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Centrally acting hypotensive agents with affinity for 5-HT_{1A} binding sites inhibit forskolin-stimulated adenylate cyclase activity in calf hippocampus

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1 A number of centrally acting hypotensive agents and other ligands with high affinity for 5-hydroxytryptamine$_{1A}$ (5-HT_{1A}) recognition sites have been tested on forskolin-stimulated adenylate cyclase activity in calf hippocampus, a functional model for 5-HT_{1A}-receptors.

2 Concentration-dependent inhibition of forskolin-stimulated adenylate cyclase activity was elicited by the reference 5-HT_1-receptor agonists (mean EC_{50} value, nM): 5-HT (22), 5-carboxamidotryptamine (5-CT, 3.2), 8-hydroxy-2-(di-n-propylamino)-tetralin (8-OH-DPAT, 8.6), N,N-dipropyl-5-carboxamidotryptamine (DP-5-CT, 2.3), 1-[2-(4-aminophenyl)ethyl]-4-(3-trifluoromethylphenyl)-piperazine (PAPP or LY 165163, 20), 5-methoxy-3-(1,2,3,6-tetrahydro-4-pyridinyl)-1H indole (RU 24969, 20), buspirone (65) and ipsapirone (56). E_{max} amounted to 18–20% inhibition for all but the latter two agonists (14%).

3 The following hypotensive agents with high affinity for 5-HT_{1A} sites were potent agonists in this system (mean EC_{50} value, nM): flesinoxan (24), indorenate (99), erythro-1-{1-[2-(1,4-benzodioxan-2-yl)-2-hydroxyethyl]-4-piperidyl}-2-benzimidazolinone (R 28935, 2.5), urapidil (390) and 5-methyl-urapidil (3.5). The first two agents were full agonists, whereas the latter three acted as partial agonists with 60–80% efficacy.

4 Metergoline and methysergide behaved as full agonists and cyanopindolol as a partial agonist with low efficacy. Spiroxatrine and 2-(2,6-dimethoxyphenoxyethyl)aminomethyl-1,4-benzodioxane (WB 4101) which bind to 5-HT_{1A} sites with nanomolar affinity, were agonists and inhibited potently forskolin-stimulated adenylate cyclase in calf hippocampus, showing mean EC_{50} values of 23 and 15 nM, respectively. Spiroxatrine and WB 4101 yielded 90% and 50% efficacy, respectively.

5 Spiperone and methiothepin (each 1 μM) caused rightward shifts of the concentration-effect curve to 8-OH-DPAT, without loss of the maximal effect, as did the partial agonist cyanopindolol (0.1 μM) and the (−)- and (+)-enantiomers of pindolol (1 μM and 0.1 mM, respectively).

6 There was an excellent correlation ($r = 0.90$, $P = 0.0001$) between the pEC_{50} values (ranging from 6.4 to 8.7) of the 19 agonists tested at adenylate cyclase and their pK_D for 5-HT_{1A} recognition sites. Apparent pK_B values of antagonists at adenylate cyclase and their pK_D values for 5-HT_{1A} binding sites were also significantly correlated.

7 This study further indicates that the 5-HT_{1A} recognition site and the 5-HT receptor mediating inhibition of adenylate cyclase in hippocampus are the same. The data show that a number of centrally acting hypotensive agents with high affinity for the 5-HT_{1A} site are potent agonists in this model, suggesting an involvement of central 5-HT_{1A}-receptors in the control of blood pressure.

Introduction

When administered parenterally to rats, 5-hydroxytryptamine (5-HT) usually exhibits a complex, triphasic effect on blood pressure; the first phase is characterized by a very rapid and marked fall in blood pressure, a second phase consists of a transient increase in blood pressure and the third phase consists of a long-lasting hypotensive response. These effects have been ascribed to stimulation of 5-HT_3-, 5-HT_2- and '5-HT_1-like'-receptors (Kalkman *et al.*, 1983; 1984), according to the

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description by Bradley *et al.* (1986) and are peripherally mediated, as 5-HT does not cross the blood-brain barrier. Since then, some 5-HT receptor agonists have been shown to evoke hypotension by a central mechanism of action, probably via 5-HT_{1A}-receptors (Bevan *et al.*, 1986a,b; Fozard *et al.*, 1987; Ramage & Fozard, 1987; Doods *et al.*, 1988).

5-HT_1 binding sites, which were originally described in the brain by Peroutka & Snyder (1979), have been subdivided into subtypes, termed 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C} and 5-HT_{1D}, on the basis of radioligand binding studies (Pedigo *et al.*, 1981; Pazos *et al.*, 1984; Hoyer *et al.*, 1985b; Heuring & Peroutka, 1987). Several functional models have been proposed to reflect activation of 5-HT_{1A} recognition sites (see Hoyer, 1988). In particular, 5-HT_{1A}-receptors mediate both stimulation (Shenker *et al.*, 1985; 1987; Markstein *et al.*, 1986) and inhibition of adenylate cyclase activity in guinea-pig and rat hippocampus (De Vivo & Maayani, 1985; 1986; Bockaert *et al.*, 1987).

High affinity ligands for 5-HT_{1A}-receptors are now available. In particular, 8-hydroxy-2-(di-n-propylamino)-tetralin (8-OH-DPAT) has emerged as a potent and selective agent at 5-HT_{1A} binding sites (Middlemiss & Fozard, 1983; Gozlan *et al.*, 1983). 8-OH-DPAT causes sustained hypotension in the rat (Gradin *et al.*, 1985; Martin & Lis, 1985; Fozard *et al.*, 1987) and in the cat (Ramage & Fozard, 1987; Doods *et al.*, 1988), an effect thought to be mediated centrally (Gradin *et al.*, 1985; Fozard *et al.*, 1987; Ramage & Fozard, 1987). Other hypotensive agents acting by a central mechanism, like flesinoxan (Bevan *et al.*, 1986a; Calis *et al.*, 1986), urapidil (Sanders & Jurna, 1985; van Zwieten *et al.*, 1985), indorenate (Safdy *et al.*, 1982) and erythro-1-{1-[2-(1, 4-benzodioxan-2-yl)-2-hydroxyethyl]-4-piperidyl}-2-benzimidazolinone (R 28935; Timmermans *et al.*, 1982) are potent and selective ligands at 5-HT_{1A} sites (Hoyer *et al.*, 1985b; Bevan *et al.*, 1986b; Gross *et al.*, 1987; Fozard & Mir, 1987; Doods *et al.*, 1988). Central 5-HT_{1A}-receptors have therefore been suspected to represent the target sites of these 5-HT-receptor ligands. The finding that 8-methoxy-2-(N-2-chloroethyl-N-n-propyl) aminotetralin (8-MeO-ClEPAT, Fozard *et al.*, 1987), which potently and selectively binds to 5-HT_{1A} sites, antagonized 8-OH-DPAT-induced cardiovascular effects further strengthens this hypothesis. Finally, several other compounds with intermediate to high affinity for 5-HT_{1A} recognition sites, like N,N-dipropyl-5-carboxamidotryptamine (DP-5-CT, Markstein *et al.*, 1986) and ipsapirone (Peroutka, 1985), have been shown to be potent and (at least partly) centrally acting hypotensive agents in the cat (Ramage & Fozard, 1987; Doods *et al.*, 1988).

The aim of the present study was two fold: first, to demonstrate that the 5-HT receptor negatively coupled to adenylate cyclase in calf hippocampus is of the 5-HT_{1A} type; second, using this model, to demonstrate that a series of putative centrally acting hypotensive drugs shown to have affinity for the 5-HT_{1A} sites are in fact 5-HT_{1A}-receptor agonists.

Methods

Adenylate cyclase activity

The brains of 10 calves were obtained from a local slaughterhouse and kept on ice until the hippocampus was dissected and transferred to 10 volumes of ice-cold Tris-sucrose buffer (composition in mM: Tris-HCl 20, sucrose 300, EGTA 1, Na_2EDTA 5 and dithiothreitol 5, pH 7.4). Tissues were then homogenized by hand using a glass/glass Potter apparatus. After a first centrifugation at about 50 *g* for 5 min, the pellet was discarded and the supernatant centrifuged again at 40,000 *g* for 10 min. The pellet of this second centrifugation was resuspended in 4 volumes of Tris-sucrose buffer and 1 ml aliquots were stored at −70°C until used for the assay of adenylate cyclase activity. No significant changes in activity were observed for at least three months after the preparation. Adenylate cyclase activity was determined by measuring the formation of [^{32}P]-cyclic AMP from [α-^{32}P]-ATP as described by Bockaert *et al.* (1987) with some minor modifications. The incubation medium contained Tris-HCl 80 mM (pH 7.4), MgATP 0.1 mM, $MgCl_2$ 2 mM, GTP 10 μM, cyclic AMP 1 mM, NaCl 100 mM, 3-isobutyl-1-methylxanthine 2 mM, ascorbic acid 0.25 mM, phosphocreatine 5 mM, creatine phosphokinase 0.2 mg ml^{-1}, [α-^{32}P]-ATP (30 Ci mmol^{-1}) about 1 μCi per assay tube and the indicated substances. The reaction was started by addition of membrane proteins (about 50 μg) to the incubation medium after a 2 min equilibration period at 30°C. The assays were conducted in triplicate, typically for 10 min at 30°C, in a final volume of 200 μl. After stopping the reaction by addition of 0.6 ml of 120 mM $Zn(CH_3COO)_2$, the assay tubes were supplemented with 30 nCi of [^{3}H]-cyclic AMP (23 Ci mmol^{-1}) for estimation of recovery. Cyclic AMP was purified by co-precipitation of other nucleotides with $ZnCO_3$ formed by addition of 0.5 ml of 144 mM Na_2CO_3. Further purification was obtained by passage through the double column system (cation exchanger AG50W-X4 from Biorad and neutral alumina) of Salomon *et al.* (1974). The recovery of cyclic AMP amounted to 75–80%. The protein content was measured according to Bradford (1976) using bovine serum albumin as a standard.

Radioligand binding studies

5-HT$_{1A}$ binding studies were carried out in pig cortical membranes as previously described (Hoyer *et al.*, 1985b), using [^{3}H]-8-OH-DPAT as radioligand and 10 μM 5-HT for determination of non-specific binding.

Analysis of data

Concentration-effect curves were analysed using SCTFIT, a non-linear regression computer programme (De Lean *et al.*, 1980). Values of E_{max} (maximal effect) and EC_{50} (concentration producing the half-maximal effect) were derived from this analysis. Dissociation constants (K_B) of antagonists were calculated according to the formula: $K_B = [B]/[A'/A) - 1]$, where [B] is the concentration of antagonist, A' and A the EC_{50} values of agonist measured respectively in the presence and in the absence of antagonist (Furchgott, 1972). The same procedure was applied when a partial agonist was used as an antagonist (Kenakin, 1984). Results are given as means ± s.e.mean. The statistical significance of the correlation was estimated by Student's *t* test.

Drugs

5-Hydroxytryptamine creatinine sulphate (5-HT), forskolin and biochemicals used in adenylate cyclase assay were from Sigma, Saint Louis, U.S.A.; 5-carboxamidotryptamine hydrogenmaleate (5-CT), N,N-dipropyl-5-carboxamidotryptamine (DP-5-CT), cyanopindolol fumarate (racemic form), (+)-pindolol, (−)-pindolol, methysergide hydrogenmaleate, and 8-methoxy-2-(N-2-chloroethyl-N-n-propyl)amino-tetralin hydrochloride (8-MeO-ClEPAT) were synthesized at Sandoz, Basel, Switzerland. Metergoline was supplied by Farmitalia, Milano, Italy; methiothepin by Hoffmann-La Roche, Basel, Switzerland; buspirone hydrochloride and ipsapirone by Bristol-Myers, New York, U.S.A. and Troponwerke, Köln, F.R.G., respectively; spiperone, spiroxatrine and erythro-1-{1-[2-(1,4-benzodioxan-2-yl)-2-hydroxyethyl]-4-piperidyl}-2-benzimidazolinone (R 28935) by Janssen, Beerse, Belgium. Urapidil and 5-methylurapidil were gifts from Byk-Gulden, Konstanz, F.R.G.; 2-(2,6-dimethoxyphenoxyethyl)aminomethyl-1, 4-benzodioxane (WB 4101) from Ward Blenkinsop, London, indorenate hydrochloride from Miles Laboratories, Elkhart IN, U.S.A., flesinoxan hydrochloride from Duphar, Weesp, The Netherlands and 5-methoxy-3-(1,2,3,6-tetrahydro-4-pyridinyl)-1H indol succinate (RU 24969) from Roussel-Uclaf, Romainville, France. 8-Hydroxy-2-(di-n-propylamino)tetralin hydrobromide (8-OH-DPAT) and 1-[2-(4-aminophenyl)ethyl]-4-(3-trifluoromethylphenyl)piperazine (PAPP or LY 165163) were purchased from Research Biochemicals Inc., Natick MA, U.S.A. Radioactive materials were from Amersham (U.K.). Forskolin was prepared as a stock solution (10 mM) in absolute ethanol and stored at 4°C. 5-HT and all 5-HT-related compounds were prepared freshly at 10 mM. 5-HT was dissolved in distilled water and other compounds in a mixture of 1-methyl-2-pyrrolidone: ethanol: water (1:1:2) containing 10 mg ml^{-1} ascorbic acid. Subsequent dilutions were made in distilled water. All vehicles were devoid of agonist or antagonist activity under the assay conditions.

Results

Forskolin-stimulated adenylate cyclase activity in calf hippocampal membranes

In the presence of 10 μM GTP, adenylate cyclase formed 47.8 ± 0.9 pmol cyclic AMP mg^{-1} protein min^{-1} ($n = 48$) in membranes from calf hippocampus. Forskolin (0.1 μM to 0.1 mM) stimulated this activity concentration-dependently. In the presence of 10 μM forskolin, adenylate cyclase activity was 243 ± 4 pmol cyclic AMP mg^{-1} protein min^{-1} ($n = 49$), that is an approximate 5 fold increase. Forskolin (10 μM)-stimulated activity was a linear function of protein concentration and incubation time under the assay conditions (data not illustrated). Subsequent experiments were performed in the presence of 10 μM forskolin.

Inhibition of forskolin-stimulated adenylate cyclase activity in calf hippocampus by reference ligands for 5-HT$_{1A}$ binding sites

5-HT (1 nM–0.1 mM) did not alter basal adenylate cyclase activity, unstimulated by forskolin, as measured in the absence of NaCl. 5-HT, 5-CT, DP-5-CT, 8-OH-DPAT and PAPP inhibited forskolin (10 μM)-stimulated adenylate cyclase activity in calf hippocampal membranes. In each case, the effect was concentration-dependent in the 1 nM–1 μM range (Figures 1–3) and maximal inhibition (E_{max}) amounted to about 20%. The respective EC_{50} values were 22 ± 7 nM ($n = 13$), 3.2 ± 0.9 nM ($n = 7$), 2.3 ± 0.6 nM ($n = 3$), 8.6 ± 2.5 nM ($n = 18$) and 20 ± 7 nM ($n = 3$). The potent but non-selective 5-HT$_1$-receptor agonist, RU 24969 showed efficacy and potency similar to that of 5-HT (Figure 1; EC_{50} value = 20 ± 6 nM, $n = 4$). Buspirone and ipsapirone were partial agonists in this model; their E_{max} values were approximately 75% of that of 5-HT (Figure 1)

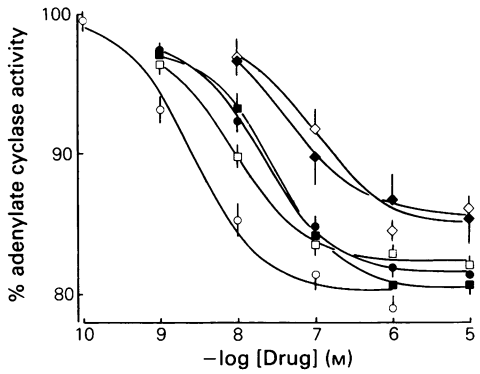

Figure 1 Concentration-effect curves for 5-hydroxytryptamine (●), 5-carboxamidotryptamine (○), 8-hydroxy-2-(di-n-propylamino)-tetralin (□), RU 24969 (■), busipirone (◇) and ipsapirone (◆) induced inhibition of forskolin-stimulated adenylate cyclase activity in calf hippocampus. Data are means, with s.e.mean represented by vertical bars, of values from 4–18 individual experiments.

and their respective EC_{50} values were 65 ± 23 nM and 56 ± 35 nM ($n = 4$ in both cases).

Inhibition of forskolin-stimulated adenylate cyclase activity by hypotensive agents binding with high affinity to 5-HT_{1A} recognition sites

Flesinoxan and indorenate acted as full agonists on forskolin-stimulated adenylate cyclase activity of calf hippocampus (Figure 2), with respective EC_{50} values of 24 ± 7 nM ($n = 4$) and 99 ± 42 nM ($n = 4$). R 28935, urapidil and 5-methyl-urapidil were partial agonists, exhibiting respective E_{max} values of about 80%, 80% and 60% of that of 5-HT (Figure 2), and EC_{50} values of 2.5 ± 1.0 nM ($n = 3$), 390 ± 190 nM ($n = 4$) and 3.5 ± 0.8 nM ($n = 5$), respectively.

Other 5-HT_{1A} ligands

Figure 3 shows the concentration-dependent inhibition of forskolin-stimulated adenylate cyclase activity of calf hippocampus by other drugs that bind with high affinity to 5-HT_{1A} sites. The two ergoline derivatives metergoline and methysergide were full agonists in this system. The EC_{50} values of these drugs were 29 ± 7 nM ($n = 3$) and 480 ± 140 nM ($n = 4$), respectively. The β-adrenoceptor antagonist and 5-HT_{1A}-receptor ligand cyanopindolol displayed about 40% efficacy but high affinity (EC_{50} value = 8.7 ± 3.0 nM, $n = 4$). Spiroxatrine (Nelson & Taylor, 1986) displayed agonist activity with more than 90% efficacy and an EC_{50} value of 23 ± 10 nM ($n = 4$). 8-MeO-ClEPAT, conceived as a putative irreversible antagonist at the 5-HT_{1A}-receptor subtype (Fozard *et al.*, 1987), behaved in the present model as a partial agonist, with an EC_{50} value of 120 ± 44 nM ($n = 3$) and 80% efficacy. The mixed α_1-adrenoceptor antagonist and 5-HT_{1A}-receptor ligand WB 4101 (Norman *et al.*, 1985) acted as a rather potent agonist (EC_{50} value = 15 ± 2 nM, $n = 3$) with about half-maximal efficacy.

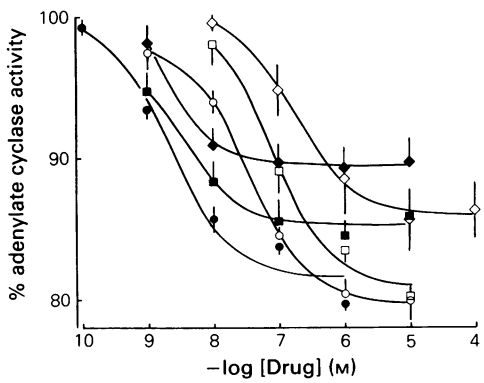

Figure 2 Concentration-effect curves for flesinoxan (○), indorenate (□), N,N-dipropyl-5-carboxamidotryptamine (●), R 28935 (■), urapidil (◇) and 5-Me-urapidil (◆) induced inhibition of forskolin-stimulated adenylate cyclase activity in calf hippocampus. Data are means, with s.e.mean represented by vertical bars, of values from 3–5 individual experiments.

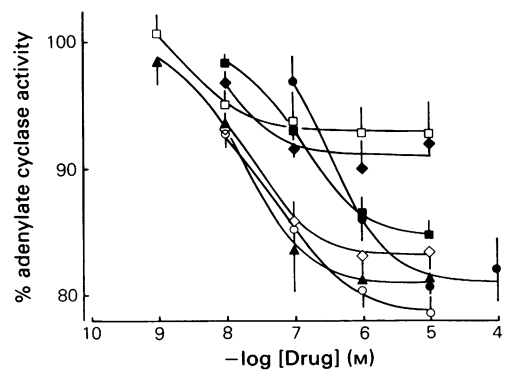

Figure 3 Concentration-effect curves for metergoline (○), methysergide (●), cyanopindolol (□), 8-methoxy-2-(N-2-chloroethyl-N-n-propyl)aminotetralin (■), spiroxatrine (◇), WB 4101 (◆) and PAPP (▲) induced inhibition of forskolin-stimulated adenylate cyclase activity in calf hippocampus. Data are means, with s.e.mean represented by vertical bars, of values from 3–4 individual experiments.

Antagonism of the 8-OH-DPAT-induced effect on adenylate cyclase activity

Spiperone, methiothepin, (−)-pindolol and (+)-pindolol did not inhibit forskolin-stimulated adenylate cyclase activity in calf hippocampus over the concentration range 10 nM to 0.1 mM. The highest concentrations ($\geq 10\,\mu$M) of the former two substances tended to increase forskolin (10 μM)-stimulated adenylate cyclase activity further. When tested as antagonists of 8-OH-DPAT, spiperone and methiothepin (each 1 μM) displaced the concentration-effect curve of the agonist to the right in a parallel manner, with no decrease of the maximal effect (Figure 4). Respective dissociation constants of 98 ± 45 nM ($n = 5$) and 23 ± 7 nM ($n = 4$) for spiperone and methiothepin were derived from these shifts. Stereoselectivity at the receptor site mediating inhibition of forskolin-stimulated adenylate cyclase activity was assessed by the use of the two enantiomers (−)-pindolol and (+)-pindolol (Figure 5). (−)-Pindolol (1 μM) and (+)-pindolol (0.1 mM) caused rightward shifts of the 8-OH-DPAT curve, without affecting the E_{max}. Dissociation constants of 14 ± 3 nM ($n = 3$) and $3.5 \pm 1.0\,\mu$M ($n = 3$) were calculated for (−)- and (+)-enantiomers of pindolol, respectively, indicating an approximately 250 fold difference in potency between the two enantiomers. The partial agonist cyanopindolol (0.1 μM) antagonized the effect of 8-OH-DPAT in an apparently competitive manner

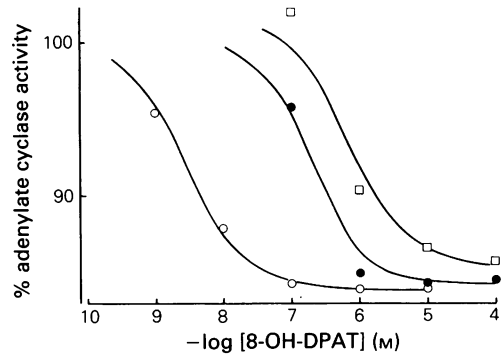

Figure 4 Antagonism of 8-hdyroxy-2-(di-n-propylamino)-tetralin (8-OH-DPAT)-induced inhibition of adenylate cyclase activity in calf hippocampus by spiperone and methiothepin. 8-OH-DPAT concentration-effect curves are represented in the absence (○) and in the presence of spiperone (1 μM, ●) or methiothepin (1 μM, □). Data are results from 1 typical experiment out of 5 for spiperone and out of 4 for methiothepin.

(Figure 5), yielding a dissociation constant of 8.0 ± 1.5 nM ($n = 3$).

Correlation between adenylate cyclase data in calf hippocampus and 5-HT$_{1A}$ binding data

Tables 1 and 2 summarize data obtained for adenylate cyclase activity in calf hippocampus, in terms of

Table 1 Parameters of agonist action for inhibition of forskolin-stimulated adenylate cyclase activity in calf hippocampus and of binding at 5-HT$_{1A}$ recognition sites

Drug	E_{max} (%)	pEC_{50}	n	pK_D
5-HT	18.4 ± 0.7	7.83 ± 0.11	13	8.51
5-CT	19.9 ± 1.0	8.59 ± 0.11	7	9.53
8-OH-DPAT	17.6 ± 0.6	8.22 ± 0.08	18	8.74
RU 24969	19.3 ± 0.8	7.81 ± 0.22	4	8.11
Buspirone	14.2 ± 1.0	7.32 ± 0.22	4	7.58
Ipsapirone	14.1 ± 1.6	7.48 ± 0.25	4	7.73
DP-5-CT	18.3 ± 0.1	8.67 ± 0.10	3	9.54
Flesinoxan	19.6 ± 1.5	7.68 ± 0.14	4	8.32
Indorenate	19.0 ± 0.9	7.15 ± 0.21	4	7.80
R 28935	14.7 ± 1.1	8.68 ± 0.17	3	9.21
Urapidil	14.4 ± 1.8	6.61 ± 0.26	4	7.18
5-Methyl-urapidil	10.7 ± 0.1	8.52 ± 0.12	5	9.14
Metergoline	20.5 ± 1.9	7.58 ± 0.13	3	8.10
Methysergide	19.4 ± 1.4	6.40 ± 0.17	4	7.63
PAPP	19.1 ± 2.6	7.74 ± 0.12	3	8.17
Cyanopindolol	8.0 ± 1.5	8.16 ± 0.18	4	8.27
Spiroxatrine	16.8 ± 1.4	7.75 ± 0.17	4	8.05
8-MeO-ClEPAT	15.4 ± 1.4	6.98 ± 0.17	3	7.89
WB 4101	9.0 ± 0.8	7.85 ± 0.07	3	7.93

E_{max} (as % inhibition of forskolin-stimulated adenylate cyclase activity) and pEC_{50} (negative logarithm of molar EC_{50}) are given as means ± s.e.mean of n values. Negative logarithms of dissociation constants (pK_D) at 5-HT$_{1A}$ recognition sites (determined in pig cortex) are the mean of at least 3 determinations (taken from Hoyer *et al.*, 1985b; Markstein *et al.*, 1986; Hoyer, 1988).

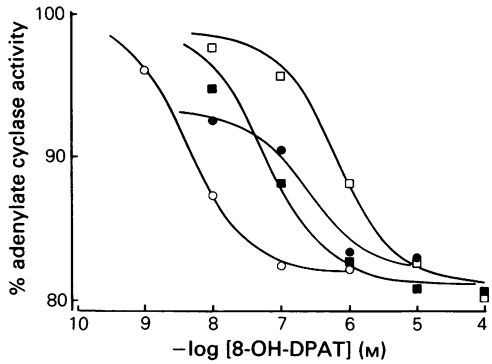

Figure 5 Antagonism of 8-hydroxy-2-(di-n-propylamino)-tetralin (8-OH-DPAT)-induced inhibition of adenylate cyclase activity in calf hippocampus by cyanopindolol, (−)-pindolol and (+)-pindolol. 8-OH-DPAT concentration-effect curves are represented in the absence (○) and in the presence of cyanopindolol (0.1 μM, ●), (−)-pindolol (1 μM, □) or (+)-pindolol (0.1 mM, ■). Data are results from 1 typical experiment out of 3.

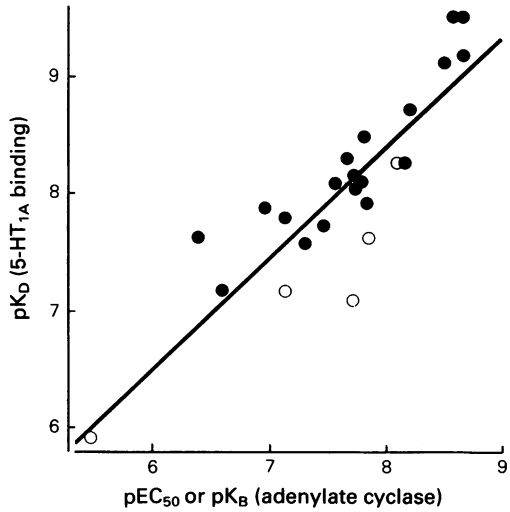

Figure 6 Correlation between pEC$_{50}$ and pK$_B$ values of agonists (●) and antagonists (○) on adenylate cyclase activity in calf hippocampus and their pK$_D$ values at 5-HT$_{1A}$ binding sites (determined in pig cortex). Data are taken from Tables 1 and 2.

% maximal inhibition (E_{max}) and negative logarithm of molar EC$_{50}$ value (pEC$_{50}$) for agonists and of negative logarithm of dissociation constant (pK$_B$) for antagonists. Negative logarithm of affinity constants (pK$_D$) at 5-HT$_{1A}$ binding sites are also listed. They were derived from radioligand binding studies in pig brain cortical membranes. When agonist pEC$_{50}$ values (adenylate cyclase) were plotted against pK$_D$ values (5-HT$_{1A}$ binding) a highly significant ($r = 0.90$, $P = 0.0001$) correlation was found between both parameters (Figure 6). Values of pK$_B$ (adenylate cyclase) and pK$_D$ (5-HT$_{1A}$ binding) of the 5 antagonists tested were also significantly ($r = 0.93$, $P = 0.022$) correlated.

Table 2 Negative logarithms of dissociation constants of antagonists for 8-hydroxy-2-(di-n-propylamino)-tetralin-induced inhibition of adenylate cyclase (pK$_B$) and for 5-HT$_{1A}$ recognition sites (pK$_D$)

Drug	pK_B	n	pK_D
Spiperone	7.15 ± 0.16	5	7.18
Methiothepin	7.73 ± 0.19	4	7.10
(−)-Pindolol	7.87 ± 0.11	3	7.63
(+)-Pindolol	5.49 ± 0.13	3	5.92
Cyanopindolol	8.11 ± 0.09	3	8.27

pK$_B$ are given as means ± s.e.mean of n values. pK$_D$ are the means of at least 3 determinations.

Discussion

Inhibition of forskolin-stimulated adenylate cyclase activity by 5-HT and related agonists has been proposed to be mediated by 5-HT$_{1A}$-receptors in rat and guinea-pig hippocampus (De Vivo & Maayani, 1985; 1986; Bockaert *et al.*, 1987). Furthermore, this has been corroborated by studies determining cyclic AMP production in intact neurones from the mouse (Bockaert *et al.*, 1987). The present data confirm this view and extend it to calf hippocampus.

A variety of structurally different drugs with moderate to high affinity (pK$_D$ values >7) for 5-HT$_{1A}$ binding sites were characterized as agonists inhibiting forskolin-stimulated adenylate cyclase activity in calf hippocampus with a rank order of potency typical of a 5-HT$_{1A}$-receptor mediated effect. The 8-OH-DPAT-induced effect was antagonized by 5 substances known to be 5-HT$_1$-receptor antagonists (including a partial agonist). Stereoselectivity at the receptor involved was demonstrated by the 250 fold potency ratio between the dissociation constants of (−)-pindolol and (+)-pindolol. The results from the adenylate cyclase experiments were in good agreement with the binding data at 5-HT$_{1A}$ sites, as reflected by the highly significant correlation between both parameters. Together the data strongly support the co-identity of 5-HT$_{1A}$ recognition sites and the 5-HT-receptor mediating inhibition of cyclase activity in calf hippocampus. There was no significant correlation (data not illustrated) between adenylate

cyclase effects and 5-HT_{1B}, 5-HT_{1C} or 5-HT_{1D} binding (Hoyer *et al.*, 1985a,b; Heuring & Peroutka, 1987). Reference substances displayed activities in calf hippocampus similar to those obtained earlier in rat and guinea-pig hippocampal preparations (De Vivo & Maayani, 1986; Bockaert *et al.*, 1987). That is, 5-CT, 5-HT, 8-OH-DPAT and RU 24969 were full and potent (EC_{50} values <20 nM) agonists, the rank order of their potencies being 5-CT > 8-OH-DPAT > 5-HT = RU 24969 and buspirone and ipsapirone acted as partial but still potent (EC_{50} values <50 nM) agonists. That the ergoline derivatives methysergide and metergoline were full agonists is perhaps not surprising since similar results were obtained in guinea-pig hippocampus (De Vivo & Maayani, 1986). On the other hand, spiperone and methiothepin were potent antagonists of the 8-OH-DPAT-induced effect; again in agreement with results from rat and guinea-pig hippocampus.

It may be noticed that pEC_{50} values obtained in the adenylate cyclase experiments are lower than pK_D values derived from radioligand binding studies. Adenylate cyclase activity is measured in the presence of GTP, whereas binding studies are routinely carried out in its absence, and measure the affinity of agonists for the high affinity state of the agonist receptor complex. When 5-HT_1-receptor binding is carried out in the presence of GTP (Norman *et al.*, 1985; Hamblin *et al.*, 1987; Heuring & Peroutka, 1987) affinity values for agonists are decreased. The discrepancy between adenylate cyclase and binding data has also been observed for 5-HT_{1B}- and 5-HT_{1D}-receptors (Schoeffter *et al.*, 1988; Bouhelal *et al.*, 1988) and other receptors coupled to adenylate cyclase, e.g. β-adrenoceptors.

In addition to 5-HT_{1A}-receptors, both 5-HT_{1B} (Bouhelal *et al.*, 1988) and 5-HT_{1D} sites (Hoyer & Schoeffter, 1988; Schoeffter *et al.*, 1988) have been shown to be negatively coupled with adenylate cyclase. 5-HT_{1B} sites could not be characterized in the brain of species other than the rat and the mouse (Hoyer *et al.*, 1985a,b; 1986; Hoyer, 1988; Heuring *et al.*, 1986; Hamblin *et al.*, 1987). Moreover, according to Heuring & Peroutka (1987) and to our own data (unpublished), hippocampus from calf and other species has relatively few 5-HT_{1D} sites, whereas it has a high concentration of 5-HT_{1A} recognition sites (Pazos *et al.*, 1987; Waeber *et al.*, 1988a,b). 5-HT, 5-CT, the ergoline derivatives metergoline and methysergide and the antagonist methiothepin do not discriminate between 5-HT_{1A}- and 5-HT_{1D}-receptors. However, 8-OH-DPAT, DP-5-CT, ipsapirone, buspirone and spiperone were at least 100 times more potent and RU 24969 and cyanopindolol more than 10 times more potent at inhibiting adenylate cyclase activity in calf hippocampus (this study) than at the 5-HT_{1D} site which mediates inhibition of adenylate cyclase activity in calf substantia nigra (Schoeffter *et al.*, 1988). Thus, 5-HT_{1D} sites are unlikely to be involved in inhibition of adenylate cyclase activity by 5-HT-related drugs in calf hippocampus.

The novelty of the present work resides in the observation that (1) 5-HT_{1A}-receptors are negatively linked to adenylate cyclase activity in calf hippocampus, (2) ligands other than the reference compounds mentioned above are potent agonists in this model, (3) stereoselective antagonism can be demonstrated for the enantiomers of pindolol and (4) cyanopindolol has the properties of a mixed agonist/antagonist at the 5-HT_{1A}-receptor. Agonist activity of the 5-HT_{1A}/5-HT_{1B}-receptor antagonist cyanopindolol (Engel *et al.*, 1986) has been described in other models (Maura *et al.*, 1987; Schoeffter *et al.*, 1988). Similarly, partial agonist activity was demonstrated in the present study for two drugs introduced as selective 5-HT_{1A}-receptor antagonists, spiroxatrine (Nelson & Taylor, 1986) and 8-MeO-ClEPAT (Fozard *et al.*, 1987). Previous observations, nevertheless, suggested that these two drugs act as agonists (Nelson *et al.*, 1987; Fozard *et al.*, 1987). Like spiroxatrine and 8-MeO-ClEPAT, WB 4101 and PAPP bind with nanomolar affinity to 5-HT_{1A} sites (Norman *et al.*, 1985; Asarch *et al.*, 1985) and these observations have been confirmed in this study. There has been no previous study on the intrinsic activity of WB 4101 at 5-HT_{1A}-receptors. Despite being an antagonist at α_1-adrenoceptors (U'Prichard *et al.*, 1977), the characteristics of [^{3}H]-WB 4101 binding in rat cortex (in the presence of prazosin) are consistent with this drug exhibiting agonist activity at 5-HT_{1A}-receptor sites (e.g. the binding is sensitive to guanine nucleotides; Norman *et al.*, 1985). The partial agonist action of WB 4101 at inhibiting adenylate cyclase activity in calf hippocampus supports this interpretation. PAPP is a N-substituted phenylpiperazine (Asarch *et al.*, 1985), a chemical family whose representatives displaced [^{3}H]-lysergic acid diethylamide binding preferentially to [^{3}H]-5-HT binding in rat brain, which was interpreted as these substances being antagonists rather than agonists (Fuller *et al.*, 1981). Since then, however, there has been functional, behavioural and electrophysiological evidence that PAPP acts as an agonist at 5-HT_{1A}-receptors (Hutson *et al.*, 1987; Ram *et al.*, 1987; Sprouse & Aghajanian, 1987). The full agonist activity of PAPP in the present study reinforces these observations.

The present study establishes inhibition of forskolin-stimulated adenylate cyclase in calf hippocampus as a convenient model for studying the interaction of agonists and antagonists with 5-HT_{1A}-receptors. Indeed, other proposed functional correlates to 5-HT_{1A} recognition sites are either still

questionable, e.g. contraction of canine basilar artery (Peroutka *et al.*, 1986; Taylor *et al.*, 1986), or less well documented, like inhibition of transmitter release from guinea-pig enteric cholinergic neurones (Fozard & Kilbinger, 1985), decrease in population spike amplitude in CA1 hippocampal cells (Beck *et al.*, 1985) and suppression of spontaneous firing in dorsal raphe 5-hydroxytryptaminergic neurones (Sprouse & Aghajanian, 1987). 5-HT_{1A}-receptor agonists induce behavioural effects in rats, e.g. reciprocal forepaw treading and flat body posture (Tricklebank, 1985). However, this test relies heavily on the capacity of drugs to enter the brain and therefore, its use is limited to compounds capable of crossing the blood-brain barrier. Stimulation of adenylate cyclase activity (in the absence of forskolin) in rat and guinea-pig hippocampus appears to provide another well-characterized 5-HT_{1A} functional correlate (Markstein *et al.*, 1986; Shenker *et al.*, 1987). However, the pharmacological analysis in these tissues is hampered by the presence of a low affinity receptor site, unrelated to the 5-HT_{1A} site. In addition, measurements of 5-HT-induced increases in basal (relatively low) adenylate cyclase activity are less precise than those of 5-HT-induced inhibition of the forskolin-stimulated enzymatic activity, due to methodological considerations. For these reasons and because calf hippocampus is a convenient material (large amount of tissue, membranes able to be stored at −70°C), it is believed that inhibition of forskolin-stimulated adenylate cyclase activity in this tissue constitutes a good test for the determination of intrinsic activity of 5-HT_{1A} ligands. Finally, another potential advantage of this method is that it does not rely on the use of laboratory animals.

Special attention was paid in this study to certain centrally acting hypotensive agents with high affinity for 5-HT_{1A} sites. Indeed, evidence has been presented that urapidil (van Zwieten *et al.*, 1985; Sanders & Jurna, 1985; Doods *et al.*, 1988), 5-methyl-urapidil (Kolassa *et al.*, 1986), R 28935 (Timmermans *et al.*, 1982; Doods *et al.*, 1988), DP-5-CT (Doods *et al.*, 1988), flesinoxan (Bevan *et al.*, 1986a) and indorenate (Safdy *et al.*, 1982), like 8-OH-DPAT and ipsapirone (Fozard *et al.*, 1987; Ramage & Fozard, 1987), lower blood pressure in cats and rats, and that this effect is, at least partly, mediated centrally. An action at central 5-HT_{1A}-receptor sites seems to be relevant to the central component of this hypotensive effect, since all these drugs are potent ligands at these sites (Hoyer *et al.*, 1985b; Bevan *et al.*, 1986b; Markstein *et al.*, 1986; Gross *et al.*, 1987; Fozard & Mir, 1987; Mir *et al.*, 1987; Doods *et al.*, 1988; this study) and their hypotensive effects (except for urapidil, otherwise known as an α_1-adrenoceptor antagonist with rather low potency; van Zwieten *et al.*, 1985; Fozard & Mir, 1987) cannot be rationally explained in any other way. Furthermore, putative antagonists at the 5-HT_{1A}-receptor inhibit the cardiovascular effects of 8-OH-DPAT, DP-5-CT, and R 28935 (Fozard *et al.*, 1987; Doods *et al.*, 1988), which strongly supports an agonist action at central 5-HT_{1A}-receptors as the basis of the hypotensive property at 8-OH-DPAT, ipsapirone, flesinoxan, R 28935, indorenate, urapidil and 5-methyl-urapidil. This assumption is reinforced by the present results demonstrating that these drugs do indeed act as agonists for the inhibition of adenylate cyclase activity in calf hippocampus. The increase in potency at inhibiting the cyclase activity along with the gain in hypotensive activity (Kolassa *et al.*, 1986) and in affinity at 5-HT_{1A} sites (Gross *et al.*, 1987; this study) upon methyl substitution at position 5 of urapidil structure lends further support to this view. An agonist action at central 5-HT_{1A}-receptors would also provide an explanation for the blood pressure lowering effect of methysergide in cats, which is mediated centrally (Antonaccio & Taylor, 1977) and associated with sympathoinhibition (Ramage, 1985), as are the hypotensive effects of urapidil (Schoetensack *et al.*, 1977; Sanders & Jurna, 1985; Ramage, 1986), flesinoxan (Bevan *et al.*, 1986a), 8-OH-DPAT and ipsapirone (Ramage & Fozard, 1987). It is worth mentioning, too, that WB 4101 reduced sympathetic nerve discharge in baroreceptor denervated cats, an indication of a central component as part of its hypotensive action besides the peripheral blockade of α_1-adrenoceptors (McCall & Humphrey, 1981).

In conclusion, calf hippocampus represents a convenient tissue source for studying the effects of agonists and antagonists on 5-HT_{1A}-receptor coupled adenylate cyclase activity. Using this model, we have shown that a variety of drugs characterized by their affinity for 5-HT_{1A} sites and their potential to reduce blood pressure at a presumably central site of action, are agonists at 5-HT_{1A}-receptors.

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