5-HT_{1A} agonists increase and 5-HT₃ agonists decrease acetylcholine efflux from the cerebral cortex of freely-moving guinea-pigs

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- 1 The influence of 5-hydroxytryptamine_{1A} (5-HT_{1A}), 5-HT₂ and 5-HT₃ agonists and antagonists on acetylcholine (ACh) release from the cerebral cortex was studied in freely moving guinea-pigs.
- 2 8-Hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT, 0.01–1 mg kg⁻¹, s.c.) caused the 5-HT syndrome and dose-dependently increased ACh release. Ru 24969 (1–10 mg kg⁻¹, s.c.) shared the same effects, but it was less potent. (–)-Propranolol (5 mg kg⁻¹) and metitepine (2 mg kg⁻¹) prevented these behavioural and neurochemical responses.
- 3 (±)-1(4-Iodo-2,5-dimethoxyphenyl)-2-aminopropane (DOI) up to 2 mg kg⁻¹ did not modify ACh release and ketanserin (0.5 mg kg⁻¹) was ineffective on 5-HT-induced changes of ACh outflow.
- 4 2-Methyl-5-HT (500 µg, i.c.v.) and 5-HT (500 µg, i.c.v.) plus metitepine (2 mg kg⁻¹, s.c.) inhibited the gross behaviour and ACh release. ICS 205-930 (0.5 mg kg⁻¹) prevented these responses.
- 5 2-Methyl-5-HT, up to 10 µmol l⁻¹, and 8-OH-DPAT, up to 0.1 µmol l⁻¹, (like 5-HT) did not change [³H]-choline efflux from cerebral cortex slices.
- 6 These results suggest that exogenous 5-HT and related selective agonists modulate guinea-pig cortical cholinergic structures through 5-HT_{1A} and 5-HT₃ receptors. The stimulation of 5-HT_{1A} autoreceptors may lead to disinhibition of the cholinergic cells, tonically inhibited by the tryptaminergic control. Conversely, the stimulation of 5-HT₃ receptors inhibits ACh release, possibly through an interneurone. No direct 5-HT modulation of the cholinergic nerve endings was found.

Introduction

There is general agreement that 5-hydroxytryptamine (5-HT) exerts an inhibitory control on the cholinergic system in the brain. 5-HT or 5-HT agonists increase acetylcholine (ACh) content *in vivo* and reduce ACh release from different brain areas *in vitro* (Envrard *et al.*, 1977; Samanin *et al.*, 1978; Consolo *et al.*, 1980; Vizi *et al.*, 1981; Gillet *et al.*, 1985; Bianchi *et al.*, 1989).

On the other hand it has been demonstrated that 5-HT, injected into the lateral ventricles, increases ACh release from the cerebral cortex of freely moving guinea-pigs. This effect has been attributed to the activation of 5-HT autoreceptors, because it was prevented by pretreating the animals with 5,7-DHT and was converted into inhibition by metitepine (Bianchi *et al.*, 1986).

5-HT_{1A} and 5-HT_{1B} recognition sites seem to function as autoreceptors on 5-HT neurones ascending from the brain raphe nuclei to the hippocampus and cerebral cortex (Sprouse & Aghajanian, 1987; Sharp *et al.*, 1989a,b) while activation of 5-HT₃ receptors reduces ACh release from the rat entorhinal cortex *in vitro* (Barnes *et al.*, 1989). Thus, experiments have been performed, with the now available selective 5-HT agonists and antagonists, to obtain confirmatory evidence that the autoreceptors are of the 5-HT_{1A} subtype and that 5-HT exerts a tonic inhibitory control on cholinergic cells through 5-HT₃ receptors in the guinea-pig.

Methods

Experiments *ex vivo*

Guinea-pigs of either sex weighing 400–500 g were anaesthetized with pentobarbitone sodium (30 mg kg⁻¹, i.p.) plus morphine sulphate (2 mg kg⁻¹, i.p.) immediately prior to and 3 h after the barbiturate. The implantation of an epidural cup

on the parietal cortex and of a guide cannula in the lateral ventricle (for i.c.v. injections) were made as previously described (Beani *et al.*, 1968; 1978). One day after surgery, the animals were trained to be gently handled. The efflux of ACh from the parietal cortex was measured 2–3 days after surgery by filling the cup with 0.25 ml of Ringer solution containing physostigmine 0.3 mmol l⁻¹. The solution was collected and renewed every 30 min. As a rule the experiments consisted of 3 collection periods before (controls) and 4–6 periods after treatment.

The ACh present in the samples was bioassayed on tetrodotoxin-pretreated guinea-pig ileum (Beani *et al.*, 1978). This method is of high sensitivity (0.5 pmol) and gives results similar to those obtained with mass spectrometry analysis (Beani *et al.*, 1988). The release of ACh from the cerebral cortex was expressed as pmol cm⁻² 30 min⁻¹ and the effect of treatment was evaluated as a percentage of the mean pretreatment value.

Experiments *in vitro*

In another set of experiments 5-HT agonists and antagonists were tested *in vitro* in superfused slices of parietal cortex preloaded with [³H]-choline (0.1 µmol l⁻¹, specific activity 80 Ci mmol⁻¹) and submitted to electrical stimulation as previously described (Beani *et al.*, 1984). The cortical slices were transferred into perfusion chambers (0.9 ml vol) and perfused at a rate of 0.5 ml min⁻¹ with a Krebs solution containing hemicholinium-3 10 µmol l⁻¹, gassed with O₂ (95%) and CO₂ (5%). The tissue was electrically stimulated for 2 min (2 Hz, alternate pulses of 5 ms duration, strength 30 mA cm⁻²) at 45 min (S₁) and 75 min (S₂) min from the start of the superfusion. Drugs were added 10 min before S₂ and their effects were expressed as changes in the S₂/S₁ ratio (Beani *et al.*, 1984).

Drugs

The compounds used were obtained from the following sources: 5-hydroxytryptamine creatine sulphate, methysergide

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maleate and tetrodotoxin (Sigma); metitepine (Hoffman La Roche); (–)-propranolol HCl (ICI); ketanserin (Janssen, Belgium); 8-hydroxy-2-(di-n-propylamino) tetralin HBr (8-OH-DPAT) and (±)-1-(4-iodo-2,5-dimethoxyphenyl)-2-aminopropane · HCl (DOI) (RBI, U.S.A.); 5-methoxy-3-(1,2,3,6-tetrahydro-4-pyridinyl)-1H-indole (RU24969, Roussel-UCLAF, France); (3a-tropanyl)-1H-indole-3-carboxylic acid ester (ICS 205-930) and 2-methyl-5-HT (Sandoz, Switzerland).

Statistical analysis

The differences in ACh release before and after drug treatment, as well as differences after individual and paired treatments, were analysed by analysis of variance and Student's *t* test or with the Wilcoxon two sample test, depending on the experimental protocol as specified in the table and figure legends.

Results

Effect of 5-HT₁ agonists and antagonists

8-OH-DPAT, a selective 5-HT_{1A} receptor agonist, injected i.c.v. or s.c. caused the 5-HT behavioural syndrome (Goodwin *et al.*, 1987) and dose-dependently increased ACh release (Figure 1a,b,d). The effect reached its maximum at 60 min and lasted for more than 2 h. RU-24969, a mixed 5-HT_{1A} and 5-HT_{1B} agonist was also effective but less potent (Figure 1c and d).

(–)-Propranolol, a non selective 5-HT₁ antagonist (Nahorski & Willcocks, 1983), had no direct effect on normal behaviour or on ACh release up to 5 mg kg^{−1} i.p. but, as

Figure 1 Effect of 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) and RU 24969 on acetylcholine (ACh) release from the cerebral cortex of freely moving guinea-pigs. At the arrow: (a) 8-OH-DPAT injected i.c.v.; (b) 8-OH-DPAT injected s.c.; (c) RU 24969 injected s.c.; (d) relationship between s.c. doses and maximum % effect of 8-OH-DPAT (●) and RU 24969 (▲). Each point is the mean of 5–6 values; vertical lines: s.e.mean. The absolute pre-drug mean values of ACh release (pmol cm^{−2} 30 min^{−1}) were: 118.5 ± 2.2. * $P < 0.05$, ** $P < 0.01$, significantly different from pre-drug values, according to ANOVA and Student's *t* test for paired data.

expected, it prevented the facilitatory effect of 8-OH-DPAT both on behaviour and on ACh release (Figure 2a). Pretreatment of guinea-pigs with metitepine (2 mg kg^{−1}) increased ACh release. 8-OH-DPAT, given 30 min after metitepine, did not further increase nor did it inhibit the ACh release (Figure 2b). This result contrasts with the effect of 5-HT which inhibited the increased ACh release caused by metitepine (Figure 2b, see also Bianchi *et al.*, 1986).

The possible role of 5-HT_{1C} receptors in the reduction of ACh outflow caused by 5-HT after metitepine was assessed in mesulergine pretreated guinea-pigs. This 5-HT_{1C} antagonist, (Pazos *et al.*, 1985) up to 0.5 mg kg^{−1} i.p., did not primarily change ACh release (which was 98.6 ± 5.6% of basal values 60 min after drug injection, mean ± s.e.mean of 5 expts.) and did not modify the effect of 5-HT after metitepine. In fact, the percentage changes of transmitter release (with respect to the antagonists alone) calculated 60 min after 5-HT (500 µg, i.c.v.) was 57 ± 10% after metitepine plus mesulergine and 55 ± 8% after metitepine alone (Figure 5).

Effect of 5-HT₂/5-HT_{1C} agonists and antagonists

To examine whether the inhibitory modulation of the cholinergic structures by 5-HT might be related to 5-HT₂ or 5-HT_{1C} receptor activation, the effect of DOI and ketanserin were tested.

As shown in Figure 3a, DOI, injected s.c. at 2 mg kg^{−1}, did not significantly change ACh release. Ketanserin (0.5 mg kg^{−1}, i.p.), *per se* was ineffective (112% ± 8.8 with respect to pre-drug release 30 min after injection, 5 expts), and did not change the effect of 5-HT either in normal or in metitepine-pretreated guinea-pigs (Figure 3a and Figure 5).

Effect of 5-HT₃ agonists and antagonists

In view of the findings that 5-HT₁ and 5-HT₂/5-HT_{1C} receptors did not seem to be involved in the inhibitory control of the cholinergic neurones, the role of 5-HT₃ receptors on ACh release was tested. 2-Methyl-5-HT, a relatively selective 5-HT₃ agonist (Richardson & Engel, 1986), injected i.c.v. did not modify ACh release at 250 µg but significantly inhibited it at 500 µg causing behavioural depression (Figure 4). These effects were prevented by ICS 205-930, a selective 5-HT₃ antagonist (Richardson *et al.*, 1985), at 0.5 mg kg^{−1} s.c. This drug, *per se*, transiently increased ACh release and caused a delayed ACh

Figure 2 (a) Effect of 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) 0.1 mg kg^{−1} s.c. (at the arrow) on acetylcholine (ACh) release from the cerebral cortex of freely moving guinea-pigs pretreated (30 min before) with (–)-propranolol 5 mg kg^{−1} i.p. (●). The effects of 8-OH-DPAT (■) and (–)-propranolol (▲) given alone are also shown. (b) Effect of 5-hydroxytryptamine (5-HT) 500 µg i.c.v. (●) or 8-OH-DPAT 0.1 mg kg^{−1} s.c. (▲) (at the arrow) on ACh release from the cerebral cortex of guinea-pigs pretreated (30 min before) with metitepine 2 mg kg^{−1} i.p. The effect of metitepine given alone (■) is also shown. Each point is the mean of 5–6 values; vertical lines: s.e.mean. The absolute pre-drug mean values of ACh release (pmol cm^{−2} 30 min^{−1}) were: 130 ± 3.2. * $P < 0.05$, ** $P < 0.01$, significantly different from metitepine alone, according to ANOVA and Student's *t* test for non-paired data.

Figure 3 (a) Effect of ($\pm$)-1-(4-iodo-2,5-dimethoxyphenyl)-2-amino-propane (DOI) 2 mg kg^{-1} i.p. (at the arrow) on acetylcholine (ACh) release from the cerebral cortex of freely moving guinea-pigs. (b) Effect of 5-hydroxytryptamine (5-HT) $500 \mu\text{g}$ i.c.v. (at the arrow) on ACh release from the cerebral cortex of freely moving guinea-pigs pretreated (30 min before) with ketanserin 0.5 mg kg^{-1} i.p. (▲). The effect of 5-HT given alone is also shown (■). Each point is the mean of 5–6 values; vertical lines: s.e.mean. The absolute pre-drug mean values of ACh release ($\text{pmol cm}^{-2} 30 \text{ min}^{-1}$) were: 133 ± 2.9 . * $P < 0.05$ significantly different from ketanserin alone, according to ANOVA and Student's *t* test for paired data.

increase in the presence of 2-methyl-5-HT (Figure 4). These two effects, however, were not significant. As shown in Figure 5, ICS 205-930, unlike ketanserin and propranolol, fully prevented the inhibition of ACh release caused by 5-HT in metitepine-pretreated animals. 2-Methyl-5-HT ($500 \mu\text{g}$, i.c.v.), like 5-HT, induced a deep depression of transmitter release in metitepine-pretreated guinea-pigs. The percentage changes of ACh release (with respect to antagonist alone) at 60 and 90 min after 2-methyl-5-HT were 50 ± 1.5 and 57 ± 9 respectively (mean $\pm$ s.e.mean of 4 animals, $P < 0.05$).

Effect of 5-HT agonists on ACh release from cerebral cortex slices

As shown in Table 1, 2-methyl-5-HT, applied 15 min before S_2 , did not change [^3H]-choline efflux induced by electrical pulses. Only at $100 \mu\text{mol l}^{-1}$ did 2-methyl-5-HT moderately, but significantly reduce the S_2/S_1 ratio. However, ICS 205-930

Figure 4 Effect of 2-methyl-5-HT (▲) $500 \mu\text{g}$ i.c.v. (at the arrow) on acetylcholine (ACh) release from the cerebral cortex of freely moving guinea-pigs and antagonism by ICS 205-930 (■) 0.5 mg kg^{-1} injected s.c. 30 min before. The effect of ICS 205-930 given alone is also shown (★). Each point is the mean of 5 values; vertical lines: s.e.mean. The absolute pre-drug mean values of ACh release ($\text{pmol cm}^{-2} 30 \text{ min}^{-1}$) were: 118.5 ± 2.5 . * $P < 0.05$, significantly different from pre-drug values, according to ANOVA and Student's *t* test for paired data.

Figure 5 Effect of 5-hydroxytryptamine (5-HT, $500 \mu\text{g}$ i.c.v., arrow) on acetylcholine (ACh) release from the cerebral cortex of freely moving guinea-pigs pretreated (30 min before) with metitepine 2 mg kg^{-1} i.p. (■); metitepine plus ketanserin 0.5 mg kg^{-1} i.p. (●); metitepine plus (–)-propranolol 5 mg kg^{-1} i.p. (★); metitepine plus ICS 205-930 0.5 mg kg^{-1} s.c. (▲). The values after 5-HT are expressed as % of the effect of the antagonists alone. Each point is the mean of 5–7 values; vertical lines: s.e.mean. The absolute pre-drug mean values of ACh release ($\text{pmol cm}^{-2} 30 \text{ min}^{-1}$) were: 123 ± 2.7 . * $P < 0.05$ significantly different from other treatments, according to ANOVA and Student's *t* test for non-paired data.

($1 \mu\text{mol l}^{-1}$) did not prevent this inhibition. Also in the presence of ritanserin $1 \mu\text{mol l}^{-1}$, 2-methyl-5-HT did not inhibit ACh release (Table 1).

In another set of experiments, the effect of 8-OH-DPAT was checked. The results showed that even this 5-HT_{1A} agonist, up to $0.1 \mu\text{mol l}^{-1}$, did not modify [^3H]-choline efflux (S_2/S_1 ratio: controls, 1.01 ± 0.04 ; 8-OH-DPAT, 1.02 ± 0.05 ; mean of 5 experiments $\pm$ s.e.mean). Thus any direct action of 2-methyl-5-HT and of 8-OH-DPAT on the electrosecretory coupling process has been ruled out.

Discussion

The present study demonstrates that the administration of 8-OH-DPAT (i.c.v. or s.c.) and RU 24969 (s.c.) increases ACh release and that 2-methyl-5-HT, injected i.c.v., reduces ACh release from the cerebral cortex of freely moving guinea-pigs.

The increased transmitter release induced by 8-OH-DPAT may depend upon the activation of somatodendritic 5-HT_{1A}

Table 1 Effect of 2-methyl-5-hydroxytryptamine (2-Me-5-HT) on electrically-evoked (2 Hz) [^3H]-choline efflux from parietal cortex slices of the guinea-pig

Experimental conditions	S_2/S_1	No. of expts.
Control	0.99 ± 0.03	9
2-Me-5-HT $3 \mu\text{mol l}^{-1}$	0.97 ± 0.10	3
2-Me-5-HT $10 \mu\text{mol l}^{-1}$	1.00 ± 0.07	3
2-Me-5-HT $100 \mu\text{mol l}^{-1}$	$0.80 \pm 0.02^*$	5
2-Me-5-HT $100 \mu\text{mol l}^{-1}$ + ICS 205-930 $1 \mu\text{mol l}^{-1}$	$0.82 \pm 0.06^*$	4
2-Me-5-HT $3 \mu\text{mol l}^{-1}$ + ritanserin $1 \mu\text{mol l}^{-1}$	0.90 ± 0.03	7

2-Me-5-HT was added 10 min before S_2 . The antagonists were added to Krebs solution from the beginning of the experiments. The S_2/S_1 ratios in control and treated slices are given.

* Significantly different from controls, $P < 0.05$, according to the Wilcoxon two sample test.

autoreceptors. They are known to reduce the firing rate of 5-HT neurones (De Montigny *et al.*, 1984; Van der Maelen *et al.*, 1986; Blier & De Montigny, 1987; Sprouse & Aghajanian, 1987) and to reduce 5-HT release *in vivo* (Sharp *et al.*, 1989a,b), but less so *in vitro* (Middlemiss, 1984a,b; Engel *et al.*, 1986; Maura & Raiteri, 1986). Consequently, 5-HT_{1A} activation in the whole animal reduces the tonic, inhibitory control on cholinergic cells, as previously inferred from indirect evidence (Bianchi *et al.*, 1986) and leads to increased cortical ACh release, as demonstrated by the present finding.

A direct enhancing effect of 5-HT_{1A} agonists on ACh release from the nerve endings has been ruled out as shown by the negative results from electronically driven neocortical slices, known to contain cholinergic axons. However, a disinhibition of cholinergic transmission via 5-HT_{1A} receptors located on inhibitory cortical interneurons remains a possible mechanism in the whole animal.

The experiments carried out to test whether 5-HT inhibition was due to 5-HT_{1C} or 5-HT₂ receptors proved negative. In fact, DOI, a putative 5-HT₂/5-HT_{1C} agonist (Shannon *et al.*, 1984) did not change ACh release and ketanserin or mesulergine, which have antagonistic activity at both 5-HT₂ and 5-HT_{1C} receptors (Leysen *et al.*, 1983; Pazos *et al.*, 1985), were ineffective against either inhibited or stimulated ACh release induced by 5-HT.

In contrast, 5-HT₃ receptors are clearly involved in the inhibition of ACh release. 2-Methyl-5-HT, a selective 5-HT₃ agonist (Richardson & Engel, 1986) inhibits ACh release both in normal and metitepine-pretreated guinea-pigs and ICS 205-930, a selective 5-HT₃ antagonist (Richardson *et al.*, 1985), prevents both 2-methyl-5-HT as well as 5-HT plus metitepine-induced inhibition. As regards the localization of this inhibitory control, it is worth noting that 2-methyl-5-HT alone or plus ritanserin, tested on guinea-pig cortical slices showed a slight inhibition but only at high concentrations and ICS 205-930 did not prevent it. These findings differ from the recent report of Barnes and coworkers (1989) demonstrating that low 2-methyl-5-HT concentrations (plus ritanserin) inhib-

ited ACh release from slices of rat entorhinal cortex. The differences in animal species, cerebral area studied and type of stimulation (KCl versus electrical stimulation) are probably responsible for this discrepancy. In any case, since 2-methyl-5-HT is ineffective in guinea-pig cortical slices, it is conceivable that 5-HT inhibits, through 5-HT₃ receptors, the cholinergic cells either directly or via interneurons.

It is generally accepted that 5-HT₃ receptors exert stimulatory effects on the target neurones and facilitate the release of transmitters (Gaddum & Piccarelli, 1957; Fozard & Mobarak Ali, 1978; Hagan *et al.*, 1987; Carboni *et al.*, 1989). This evidence opens the question as to whether 5-HT₃-induced ACh inhibition is direct or mediated through other neuronal pools. The available data lead one to rule out dopamine involvement. Previous studies, in fact, have demonstrated that dopamine release is associated with increased ACh outflow from the cortex (Bianchi *et al.*, 1979). Experiments are in progress to ascertain whether other neuronal systems known to inhibit ACh release, such as the noradrenergic, the GABAergic and enkephalinergic neurones, are involved (but see, Barnes *et al.*, 1989).

In conclusion, exogenous 5-HT can modulate the guinea-pig corticopetal cholinergic structures: (i) by stimulating 5-HT_{1A} somatodendritic autoreceptors: this leads to disinhibition of the cholinergic cells and increased ACh release; (ii) by activating 5-HT₃ receptors: they probably trigger other neuronal pools which, in turn, inhibit the cholinergic cells. Since the subclasses of 5-HT receptors are topographically and functionally different in different animals (Peroutka, 1988; Hoyer, 1989; Hoyer & Middlemiss, 1989; Burridge *et al.*, 1989; Siniscalchi *et al.*, 1990), caution must be taken in extrapolating these conclusions to other species, including man. In any case, the present data stress the physiological inhibitory role played by 5-HT through 5-HT₃ receptors on the cholinergic system projecting into the cerebral cortex.

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