LSN 3612

European Journal of Pharmacology, 106 (1985) 539–546 N°3
Elsevier

THE BINDING OF SEROTONERGIC LIGANDS TO THE PORCINE CHOROID PLEXUS: CHARACTERIZATION OF A NEW TYPE OF SEROTONIN RECOGNITION SITE

ANGEL PAZOS, DANIEL HOYER and JOSE M. PALACIOS *

Preclinical Research, Sandoz Ltd., Basle, CH-4002, Switzerland

Received 2 May 1984, revised MS received 10 August 1984, accepted 10 September 1984

A. PAZOS, D. HOYER and J.M. PALACIOS, *The binding of serotonergic ligands to the porcine choroid plexus: characterization of a new type of serotonin recognition site*, European J. Pharmacol. 106 (1985) 539–546.

The kinetic and pharmacological characteristics of the binding of [^{3}H]5-HT (serotonin), [^{3}H]8-OH-DPAT (8-OH-2-di-n-propylaminotetraline), [^{3}H]LSD, [^{3}H]ketanserin and [^{3}H]mesulergine to membranes from frontal cortex, hippocampus and choroid plexus of pig brain were studied. The binding of these ligands to frontal cortex and hippocampus demonstrated the presence of 5-HT$_1$ and 5-HT$_2$ sites in both tissues, although hippocampus was richer in 5-HT$_1$ (subtype 5-HT$_{1A}$) sites. [^{3}H]5-HT, [^{3}H]mesulergine and [^{3}H]LSD labeled the pig choroid plexus with high affinity. The pharmacological profiles of [^{3}H]5-HT and [^{3}H]mesulergine binding to this tissue were closely comparable. Ligands reported as selective for 5-HT$_{1A}$, 5-HT$_{1B}$ or 5-HT$_2$ subtypes did not show high affinity for these binding sites. Therefore, these 5-HT binding sites in pig choroid plexus could be named 5-HT$_{1C}$. Other drugs with a high affinity for these sites were methysergide and mianserine. In pig frontal cortex, [^{3}H]5-HT labeled the different subtypes of 5-HT$_1$ sites. In contrast, [^{3}H]mesulergine bound in pig frontal cortex to a small population of sites with pharmacological properties similar to those of the choroid plexus 5-HT$_{1C}$ sites. Possible physiological functions in which these sites might be involved are discussed.

5-HT$_1$ recognition sites Serotonin Mesulergine LSD Choroid plexus Frontal cortex

1. Introduction

The presence of multiple serotonin recognition sites has been supported by pharmacological, biochemical and electrophysiological findings (Aghajanian, 1981; Nelson et al., 1980). The classification of 5-HT receptors proposed by Peroutka and Snyder (1979), naming 5-HT$_1$ sites those labeled by [^{3}H]5-HT with nanomolar affinity and 5-HT$_2$ sites those labeled by [^{3}H]spiperone, has gained widespread use.

In recent years, evidence has accumulated in support of the existence of different subtypes of 5-HT$_1$ recognition sites. Some neuroleptics (Pedigo et al., 1981) and β-blockers (Middlemiss et al., 1977; Nahorski and Willcocks, 1983; Hoyer et al., in preparation) displace [^{3}H]5-HT binding in a biphasic manner. Two serotonergic agonists, 8-OH-DPAT (8-OH-2-di-n-propylaminotetraline) (Hjorth et al., 1982) and RU-24 969 (5-methoxy-3[1,2,3,6-tetrahydropyridin-4-yl]1H-indole) (Euvrard and Boissier, 1980) also show high affinity for only a portion of the [^{3}H]5-HT binding sites (Hunt and Oberlander, 1981; Middlemiss and Fozard, 1983; Gozlan et al., 1983). Autoradiographic studies have also demonstrated the heterogeneity of the sites labeled by [^{3}H]5-HT (Deshmukh et al., 1983; Marcinkiewicz et al., 1984; Cortés et al., 1984). These results have suggested the presence of at least 2 subtypes of 5-HT$_1$ sites named 5-HT$_{1A}$ and 5-HT$_{1B}$ (Pedigo et al., 1981). Whether or not the different subtypes of 5-HT$_1$ recognition sites do represent actual 5-HT receptor subtypes awaits the demonstration of a functional role for these sites (see Fozard (1983) for a recent discussion).

* To whom correspondence should be addressed.

By using in vitro quantitative autoradiography, we confirmed (Cortés et al., 1984) that one of the areas labeled with high affinity by [^{3}H]5-HT in the rat was the choroid plexus as previously reported by several authors (Young and Kuhar, 1980; Meibach et al., 1980; Biegon et al., 1982; Marcinkiewicz et al., 1984). The presence of 5-HT neurons and fibers in choroid plexus has also been described (Chan-Palay, 1976; Steinbush, 1981). In our study, we found that these 5-HT receptors in the rat choroid plexus were labeled not only by [^{3}H]5-HT and [^{3}H]LSD, but, surprisingly, also by the reputed 'selective' 5-HT$_2$ ligand [^{3}H]mesulergine (Closse, 1983). In addition, spiperone, β-blockers, RU-24 969 and 8-OH-DPAT (proposed ligands for 5-HT$_{1A}$ and 5-HT$_{1B}$ sites, see above) did not displace these sites with high affinity. The 5-HT$_2$ ligand [^{3}H]ketanserin (Leysen et al., 1982) did not label them. These results showed that the 5-HT receptors localized in the choroid plexus could not be classified with the subtypes already described. Therefore, we named these receptors 5-HT$_{1C}$ sites (Cortés et al., 1984).

In order to obtain more detailed information on the pharmacological characteristics of these sites, we did membrane binding studies, and because of the amount of tissue required we used porcine choroid plexus. The properties of the serotonergic binding sites in the choroid plexus were compared to those of the hippocampus and frontal cortex, areas rich in the other subtypes.

We report here (i) the kinetic characteristics of the binding of [^{3}H]5-HT, [^{3}H]LSD, [^{3}H]8-OH-DPAT, [^{3}H]ketanserin and [^{3}H]mesulergine to pig frontal cortex, hippocampus and choroid plexus, (ii) the pharmacological profile of the [^{3}H]5-HT and [^{3}H]mesulergine binding to so-called '5-HT$_{1C}$' sites, and (iii) evidence that these subtypes are not only located in the choroid plexus.

2. Materials and methods

Pig brains were obtained from a local slaughterhouse and placed on ice immediately after death. Choroid plexuses were carefully removed from the ventricular system. The hippocampus and the frontal pole of the cortex were dissected. Choroid plexuses from 4-6 animals were used in each saturation or competition experiment.

Membranes from the 3 tissues were prepared as described (Pazos et al., 1984). Briefly, tissues were homogenized in 0.32 M sucrose and this homogenate was centrifuged at 70000 $\times g$ for 15 min. The pellet was resuspended, incubated at 37°C for 15 min then centrifuged again. The final pellet was resuspended in 50 mM Tris-HCl buffer (pH 7.7) containing 4 mM $CaCl_2$ and 0.1% ascorbic acid and stored in liquid nitrogen till used.

Binding assays were carried out by adding 750 μl of tissue suspension to tubes containing 50 μl of ^{3}H-ligands and 200 μl of different drugs all dissolved in the same Tris-HCl buffer, containing in addition 10 μM pargyline. The concentrations of labeled ligands in the competition experiments were 4 nM [^{3}H]5-HT and 1 nM [^{3}H]mesulergine. The final tissue concentrations were 20 mg/ml for choroid plexus and 40 mg/ml for hippocampus and frontal cortex. The tubes were incubated at 37°C for 30 min and rapidly filtered under reduced pressure through Whatman GF/B filters. The filters were rapidly washed twice with ice-cold Tris buffer and transferred to plastic counting vials containing 10 ml of Lumagel. Extraction of radioactivity from the filters was carried out by incubation at 60°C for 30 min and subsequent cooling. Radioactivity on the filters was counted by liquid scintillation spectrometry (67% efficiency). To define non-specific binding, 1 μM 5-HT was used in the case of 5-HT$_1$ sites while mianserin (1 μM) was used for the 5-HT$_2$ sites. As a rule, all incubations were carried out in triplicate. Experiments were repeated 2 or 3 times.

Protein concentration was determined according to the method described by Bradford (1976). Binding parameters for saturation kinetics and drug competition studies were calculated according to the SCTFIT program (De Lean et al., 1980), using a computer-assisted system.

Means $\pm$ S.E.M. are given for the B_{max} and K_D values obtained with all ligands in the 3 tissues. Linear regressions and correlation coefficients were calculated in order to quantify any correlations between the affinities of different drugs.

[^{3}H]5-HT (26.8 Ci/mmol), [^{3}H]LSD (46.7 Ci/mmol) and [^{3}H]ketanserin (64 Ci/mmol) were

obtained from New England Nuclear (Dreieich, F.R.G.). [^{3}H]8-OH-DPAT (105 Ci/mmol) was obtained from CEA, Saclay (France). [^{3}H]Mesulergine (85 Ci/mmol) was synthesized and labeled by the Biopharmaceutical Department, Sandoz Ltd. (Basle, Switzerland). Other drugs used were either purchased or kindly provided by the manufacturers and pharmaceutical companies.

3. Results

3.1. Saturation kinetics of the binding of several 5-HT ^{3}H-ligands in several pig brain areas

Table 1 is a summary of the values for maximal capacities (B_{max}) and apparent dissociation constants (K_D) for [^{3}H]5-HT, [^{3}H]LSD, [^{3}H]8-OH-DPAT, [^{3}H]ketanserin and [^{3}H]mesulergine in pig cortex, hippocampus and choroid plexus. Specific binding was defined using 1 μM 5-HT when [^{3}H]5-HT or [^{3}H]8-OH-DPAT were used as ligands. For [^{3}H]ketanserin, 1 μM mianserin was used. When [^{3}H]mesulergine was assayed either 1 μM mianserin or 1 μM 5-HT were used independently to define non-specific binding (see below). Finally, when [^{3}H]LSD binding experiments were carried out, non-specific binding was defined by a mixture of both 1 μM 5-HT and 1 μM mianserin.

In the frontal cortex [^{3}H]LSD, a ligand of both 5-HT_1 and 5-HT_2 sites, labeled the largest number of sites. The population of sites labeled by [^{3}H]5-HT was lower than that obtained with [^{3}H]LSD, but clearly larger than that labeled by [^{3}H]8-OH-DPAT, a 5-HT_{1A} ligand. Of the selective 5-HT_2 ligands, [^{3}H]ketanserin marked a significantly larger number of sites ($P < 0.05$) than [^{3}H]mesulergine (table 1).

In the hippocampus [^{3}H]LSD also had the largest B_{max}. The number of sites recognized by

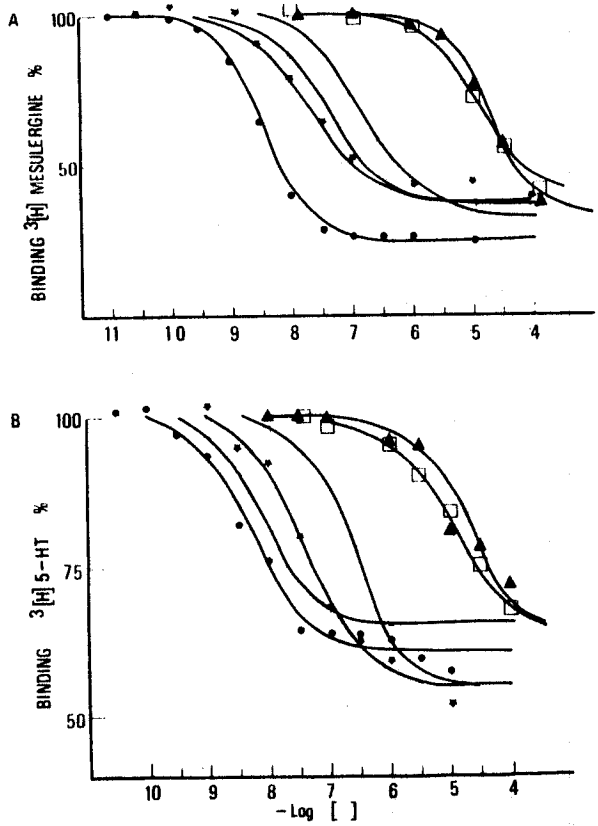

Fig. 1. Competition curves of several drugs for [^{3}H]mesulergine (A) and [^{3}H]5-HT (B) binding to pig choroid plexus. LSD (○), methysergide (●), ketanserin (□), (−)21 009 (□), mianserin (★) and 8-OH-DPAT (▲). The curves shown are from one representative experiment. Binding assays and curves fitting were carried out as described in Materials and methods.

TABLE 1

B_{max} and K_D values of the binding of several serotonergic ligands in different brain areas in the pig.

Ligand	Cortex		Hippocampus		Choroid plexus	
	B_{max} [a] (fmol/mg prot)	K_D [a] (nM)	B_{max} (fmol/mg prot)	K_D (nM)	B_{max} (fmol/mg prot)	K_D (nM)
[^{3}H]5-HT [b]	212 ± 41.2	4.9 ± 1.0	212 ± 21.9	10.8 ± 3.9	347 ± 91	6.5 ± 2.8
[^{3}H]LSD	368 ± 37.8	5.5 ± 0.8	290 ± 17.3	6.0 ± 0.7	602 ± 82.3	9.7 ± 2.5
[^{3}H]8-OH-DPAT [c]	75 ± 7.7	4.2 ± 1.3	123 ± 17.6	3.1 ± 2.2	ND [d]	–
[^{3}H]Ketanserin	130 ± 21.0	2.6 ± 0.7	53 ± 10.0	3.1 ± 1.0	ND	–
[^{3}H]Mesulergine	34 ± 9.5	1.7 ± 0.3	19 ± 4.6	2.2 ± 0.7	300 ± 63.7	1.1 ± 0.1

[a] Mean ± S.E.M. (n = 3-4). [b] [^{3}H]Serotonin. [c] [^{3}H]8-OH-2-di-n-propylaminotetraline. [d] Non detectable.

[^{3}H]5-HT was somewhat higher than that labeled by [^{3}H]8-OH-DPAT. On the other hand, [^{3}H]ketanserin labeled a small population of sites although one clearly larger than that labeled by [^{3}H]mesulergine (table 1). Finally, no binding of [^{3}H]8-OH-DPAT and [^{3}H]ketanserin was detected in the choroid plexus. [^{3}H]5-HT and [^{3}H]mesulergine labeled a similar population of receptors, although less than with [^{3}H]LSD (table 1).

Apparent dissociation constants were similar for each ligand in the 3 tissues (table 1).

3.2. Competition by different drugs for [^{3}H]mesulergine and [^{3}H]5-HT binding sites in pig choroid plexus membranes

$-\log K_i$ values for the different drugs in pig choroid plexus for two proposed 5-HT_{1C} ligands (see Introduction) are summarized in table 2. The competition curves of some of these drugs are shown in fig. 1. These curves as well as the data in table 2 were obtained by computer-assisted analysis as described above. When the best fit obtained was to a two-site population model, the values for the $-\log K_i$s and the proportion of sites are given in table 2.

In the choroid plexus, [^{3}H]mesulergine binding was inhibited in a monophasic manner by all the drugs used (table 2 and fig. 1A). The amount of non-specific binding defined using 1 μM 5-HT was comparable for the different drugs and represented 25-35% of the total binding (fig. 1A). No difference was found when 1 μM mianserin instead of 5-HT was used to define non-specific binding.

With the exception of metitepin and pirenperone, the inhibition of [^{3}H]5-HT binding to choroid plexus by the drugs tested was also best fitted by a single site analysis (table 2, fig. 1B). Similar amounts of non-specific binding, corre-

TABLE 2

Comparison of the affinities of several drugs for [^{3}H]mesulergine and [^{3}H]5-HT binding sites in cortex and choroid plexus in the pig.

Drug	[^{3}H]Mesulergine		[^{3}H]5-HT	
	Cortex $-\log K_i$ [a]	Choroid plex. $-\log K_i$	Cortex $-\log K_i$	Choroid plex. $-\log K_i$
Mianserin	7.9 ± 0.05	8.2 ± 0.17	5.9 ± 0.40	7.9 ± 0.30
LSD	8.1 ± 0.05	8.1 ± 0.36	8.2 ± 0.01	8.3 ± 0.10
Methysergide	8.7 ± 0.04	8.7 ± 0.02	8.9 ± 0.50 (19%)	8.5 ± 0.08
			7.3 ± 0.16 (81%)	
5-HT	7.4 ± 0.09	7.6 ± 0.07	8.3 ± 0.10	7.7 ± 0.09
Metitepin	7.9 ± 0.50	7.8 ± 0.40	7.7 ± 0.26 (58%)	8.5 ± 0.02 (50%)
			6.0 ± 0.27 (42%)	6.7 ± 0.27 (50%)
Ketanserin	7.0 ± 0.22	7.3 ± 0.04	5.5 ± 0.21	6.8 ± 0.09
Pirenperone	7.7 ± 0.40	7.6 ± 0.29	8.3 ± 0.40 (36%)	7.9 ± 0.50 (58%)
			5.3 ± 0.26 (64%)	6.0 ± 0.57 (42%)
Cinanserin	6.8 [c]	6.7 ± 0.11	5.5 ± 0.10	5.8 ± 0.01
(+)Butaclamol	6.7 ± 0.03	6.8 ± 0.09	7.0 ± 0.10	6.8 ± 0.12
RU-24 969 [b]	6.2 ± 0.30	6.7 ± 0.28	9.6 ± 0.42 (26%)	6.8 ± 0.51
			7.4 ± 0.03 (74%)	
8-OH-DPAT [c]	5.3 ± 0.35	5.3 ± 0.20	8.4 ± 0.31 (31%)	5.3 ± 0.2
			6.0 ± 0.03 (69%)	
(−)21 009 [d]	5.7 ± 0.04	5.7 [e]	8.4 ± 0.02 (42%)	5.7 ± 0.20
			6.1 ± 0.20 (58%)	
Spiperone	5.9 ± 0.01	6.0 ± 0.20	7.1 ± 0.31 (52%)	5.7 ± 0.30
			5.3 ± 0.38 (48%)	
Mesulergine	8.7 ± 0.09	8.9 ± 0.10	8.2 ± 0.26 (37%)	8.4 ± 0.28
			5.5 ± 0.20 (63%)	

[a] Mean ± S.E.M. [b] 5-Methoxy-3[1,2,3,6-tetrahydropyridin-4-yl]1H-indole. [c] 8-OH-2-di-n-propylaminotetraline. [d] 4[3-ter-butyl-amino-2-hydroxypropoxy]-indol-2-carbonic-acid-isopropylester. [e] Data from 1 experiment.

sponding to 55-65% of the total binding, were obtained with the different drugs (fig. 1B).

A very good correlation was found between the affinities of the different drugs for [^{3}H]mesulergine and [^{3}H]5-HT binding sites in pig choroid plexus (fig. 2A). Besides 5-HT and mesulergine, methysergide, LSD, mianserin and metitepin showed high affinity for these sites (table 2, fig. 1A, B). Spiperone, cinanserin or ketanserin, described as selective 5-HT$_2$ ligands, had a poor affinity for these sites (table 2, fig. 1A, B). Other drugs with low affinity for these 5-HT$_{1C}$ sites were 8-OH-DPAT, RU-24 969, spiperone and the β-blocker (−)21 009 (4[3-ter-butyl-amino-2-hydroxypropoxy]-indol-2-carbonic acid-isopropylester), ligands selective for 5-HT$_{1A}$ or 5-HT$_{1B}$ sites (table 2, fig. 1A, B).

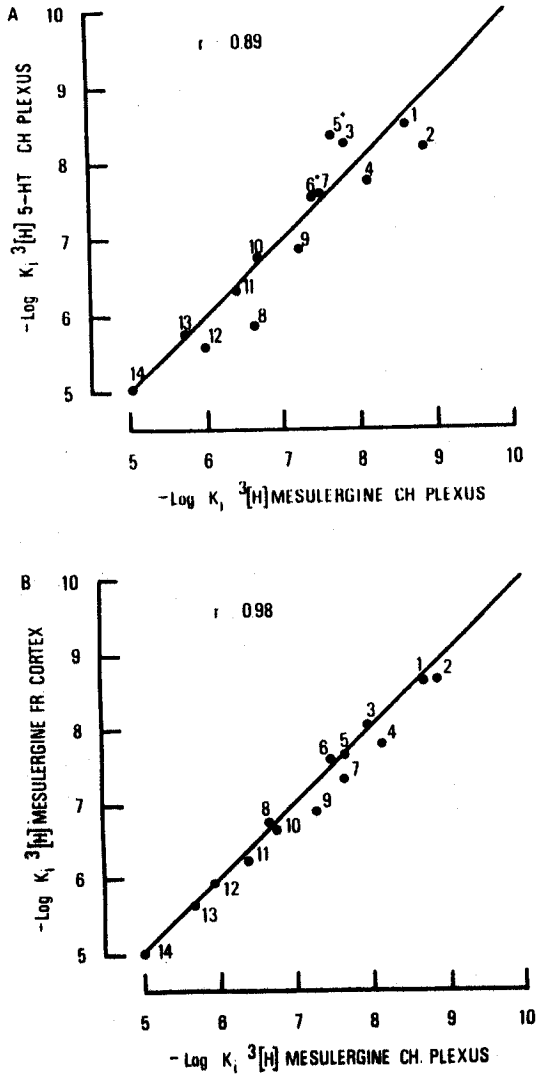

Fig. 2. Correlation between drug affinities (−log K$_i$) for [^{3}H]mesulergine and [^{3}H]5-HT binding to pig choroid plexus (A) and for [^{3}H]mesulergine binding to pig cortex and choroid plexus (B). Numbers correspond to the drugs listed below. Data were compared by linear regression analysis and the correlation coefficient is given in the figure. * High affinity site was taken for the correlation. 1 Methysergide, 2 Mesulergine, 3 LSD, 4 Mianserin, 5 Metitepin, 6 Pirenperone, 7 5-HT, 8 Cinanserine, 9 Ketanserin, 10 Butaclamol, 11 RU-24 969, 12 Spiperone, 13 (−)21 009, 14 8-OH-DPAT.

3.3. Competition by different drugs for [^{3}H]mesulergine and [^{3}H]5-HT binding sites in pig frontal cortical membranes

Table 2 also summarizes the affinities of the different drugs for [^{3}H]mesulergine and [^{3}H]5-HT binding sites in pig frontal cortex. The competition curves obtained for [^{3}H]mesulergine binding to cortical membranes were again monophasic (table 2). The amount of non-specific binding, defined as before, was comparable for the different drugs and much higher than that obtained in choroid plexus i.e. about 50% of the total binding.

The affinities of the drugs for these binding sites were similar to those found in the choroid plexus, as illustrated by the very good correlation between the K$_i$s for [^{3}H]mesulergine binding in both tissues (fig. 2B).

In contrast, the profile of the competitive blockade of [^{3}H]5-HT binding to cortical membranes was completely different from that described before. Most of the drugs showed biphasic competition curves (table 2). No correlation was found between K$_i$s of the drugs in this tissue and those observed either in the choroid plexus with [^{3}H]5-HT itself or in both tissues using [^{3}H]mesulergine as a ligand (not shown).

4. Discussion

The data presented here extend and confirm our previous report of the existence of a new subtype of 5-HT$_1$ binding site, richest in the mam-

malian choroid plexus, and which we have named 5-HT_{1C} (Cortés et al., 1984). In addition we show that this subtype is also present in frontal cortical membranes.

Multiple serotonin recognition sites have been described. Peroutka and Snyder (1979) classified them as 5-HT_1 (labeled with high affinity by [^{3}H]5-HT) and 5-HT_2 (labeled by [^{3}H]spiperone with high affinity). 5-HT_{1A} and 5-HT_{1B} subtypes have been proposed (Pedigo et al., 1981). We have suggested a third subtype of 5-HT_1 site, 5-HT_{1C} (Cortés et al., 1984; this paper).

Our saturation studies with pig brain (table 1) have demonstrated the existence of this multiplicity of sites in frontal cortex and hippocampus, as described for other mammalian species (Peroutka and Snyder, 1979; Pedigo et al., 1981; Marcinkiewicz et al., 1984). Frontal cortex seems to contain all the 5-HT subtypes described. This is illustrated by the fact that [^{3}H]LSD, a 5-HT_1 and 5-HT_2 ligand (Peroutka and Snyder, 1979), labeled a greater number of sites than did [^{3}H]5-HT. In addition [^{3}H]5-HT labeled a significantly higher population of sites ($P < 0.05$) than did the 'selective' 5-HT_{1A} ligand [^{3}H]8-OH-DPAT (Gozlan et al., 1983), showing the presence of different subtypes of 5-HT_1 sites in this structure. Sites labeled by [^{3}H]ketanserin (Leysen et al., 1982) had the pharmacological characteristics of 5-HT_2 receptors, as has been shown elsewhere (Pazos et al., 1984). Finally, as discussed here, sites labeled by [^{3}H]mesulergine displayed the properties of the 5-HT_{1C} subtype (table 1).

A similar analysis of the data obtained with pig hippocampus, reveals that this tissue is strongly enriched in 5-HT_{1A} sites and has very few 5-HT_{1B} and 5-HT_{1C} sites (table 1). Autoradiography has shown the same to be true in the rat (Cortés et al., 1984).

Only [^{3}H]5-HT, [^{3}H]LSD and [^{3}H]mesulergine labeled the choroid plexus while [^{3}H]8-OH-DPAT and [^{3}H]ketanserin did not show any detectable binding. This is consistent with our initial autoradiographic findings in the rat (Cortés et al., 1984). The number of sites labeled by [^{3}H]5-HT and [^{3}H]mesulergine was quite comparable, although the number of sites labeled by [^{3}H]LSD was higher (table 1), a fact which we cannot yet explain.

[^{3}H]5-HT and [^{3}H]mesulergine labeled a site that seems to be the same, as indicated by (1) the maximal number of sites obtained with both ligands (table 1), and (2) the very good correlation found between K_i of drugs for [^{3}H]5-HT and [^{3}H]mesulergine (fig. 2A). Ligands selective for 5-HT_{1A} and 5-HT_{1B}, e.g. spiperone (Pedigo et al., 1981), 8-OH-DPAT (Gozlan et al., 1983), RU-24 969 (Hunt and Oberlander, 1981) or the β-blocker (−)21 009 (Hoyer et al., in preparation) showed a low affinity for these sites (table 2, fig. 1A, B). On the other hand, it is not a 5-HT_2 site, because it is not recognized by [^{3}H]ketanserin and because drugs such as spiperone, butaclamol, cinanserine or ketanserin (Leysen et al., 1982) had a low affinity (table 2). Therefore, this subtype cannot be classified as a 5-HT_{1A}, 5-HT_{1B} or 5-HT_2 site. Applying the criteria of Peroutka and Snyder (1979), we proposed that this site be called 5-HT_{1C} (Cortés et al., 1984), because 5-HT exhibits nanomolar affinity for it (table 1, table 2). In addition to the drugs mentioned above methysergide, LSD and mianserin showed high affinities for these 5-HT sites (table 2, fig. 1A,B). Interestingly, metitepin and pirenperone also had good affinity although they displaced [^{3}H]5-HT biphasically (table 2).

The characteristics of the [^{3}H]mesulergine binding sites in porcine frontal cortex membranes closely resembled those described in choroid plexus, as illustrated by the very good correlation for the affinity of several drugs in both tissues (fig. 2B). On the other hand, this confirms that [^{3}H]mesulergine did not label 5-HT_2 receptors in pig cortex, but rather the 5-HT_{1C} receptors as we have reported (Pazos et al., 1984).

In contrast to the results from choroid plexus, the binding of [^{3}H]5-HT to porcine cortical membranes corresponded to that expected for a 5-HT_1 ligand. The fact that drugs such as RU-24 969, 8-OH-DPAT, spiperone or (−)21 009 displaced these binding sites biphasically (table 2) could suggest the existence of 5-HT_{1A} and 5-HT_{1B} in this tissue. The biphasic competition curves generated by methysergide and mesulergine (table 2) might be indicative of the labeling of 5-HT_{1C} sites, but more specific experiments are required to clarify this point.

What is the functional significance of these 5-HT_{1C} sites? The main physiological role of the choroid plexus is the control of the volume and composition of the cerebrospinal fluid (CSF), (Davson, 1967). The involvement of several neurotransmitters in the regulation of the activity of the choroid plexus has been suggested on the basis of morphological and physiological evidence. Noradrenergic and cholinergic innervation, as well as the effects of adrenergic and cholinergic drugs on CSF production have been documented (Lindvall et al., 1977; 1978). It is of relevance to the present findings that serotonergic fibers have also been found in the choroid plexus of the rat (Chan-Palay, 1976; Steinbush, 1981). These fibers arise from neurons within the dorsal and medial raphe nuclei (Moskowitz et al., 1979) and most of them end on the blood vessels that supply the plexus (Napoleone et al., 1982). Serotonergic fibers also emanating from the median and dorsal raphe nuclei have been described on the supraependymal surface of the ventricular system in rat and human (Richards et al., 1980, 1981) and in the pial vessels of the brain (Edvinsson et al., 1983). Taken together, these results support the existence of 5-HT innervation in the choroid plexus, closely related to the vascular component and the epithelial cells. This fact, in agreement with the vascular nature of the choroid plexus, may indicate that this serotonergic innervation and, therefore, the 5-HT_{1C} sites described here may play a role in the regulation of the production of CSF, the blood flow in the plexus and/or in the cerebral circulation (Edvinsson et al., 1983). No data are yet available on the physiological role of 5-HT_{1C} sites but these sites could be related to the reported involvement of 5-HT in the physiopathology of some nervous disorders, such as migraine or stroke. In particular, although the pathogenesis of migraine is not well clarified, alterations in vascular central tone or neuronal activity are currently invoked and a role in these mechanisms has been claimed for 5-HT (Raskin, 1981; Caviness and O'Brian, 1980). A prophylactic effect of some 5-HT antagonists, mainly methysergide, has been reported in some types of migraine (see Lance, 1982). Therefore, 5-HT_{1C} sites found in the choroid plexus might be involved in some way in the mediation of this disease. Further studies are required to support this hypothesis.

Because of the influence of CSF production on central nervous system activity, one cannot disregard the possible involvement of 5-HT_{1C} recognition sites in other central functions known to be partially regulated by serotonergic tone, such as analgesia (Basbaum and Fields, 1978), cardiovascular control (Kuhn et al., 1980) or sleep (Coulter et al., 1971). It must be pointed out that no effect related to these topics has been found so far in the clinical trials performed using mesulergine (Jellinger, 1982; Schneider et al., 1983).

The present results, as well as those of autoradiographic studies in progress in our laboratory, show the presence of 5-HT_{1C} sites in the frontal cortex and several other brain areas. Systematic studies are needed in order to correlate the affinities of the different drugs for these sites and their activity in several behavioral tests before any physiological role can be suggested.

Acknowledgement

The authors wish to thank Dr. P.H. Kelly for critical reading of the manuscript.

References

Aghajanian, G.K., 1981, The modulatory role of serotonin at multiple receptors in brain, in: Serotonin Neurotransmission and Behaviour, eds. B.L. Jacobs and Gelperin, A. (MIT Press, Cambridge, MA) p. 156.

Basbaum, A.I. and H.L. Fields, 1978, Endogenous pain control mechanism: review and hypothesis, Ann. Neurol. 4, 451.

Biegon, A., T.C. Rainbow and B.S. McEwen, 1982, Quantitative autoradiography of serotonin receptors in the rat brain, Brain Res. 242, 197.

Bradford, M.M., 1976, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, Anal. Biochem. 72, 248.

Caviness, V.S. and P. O'Brian, 1980, Headache, N. Engl. J. Med. 302, 446.

Chan-Palay, V., 1976, Serotonin axons in the supra- and subependymal plexuses and in the leptomeninges; their roles in local alterations of cerebro-spinal fluid and vasomotor activity, Brain Res. 102, 103.

Closse, A., 1983, [^{3}H]Mesulergine, a selective ligand for serotonin-2 receptors, Life Sci. 32, 2485.

Cortés, R., J.M. Palacios and A. Pazos, 1984, Visualization of multiple serotonin receptors in the rat brain by autoradiography, Br. J. Pharmacol. (in press).

Coulter, J.D., B.K. Lester and H.L. Williams, 1971, Reserpine and sleep, Psychopharmacologia 19, 134.

Davson, H., 1967, The Physiology of the Cerebrospinal Fluid (Little Brown, Boston, MA).

De Lean, A., J. Stadel and R.J. Lefkowitz, 1980, A ternary complex model explains the agonist-specific binding properties of the adenylate cyclase-coupled β-adrenergic receptor, J. Biol. Chem. 255, 7108.

Deshmukh, P.P., H.I. Yamamura, L. Woods and D.L. Nelson, 1983, Computer-assisted autoradiographic localization of subtypes of serotonin-1 receptors in rat brain, Brain Res. 288, 338.

Edvinsson, L., A. Degueurce, D. Duverger, E.T. Mackenzie and B. Scatton, 1983, Central serotonergic nerves project to the pial vessels of the brain, Nature 306, 55.

Euvrard, C. and J.R. Boissier, 1980, Biochemical assessment of the central 5-HT agonist activity of RU-24 969 (a piperidinylindole), European J. Pharmacol. 63, 65.

Fozard, J.R., 1983, Functional correlates of 5-HT_1 recognition sites, Trends Pharmacol. Sci. 4, 288.

Gozlan, H., S. El Mestikawy, L. Pichat, J. Glowinski and M. Hamon, 1983, Identification of presynaptic serotonin autoreceptors using a new ligand: ^{3}H-PAT, Nature 305, 140.

Hjorth, S., A. Carlsson, P. Lindberg, D. Sanchez, H. Wikström, L.E. Arvidson, U. Hacksell and J.L.G. Nilsson, 1982, 8-Hydroxy-2-(di-n-propylamino)tetraline, 8-OH-DPAT, a potent and selective simplified ergot congener with central 5-HT receptor stimulating activity, J. Neural Transm. 55, 169.

Hunt, P. and C. Oberlander, 1981, The interaction of indole derivatives with the serotonin receptor and non-dopaminergic circling behaviour, in: Serotonin – Current Aspects of Neurochemistry and Function, ed. B. Haber (Plenum Publ. Corp.) p. 547.

Jellinger, K., 1982, Adjuvant treatment of Parkinson's disease with dopamine agonists: open trial with bromocriptine and CU-32 085, J. Neurol. 227, 75.

Kuhn, D.M., W.A. Wolf and W. Lovenberg, 1980, Review of the role of the central serotonergic neuronal system in blood pressure regulation, Hypertension 2, 243.

Lance, J.W., 1982, Mechanism and Management of Headache, 4th ed. (Butterworths, Boston).

Leysen, J.E., C.J.E. Niemegeers, J.M. Van Nueten and P.M. Laduron, 1982, [^{3}H]Ketanserin (R 41 468), a selective ^{3}H-ligand for serotonin$_2$ receptor binding sites, Mol. Pharmacol. 21, 301.

Lindvall, M., L. Edvinsson and C. Owman, 1977, Histochemical study on regional differences in the cholinergic nerve supply of the choroid plexus from various laboratory animals, Exp. Neurol. 55, 152.

Lindvall, M., L. Edvinsson and C. Owman, 1978, Sympathetic nervous control of cerebrospinal fluid production from the choroid plexus, Science 201, 176.

Marcinkiewicz, M., D. Vergé, H. Gozlan, L. Pichat and M. Hamon, 1984, Autoradiographic evidence for the heterogeneity of 5-HT_1 sites in the rat brain, Brain Res. 291, 159.

Meibach, R.C., S. Maayani and J.P. Green, 1980, Characterization and autoradiography of [^{3}H]LSD binding by rat brain slices in vitro: the effect of 5-hydroxytryptamine, European J. Pharmacol. 67, 371.

Middlemiss, D.N., L. Blakeborough and S.R. Leather, 1977, Direct evidence for an interaction of β-adrenergic blockers with the 5-HT receptor, Nature 267, 289.

Middlemiss, D.N. and J. Fozard, 1983, 8-Hydroxy-2-(di-n-propylamino)-tetraline discriminates between subtypes of the 5-HT_1 recognition site, European J. Pharmacol. 90, 151.

Moskowitz, M.A., J.E. Liebmann, J.F. Reinhard and A. Schlosberg, 1979, Raphé origin of serotonin-containing neurons within choroid plexus of the rat, Brain Res. 169, 590.

Nahorski, S.R. and A.L. Willcocks, 1983, Interactions of β-adrenoceptor antagonists with 5-hydroxytryptamine receptor subtypes in rat cerebral cortex, Br. J. Pharmacol. 78 (Suppl.), 107.

Napoleone, P., G. Sancessario and F. Amenta, 1982, Indolaminergic innervation of rat choroid plexus: a fluorescence histochemical study, Neurosci. Lett. 34, 143.

Nelson, D.L., N.W. Pedigo and H.I. Yamamura, 1980, Multiple types of serotonin receptors, in: Psychopharmacology and Biochemistry of Neurotransmitter Receptors, eds. H.I. Yamamura, R.W. Olsen and E. Usdin (Elsevier North Holland Inc.) p. 325.

Pazos, A., D. Hoyer and J.M. Palacios, Mesulergine, a selective serotonin-2 ligand in the rat cortex, does not label these receptors in porcine and human cortex: evidences for species differences on brain serotonin-2 receptors, European J. Pharmacol. 106, 531.

Pedigo, N.W., H.I. Yamamura and D.L. Nelson, 1981, Discrimination of multiple [^{3}H]5-hydroxytryptamine binding sites by the neuroleptic spiperone in rat brain, J. Neurochem. 36, 220.

Peroutka, S.J. and S.H. Snyder, 1979, Multiple serotonin receptors: differential binding of [^{3}H]5-hydroxytryptamine, [^{3}H]lysergic acid diethylamide and [^{3}H]spiroperidol, Mol. Pharmacol. 16, 687.

Raskin, N.H., 1981, Pharmacology of migraine, Ann. Rev. Pharmacol. Toxicol. 21, 463.

Richards, J.G., H.P. Lorez, V.E. Colombo, R. Guggenheim and D. Kiss, 1980, Supraependymal nerve fibres in human brain: correlative transmission and scanning electron microscopical and fluorescence histochemical studies, Neuroscience 5, 1489.

Richards, J.G., H.P. Lorez, V.E. Colombo, R. Guggenheim, D. Kiss and J.Y. Wu, 1981, Demonstration of supraependymal 5-HT nerve fibres in human brain and their immunohistochemical identification in rat brain, J. Physiol. (Paris) 77, 219.

Schneider, E., K. Hubener and P.A. Fischer, 1983, Treatment of Parkinson's disease with 8-alpha-amino-ergoline, CU-32 085, Neurology 33, 468.

Steinbush, H.W.M., 1981, Distribution of serotonin-immunoreactivity in the central nervous system of the rat cell bodies and terminals, Neuroscience 6, 557.

Young, W.S. and M.J. Kuhar, 1980, Serotonin receptor localization in rat brain by light microscopic autoradiography, European J. Pharmacol. 62, 237.