and methiothepin, although at high concentrations, prevented 5-HT inhibition at rest. These results do not allow for identification of the receptor involved. In fact, ritanserin, a specific ligand of 5-HT_2 receptor sites (Janssen, 1985; Bradley *et al.*, 1986), and (−)-propranolol which stereospecifically displaces [^{3}H]-5-HT from 5-HT_1, sites (Nahorski & Willcocks, 1983) were both ineffective. The antagonism by methysergide and methiothepin of the inhibition of tritium efflux caused by 5-HT may be due to stimulation of 5-HT_1 or 5-HT_2 sites other than those classified or recognized to date.

Effect of 5-HT on electrically-evoked tritium efflux

The present data concerning the effect of 5-HT on tritium efflux induced by electrical stimulation shows

that, in accordance with previous findings (Vizi *et al.*, 1981; Gillet *et al.*, 1985), 5-HT inhibits tritium outflow and that methysergide or methiothepin significantly reduce its action. However, the effect is evident only at high doses of 5-HT ($100\,\mu mol\,l^{-1}$) and is not changed even by a reuptake inhibitor such as paroxetine (Table 1). This pattern of action differs from that reported by other authors (De Belleroche & Gardiner, 1982; Gillet *et al.*, 1985) who demonstrated that 5-HT even at low concentrations of 1–$10\,\mu mol\,l^{-1}$, inhibited tritium efflux induced by potassium ($25\,mmol\,l^{-1}$) in rat brain slices. The different animal species (guinea-pig versus rat) and the different kind of stimulation (electrical stimulation vs KCl depolarization) are probably responsible for this discrepancy. Since the inhibition of electrically evoked tritium outflow is unchanged in slices obtained from dopamine-depleted guinea-pigs, it is reasonable to assume that 5-HT directly inhibits the tritium efflux, as already described by Gillet *et al.* (1985). It is worth noting, however, that in the dopamine-depleted slices the net extra release increases, suggesting a tonic control by the amine of the cholinergic cells (Bianchi *et al.*, 1979; Hertting *et al.*, 1980). In conclusion, 5-HT seems to exert a dual effect on the spontaneous tritium efflux: i.e. first a transient enhancement and, later, a dopamine-mediated inhibition of efflux. Thus, the physiological effects of 5-HT might at first be to activate and then inhibit the cholinergic neurones. Clearly the prolonged release of endogenous 5-HT in the striatum inhibits the electrosecretory coupling of the cholinergic structures as previously reported by others.

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