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Pharmacology of 5-Hydroxytryptamine-1A Receptors Which Inhibit cAMP Production in Hippocampal and Cortical Neurons in Primary Culture

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SUMMARY

Serotonin (5-hydroxytryptamine, 5-HT) inhibited the formation of cAMP promoted by vasoactive intestinal polypeptide, plus forskolin, in mouse hippocampal and cortical neurons in primary culture. The rank order of potencies of classical 5-HT_1 agonists in inhibiting cAMP formation in hippocampal neurons was 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) > 5-carboxamidotryptamine (5-CT) > *d*-lysergic acid diethylamide > 5-HT > 5-methoxy-*N*,*N*-dimethyltryptamine (5-MeO-N,N-DMT) > RU 24969 > ipsapirone > bufotenine > buspirone [half-maximal efficacy (EC_{50}) = 7, 18, 30, 52, 90, 102, 100, 110, and 128 nM, respectively]. All the tryptamine derivatives substituted in position 5 of the indol were potent agonists [5-HT, 5-CT, 5-MeO-N,N-DMT, 5-methoxytryptamine, and bufotenine], whereas tryptamine, *N*-methyltryptamine, and *N*,*N*-dimethyltryptamine were poor agonists. The most potent antagonists tested were spiperone, (±)-pindolol, (±)-cyanopindolol, WB4101, and methiothepin, the affinity of spiperone for this receptor being 22 nM. In contrast, ketanserin, a specific 5-HT_2 antagonist, and 5-HT_3-selective drugs (ICS 205 930 and MDL 72222) were very weak in antagonizing the 5-HT-inhibited cAMP formation. The pharmacological profiles of 5-HT receptors mediating the inhibition of cAMP formation indicate that these receptors correspond to the 5-HT_{1A}-binding site subtypes. Experiments with the *Bordetella pertussis* toxin indicate that the 5-HT_{1A} receptor mediating inhibition of cAMP production involves a pertussis toxin-sensitive GTP-binding protein. In the absence of VIP, cAMP formation could be stimulated through a 5-HT receptor, but the specific 5-HT_{1A} agonists, 8-OH-DPAT and RU 24969 did not stimulate cAMP production. These results suggest that in mouse embryonic hippocampal neurons, the 5-HT_{1A} receptors, which are negatively coupled to adenylate cyclase, are distinct from the receptor positively coupled to this enzyme. The pharmacological characterization of the 5-HT receptor negatively coupled to adenylate cyclase in mouse embryonic cortical neurons indicates that it differs from the 5-HT_{1A} receptor found in hippocampal neurons. Its main differences with the 5-HT_{1A} receptor in hippocampal neurons are as follows: 1) 8-OH-DPAT was only a poor partial agonist in cortical neurons, whereas it was the best full agonist in hippocampal neurons; and 2) metergoline and methysergide as well as the anxiolytic drugs, ipsapirone and buspirone, which were potent agonists in hippocampal neurons, were competitive antagonists in cortical neurons. Since 5-HT receptors inhibiting cAMP formation in embryonic cortical neurons share many other pharmacological characteristics with a typical 5-HT_{1A} receptor, we can conclude that it is likely to be a nontypical 5-HT_{1A} receptor.

Direct evidence for the presence of multiple 5-HT receptors in mammalian CNS and peripheral nervous system has been accumulating over the last 10 years.

Two basic types of 5-HT receptors have been shown to exist in the CNS (1): a 5-HT_1 type which has a higher affinity for agonists (such as 5-HT, RU 24969, or 5-CT) than for antagonists (1–3) and a 5-HT_2 type which has a high affinity for antagonists like ketanserin or spiperone (1, 4) and for the putative agonist DOB (5). Finally, a third type of receptor termed 5-HT_3 has been characterized mainly in the peripheral nervous system (6). The 5-HT_3 receptors recognize 5-HT agonists when they are derivatives of tryptamine, and a series of new antagonists, such as ICS 205 930 or MDL 72222 (7, 8).

Recent studies based on the displacement of more selective radioligand and autoradiography show the great heterogeneity of 5-HT_1-binding sites (9). The existence of four pharmacologically distinct subtypes of 5-HT_1-binding sites has been established. 5-HT_{1A}-binding sites have a high affinity for agonists

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ABBREVIATIONS: 5-HT, 5-hydroxytryptamine; 5-CT, 5-carboxamidotryptamine; DOB, 4-bromo-2,5-dimethoxyphenylisopropylamine; bufotenine, 5-hydroxy-*N*,*N*-dimethyltryptamine; 5-MeOT, 5-methoxytryptamine; 5-MeO-*N*,*N*-DMT, 5-methoxy-*N*,*N*-dimethyltryptamine; N,N-DMT, *N*,*N*-dimethyltryptamine; N-MT, *N*-methyltryptamine; mCPP, 1-(*m*-chlorophenyl)piperazine; TFMPP, 1-(*m*-trifluoromethylphenyl)piperazine; 8-OH-DPAT, 8-hydroxy-2-(di-*n*-propylamino)tetralin; ipsapirone (TVXQ 7821), 2-(4-4-2-pyramidinyl, 1,2-benzoisothiazol-3-(2H)-one-1,1 dioxide hydrochloride; d-LSD, d-lysergic acid diethylamide; VIP, vasoactive intestinal polypeptide; CNS, central nervous system; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; *E*, efficacy; EC_{50}, half-maximal efficacy.

such as 5-HT, 5-CT, RU 24969, 8-OH-DPAT, or ipsapirone and a moderate affinity (about 100 nM) for the antagonist spiperone (10–13). The 5-HT_{1B}-binding sites, which appear to be present in rat and mouse brains, but not in human, bovine, or pig brains, are defined as having a high affinity for 5-HT, 5-CT, and RU 24969 but a very poor affinity (> 1 μM) for 8-OH-DPAT, ipsapirone, and spiperone (10, 14). The 5-HT_{1C}-binding sites have a high affinity for 5-HT and the antagonists mianserin and mesulergine, but a low affinity for RU 24969, 8-OH-DPAT, and spiperone. They are concentrated in the choroid plexus and in the cortex and represent only 10% of the 5-HT_1 receptors in CNS (15–18). Finally, novel [^{3}H]-5-HT-binding sites have been described in bovine brain membranes and designated as the 5-HT_{1D} subtype of the 5-HT_1 receptors. The 5-HT_{1D} receptors have high affinity for 5-HT and 5-CT, and a low affinity for RU 24969 and spiperone (19).

Some physiological and behavioral functions such as memory, sleep, anxiety, depression, pain perception, sexual behavior, and endocrine secretions are thought to be linked to serotoninergic systems (20, 21). It remains unclear which serotoninergic systems are involved in these multiple functions. However, recently, a number of cellular physiological responses have been shown to be mediated by well characterized 5-HT receptors. For instance, 5-HT_{1B} receptors mediate inhibition of neuronal transmitter release (22), whereas the 5-HT_{1A} receptors decrease spontaneous firing in hippocampal pyramidal cells and raphe neurons (23, 24). The cellular physiological responses mediated by the stimulation of 5-HT_2 receptors include contraction of various smooth muscles, platelet aggregation, and neuronal depolarization (25). 5-HT_3 receptors have been found to increase neurotransmitter release in peripheral neurons (6, 25).

Biochemical studies both at the periphery and in the CNS have established that 5-HT_2 receptors act through the inositol trisphosphate-diacylglycerol system (26–30). A similar coupling mechanism has also been proposed for the mechanism of action of 5-HT_{1C} receptors in the choroid plexus (31). In contrast, 5-HT_1 receptors appear to modulate cAMP levels. In neurons in primary cultures, 5-HT_1 receptors mediate the inhibition of cAMP produced in the presence of VIP plus forskolin (32). Recent reports indicate that in these neurons, as well as in hippocampal membranes, the 5-HT_1 receptors that inhibit stimulated adenylate cyclase are of the 5-HT_{1A} subtype (33, 34). However, under basal conditions, a 5-HT_{1A} receptor has been shown to mediate stimulation of adenylate cyclase in hippocampal membranes (35–37). In addition to this 5-HT_{1A} receptor, which stimulates adenylate cyclase, there is another 5-HT receptor in adult guinea pig hippocampal membranes that also stimulates this enzyme. This receptor has a lower affinity for 5-HT and does not have the 5-HT_1 pharmacological profile (35, 37). It is also present in the neurons of young mice (32) and rats (38).

In this report, we have extensively studied and compared the pharmacological profile of 5-HT_1 receptors that inhibit cAMP produced in the presence of VIP plus forskolin in hippocampal and cortical neurons in primary cultures. Our results indicate that, although 5-HT receptors involved in hippocampal and cortical neurons belong to the 5-HT_{1A} receptor family, they are not identical.

Materials and Methods

Neuronal cell cultures. Neuronal cell cultures generated from cerebral cortex and hippocampus of 14- to 15-day-old and 16- to 17-day-old Swiss mouse embryos, respectively, were grown for 6 days. These cultures were prepared as previously described (39). Briefly, cortex and hippocampal cells (10^6) were mechanically dissociated and plated in the absence of fetal calf serum, in 12-well Costar plates previously coated with 1.5 μg/ml poly-L-ornithine. The culture medium consisted of a mixture (1:1) of Dulbecco's modified Eagle's medium and F12 nutrient (Gibco), supplemented with glucose (33 mM), glutamine (2 mM), sodium bicarbonate (3 mM), Hepes (5 mM). In the place of serum, a defined hormone and salt mixture including insulin (25 μg/ml), transferrin (100 μg/ml), progesterone (20 nM), estradiol (10^{-12} M), and putrescine (60 μM) was added.

Formation of cAMP. The cAMP content of cells was measured by the prelabeling technique previously described (40). On the sixth day, the cells were washed and incubated at 37° (5% CO_2/95% air mixture) with 2 μCi/ml of [^{3}H]adenine (24 Ci/mol, Amersham, UK). After 2 hr, the cultures were washed and incubated with 0.75 mM isobutylmethylxanthine and test agents (all prepared in culture medium) in a volume of 1 ml, for 5 min at 37°. The reaction was stopped by aspiration of the media and addition of 1 ml of ice-cold 5% trichloroacetic acid. Cells were scraped with the aid of a rubber policeman and 100 μl of 5 mM ATP and 5 mM cAMP were added to the mixture. Cellular protein was centrifuged at 5000 × *g*. [^{3}H]ATP and [^{3}H]cAMP were separated by sequential chromatography on Dowex and alumina columns. cAMP formation is expressed as percentage conversion:

$$\frac{[^3\text{H}]\text{cAMP}}{[^3\text{H}]\text{cAMP} + [^3\text{H}]\text{ATP}} \times 100$$

***Bordetella pertussis* toxin pretreatment of cells.** The pertussis toxin, generously donated by A. García-Sainz, was dissolved in a buffer containing 0.1 M potassium phosphate, pH 7.4, 2 M urea, and 1 mg/ml bovine serum albumin at a concentration of 5 mg/ml. On the fifth day the cells were incubated with the toxin at different concentrations: 0.001–10 μg/ml diluted in serum-free culture medium. After 24 hr pretreatment with increasing doses of the toxin, cells were washed and incubated with [^{3}H]adenine for cAMP determination, as described above.

Data analysis. For each drug concentration, we calculated the percentage inhibition taking the maximal activity of 5-HT determined in the same experiment as 100%. In the figures, the mean values of three to five experiments have been given. The error bars corresponding to these percentages are only given in Figs. 1 and 4 to indicate the measurement variations. In the other figures, the standard errors were not included to avoid confusion in the drawing. EC_{50} refers to the agonist concentration yielding 50% of the maximal inhibition determined directly on each concentration response curve. The EC_{50} values given in Table 1 and under Results are the means ± standard errors of three to five experiments performed in duplicate.

The K_i values of antagonists were determined from the concentration of the drug reversing the inhibition obtained with 5-HT (1 μM) by 50%, using the Cheng-Prusoff equation (41).

All of the concentration response curves were considered to follow a simple mass action kinetic. Considering the difficulty of the method used (each data point is determined on one individual primary neuronal culture), it was not possible to generate enough measurements to verify this assumption. Significance probabilities (p) for the correlation coefficient (r) were taken from Table VI of Fisher and Yates (42).

Drugs. The following drugs were generously donated: ipsapirone (TVQX 7821) (J. Traber, Troponwerke GmbH and Co., Cologne, FRG); buspirone (F. D. Yocca of Bristol Myers Company); RU 24969, RU 28253, and RU 26109 (J. F. Pujol of Roussel-Uclaf, Romainville, France); 5-CT (P. P. A. Humphrey, Glaxo Group Research, Hertfordshire, UK); metergoline, methysergide, and pizotifen (H. Gozlan, Faculté de Médecine Pitié-Salpêtrière, Paris, France); spiperone (spiro-

peridol) and ketanserin (J. Leysen, Janssen Pharmaceutica, Beerse, Belgium); haloperidol (Rhône-Poulenc Santé, France); ICS 205930; mesulergine, and *d*-LSD (G. Engel, Sandoz Ltd., Basel, Switzerland); MDL 72222 (J. Fozard, Merrell Dow Research Institute, Strasbourg, France), cocaine hydrochloride (J. Glowinski, Collège de France, Paris, France); WB4101 (Ward Blenkinsop); and Prazosin hydrochloride (Pfizer, Brooklyn, NY).

The purchased drugs were: 5-hydroxytryptamine creatine sulfate (5-HT), tryptamine hydrochloride, N-MT, N,N-DMT, 5-MeOT, 5-MeO-N,N-DMT, and (−)-propranolol base, all from Sigma Chemical Co. (St. Louis, MO); 8-OH-DPAT, Research Biochemical Inc., Wayland, MA; TFMPP and mCPP, Aldrich Chemical Co., Milwaukee, WI; mianserine hydrochloride, Organon, Oss, Holland; bufotenine, Regis, Morton Grove, IL; and (±)-butaclamol, Ayerst Laboratories.

Results

Effects of 5-HT, tryptamine derivatives, and 8-OH-DPAT on cAMP produced by VIP plus forskolin. Except where otherwise stated, experiments reported here were performed in the presence of VIP (0.1 μM) plus forskolin (1 μM). It has been shown previously that in primary cultures of cortical and striatal neurons when present together, VIP plus forskolin stimulated the basal cAMP production by 10-fold and that no further stimulation could be obtained with other agonists known to stimulate cAMP production (40). Under these conditions we have reported that 5-HT was able to inhibit cAMP production through specific 5-HT_1 receptors (32).

Fig. 1 shows the inhibition of cAMP produced by VIP plus forskolin by a series of 5-HT agonists. As previously described (34), 5-CT, a specific 5-HT_1 agonist, and 8-OH-DPAT, a specific 5-HT_{1A} agonist, were slightly more active than 5-HT in inhibiting cAMP production in hippocampal neurons (EC_{50} = 52 ± 18 nM, 18 ± 4 nM, and 7 ± 3 nM for 5-HT, 5-CT, and 8-OH-DPAT, respectively). In cortical neurons 5-HT and 5-CT were still fully potent agonists (EC_{50} = 98 ± 28 nM and 38 ± 10 nM, respectively), whereas 8-OH-DPAT was only a partial agonist, having an EC_{50} of 277 ± 22 nM, and eliciting 50% of the maximal activity of 5-HT.

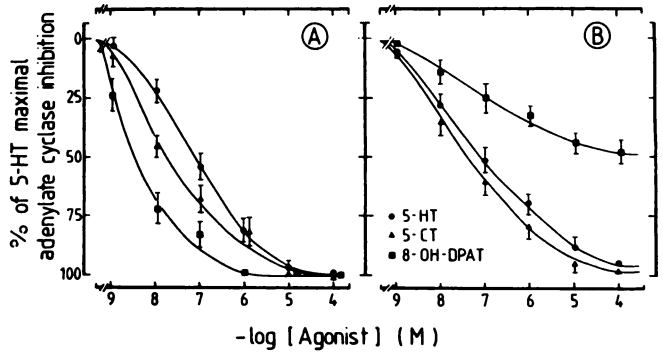

Fig. 1. Effects of 5-HT, 5-CT, and 8-OH-DPAT on VIP plus forskolin-stimulated cAMP formation in hippocampal (A) and cortical (B) neurons in primary culture. Cells were incubated in the presence of 0.1 μM VIP and 1 μM forskolin and increasing concentrations of agonists. Conversion of [^{3}H]ATP to [^{3}H]cAMP was determined after a 5-min incubation period at 37° (see Materials and Methods). In the absence of 5-HT, the per cent conversion was 4.0 ± 0.1% (n = 10) and 4.4 ± 0.3% (n = 12), whereas in the presence of 10^{-5} M 5-HT, the per cent conversion was 3.0 ± 0.1% (n = 10) and 3.3 ± 0.2% (n = 12) in hippocampal and cortical neurons, respectively. The inhibition of cAMP formation is expressed as a percentage of the maximal inhibitory effect of 5-HT. The values are the means ± standard errors of three to five separate experiments, each performed with duplicate determinations.

Tryptamine derivatives like 5-MeO-N,N-DMT and bufotenine, which are known to display a higher affinity for the 5-HT_{1A} than for the 5-HT_{1B} subtype (43, 44), were potent agonists both in hippocampal neurons (EC_{50} = 90 ± 20 nM and 110 ± 25 nM for 5-MeO-N,N-DMT and bufotenine, respectively) and in cortical neurons (32 ± 12 nM and 100 ± 30 nM for the same drugs, respectively). 5-MeOT, a potent 5-HT_1 agonist, but with a rather low selectivity for both 5-HT_{1A} and 5-HT_{1B} subtypes (44), was a fully potent agonist in hippocampal neurons (100 ± 22 nM). In cortical neurons 5-MeOT was a potent agonist but elicited only 70% of the maximal activity of 5-HT (90 ± 37 nM). Therefore, all the tryptamine derivatives substituted in position 5 of the indol were very potent agonists in inhibiting adenylate cyclase in hippocampal neurons as well as in cortical neurons (5-HT, 5-CT, 5-MeO-N,N-DMT, 5 MeOT, and bufotenine). The removal of the substitution in position 5 decreases the agonist efficiency of tryptamine derivatives such as tryptamine, *N*-methyltryptamine, and *N*,*N*-dimethyltryptamine in both hippocampal and cortical neurons (Fig. 2, Table 1).

Effect of tetrahydropyridoindole derivatives RU 24969, RU 28253, and RU 26109. No evident difference was found between the affinity of RU 24969, RU 28253, and RU 26109 for 5-HT receptors that inhibit cAMP production in hippocampal and cortical neurons (Fig. 3). They were all nearly as potent as 5-HT: RU 24969, EC_{50} = 102 ± 45 and 112 ± 50 nM; RU 28253, EC_{50} = 158 ± 45 and 160 ± 30 nM; RU 26109, EC_{50} = 100 ± 24 and 140 ± 52 nM, in hippocampal and cortical neurons, respectively.

Effect of arylpiperazine derivatives. The agonist potencies of two distinct classes of arylpiperazine derivatives were tested: 1) the arylpiperazine substitued with long chain such as ipsapirone (TVXQ 7821) and buspirone, and 2) the arylpiperazine substitued with short chain, such as TFMPP. On the one hand, as previously reported (34), cAMP production was partially inhibited by the two classes of arylpiperazines in hippocampal neurons (Fig. 4A, Table 1); the intrinsic activities were, respectively, 77, 66, and 35% of the maximal activity obtained with 5-HT. On the other hand, in cortical neurons, all of the arylpiperazines failed to significantly inhibit cAMP production (Fig. 4B).

In order to determine whether these drugs were antagonists rather than partial agonists on 5-HT receptors in cortical

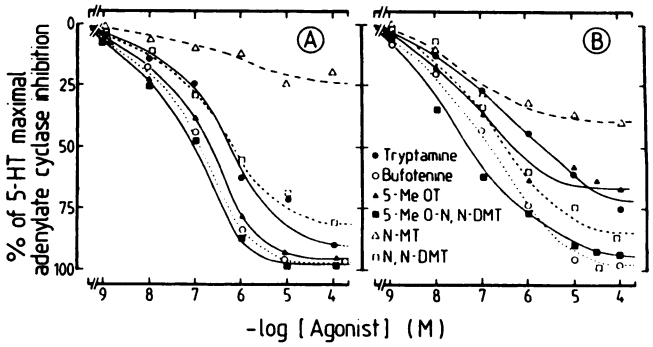

Fig. 2. Inhibitory effect of six tryptamine derivatives on VIP plus forskolin-stimulated cAMP formation in hippocampal (A) and cortical (B) neurons in primary culture. For details, see legend to Fig. 1 and Materials and Methods. Results are expressed as a percentage of maximal inhibitory effects produced by 5-HT. The values are the means of duplicate determinations of three to five separate cultures. The variations of the data points averaged 5% for each drug (not indicated to simplify the figure).

TABLE 1

Pharmacological characteristics of a series of 5-HT agonists on 5-HT receptors inhibiting cAMP production in hippocampal and cortical neurons: Comparison with those of 5-HT$_{1A}$- and 5-HT$_{1B}$-binding sites

The effects of agonists on adenylate cyclase activity were determined as described under Materials and Methods. Data are expressed as pEC$_{50}$, means ± standard errors of duplicate determinations performed on at least three separate cultures. The efficacy (*E*) of the agonist is the maximal inhibitory effect on adenylate cyclase activity as a percentage of the maximal inhibitory effect of 5-HT. The pK_D *values of the agonists at 5-HT$_{1A}$- and 5-HT$_{1B}$-binding sites were all taken from the literature (36, 44–47) as indicated in the footnote to Table 1. In binding studies in which we have taken* pK_D values, 5-HT$_{1A}$-binding sites were labeled using an agonist: [^{3}H]-8-OH-DPAT. No differences have been reported in the literature between the pharmacological profiles of 5-HT$_{1A}$-binding sites (labeled with [^{3}H]-8-OH-DPAT) of different species and different brain areas (36). Therefore, 5-HT$_{1A}$- and 5-HT$_{1B}$-binding studies were routinely carried out on rat or pig cortical membranes (36, 44–46). 5-HT$_{1B}$-binding sites were labeled using an antagonist, [^{125}I]cyanopindolol, in the presence of 30 μM (−)-isoprenaline in order to suppress binding to β-adrenocepotors in rat cortex (36, 45, 46).

Agonists	5-HT receptors negatively coupled to adenylate cyclase				5-HT-binding sites (pK_D)*	
	Hippocampal neurons		Cortical neurons			
	pEC$_{50}$	*E*%	pEC$_{50}$	*E*%	5-HT$_{1A}$	5-HT$_{1B}$
1. 5-HT	7.30 ± 0.10	100	7.00 ± 0.16	100	8.50 (*a*)	7.60 (*a*)
2. 8-OH-DPAT	8.10 ± 0.15	100	6.60 ± 0.40	50 ± 5	8.74 (*a*)	4.20 (*a*)
3. 5-CT	7.70 ± 0.14	100	7.50 ± 0.18	100	9.53 (*a*)	8.30 (*a*)
4. 5-MeOT	7.00 ± 0.18	100	7.10 ± 0.25	70 ± 8	8.04 (*a*)	6.40 (*a*)
5. 5-MeO-N,N-DMT	7.30 ± 0.13	100	7.40 ± 0.18	92 ± 8	7.89 (*b*)	6.24 (*b*)
6. Bufotenine	7.06 ± 0.12	100	7.02 ± 0.20	100	7.60 (*c*)	6.04 (*c*)
7. Tryptamine	6.34 ± 0.30	85 ± 6	6.50 ± 0.15	75 ± 10	6.77 (*c*)	4.99 (*c*)
8. N-MT	6.30 ± 0.18	20 ± 12	6.20 ± 0.40	36 ± 8		
9. N,N-DMT	6.35 ± 0.18	75 ± 10	6.72 ± 0.25	92 ± 3	7.60 (*c*)	6.04 (*c*)
10. RU 24969	7.16 ± 0.22	100	6.95 ± 0.20	100	8.11 (*a*)	8.42 (*a*)
11. RU 28253	6.80 ± 0.25	100	6.80 ± 0.28	100		
12. RU 26109	7.00 ± 0.12	100	6.80 ± 0.20	100		
13. Ipsapirone	7.10 ± 0.30	77 ± 7	antagonist		7.61 (*b*)	4.77 (*b*)
14. Buspirone	6.90 ± 0.15	66 ± 6	inactive as agonist			
15. TFMPP	6.93 ± 0.10	35 ± 7	inactive as agonist			
16. mCPP	6.95 ± 0.15	55 ± 8	inactive as agonist			
17. *d*-LSD	7.40 ± 0.22	100	7.50 ± 0.10	100	8.59 (*a*)	6.82 (*a*)
18. Methysergide	6.35 ± 0.29	92 ± 6	antagonist			
19. Metergoline	6.90 ± 0.22	94 ± 5	antagonist			

* The pK_D values are taken from: (*a*) Markstein *et al.* (36), (*b*) Hamon *et al.* (44), or (*c*) Engel *et al.* (45).

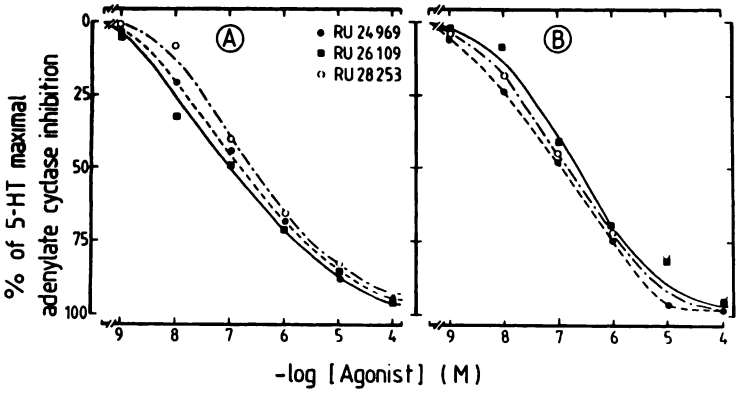

Fig. 3. Concentration response curves for three tetrahydropyridoindol derivatives: RU 24969, RU 28253, and RU 26109 on VIP plus forskolin-stimulated cAMP formation in hippocampal (A) and cortical (B) neurons in primary culture. For details, see legend to Figure 1 and Materials and Methods. The values are the means of duplicate determinations on three separate cultures. The coefficient of variations of measurements (standard errors) averaged less then 5%.

neurons, ipsapirone, the more potent arylpiperazine derivative, was tested for its antagonist activity on cortical neurons. As reported in Fig. 4B (*inset*), the agonist effect of 1 μM 5-HT was reversed at high ipsapirone concentrations (pK_i = 6.4).

Effect of ergoline derivatives. As reported in Fig. 5, *d*-LSD was as potent an agonist as 5-HT in both hippocampal and cortical neurons (Fig. 5, Table 1). It is known that *d*-LSD displays a very high affinity but a rather low selectivity for 5-HT-binding sites. In contrast, methysergide and metergoline were both total agonists in hippocampal neurons, whereas they were devoid of agonist activity in cortical neurons. In addition, Table 2 shows that these two ergolines were potent antagonists in cortical neurons.

Effect of antagonists on 5-HT inhibition of cAMP in neurons. As reported in Table 2, both in cortical and hippocampal neurons, the selective 5-HT$_2$ antagonists, ketanserine and cyproheptadine, were very weak in blocking the inhibition of cAMP produced by 1 μM 5-HT. Selective 5-HT$_3$ antagonists, such as ICS 205 930, MDL 72222, and cocaine failed to reverse 5-HT inhibition. These results led to the assumption that 5-HT receptors present in cortical and hippocampal neurons are neither 5-HT$_2$ nor 5-HT$_3$. Furthermore, spiperone, a rather selective 5-HT$_{1A}$ antagonist, entirely reversed the response to 1 μM 5-HT with high affinity, in both systems (Table 2). Metergoline, a potent 5-HT$_1$ ligand having a higher affinity for 5-HT$_{1A}$ and 5-HT$_{1C}$ than for 5-HT$_{1B}$, markedly inhibited the agonist effect only in cortical neurons (pK_i = 7.9); it was a good agonist in hippocampal neurons (EC$_{50}$ = 125 ± 40 nM). The 5-HT effect on cAMP production could also be antagonized by methiothepin, a 5-HT$_1$ antagonist without selectivity. (±)-Cyanopindolol, a nonselective 5-HT$_1$ antagonist (44, 45), was found to have a high affinity for 5-HT receptors in hippocampal

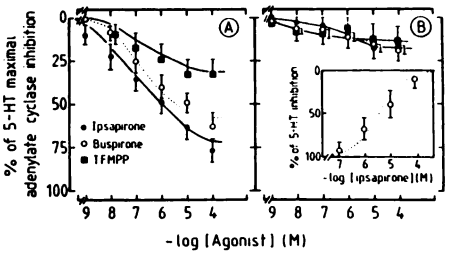

Fig. 4. Inhibitory effects of three arylpiperazine derivatives: ipsapirone, buspirone, and TFMPP, on VIP plus forskolin-stimulated cAMP formation in hippocampal (A) and cortical (B) neurons in primary culture. The experiments were carried out as described in the legend to Fig. 1 and under Materials and Methods. The data points, expressed as the percentage of maximal inhibitory effect produced by 5-HT, are the means ± standard errors of duplicate determinations on three separate cultures. *Inset*: the inhibition produced by 5-HT (1 μM) in cortical neurons by ipsapirone. The data show cumulative results from two experiments, each performed in duplicate.

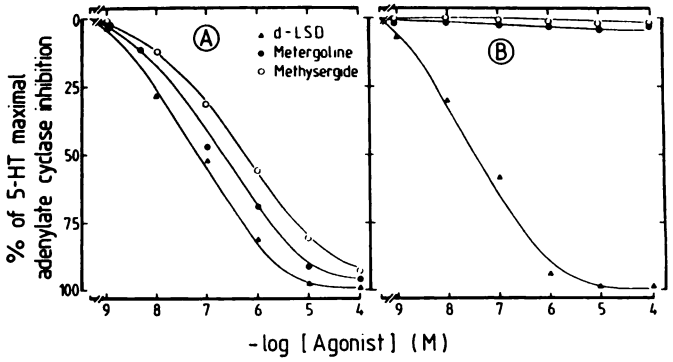

Fig. 5. Effects of various concentrations of three ergolines: *d*-LSD, metergoline, and methysergide, on VIP plus forskolin-stimulated cAMP formation in hippocampal (A) and cortical (B) neurons in primary culture. The experiments were carried out as described in the legend to Fig. 1 and under Materials and Methods. Results are expressed as a percentage of maximum effect of 5-HT. Values are the means of at least three determinations.

neurons (pK_i = 7.8) similar to that found in binding experiments (pK_D = 8.27) (45) (Table 1); this antagonist was almost 50 times less active on 5-HT receptors in cortical neurons (pK_i = 6.3) (Table 2). Similarly, a 10-fold difference was noted between the pK_i of (−)-propranolol and those of WB4101 for the 5-HT receptors inhibiting cAMP production in hippocampal and cortical neurons, respectively (Table 2). The poor affinity of mianserin and mesulergine indicates that the receptors studied are not of the 5-HT_{1C} subtype in hippocampal neurons, or in cortical neurons.

Relationships between the pharmacological profiles of 5-HT_1 receptors that inhibit cAMP production in hippocampal and cortical neurons and those of 5-HT_{1A}- and 5-HT_{1B}-binding sites. We have compared the pK_D of a series of 10 agonists and 11 antagonists for the 5-HT_{1A}- and 5-HT_{1B}-binding sites obtained either from rat or pig cortical membranes (36, 44–46) or with rat hippocampal membranes (47) with the pEC_{50} and pK_i of those drugs to inhibit or reverse the 5-HT inhibition of cAMP production in hippocampal and cortical neurons. As seen in Fig. 6, A and B, significant correlations (r agonists = 0.82, $p < 0.01$; r antagonists = 0.93, $p < 0.001$) were found between the affinities of 5-HT agonists and antagonists for 5-HT_{1A}-binding sites and their affinities for the 5-HT receptors that inhibit cAMP production in hippocampal neurons. On the contrary, poor correlations were obtained between the affinities of these agonists and antagonists for the 5-HT_{1B}-binding sites and the 5-HT receptor, negatively coupled with an adenylate cyclase in hippocampal neurons (r agonists = 0.16, $p < 0.1$; r antagonists = 0.65, $p < 0.05$) (Fig. 6, C and D). We also compared the same pK_D values for 5-HT_1-binding sites (5-HT_{1A} and 5-HT_{1B}) and the pEC_{50} and pK_i values of the same drug to inhibit or reverse the 5-HT inhibition of cAMP production in mouse embryonic cortical neurons. The pEC_{50} values obtained with cortical neurons do not correlate well with pK_D values for 5-HT_{1A} measured in competition binding experiments with rat cortical membranes (36, 44–46) or rat hippocampal membranes (47), r agonists = 0.6, $p < 0.1$. However, if we omit 8-OH-DPAT, the source of poor correlation, r agonist, becomes 0.78, $p < 0.01$. As in hippocampal neurons, there was poor correlation between stimulation of adenylate cyclase activity by the agonists in cortical neurons and their affinity for 5-HT_{1B}-binding sites, r = 0.58 ($p < 0.1$). When we omitted 8-OH-DPAT, r was 0.46 ($p < 0.1$). Poor correlations were found between the affinities of 5-HT antagonists for 5-HT_{1A}-binding sites and their affinities for the 5-HT receptors that inhibit cAMP production in cortical neurons (r = 0.39, $p < 0.1$). Similarly, poor correlations were also found between the affinities of these antagonists for 5-HT_{1B} binding and for inhibiting the 5-HT receptors in cortical neurons (r = 0.33, $p < 0.1$).

Effect of *Bordetella pertussis* toxin on the coupling of 5-HT receptors to adenylate cyclase. After a pretreatment of hippocampal and cortical cultures during 24 hr with various concentrations of pertussis toxin (0.001 to 10 μg/ml), we observed that the inhibition of cAMP production by 5-HT was abolished (Fig. 7).

A 24-hr pretreatment with the toxin did not significantly affect the control VIP-forskolin stimulation of cAMP. Only a modest stimulatory effect was observed at low concentrations which remained constant at higher concentrations.

Effect of a series of 5-HT agonists on cAMP production in the absence of VIP in hippocampal neurons. In the absence of VIP and in the presence of very low forskolin concentrations (10^{-7} M), 5-HT stimulated cAMP production in a concentration-dependent manner (EC_{50} = 120 ± 15 μM). This receptor is not the 5-HT_{1A} receptor which stimulates adenylate cyclase in guinea pig and in rat hippocampal membranes described by Shenker *et al.* (35, 37) and Markstein *et al.* (36), since it was not stimulated either by 8-OH-DPAT, a specific 5-HT_{1A} agonist (11), or by RU 24969, a potent 5-HT_{1A} and 5-HT_{1B} agonist (44).

Discussion

The aim of this study was to extend the pharmacological characterization of those 5-HT receptors that mediate inhibition of VIP-forskolin-stimulated adenylate cyclase activity in mouse hippocampal neurons and to compare it with the pharmacology of 5-HT receptors mediating inhibition of adenylate cyclase in mouse cortical neurons.

Our results confirm those of our initial studies (34), that the inhibitory effect of 5-HT agonists (Table 1) in mouse hippocampal neurons are mediated through 5-HT_{1A} receptors.

Indeed, we found a significant correlation between the affinities of a series of 10 agonists (r = 0.82) and 11 antagonists (r = 0.93) for the 5-HT receptor, negatively coupled with an adenylate cyclase in hippocampal neurons, and their affinity for 5-HT_{1A}-binding sites already reported in the literature (36,

TABLE 2

Pharmacological characteristics of a series of 5-HT antagonists on 5-HT receptors reversing the inhibition of cAMP obtained with 5-HT in hippocampal and cortical neurons: Comparison with those of 5-HT_{1A}- and 5-HT_{1B}-binding sites

The effects of antagonists were determined as described under Materials and Methods. Data are expressed as pK_i, means ± standard errors of duplicate determinations performed on at least three separate cultures. In cAMP experiments, concentrations of the drug reversing by 50% the inhibition obtained with 5-HT (1 μM) were used, as measured by the Cheng and Prusoff equation (41) as indicated under Materials and Methods. The pK_D values of antagonists at 5-HT_{1A}- and 5-HT_{1B}-binding sites were all taken from the literature as indicated in the footnote to Table 2.

Antagonists	5-HT receptors negatively coupled to adenylate cyclase (pK_i)		(pK_D)*	
	Hippocampal neurons	Cortical neurons	5-HT_{1A}	5-HT_{1B}
20. Spiperone	7.60 ± 0.15	7.55 ± 0.10	7.18 (*a*)	5.27 (*a*)
21. WB4101	7.55 ± 0.10	6.50 ± 0.22		
22. Methiothepin	7.55 ± 0.17	7.20 ± 0.13	7.10 (*a*)	7.28 (*a*)
23. Methysergide	agonist	7.00 ± 0.25		
24. Metergoline	agonist	7.90 ± 0.10		
25. Ipsapirone	agonist	6.40 ± 0.28	7.61 (*a*)	4.77 (*b*)
26. (±)-Pindolol	7.90 ± 0.12	7.24 ± 0.20	7.49 (*b*)	6.28 (*b*)
27. (−)-Propranolol	6.40 ± 0.14	7.40 ± 0.15	6.67 (*d*)	7.33 (*d*)
28. (±)-Cyanopindolol	7.80 ± 0.18	6.30 ± 0.22	8.27 (*c*)	8.28 (*c*)
29. Haloperidol	6.00 ± 0.25	6.55 ± 0.30	5.45 (*e*)	
30. Mianserin	5.80 ± 0.10	6.60 ± 0.18	5.94 (*d*)	5.33 (*d*)
31. Mesulergine	6.20 ± 0.24	5.90 ± 0.25	6.23 (*a*)	4.88 (*a*)
32. Ketanserine	5.56 ± 0.20	5.50 ± 0.10	5.86 (*a*)	5.72 (*a*)
33. Cyproheptadine	5.90 ± 0.18	5.40 ± 0.25	6.45 (*c*)	5.32 (*c*)
34. ICS 250 930	5 <	5 <	4.70 (*a*)	4.35 (*a*)
35. MDL 72222	5 <	5 <		
36. (−)-Cocaine	5 <	5 <		
37. (+)-Butaclamol	not determined	5.95		
38. (−)-Butaclamol	not determined	6.40		
39. Pizotifen	inactive	inactive		
40. Prazosin	inactive	inactive		
41. Rauwolsine	inactive	inactive		

* The pK_D values are taken from: (*a*) Markstein *et al.* (36), (*b*) Hamon *et al.* (44), (*c*) Engel *et al.* (45), (*d*) Hoyer *et al.* (46), or (*e*) Gozlan *et al.* (47).

44–47) (Fig. 6, A and B). In contrast, no correlation was observed when the comparison was made with the affinities of these drugs for the 5-HT_{1B}-binding sites (Fig. 6, C and D). In particular, the 5-HT receptor of hippocampal neurons has a high affinity for the selective 5-HT_{1A} ligands, which have a low affinity for 5-HT_{1B}-binding sites such as 8-OH-DPAT, tryptamine, 5-MeO-N,N-DMT, bufotenine, ipsapirone, buspirone, *d*-LSD, and spiperone (Figs. 1–5, Tables 1 and 2). In general, this 5-HT_{1A} receptor of hippocampal neurons has a high affinity for tryptamine derivatives substituted in position 5, for tetrahydropyridoindole derivatives (RU 24969, RU 28253, and RU 26109) and for long chain substituted arylpiperazine derivatives (ipsapirone and buspirone).

The pharmacological characteristics of the 5-HT_{1C}-binding sites as reported by Bradley *et al.* (25) are not consistent with those described in the present report because mesulergine and mianserin, two specific 5-HT_{1C} antagonists, have a low affinity for the receptor present in hippocampal neurons (Table 2). Furthermore, a poor correlation also exists between the 5-HT receptor studied here and the 5-HT_{1D}-binding sites recently described by Heuring and Peroutka (19). Indeed, the 5-HT_{1D}-binding sites have a low affinity for selective 5-HT_{1A} ligands, such as spiperone, ipsapirone, buspirone, (±)-pindolol, and 8-OH-DPAT.

Our results are congruent with those of De Vivo and Maayani (33), who reported that the inhibition of forskolin-stimulated adenylate cyclase activity in guinea pig and rat hippocampal membranes is mediated by a receptor with the characteristics of the 5-HT_{1A}-binding site. Although less extensive than the one reported here, the pharmacology reported by De Vivo and Maayani (33) is very similar and suggests that there is no difference between the 5-HT_{1A} receptor present in intact hippocampal neurons in primary culture generated from mouse embryos and adult guinea pig or rat hippocampal membranes.

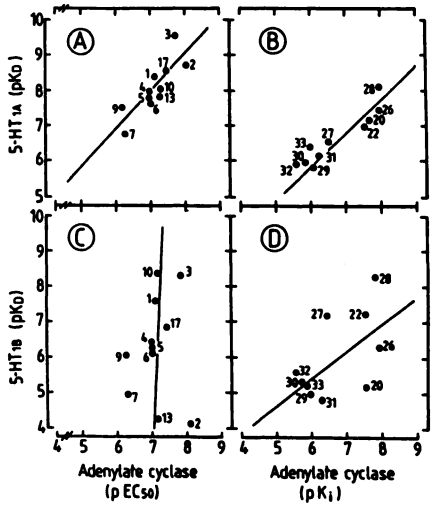

Fig. 6. Correlation between the affinities of 5-HT agonists and antagonists for the 5-HT receptor inhibiting adenylate cyclase activity in hippocampal neurons and their affinities for 5-HT_{1A} and 5-HT_{1B} recognition sites. In A and C, the pEC_{50} values of 11 agonists (on the *x* axis) for inhibiting cAMP production are correlated with the pK_i values of the same agonists for the 5-HT_{1A} (A)- and 5-HT_{1B}-binding sites (on the *y* axis) (C) (see Table 1 for values) (36, 44, 45). The correlation coefficients (*r*) are 0.82 in A and 0.16 in C. In B and D, the pK_i values of 10 antagonists to block 5-HT receptors inhibiting cAMP production (on the *x* axis) are correlated with the pK_D values of the same antagonist for 5-HT_{1A} (B) and 5-HT_{1B} (D) recognition sites (on the *y* axis) (see Table 2 for values) (36, 44–47). The correlation coefficients (*r*) are 0.93 in B and 0.65 in D.

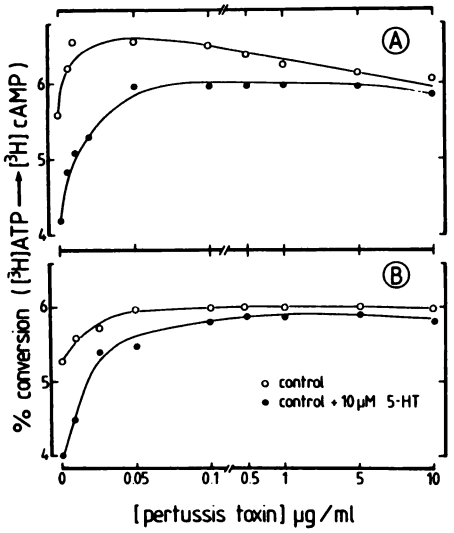

Fig. 7. Effects of pertussis toxin on the 5-HT inhibition of VIP plus forskolin-stimulated cAMP formation in hippocampal (A) and cortical (B) neurons in primary culture. Neurons in primary culture were pretreated for 24 hr with increasing concentrations of pertussis toxin (0.001–10 μg/ml). After 24 hr pretreatment, the cells were incubated with [^{3}H]adenine, and the inhibitory effect of 10 μM 5-HT was measured on VIP-forskolin-stimulated cAMP formation as described under Materials and Methods and compared to the control. Results are expressed as a percentage conversion of [^{3}H]ATP to [^{3}H]cAMP. The values are the means of four separate experiments, each performed with duplicate determinations.

This suggests that the receptors mediating the response in hippocampus are present in an early stage of life and that no structural evolution seems to occur during the course of differentiation. Our results are also consistent with autoradiographic studies which show that 5-HT_{1A}-binding sites are densely present in hippocampus (48).

In vivo, 5-HT_{1A}-binding sites are postsynaptically localized in hippocampus, since lesions of 5-HT neurons do not reduce their density (49). The description here of 5-HT_{1A} receptors in intrinsic hippocampal neurons in culture confirms their postsynaptic localization. 5-HT_{1A} receptors are localized both in intrinsic hippocampal neurons and on cell bodies of 5-HT raphe neurons (14, 48, 49). Electrophysiological studies have confirmed these two localizations. 5-HT_{1A} agonists, including ipsapirone, inhibit the firing of 5-HT raphe neurons (24) and also inhibit the firing of pyramidal neurons in hippocampus (23). The effects of 5-HT_{1A} receptors on pyramidal neurons, like those of 5-HT_{1A} receptors that inhibit cAMP production described here, are blocked by pertussis toxin (23). However, Andrade *et al.* (23) suggested that in hippocampal pyramidal cells, 5-HT_{1A} receptors are directly coupled with potassium channels through a pertussis toxin-sensitive GTP-binding protein. They excluded the possibility that inhibition of cAMP production by 5-HT_{1A} receptors could be involved in the opening of K^+ channels induced by these receptors. Therefore, 5-HT_{1A} receptors are going to rejoin the category of receptors which are possibly coupled with several transduction mechanisms (50). Since at least three pertussis toxin-sensitive GTP-binding proteins are present in neurons (51), further work is required to establish whether the effects of 5-HT_{1A} receptors on K^+ channels and adenylate cyclase are mediated by the same GTP-binding protein.

In guinea pig and in rat hippocampal membranes, two effects of 5-HT_{1A} receptors have been described. One is the stimulation of adenylate cyclase under basal conditions (35–37) and the other is the inhibition of adenylate cyclase (33, 34). It is interesting to know whether or not 5-HT_{1A} receptors can be coupled to adenylate cyclase through the stimulatory G protein (G_s) under basal conditions and to the inhibitory G protein (pertussis toxin-sensitive) in the presence of forskolin (51). Whereas it is not easy to answer this question, our observation that, in intact hippocampal embryonic neurons, there are 5-HT_{1A} receptors negatively coupled with stimulated adenylate cyclase and no 5-HT_{1A} receptors positively coupled with this enzyme under basal conditions, suggests that the stimulatory and inhibitory 5-HT_{1A} receptors are different (Fig. 8). The stimulatory 5-HT receptors observed here under basal conditions (Fig. 8) are not of the 5-HT_{1A} subtype and appear to be similar to those we described 10 years ago in rat colliculi homogenates (38). The pharmacology of these receptors is different from those of all binding sites described so far in CNS.

In culture of cortical embryonic mouse neurons, it is clear that the 5-HT receptor subtype involved is more difficult to establish. Since we have clearly determined that in hippocampal neurons, the 5-HT receptor which inhibits cAMP production is the 5-HT_{1A} receptor, one way of analyzing this problem is to look at the difference between hippocampal and cortical receptors.

Considering 5-HT agonists, most of them have the same efficiency in inhibiting cAMP production in hippocampal and cortical neurons. This includes 5-HT itself, most of the tryptamines, and the tetrahydropyridoindole derivatives (Figs. 1–3). The main difference between these two receptors was seen with non-tryptamine compounds such as 8-OH-DPAT, arylpiperazine derivatives, and ergolines (methysergide and metergoline). As seen in Fig. 1, 8-OH-DPAT, which was the most potent full agonist of the 5-HT receptor in hippocampal neurons (EC_{50} = 7 nM), was a poor partial agonist (EC_{50} = 277 nM) in cortical neurons. Furthermore, methysergide and metergoline, which were full agonists in hippocampal neurons, were antagonists in cortical neurons (Tables 1 and 2). A similar

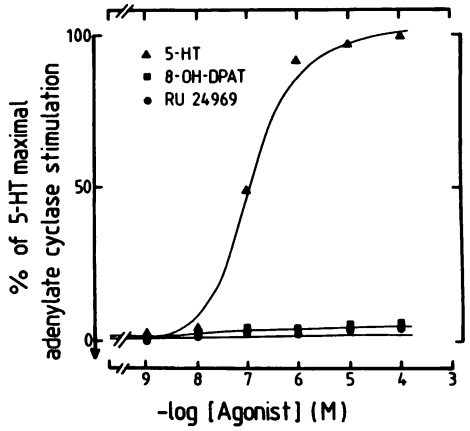

Fig. 8. Effect of various concentrations of 5-HT, 8-OH-DPAT, and RU 24969 on the stimulation of cAMP production in hippocampal neurons in primary culture. Cells were incubated at low concentrations of forskolin (0.1 μM) and increasing concentrations of agonists. Conversion of [^{3}H]ATP to [^{3}H]cAMP was determined after 5 min at 37° (see Materials and Methods). In the absence of 5-HT, the percentage conversion was 0.15 ± 0.02% (n = 3) and 0.34 ± 0.03% in the presence of 10^{-5} M 5-HT. The results are expressed as the percentage of the maximal stimulatory effect of 5-HT. Values are the means of duplicate determinations performed on three separate neuronal cultures with less then 3% variations.

situation was found for arylpiperazine derivatives such as buspirone, ipsapirone, TFMPP, and mCPP (Fig. 4, Tables 1 and 2). Two simple hypotheses can be proposed to account for the difference observed in cortical and hippocampal neurons: 1) 8-OH-DPAT, arylpiperazine, methysergide, and metergoline are partial agonists or antagonists of 5-HT receptors in cortical neurons because there are less receptors in those neurons than in hippocampal neurons. The amplification between receptor occupancy and cAMP inhibition is not as great in cortical as in hippocampal neurons, and, therefore, poor agonists become antagonists. 2) These two receptors are of the same family but not identical. Whereas the first hypothesis cannot be excluded, we think that three main arguments are against it. 1) If such a difference in receptor number influences the amplification between receptor occupancy and cAMP production, the EC_{50} for full agonists (such as 5-HT itself, 5-CT, RU compounds, and *d*-LSD) should be lower in hippocampal than in cortical neurons. This was not the case for these and for all full agonists tested (Figs. 1–3, Table 1). 2) In the same line, since the EC_{50} values for 5-HT are the same in both systems, a simple difference in the coupling does not fit with the observation that some good agonists in hippocampus (such as metergoline and methysergide) are good antagonists in cortex. 3) Some antagonists (such as (±)-cyanopindolol, (−)-propranolol, and WB4101) have different affinities for hippocampal and cortical 5-HT receptors, respectively (Table 2). We should therefore conclude that the 5-HT receptor in cortical neurons is not similar to the 5-HT_{1A} receptor in hippocampal neurons.

We can also exclude that this cortical receptor is not of the 5-HT_{1B} or 5-HT_{1D} subtype since it has a high affinity for spiperone (Table 2). It is not a 5-HT_{1C} receptor since its affinity for mesulergine is poor.

Therefore, we proposed that the 5-HT receptor which inhibits cAMP production in embryonic cortical neurons is a 5-HT_{1A}-"like" receptor. The 5-HT_{1A} receptors are possibly members of a family of homologous proteins. Of course, we still have no proof yet that this cortical 5-HT_{1A}-"like" receptor exists in adult animals. It could be an embryonic form of the 5-HT_{1A} receptor, and experiments are under way to find such a receptor in adult animals. If this 5-HT_{1A}-"like" receptor exists in adults and especially in human brain, one interesting remark could be made. 5-HT_{1A} receptors, having a pharmacology similar to those inhibiting adenylate cyclase in hippocampal neurons and firing 5-HT raphe neurons, are likely to be the site of action of new anxiolytic drugs of the arylpiperazine type, such as ipsapirone and buspirone. If these drugs are weak agonists or are even antagonists on 5-HT_{1A} receptors of cortical neurons, this could explain why these anxiolytic drugs have relatively few undesirable effects.

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