European Journal of Pharmacology, 129 (1986) 333–337
Elsevier

EJP 00529

Cholinergic terminals in rat hippocampus possess 5-HT_{1B} receptors mediating inhibition of acetylcholine release

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Received 17 April 1986, revised MS received 1 July 1986, accepted 15 July 1986

The effects of 5-hydroxytryptamine (5-HT) on the release of [^{3}H]acetylcholine ([^{3}H]ACh) from rat hippocampal nerve endings were investigated using synaptosomes labelled with [^{3}H]choline and depolarized in superfusion with 15 mM KCl. The release of [^{3}H]ACh was concentration dependently inhibited by exogenous 5-HT. The concentration-response curve of 5-HT was shifted to the right in a parallel way by methiothepin. The 5-HT_2 antagonists ketanserin or methysergide did not antagonize the effect of 5-HT. The 5-HT_1 agonist 5-methoxy-3-[1,2,3,6-tetrahydropyridin-4-yl]-1H-indole (RU 24969) mimicked 5-HT, whereas the 5-HT_{1A} selective agonist 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) was ineffective. When used as a 5-HT_{1A}/5-HT_{1B} antagonist, (−)propranolol antagonized 5-HT whereas spiperone (a 5-HT_{1A} displacer) did not. The 5-HT_{1C} selective antagonist mesulergine was also ineffective towards 5-HT. It can be concluded that hippocampal cholinergic terminals are endowed with inhibitory 5-HT receptors which appear to belong to the 5-HT_{1B} subtype.

5-HT_1 receptor subtypes; ACh release; Hippocampus; Presynaptic receptors; (Rat, Superfused synaptosomes)

1. Introduction

The hippocampus receives serotonergic projections from both the median raphe nucleus and the dorsal raphe nucleus (Azmitia, 1978). The same brain area also receives cholinergic projections originating in the medial septum. Interactions between acetylcholine (ACh) and 5-hydroxytryptamine (5-HT) are therefore possible in the hippocampus.

To our knowledge the effects of 5-HT on the release of hippocampal ACh have not been investigated. In fact, only few studies have considered this interaction in the central nervous system. On the basis of results obtained with rat striatal slices it has been proposed (Vizi et al., 1981; Gillet et al., 1985) that 5-HT can inhibit the release of ACh. An inhibitory effect of 5-HT on ACh release was also observed in slices of rat nucleus accumbens (De Belleroche and Gardiner, 1982). More recently, Beani et al. (1985) reported that, in slices from guinea-pig caudate, 5-HT transiently increased and subsequently inhibited the basal efflux of ACh.

We have investigated the effects of 5-HT on the release of [^{3}H]ACh from rat hippocampus synaptosomes prelabelled with [^{3}H]choline and found that 5-HT inhibitory receptors are sited on ACh nerve terminals. These receptors were characterized pharmacologically, in view of the existence of subtypes of the 5-HT receptor.

2. Materials and methods

2.1. Preparation and labelling of synaptosomes

Crude synaptosomes were prepared according to the method of Gray and Whittaker (1962) with minor modifications. Briefly, the hippocampus of

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adult male Sprague-Dawley rats (200-250 g) was homogenized in 40 volumes of 0.32 M sucrose buffered at pH 7.4 with phosphate. The homogenate was centrifuged (5 min, $1000 \times g$) to remove nuclei and debris, and synaptosomes were isolated from the supernatant by centrifugation at $12000 \times g$ for 20 min. The synaptosomal pellet was then resuspended in a physiological salt solution having the following composition (mM): NaCl 125, KCl 3, $MgSO_4$ 1.2, $CaCl_2$ 1.2, NaH_2PO_4 1.0, $NaHCO_3$ 22, glucose 10 (aeration with 95% O_2 and 5% CO_2 at 37°C), pH 7.2-7.4.

The synaptosomes were incubated 15 min at 37°C in a rotary water-bath in an atmosphere of 95% O_2 and 5% CO_2 with [^{3}H]Ch (final concentration 0.07 μM).

The protein content of the synaptosomal suspensions was determined by the method of Bradford (1976) using bovine serum albumin as standard.

2.2. Release experiments

After labelling, aliquots of the synaptosomal suspension (0.4 mg protein per filter) were distributed on 0.65 μm Millipore filters placed at the bottom of a set of parallel superfusion chambers maintained at 37°C (Raiteri et al., 1974; Raiteri and Levi, 1978) and layered by moderate vacuum filtration. Superfusion was then started at a rate of 0.6 ml/min, with standard medium (aeration with 95% O_2 and 5% CO_2) pH 7.3-7.4; duration 76 min. The superfusate was collected in 3 min samples (basal release) before and after 6 min samples (evoked release). Two 2 min periods of depolarization (15 mM KCl substituting an equimolar concentration of NaCl) were applied 38 and 66 min after the start of superfusion (S_1 and S_2, respectively). The antagonists were added 8 min before and the agonists concomitantly with high-K^+ (S_2).

[^{3}H]ACh and [^{3}H]choline were separated in one set of experiments. One milliliter of 0.1 N HCl was added to the filters to extract [^{3}H]ACh and [^{3}H]choline. Aliquots of the fractions collected (300 μl) and of the HCl extract (50 μl) were lyophilized and stored at −90°C until separation of [^{3}H]ACh from [^{3}H]choline according to Marchi et al. (1983). Under the experimental conditions described the [^{3}H]ACh expressed as a percent of the total radioactivity was: basal release, 43.7 ± 3.91; K^+-evoked release, 91.1 ± 0.81; synaptosomes, 87.5 ± 0.81.

2.3. Calculation and statistics

The amount of radioactivity released into each fraction was expressed as percentage of the total synaptosomal tritium at the start of the fraction in question. The K^+-evoked release was calculated by subtracting the percentage of tritium radioactivity present in the two 3 min fractions collected before and after the 6 min fraction containing the K^+-induced overflow. The drug effects were evaluated from the ratio (S_2/S_1) of the tritium release evoked during the two stimulation periods and were compared to the corresponding ratio obtained under control conditions. Student's t-test was used for analyzing the significance of the difference between two means.

2.4. Drugs

[Methyl-^{3}H]Choline chloride (spec. act. 80 Ci/mmol) was obtained from Amersham Int. (Amersham, U.K.); 8-OH-DPAT was purchased from RBI (Wayland, MA) and 5-HT creatinine sulfate from Calbiochem (Los Angeles, CA). The following compounds were gifts: RU 24969 (Roussel Uclaf, Romainville, France), methiothepin (Hoffman-La Roche, Basel, Switzerland), methysergide and mesulergine (Sandoz, Basel, Switzerland), ketanserin and spiperone (Janssen, Beerse, Belgium), (−)-propranolol (ICI, Macclesfield, UK).

3. Results

3.1. Effect of 5-HT on the Ca^{2+}-dependent release of [^{3}H]ACh evoked by high-K^+

Depolarization with 15 mM K^+ caused the release of [^{3}H]ACh from rat hippocampal synaptosomes prelabelled with [^{3}H]choline. The release was totally Ca^{2+}-dependent (not shown).

Figure 1 shows that the K^+-evoked release of

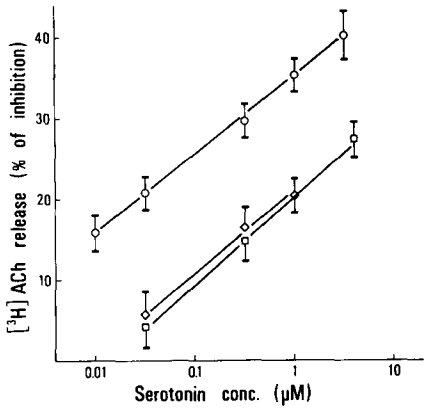

Fig. 1. Effect of methiothepin and (−)propranolol on the inhibition by 5-HT of the K^+-evoked release of [^{3}H]ACh from rat hippocampal synaptosomes. Crude synaptosomes were prepared, prelabelled with [^{3}H]choline and superfused as described under Materials and methods. Depolarization was carried out for 90 s with 15 mM KCl (S_1 and S_2). Exogenous 5-HT was added concomitantly with 15 mM KCl and the antagonists were present starting 8 min before depolarization (S_2). The overflow in S_1 and S_2 was 2.92 ± 0.26 and 1.98 ± 0.20%, respectively; the mean ratio (S_2/S_1) was 0.68 ± 0.02. Means ± S.E.M. of 4-8 experiments in triplicate are presented. All values were significantly different from the corresponding values obtained in the absence of antagonist (at least $P < 0.001$). (○) 5-HT; (□) 5-HT + 0.3 μM methiothepin; (◇) 5-HT + 1 μM (−)propranolol.

[^{3}H]ACh was inhibited in a concentration-dependent way by exogenous 5-HT added to the superfusion medium. The maximum inhibition amounted to about 50% and was obtained with 100 μM 5-HT (not shown in the figure). The pEC_{30} values (negative logarithm of the 5-HT concentration causing 30% inhibition of the K^+-evoked release) amounted to 6.55.

3.2. Effects of 5-HT antagonists

The concentration-response curve of 5-HT was shifted to the right in a parallel way when the nerve endings were exposed to methiothepin (0.3 μM) or to (−)propranolol (1 μM; fig. 1).

In contrast to methiothepin and (−)propranolol, the 5-HT antagonists ketanserin, methysergide, spiperone and mesulergine did not counteract the inhibitory effect of 5-HT on the K^+-evoked release of [^{3}H]ACh (table 1).

TABLE 1

Effects of 5-HT antagonists on the inhibition by 5-HT of the K^+-evoked release of [^{3}H]ACh from hippocampal synaptosomes. Experimental details as in the legend to fig. 1 and Materials and methods. The data are means ± S.E.M. The number of experiments is given in parentheses. [a] $P < 0.001$ vs. 0.3 μM 5-HT; [b] not significantly different from 0.3 μM 5-HT; [c] not significantly different from 0.3 μM 5-HT.

Drugs	% of inhibition
0.3 μM 5-HT	29.61 ± 2.22 (10)
0.3 μM 5-HT + 0.3 μM methiothepin	13.29 ± 2.25 (6) [a]
0.3 μM 5-HT + 1 μM (−)propranolol	16.59 ± 2.45 (10) [a]
0.3 μM 5-HT + 1 μM methysergide	28.01 ± 2.03 (4) [b]
0.3 μM 5-HT + 1 μM ketanserin	31.45 ± 2.87 (3) [b]
0.3 μM 5-HT + 1 μM mesulergine	30.09 ± 3.95 (3) [b]
0.03 μM 5-HT	21.29 ± 1.80 (5)
0.03 μM 5-HT + 0.3 μM spiperone	23.91 ± 3.02 (3) [c]

3.3. Effects of the 5-HT$_1$ agonists RU 24969 and 8-OH-DPAT

The depolarization-induced release of [^{3}H]ACh was inhibited by RU 24969 in a concentration-dependent manner ($pEC_{30} = 6.24$). In contrast, 8-OH-DPAT was ineffective when tested at 1 μM, a concentration 100 times higher than that producing, in the case of 5-HT or RU 24969, about 15% inhibition of [^{3}H]ACh release (fig. 2).

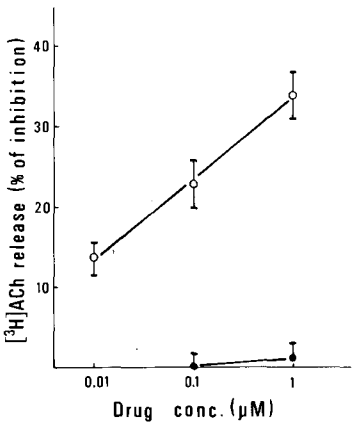

Fig. 2. Effect of RU 24969 and 8-OH-DPAT on the K^+-evoked release of [^{3}H]ACh. Experimental details as in the legend to fig. 1 and Materials and methods. The data presented are means ± S.E.M. of 3-6 experiments run in triplicate. (○) RU 24969; (●) 8-OH-DPAT.

4. Discussion

The hippocampus is a brain area relatively rich in both 5-HT and ACh nerve endings and in which interactions at the presynaptic level between the two corresponding transmitters are therefore possible. Although the effects of 5-HT on presynaptic cholinergic mechanisms have not been directly investigated in this area, lesion experiments have provided results suggesting that 5-HT neurons exert a tonic inhibitory effect on hippocampal cholinergic neurons (Robinson, 1983). In fact, the turnover of ACh in the hippocampus was significantly increased after injection of 5,7-dihydroxytryptamine into the dorsal raphe nucleus. However, since the dorsal raphe nucleus projects to the lateral septum as well as to the hippocampus, it is uncertain whether the site of 5-HT/ACh interaction is in the septum or in the hippocampus.

Since we have used nerve terminals isolated from the hippocampus, the first relevant result of the present study suggests that 5-HT interacts with cholinergic neurons through a 5-HT receptor which is localized in the hippocampus and in particular on the cholinergic terminals. An interaction in the septum also cannot be excluded.

The second result of this work concerns the pharmacological characteristics of the 5-HT receptor involved in the regulation of ACh release. Evidence has accumulated for different 5-HT receptor subtypes in the central nervous system. Two types of recognition sites, termed 5-HT_1 and 5-HT_2, were originally identified (Peroutka and Snyder, 1979). More recently, heterogeneity was proposed for the 5-HT_1 binding sites which have been subdivided into 5-HT_{1A}, 5-HT_{1B} (Pedigo et al., 1981) and 5-HT_{1C} (Pazos et al., 1984). The finding that the 5-HT receptor mediating the regulation of ACh release from hippocampal nerve endings was insensitive to ketanserin or methysergide indicates that the receptor does not belong to the 5-HT_2 type. On the other hand, its blockade by methiothepin, a non-selective 5-HT_1/5-HT_2 antagonist, together with its activation by RU 24969, a mixed 5-HT_{1A}/5-HT_{1B} agonist (Hunt and Oberlander, 1981) allows this receptor to be classed as a 5-HT_1. Moreover, the receptor could not be activated by 8-OH-DPAT, a selective 5-HT_{1A} agonist (Middlemiss and Fozard, 1983); it was not blocked by spiperone, a 5-HT_{1A} displacer (Pedigo et al., 1981; Doods et al., 1985) nor by mesulergine, a 5-HT_{1C} selective antagonist (Pazos et al., 1984). Therefore, according to the present binding nomenclature, the 5-HT receptor mediating inhibition of ACh release in rat hippocampus appears to be of the 5-HT_{1B} subtype.

In a very recent study (Maura et al., in press) we have characterized pharmacologically the 5-HT autoreceptor in the same brain area. This presynaptic receptor also appears to belong to the 5-HT_{1B} subtype. Thus, on the basis of evidence obtained with the presently available pharmacological tools, the 5-HT autoreceptors sited on 5-HT axon terminals and the 5-HT heteroreceptors located on cholinergic terminals in the rat hippocampus appear to be pharmacologically indistinguishable.

Interestingly, the effects of 5-HT on the release of ACh observed in striatal slices (Vizi et al., 1981; Beani et al., 1985; Gillet et al., 1985) were sensitive to methysergide, cyproheptadine, cinanserin or mianserin and therefore are probably mediated by 5-HT_2 receptors. Moreover, lesions of the nucleus basalis magnocellularis in the rat brain caused a decrease of [^{3}H]ketanserin binding in lamina IV of the anterior and middle cortex. This finding was taken as evidence that 5-HT_2 receptors exist on cholinergic terminals in rat cortex (Quirion et al., 1985). The inhibition by 5-HT of the release of ACh from slices of nucleus accumbens was however not affected by methysergide (De Belleroche and Gardiner, 1982) and, similarly to what we have found in the hippocampus, it may therefore involve the activation of a 5-HT_1 receptor. Interestingly, the release of [^{3}H]ACh from guinea-pig enteric neurons was inhibited by 5-HT through the activation of a receptor which was sensitive to 8-OH-DPAT and to spiperone and which, differently from the hippocampal receptor, appears therefore to belong to the 5-HT_{1A} subtype (Fozard and Kilbinger, 1985).

The hippocampus is thought to be involved in memory processes and cholinergic transmission has long been considered to play an important role. Stimulation of the septum elicits hippocampal theta activity. This activity which involves

the septal-hippocampal cholinergic neurons and may be related to memory consolidation was shown to be inhibited through a serotoninergic mechanism (McNaughton et al., 1980). Moreover, electrical stimulation of the dorsal raphe nucleus disrupted memory by a process involving 5-HT (Fibiger et al., 1978). In conclusion, the inhibitory action of 5-HT on the release of ACh in the hippocampus may be relevant to mnemonic processes.

Acknowledgements

This work was supported by grants from the Italian Ministry of Education and from the Italian National Research Council. The authors wish to thank Mrs. Maura Agate for typing the manuscript.

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