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Characterization of 5-hydroxytryptaminergic autoreceptors in the rat hypothalamus

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5-hydroxytryptamine (5-HT) (3×10^{-9} to 10^{-6} M) produced a concentration-related inhibition of potassium-evoked tritium release from slices of rat hypothalamus preloaded with [^{3}H]-5-HT. The response to 5-HT was unaffected by the presence of yohimbine (10^{-6} M), pimozide (10^{-7} M), domperidone (10^{-7} M) or tetrodotoxin (10^{-7} M), indicating that the response was not mediated via α_1- or α_2-adrenoceptors or dopamine receptors and that the receptors that were involved were located directly on the 5-HT nerve terminal. The 5-HT antagonist metergoline (10^{-8} to 3×10^{-7} M) produced a parallel rightward shift in the concentration-effect curve to 5-HT with no reduction in the size of the maximum response. The pA_{10} value for metergoline was 6·82 and the slope of the Arunlakshana-Schild plot was not significantly different from 1·0 indicating that it was a competitive antagonist. Methiothepin produced a similar effect to metergoline whilst cyproheptadine and methysergide were less potent as antagonists of 5-HT and were not competitive. Cinanserin was inactive. Thus we have characterized the 5-HT autoreceptor in the rat hypothalamus using a classical pharmacological approach and found that it has more in common with the autoreceptor which we have previously identified in the raphe nuclei of the rat than it has with the 5-HT receptor located on dopamine neuroterminals in the striatum.

The existence of autoreceptors that modulate neurotransmitter release is now well established for a number of neurotransmitters in the central nervous system. These presynaptic receptors usually have a different sensitivity to agonists and antagonists when compared with the postsynaptic receptor (Starke et al 1977). We have previously reported the characterization of two types of 5-hydroxytryptamine (5-HT) receptors in rat brain on the basis of differential agonist and antagonist potencies. These receptors were located postsynaptically on dopamine neuroterminals in the striatum (Ennis et al 1981) and presynaptically on 5-hydroxytryptaminergic neurons in the raphe nuclei of the rat (Ennis & Cox 1982). The relative orders of antagonist potencies in these studies were obtained using the rapid method of Schild (1947) to calculate pA_{10} values. However, a disadvantage of the rapid method is that it assumes competitive antagonism but does not prove it. We have now developed a method which allows analysis of the results according to the method of Arunlakshana & Schild (1959), from which it is possible to fully characterize the type of antagonism i.e. whether it is competitive.

The presence of the 5-HT autoreceptor in the hypothalamus has previously been reported by Cerrito & Raiteri (1979) but since these authors used only a small dose range and a limited number of antagonists the receptor could not be fully characterized. We have further investigated the nature of the receptor located on 5-HT neurons in the hypothalamus of the rat on the basis of relative antagonist potency under the conditions described by Furchgott (1972) and have found that it is similar to the 5-HT receptor located in the raphe nuclei which we have previously identified (Ennis & Cox 1982).

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METHODS

Four male rats (150–200 g, Alderley Park SPF strain) were decapitated, the hypothalami removed and chopped into 250×250 μm slices using a McIlwain tissue chopper. The slices of hypothalamus were incubated in Krebs-Henseleit solution containing ascorbic acid (2×10^4 M), pargyline (10^{-6} M) and [^{3}H]5-HT (10^{-7} M, specific activity 25 Ci mmol^{-1}) for 20 min at 37 °C. The slices were washed and resuspended in Krebs-Henseleit solution. Equal aliquots of the tissue suspension were placed in each of 10 superfusion chambers, approximately 25 mg tissue per chamber. The volume of the superfusion chamber was 200 μl. The slices were superfused with Krebs-Henseleit solution (containing chlorimipramine 5×10^{-5} M) at 37 °C at a rate of 0·4 ml min^{-1}. Superfusate fractions were collected every 4 min and the radioactivity in each fraction was determined by liquid scintillation counting. At the end of the

experiment the tissue slices were removed and their residual radioactivity determined.

Two pulses of Krebs-Henseleit solution containing 25 mM KCl, obtained by iso-osmotic replacement of NaCl with KCl, were administered for 4 min, 42 (P_1) and 66 (P_2) min after the start of the superfusion. Modifying drugs were added to the superfusing medium immediately after P_1. The percentage efflux of tritium was calculated as the fractional release i.e. the radioactivity in each superfusate fraction divided by the amount of radioactivity present in the slices at the start of that collection period. The percentage of radioactivity released above basal by the 2 pulses of potassium was expressed as the ratio P_2/P_1 for both control and drug treated slices. The P_2/P_1 ratio for drug-treated slices was expressed as a percentage of the control P_2/P_1 ratio.

In each of the experiments which were designed to determine agonist potency, 8 concentrations of agonist were compared with controls and an individual concentration-effect curve obtained from which the maximum response, slope and pD_2 value were calculated. In each experiment which was designed to determine antagonist potency three concentrations of agonist from the linear portion of the concentration-effect curve (i.e. between 10 and 80% of the maximum response) were tested alone and in the presence of two concentrations of antagonist and the shift in the concentration-effect curve measured at the EC_{50} level. From 4 of the above experiments, designed so that 4 different concentrations of antagonist were used, a linear regression analysis of Log (dose ratio–1) against negative log molar concentration antagonist was performed to obtain a pA_{10} value and the slope of this line.

The drugs used in this study were: ascorbic acid (BDH), chlorimipramine HCl (Ciba-Geigy Ltd), cinanserin HCl (gift from R. J. Pearce, ICI), cyproheptadine HCl (Merck, Sharp & Dohme), metergoline (Farmitalia), methiothepin oxalate (Spofa), 5-methoxytryptamine HCl (Sigma, London), methysergide bimaleate (Sandoz Ltd), pargyline HCl (Sigma, London), [^{3}H]-5-HT creatinine sulphate (specific activity 25 Ci mmol^{-1}, New England Nuclear), 5-HT creatinine sulphate (Sigma, London), tetrodotoxin (Sankyo), tyrptamine HCl (Koch-Light Ltd.) and yohimbine HCl (Sigma, London). Domperidone and pimozide were gifts from Janssen Pharmaceuticals Ltd.

RESULTS

The efflux of tritium from the hypothalamic slices was initially rapid but became constant at a rate of 0·82 ± 0·08% min^{-1} of total tissue radioactivity after 40 min. Superfusion of the slices with Krebs-Henseleit solution containing 25 mM KCl produced an increase in tritium efflux over basal to 1·83 ± 0·09% min^{-1} for P_1 and 1·56 ± 0·07% min^{-1} for P_2. The control P_2/P_1 ratio was 0·84 ± 0·07. This potassium-evoked efflux of tritium was shown to be calcium dependent. Fig. 1 is a typical efflux curve.

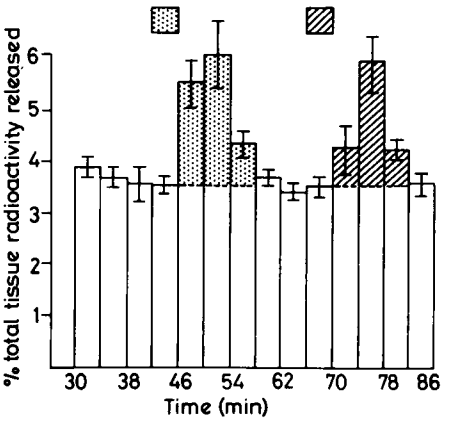

FIG. 1. Release of tritium from slices of rat hypothalamus preloaded with [^{3}H]-5-HT and superfused with Krebs-Henseleit solution. The hatched portions of the columns represent the tritium released in response to two pulses of potassium (25 mM) applied for 4 min at 42 (P_1) and 66 (P_2) min after the start of the superfusion. Each column represents the mean of 20 determinations and the vertical bars the standard error of the mean.

5-HT in the presence of chlorimipramine 5 × 10^{-6} M) produced a concentration related inhibition of potassium-evoked tritium release over the concentration range 3 × 10^{-9} to 10^{-6} M. The maximum inhibition of tritium release produced by 5-HT was 70%. Therefore each response was expressed as a percentage of this maximum. The concentration-effect curve to 5-HT is shown in Fig. 2. The pD_2 value (negative log molar concn of agonist which produces a 50% response) was 7·49 ± 0·06 (n = 14).

The 5-HT agonists tryptamine and 5-methoxytryptamine produced a similar inhibition of potassium-evoked tritium release although the maximum response elicited by these agonists was only 80% of that produced by 5-HT. The pD_2 value for tryptamine was 6·86 ± 0·09 and that for 5-methoxytryptamine was 6·80 ± 0·15. The response to 5-HT (5 × 10^{-8} M) was unaffected by the presence of tetrodotoxin (10^{-7} M) in the superfusing medium.

The 5-HT antagonist metergoline produced a parallel rightward shift in the concentration-effect curve to 5-HT over the concentration range 10^{-8} to 3 × 10^{-6} M. An example of this effect is shown in

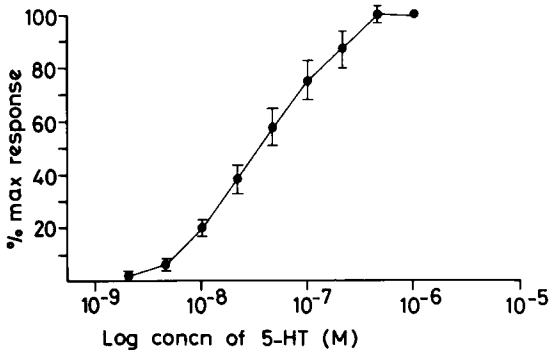

FIG. 2. Log concentration-effect curve for 5-HT for the inhibition of potassium-evoked tritium release from slices of rat hypothalamus preloaded with [^{3}H]-5-HT. Each point represents the mean response and the vertical bar the standard error of the mean of at least 4 determinations.

Fig. 3. The dose-ratio was measured at the EC50 level. Analysis of the data shown in Fig. 3 according to the method of Arunlakshana & Schild (1959) gave a pA_{10} value of 6·8 and a slope of 0·9 which was not significantly different from 1·0.

Similar experiments were performed using a number of 5-HT antagonists and the pA_{10} values and slopes of the Arunlakshana–Schild plots are shown in Table 1. At the concentrations used none of the antagonists modified potassium-evoked tritium release on their own.

The inhibition of potassium-evoked tritium release produced by 5-HT (5×10^{-8} M) was not reduced by the presence of yohimbine (10^{-6} M), pimozide (10^{-7} M) or domperidone (10^{-7} M).

DISCUSSION

The first suggestion that the release of 5-HT from rat brain slices was modulated by presynaptic autoreceptors was made by Farnebo & Hamberger (1974). The presence of a 5-HT autoreceptor in rat hypothalamus has previously been reported (Cerrito & Raiteri 1979) on the basis that the 5-HT-induced inhibition of potassium-evoked tritium release from rat hypothalamic synaptosomes preloaded with [^{3}H]-5-HT could be prevented by methiothepin (5×10^{-7} M) but not by cyproheptadine (10^{-6} M), methysergide (10^{-6} M) or mianserin (10^{-6} M). Since these authors used only single concentrations of antagonists it is not possible to obtain any information on relative potency from these studies nor whether the antagonism is competitive. Therefore we have more fully investigated this receptor using a classical approach (Furchgott 1972) measuring parallel rightward shifts in the concentration-effect curve to 5-HT.

Table 1. pA_{10} values and slopes of the Arunlakshana-Schild plots for the antagonism of 5-HT induced inhibition of potassium-evoked tritium release from slices of rat hypothalamus preloaded with [^{3}H]-5-HT. Each value was from the regression line for 4 individual experiments.

Antagonist	pA_{10} value	Slope
Metergoline	6·80	0·91
Methiothepin	6·46	0·95
Cyproheptadine	5·54	0·69*
Methysergide	4·34	0·62*
Cinanserin	Not active	

* Slopes are significantly different from 1·0.

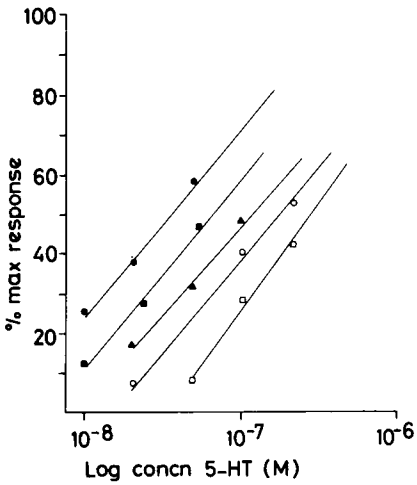

FIG. 3. An example of calculated regression lines of the log concentration-effect curves for the inhibition of potassium-evoked tritium release from slices of rat hypothalamus preloaded with [^{3}H]-5-HT for 5-HT alone (●) and in the presence of metergoline 10^{-8} M (■), 3×10^{-8} M (▲), 10^{-7} M (○) and 3×10^{-7} (□). Each line represents the results obtained in a single experiment.

All the experiments were performed in the presence of chlorimipramine (5×10^{-6} M) which had no effect alone but prevented the unlabelled 5-HT from being actively transported into the 5-HT neuroterminals which would both displace the labelled 5-HT from its storage sites in the neuroterminal and also lead to a reduction in the effective concentration of unlabelled 5-HT at the receptor.

5-HT and related compounds produced a concentration related inhibition of potassium-evoked tritium release from hypothalamic slices with a maximum inhibition of 70%. The response to 5-HT was unaffected by the presence of tetrodotoxin (10^{-7} M) indicating that the response was not dependent on the movement of sodium ions as in the propagation of action potentials thus confirming that the receptor mediating the response was situated on the 5-HT

nerve terminals. Furthermore the response to 5-HT was also unaffected by the presence of the α_2-adrenoceptor antagonist yohimbine (10^{-6} M) (Starke et al 1975), the dopamine antagonist pimozide (10^{-7} M) (Janssen et al 1968) and the dopamine antagonist domperidone (10^{-7} M) (Laduron & Leysen 1979) which also has α_1-adrenoceptor blocking activity (Ennis & Cox 1980). Thus the response to 5-HT does not involve α_1-adrenoceptors, α_2-adrenoceptors or dopamine receptors.

The 5-HT antagonist metergoline produced a parallel rightward shift in the concentration curve to 5-HT with no apparent reduction in the size of the maximum response. The slope of the Arunlakshana-Schild plot of Log (dose ratio −1) against negative log molar concentration metergoline was not significantly different from 1·0 indicating that the antagonism was competitive in nature. Similar results were obtained with methiothepin. Cyproheptadine and methysergide were both less potent than metergoline or methiothepin and the antagonism produced by these compounds was not competitive since the slopes of the Arunlakshana-Schild plots were 0·69 and 0·62 respectively. Cinanserin was inactive as an antagonist in this study.

Thus the relative order of potency for the antagonists was metergoline = methiothepin > cyproheptadine > methysergide with cinanserin inactive. This order of potency is similar to, but not identical with, the order of potency which we have previously reported for the 5-HT autoreceptor in the raphe nuclei of the rat (Ennis & Cox 1982) where metergoline and methiothepin were the most potent antagonists followed by methysergide with cyproheptadine and cinanserin inactive. Cerrito & Raiteri (1979) have suggested that methiothepin is the only compound which is an effective antagonist at 5-HT autoreceptors, however these authors did not investigate the effects of metergoline. There appears to be some controversy in the literature concerning the efficacy of metergoline as an antagonist at presynaptic 5-HT receptors since Bourgoin et al (1978) found metergoline to be an effective antagonist of LSD-induced inhibition of [^{3}H]-5-HT release from rat brain stem slices whilst Gothert (1980) using slices of frontal cortex reported that metergoline was not active in concentrations up to 10^{-5} M. However, there was a slight but non-significant shift to the right in the 5-HT response curve in these experiments.

Since all the antagonists which were effective in the present study were also effective as antagonists of 5-HT-induced inhibition of potassium-evoked [^{3}H]dopamine release from striatal slices it would appear that at present there is no known antagonist which is selective for the 5-HT autoreceptor. Therefore this raises problems in the in vivo investigation of these autoreceptors since any manipulation with antagonists would necessarily also block the postsynaptic 5-HT receptors making interpretation of the results very complex. Furthermore since 5-HT itself was only capable of producing a 70% inhibition of evoked [^{3}H]-5-HT release it is possible that these autoreceptors represent a fine control or modulation of transmitter output so that it may be difficult to observe the in vivo effects of blocking these receptors.

The method used in the present study allows a quantitative pharmacological approach to be made to the problem of the characterization of neurotransmitter receptors in the central nervous system. This technique has advantages over binding studies in that a pharmacological response is measured ensuring that receptors rather than binding sites which may have no pharmacological relevance are being studied.

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