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K^+-EVOKED [^{3}H]-5-HT RELEASE FROM RAT FRONTAL CORTEX SLICES: THE EFFECT OF 5-HT AGONISTS AND ANTAGONISTS

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Abstract—The pharmacological characteristics of a pre-junctional 5-HT autoreceptor have been studied by following the Ca^{2+}-dependent, K^+-evoked release of [^{3}H]-5-HT from preloaded rat frontal cortex slices. Added 5-HT, in the presence of the 5-HT uptake inhibitor chlorimipramine, caused a dose related inhibition of the K^+-evoked release of [^{3}H]-5-HT in this system as did the 5-HT analogues 5-methoxytryptamine, *N*-methyltryptamine, 5-methoxy-*NN'*-dimethyltryptamine, *N*-methyl 5-hydroxytryptamine and tryptamine.

The inhibitory effect of 1 μM 5-HT on the K^+-evoked release of [^{3}H]-5-HT was reversed in a dose-related manner by the 5-HT antagonist drug, methiothepin (pA_{10} value 6.7). At a concentration of 1 μM, the 5-HT antagonists drugs cinanserin and mianserin produced a small but significant reversal of the 5-HT induced inhibition of K^+-evoked [^{3}H]-5-HT release, but methysergide, metergoline and cyproheptadine were completely without effect at this concentration. The results are interpreted as evidence for a pre-junctional autoreceptor for 5-HT in the frontal cortex of the rat with a different pharmacological specificity for 5-HT antagonists from previously studied 5-HT receptors.

It has now been shown in a number of pharmacological and biochemical systems that neurotransmitters can control their own release via prejunctional autoreceptors and these receptors have been postulated to play a crucial physiological role in the control of neurotransmitter function [1]. The presence of such pre-junctional autoreceptors has been demonstrated for the neurotransmitters noradrenaline [2, 3], dopamine [4, 5], acetylcholine [6], and more recently 5-hydroxytryptamine (5-HT) [7, 8].

The present study reports the effect of putative 5-HT agonists and antagonists on the potassium evoked release of [^{3}H]-5-HT from preloaded slices of the rat frontal cortex. Our results suggest the presence of an inhibitory pre-junctional autoreceptor on 5-HT neuroterminals in this brain area of the rat. The receptor is stimulated by a number of putative 5-HT agonists and the effect of 5-HT is reversed in a dose related manner by the 5-HT antagonist drug, methiothepin.

MATERIALS AND METHODS

Materials

Drugs used were: [^{3}H]-5-HT (sp. act. 28.9 Ci/mmole, New England Nuclear); 5-hydroxytryptamine creatinine sulphate (Koch–Light Ltd.); 5-methoxytryptamine HCl (Aldrich Chem. Co.); *N*-methyl 5-hydroxytryptamine HCl (Aldrich Chem. Co.); tryptamine HCl (Koch–Light Ltd.); 5-methoxy-*NN'*-dimethyltryptamine HCl (Sigma Chemical Co.); *N*-methyl tryptamine HCl (Aldrich Chem. Co.); cinanserin HCl (synthesised by R. J. Pearce, I.C.I.); cyproheptadine HCl (Merck, Sharpe & Dohme Ltd.); metergoline (Farmitalia); methiothepin oxalate (Spofa); methysergide bimaleate (Sandoz Ltd.); mianserin HCl (Organon Labs. Ltd.); pargyline HCl (Sigma Chemical Co.); chlorimipramine HCl (Ciba–Geigy Ltd.); Phase Combining System (PCS, Radiochemical Centre, Amersham).

All other reagents used were Analar grade.

Solutions

Normal Krebs solution comprised 135 mM NaCl, 5 mM KCl, 25 mM $NaHCO_3$, 1 mM $MgSO_4 \cdot 7H_2O$, 1.25 mM KH_2PO_4, 2 mM $CaCl_2$, 10 mM glucose, and was gassed with 95%/5% O_2/CO_2 to pH 7.4.

Alterations to the K^+ ion concentration in the Krebs solution were compensated for by an equivalent reduction in the Na^+ ion concentration. Alterations to the $CaCl_2$ concentration were compensated for by isotonic substitution with $MgCl_2$. All drugs were dissolved in DMSO and then diluted with Krebs at least 1000-fold before use.

Preparation of [^{3}H]-5-*HT loaded frontal cortex slices*

The frontal cortex of two male Alderley Park strain rats (180–200 g) was rapidly dissected and chopped at 100 μm intervals in two directions using a McIlwain Tissue Chopper. The slices were transferred to 5 ml of gassed Krebs buffer containing 0.1 μM [^{3}H]-5-HT and 1 μM pargyline. After a 15 min incubation at 37°, the slices were washed three times by decantation, resuspended in 5 ml Krebs buffer and then preincubated at 37° for a period of 30 min to allow the efflux of [^{3}H]-5-HT to

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reach a constant low level, (3% to 6% of tissue stores/min).

Effect of K^+ *on* [^{3}H]-5-*HT release*

After one further wash with 5 ml Krebs at 30 min, aliquots of the slices (100 μl) were exposed (in triplicate) in a final volume of 1 ml to normal Krebs buffer or to Krebs buffer containing elevated levels of K^+. Drugs were added to the slices at 2 min (5-HT agonists) or 12 min (5-HT antagonists) before exposure to elevated levels of K^+. For all experiments involving the addition of 5-HT or 5-HT analogues, 5 μM chlorimipramine was added to the slices 12 min before the end of the preincubation period. After a 5 min incubation at 37°, the slices were separated from the incubation medium by 2 min centrifugation. The samples were then stored on ice to prevent further release from the pelleted slices and 500 μl of the incubation medium removed for liquid scintillation counting (in PCS). The remaining medium was aspirated and the tissue pellet dissolved in 500 μl of PCS. 250 μl of the resulting solution was then removed for liquid scintillation counting in PCS. All samples were counted at 58% efficiency in an LKB Liquid Scintillation Analyser.

Calculation of pA_{10} *values for 5-HT antagonists*

The pA_{10} values were obtained by a method based on that of Schild [10] described by Fozard *et al.* [11]. The molar concentration of antagonist which reduced the response of 1 μM 5-HT to that of 0.1 μM 5-HT in the absence of antagonist was determined. This concentration was then expressed as a negative logarithm, or pA_{10} value.

RESULTS

Preliminary results indicated a rapid initial efflux of tritium from the slices which by the end of the 30 min preincubation period had reached a low basal level of 3% to 6% of tissue stores/min; calculated as the per cent released over a 5 min time period. Exposure of the slices to high (23 mM) K^+ ion concentrations caused an increased and time-dependent (up to 10 min) release of tritium. The additional (K^+-evoked) release, calculated as the difference between the release evoked by 23 mM K^+ Krebs and the basal release, over a 5 min time period, was in the range of 2% to 3% min, in the absence of added 5-HT agonists or antagonists (data not shown). The per cent increase in the release of tritium over basal was dependent on the K^+ ion concentration of the Krebs solution (Fig. 1).

Isotonic substitution of Ca^{2+} in the solutions with Mg^{2+}, following the initial uptake period, in the presence or absence of 10 μM EGTA, abolished the 23 mM K^+-evoked release of tritium from the slices (Fig. 2).

The addition of 5-HT to the slices in the presence of 5 μM chlorimipramine (a 5-HT uptake blocker), for 2 min before and during a 5 min exposure to 23 mM K^+ Krebs, caused a dose-related inhibition of the K^+-evoked release of tritium (Fig. 3). Maximum reduction of the K^+-evoked release was effected by approximately 1 μM 5-HT and represented a 60 per cent reduction of control values. (Chlorimipramine at this concentration (5 μM) showed no effect on either basal or K^+-evoked release rates.) At concentrations lower than 10 μM, added 5-HT in the presence of 5 μM chlorimipra-

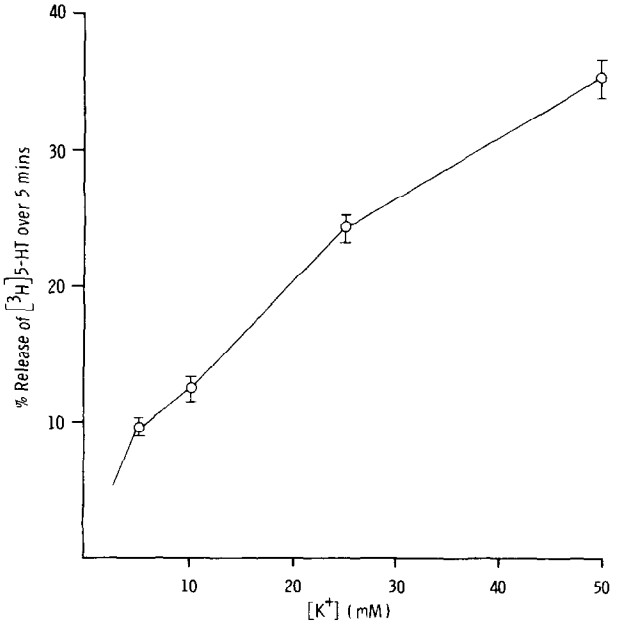

Fig. 1. Effect of [K^+] on the % release of [^{3}H]-5-HT from frontal cortex slices over a 5 min time period. % Release is calculated as detailed in Materials and Methods. Data is mean ± S.E.M. of 3 determinations.

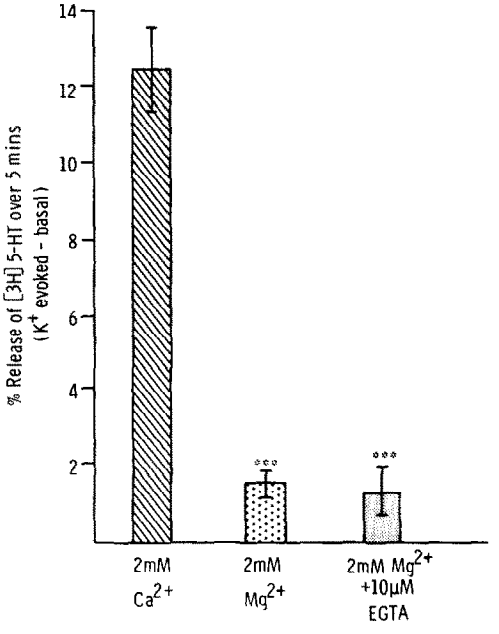

Fig. 2. Effects of [Ca^{2+}] on the % release of [^{3}H]-5-HT from frontal cortex slices evoked by exposure to 23 mM K^+ Krebs. % Release is calculated as additional release evoked by exposure to 23 mM K^+ Krebs (see Materials and Methods). Exclusion of the Ca^{2+} was compensated for by the addition of an equivalent concentration of Mg^{2+}. Data is mean ±S.E.M. of 4 determinations. Statistical significance with respect to control was assessed using a paired Student's t test (***$P < 0.001$).

mine, did not affect the basal release rate. At concentrations of 5-HT greater than 10 μM, large increases in the basal release of tritium occurred, presumably due to exchange of exogenous 5-HT with the [^{3}H]-5-HT in the slices and for this reason, higher 5-HT concentrations could not be tested.

A number of 5-HT analogues also caused dose related decreases in the K^+-evoked release of tritium (Figs. 3, 4 and 5). The maximum effect of these analogues, measured at 10 μM, represented about a 60 per cent reduction of the control values. At and below this concentration (10 μM), none of the analogues had any effect on the basal release rate in the presence of 5 μM chlorimipramine. However at concentrations greater than 10 μM, there was a marked effect on the basal release of tritium, and it was therefore not possible to determine the relative potencies of these analogues in reducing the K^+-evoked release of tritium.

The results of a series of experiments to test the effectiveness of putative 5-HT antagonists in reversing the effect of 1 μM 5-HT on the K^+-evoked release of tritium are given in Fig. 6. The slices were given a 12 min preincubation with the appropriate concentration of the antagonist before a 5 min exposure to normal or 23 mM K^+ Krebs in the presence or absence of 1 μM 5-HT. The concentrations of antagonists tested had no effect on basal or K^+-evoked release of tritium in the absence of 5-HT. The results

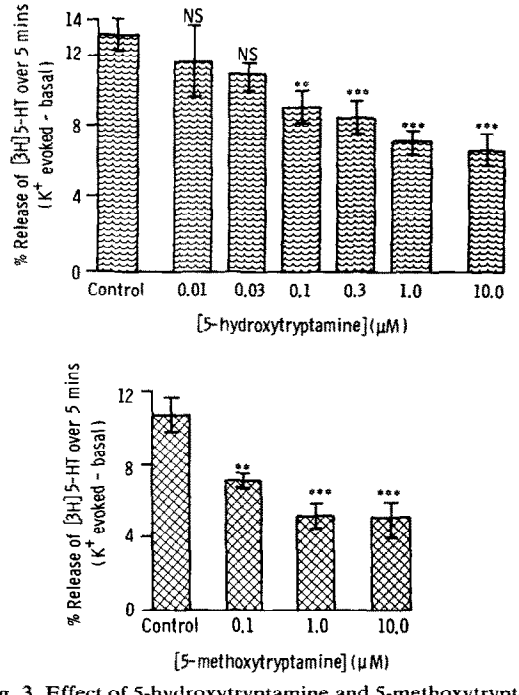

Fig. 3. Effect of 5-hydroxytryptamine and 5-methoxytryptamine on K^+-evoked release of [^{3}H]-5-HT from frontal cortex slices. Details of experimental design are given in Materials and Methods. Data is mean ±S.E.M. of 5 determinations. Statistical significance with respect to control was assessed using a paired Student's t test (**$P < 0.01$, ***$P < 0.001$).

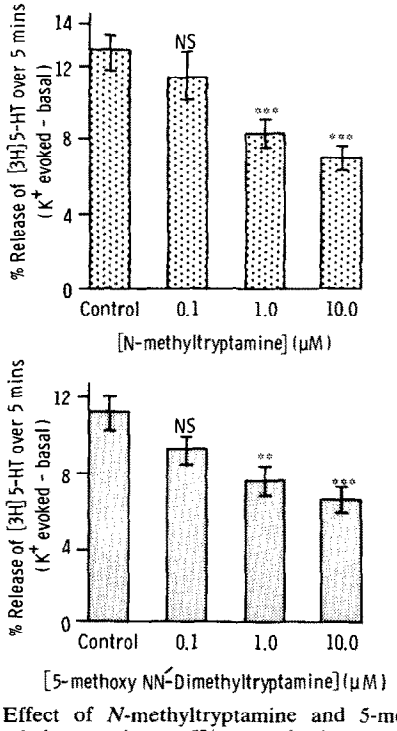

Fig. 4. Effect of *N*-methyltryptamine and 5-methoxy-*NN'*-dimethyltryptamine on K^+ evoked release of [^{3}H]-5-HT from frontal cortex slices. Details of experimental design are given in Materials and Methods. Data is mean ±S.E.M. of 5 determinations. Statistical significance with respect to control was assessed using a paired Student's t test (**$P < 0.01$, ***$P < 0.001$).

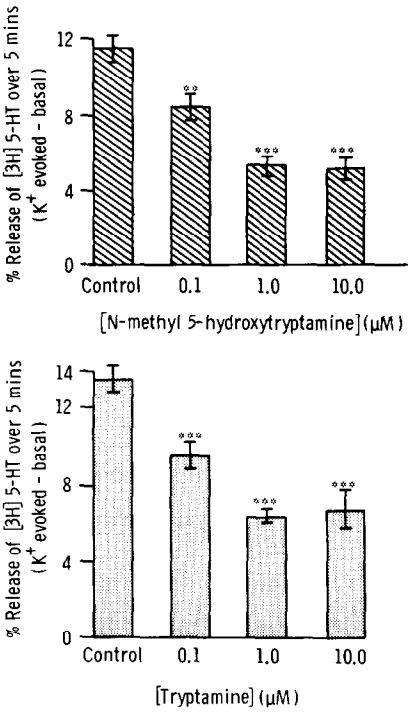

Fig. 5. Effect of *N*-methyl 5-hydroxytryptamine and tryptamine on K^+-evoked release of [^{3}H]-5-HT from frontal cortex slices. Details of experimental design are given in Materials and Methods. Data is mean ±S.E.M. of 5 determinations. Statistical significance with respect to control was assessed using a paired Student's *t* test. (**P < 0.01, ***P < 0.001).

are expressed as the percentage of the control response (in the absence of antagonist or 5-HT) over the 5 min test period.

Of the antagonists tested methiothepin was the most active, showing a significant and dose-related reversal of the effect of 5-HT down to a concentration of 0.1 μM. A pA_{10} value of 6.7 was obtained from the data using the method of Schild [10] as described by Fozard *et al.* [11]. Cinanserin and mianserin showed a small but statistically significant reversal of the effect of 1 μM 5-HT at 1 μM but were inactive at 0.5 μM. All other drugs tested (methysergide, metergoline and cyproheptadine) were inactive at 1 μM. Higher concentrations of the antagonists caused marked increases in the basal release of [^{3}H]-5-HT, and could not therefore be tested.

DISCUSSION

The present study has utilised the high affinity uptake system present in 100 μm slices of rat frontal cortex tissue, to load these slices *in vitro* with [^{3}H]-5-HT. The slices, after suitable preincubation and washing, release the tritium at a low basal rate which is enhanced by exposure to elevated K^+ ion concentrations. This K^+-evokable release of tritium is markedly Ca^{2+} ion dependent and is therefore likely to represent a physiologically relevant release of [^{3}H]-5-HT. The addition of 5-HT and a number of 5-HT analogues, causes a dose-related reduction in K^+-evoked release of tritium presumably by the interaction of these drugs with a 5-HT pre-junctional autoreceptor. We have also observed a dose-related inhibition of K^+-evoked release of [^{3}H]-5-HT by LSD

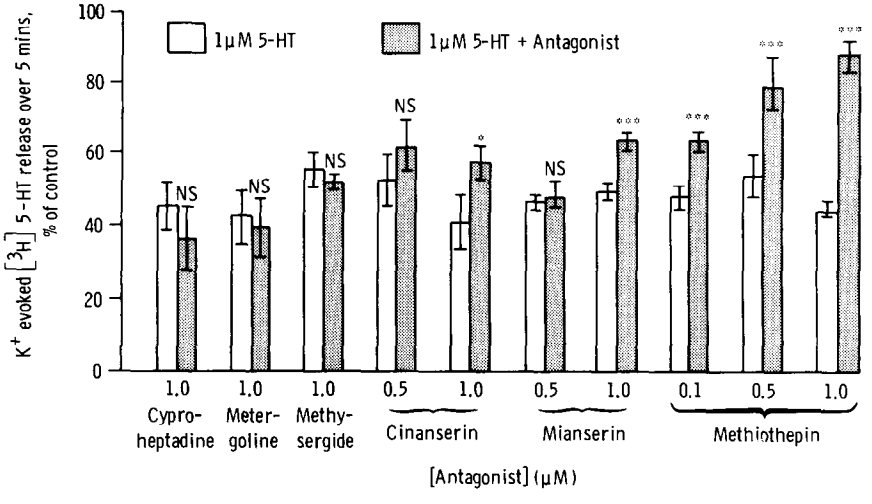

Fig. 6. Effect of 5-HT antagonists on the inhibition of K^+-evoked release of [^{3}H]-5-HT by 1 μM 5-HT. Details of experimental design are given in Materials and Methods. In each pair of histograms the unhatched column represents the % of control response in the presence of 1 μM 5-HT and the hatched column represents the % of control response in the presence of 1 μM 5-HT plus the indicated concentration of antagonist. Data is mean ±S.E.M. of 5 determinations. Statistical significance with respect to control was assessed using a paired Student's *t* test (*P < 0.05, ***P < 0.001).

(data not shown), a drug which is thought to inhibit 5-HT neuronal firing via a stimulation of pre-synaptic 5-HT receptors [9]. A similar effect of 5-HT on the K^+-evoked release of [^{3}H]-5-HT has been demonstrated in superfused hypothalmic synaptosomes, suggesting that the presence of a 5-HT interneurone may not be necessary to explain the effects observed here [7].

The effects of a number of putative 5-HT antagonists on the reversal of the effects of 1 μM 5-HT on K^+-evoked release of [^{3}H]-5-HT have also been evaluated. The only drug to show a dose related reversal of the 5-HT effect is methiothepin with a pA_{10} value of 6.7. These results for antagonists are in general agreement with data generated by other workers on the K^+ or electrically evoked release of [^{3}H]-5-HT in superfused synaptosomes or slices [7, 8]. However, one major difference in the present study is the finding that both mianserin and cinanserin do induce a weak but significant reversal of the inhibition caused by 1 μM 5-HT. We consider that the data generated here indicate that these two drugs (mianserin and cinanserin) do have weak antagonist activity on the pre-junctional autoreceptor and the lack of activity seen in other studies may be related either to the doses tested or to the pre-exposure time of the tissue slices to the drugs.

Of considerable interest in the elucidation of the receptor pharmacology of the serotoninergic system is a comparison of the potency of 5-HT agonist and antagonist drugs on pre and post-junctional 5-HT receptors. In other neurotransmitter systems, the identification of these anatomically distinct receptors has led to the development of selective pre- and post-junctional agonists and antagonists. It is interesting to compare the relative potency of the agonists and antagonists reported here on the putative pre-junctional autoreceptor with those of a similar series of 5-HT agonists and antagonists tested on the K^+-evoked release of [^{3}H]dopamine from rat striatal slices [12] where the interaction is thought to be with a 5-HT receptor which is pre-junctional to the dopamine neuroterminal but presumably post-junctional with respect to a 5-HT input onto the dopamine neurone. Of the antagonists tested in both studies, only methiothepin blocks both 5-HT receptors. Methysergide, a putative post-junctional 5-HT antagonist is highly potent ($pA_{10} = 8.3$) on the proposed post-junctional 5-HT receptor but is inactive on the pre-junctional 5-HT auto-receptor studied here. We conclude from this data that the pharmacological specificity of these two 5-HT receptors is different, and that it may be possible to develop selective antagonists for the two receptor sub-types.

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