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SHORT COMMUNICATIONS

Identification of 5-hydroxytryptamine$_{1D}$ binding sites in sheep caudate nucleus membranes

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Abstract—Radioligand binding measurements were performed in membranes of sheep caudate nucleus using [^{3}H]5-hydroxytryptamine (5-HT). [^{3}H]5-HT labeled a population of high affinity binding sites with a K_d of 1.9 ± 0.1 nM and a B_{max} of 19.8 ± 2.2 fmol/mg tissue. Combined 5-HT$_{1D/E}$ binding sites were the predominant 5-HT$_1$ subtype, accounting for 78% of the total population of 5-HT$_1$ binding sites. 5-Carboxamidotryptamine (5-CT) and sumatriptan yielded inhibition curves which best fitted a two-site model with high affinity values of 0.8 and 10.1 nM, and 1000 and 206 nM for their low affinity components. The proportion of the high affinity 5-CT and sumatriptan binding sites was 79 and 72%. The binding affinity profile of 5-HT$_{1D}$ binding sites [5-CT > 5-HT > d-LSD > 5-MeOT > sumatriptan > RU 24,969 > metergoline > tryptamine = rauwolscine = methylsergide > yohimbine = methiothepin > TFMPP = 8-OH-DPAT > 2-methyl-5-HT > mCPP = quipazine = CP 93,129 > ketanserin > (−)-propranolol = haloperidol = ipsapirone] compares well to that reported for 5-HT$_{1D}$ receptor sites in human caudate and cortex (correlation coefficient: 0.99 and 0.98). The present results indicate that sheep caudate nucleus is a valid tissue for studying interaction of compounds with 5-HT$_{1D}$ binding sites in the relative absence of 5-HT$_{1E}$ binding sites.

The existence of the 5-hydroxytryptamine$_{1D}$ (5-HT$_{1D}$*) receptor subtype was proposed by Heuring and Peroutka [1] on the basis of binding studies performed with bovine brain using [^{3}H]5-HT in the presence of compounds masking 5-HT$_{1A}$, 5-HT$_{1B}$ and 5-HT$_{1C}$ receptor subtypes, the remaining binding being defined as 5-HT$_{1D}$. Since then 5-HT$_{1D}$ binding sites have often been considered as a heterogenous population of binding sites [2]. The first evidence for 5-HT$_{1D}$ receptor heterogeneity was given by Waeber *et al.* [3]; the inhibition curves obtained for 5-carboxamidotryptamine (5-CT) in bovine, porcine and human caudate membranes were biphasic, which would correspond to two populations of receptors representing each about 50% of total 5-HT$_{1D}$ receptors. Similar results were obtained for brain tissue of dog, guinea-pig, piglet, rabbit and hamster [4–7]. This heterogeneity was also studied by Leonhardt *et al.* [8] using human cortex, showing sub-populations with high and low affinity for 5-CT. The 5-CT low affinity component (which represents 50% of the total binding in human cortex) was defined as the 5-HT$_{1E}$ receptor subtype. A further indication of the heterogeneity of 5-HT$_{1D}$ receptors, depending on the region or the species considered, is the variation in the abilities of different compounds to compete for 5-HT$_{1D}$ binding sites in calf, pig, guinea-pig and human cortex as well as caudate [6].

In order to select a brain tissue enriched in 5-HT$_{1D}$ binding sites, we found that sheep caudate nucleus contains mainly these sites relatively free of 5-HT$_{1E}$ binding sites. We report here the properties of [^{3}H]5-HT binding to sheep caudate nucleus membranes.

Materials and Methods

Sheep brains were obtained from the local slaughterhouse. The caudate nucleus was dissected and homogenized in 20 vol. of ice-cold 50 mM Tris–HCl pH 7.7. The homogenate was centrifuged for 10 min at 48,000 *g*. The pellet was rehomogenized in 20 vol. of 50 mM Tris–HCl pH 7.7, incubated for 10 min at 37° and recentrifuged for 10 min at 48,000 *g*. The pellet was stored at −80° in fractions of 0.5 g original wet weight of tissue. For binding assays, the pellet was thawed and homogenized using a Dounce homogenizer in 80 vol. of 50 mM Tris–HCl pH 7.7 containing 4 mM $CaCl_2$, 10 μM pargyline and 0.1% ascorbic acid. For measurement of the 5-HT$_{1B}$ receptor, a membrane preparation of total rat brain cortex was prepared similarly as described for the sheep caudate nucleus membrane preparation.

Incubation mixtures for [^{3}H]5-HT binding consisted of 0.8 mL of membrane preparation (10 mg original wet weight of tissue), 0.1 mL of [^{3}H]5-HT and 0.1 mL compound for inhibition or sumatriptan at a final concentration of 10 μM to determine non-specific binding. The reactions were stopped after a 30 min incubation at 25° by adding 3 mL of ice-cold 50 mM Tris–HCl pH 7.7 and rapid filtration over Whatman GF/B glass fiber filters using a Brandel harvester. The filters were rinsed twice with 3 mL of ice-cold 50 mM Tris–HCl pH 7.7, placed in scintillation vials and the radioactivity was extracted in 4 mL of Emulsifier-Safe (Packard, Warrenville, PA, U.S.A.). The radioactivity was counted in a Tri-Carb 4560 liquid scintillation counter. K_i values were calculated according to the equation

$$K_i = IC_{50}/(1 + C/K_d)$$

with C the concentration and K_d the equilibrium dissociation constant of the ^{3}H ligand. For 5-HT, 5-CT and sumatriptan, data were analysed with Ligand [9]. 5-HT$_{1A}$ and 5-HT$_{1C}$ receptors were measured with the radioligands [^{3}H]8-OH-DPAT (8-hydroxy-2(di-*n*-propilamino)tetralin) and [^{3}H]-mesulergine as described previously [10, 11]. [^{3}H]5-HT (27.3 Ci/mmol) and [^{3}H]mesulergine (80.6 Ci/mmol) were from Amersham (Les Ulis, France). [^{3}H]8-OH-DPAT (219 Ci/mmol) was from New England Nuclear (Les Ulis, France). The stock solutions of compounds were prepared in water or ethanol. Dilutions were made in 10% ethanol. The final maximal concentration of ethanol did not exceed 1%.

* Abbreviations: 5-CT, 5-carboxamidotryptamine; 5-HT, 5-hydroxytryptamine; 5-MeOT, 5-methoxytryptamine; d-LSD, lysergic and diethylamide; RU 24,969, 5-methoxy-3-1,2,3,6-tetrahydro-4-pyridinyl-1*H*-indole; TFMPP, *m*-trifluoro-methyl-phenyl-piperazine; 8-OH-DPAT, 8-hydroxy-2(di-*n*-propilamino)tetralin; mCPP, *m*-chlorophenylpiperazine; CP 93,129, 3-(1,2,5,6)-tetrahydro-4-pyridyl-5-pyrrolo(3,2-*b*)pyril-5-one.

Table 1. K_d and B_{max} values of ^{3}H-ligand binding to various 5-HT$_1$ receptor subtypes in sheep caudate nucleus membrane preparation

Receptor	^{3}H-Ligand	Occluding agents	Non-specific binding	K_d (nM)	B_{max} (fmol/mg tissue)
5-HT$_1$	5-HT	—	10 μM Sumatriptan	1.92 ± 0.07	19.84 ± 2.20
5-HT$_{1A}$	8-OH-DPAT	—	1 μM Spiroxatrine	1.42 ± 0.25	1.78 ± 0.60
5-HT$_{1B}$	5-HT	0.1 μM 8-OH-DPAT 0.1 μM Mesulergine	10 μM Sumatriptan	2.99 ± 0.89	15.62 ± 2.99
5-HT$_{1C}$	Mesulergine	—	1 μM Ritanserin	ND	ND
5-HT$_{1D/E}$	5-HT	1 μM Pindolol 0.1 μM Mesulergine	10 μM Sumatriptan	2.30 ± 0.12	15.52 ± 3.02

Concentration binding curves were performed as described in Materials and Methods. K_d and B_{max} values were determined by Scatchard analysis and are the means ± SD of values obtained in three separate experiments.
ND, not detected.

Results

In a first series of experiments, concentration binding curves of [^{3}H]5-HT were investigated in the absence or presence of occluding agents for 5-HT$_{1A}$, 5-HT$_{1B}$ and 5-HT$_{1C}$ receptor sites with a membrane preparation from sheep caudate nucleus. Table 1 summarizes the K_d and B_{max} values determined by Scatchard analysis. Sheep caudate membranes contain 19.8 fmol/mg tissue of total 5-HT$_1$ binding sites. In the presence of 0.1 μM 8-OH-DPAT and 0.1 μM mesulergine to block 5-HT$_{1A}$ and 5-HT$_{1C}$ receptors or 1 μM pindolol and 0.1 μM mesulergine to block 5-HT$_{1A}$, 5-HT$_{1B}$ and 5-HT$_{1C}$ receptors, K_d and B_{max} values were only slightly decreased. Under these conditions, Scatchard plots were linear indicating the presence of a single binding site (not shown). These data suggest that sheep caudate membranes contain minimal amounts of 5-HT$_{1A}$, 5-HT$_{1B}$ and 5-HT$_{1C}$ binding sites. 5-HT$_{1A}$ binding sites estimated with [^{3}H]8-OH-DPAT revealed only 1.8 fmol/mg tissue. No specific binding could be observed with the radioligand [^{3}H]mesulergine to estimate 5-HT$_{1C}$ binding sites.

Table 2. Apparent equilibrium inhibition constants (K_i values) of compounds for inhibition of [^{3}H]-5-HT binding to sheep caudate 5-HT$_{1D}$, rat cortex 5-HT$_{1B}$, human caudate 5-HT$_{1D}$ and human cortex 5-HT$_{1D}$ binding sites

Compounds	Sheep caudate	Rat cortex	Human caudate	Human cortex
5-CT	0.8 ± 0.3‡	0.7‡	0.9‡	—
5-HT	3.6 ± 0.7	1.4 ± 0.6	6.5	1.2
d-LSD	5.3 ± 1.9	8.0	—	14
5-MeOT	7.9	—	6.2	7.3
Sumatriptan	10.1 ± 2.6‡	3.1 ± 0.6‡	4.1*	14.1†‡
RU 24,969	12 ± 2.6	0.7 ± 0.1	43.7	23
Metergoline	19.8 ± 13.3	16.2 ± 4.4	11.7	—
Tryptamine	41.3 ± 2.5	74.5 ± 49	41.7	46
Rauwolscine	45.4 ± 21.2	1381 ± 502	758.6	—
Methysergide	48.6 ± 6.5	60.0 ± 4.0	26.9	—
Yohimbine	55.6 ± 21.9	500 ± 343	288	160
Methiothepin	57.1 ± 32.1	53.3 ± 17.5	170	17
TFMPP	243.6 ± 84.1	16.4 ± 3.5	—	210
8-OH-DPAT	294.9 ± 37.3	2857	1259	840
2-methyl-5-HT	556	—	—	1700
mCPP	612.5 ± 191.5	83.8 ± 16.5	—	1200
Quipazine	737.2 ± 328.0	314 ± 131	3715	1100
CP 93,129	826.2 ± 544.5	7.6 ± 1.8	—	—
Ketanserin	4774	—	56,234	69,000
(−)-Propranolol	>4774	85.7	5495	3700
Haloperidol	>4774	—	11,749	15,000
Ipsapirone	>4774	>4774	—	7100

Binding was performed with 3 nM [^{3}H]5-HT in the presence of 1 μM pindolol and 0.1 μM mesulergine for sheep caudate (total binding, 7559 ± 881 dpm and non-specific binding, 1941 ± 300 dpm, N = 5), and 4 nM in the presence of 0.1 μM 8-OH-DPAT and 0.1 μM mesulergine for rat cortex (total binding, 6787 ± 476 dpm and non-specific binding 3283 ± 230 dpm, N = 5) as described in Materials and Methods.

IC_{50} values from inhibition curves were converted into K_i values as described in Materials and Methods.

K_i values for the 5-HT$_{1D}$ binding sites in human caudate and human cortex are from Waeber *et al.* [3] and Peroutka *et al.* [12] with the exception of sumatriptan [(*) 13; (†) 7]. ‡, High affinity component of a displacement curve best fit to a two-site model.

The correlation between K_i values of compounds for inhibition of [^{3}H]5-HT binding to sheep caudate 5-HT$_{1D}$ binding sites versus human caudate and human cortex 5-HT$_{1D}$ binding sites was 0.99 and 0.98, respectively.

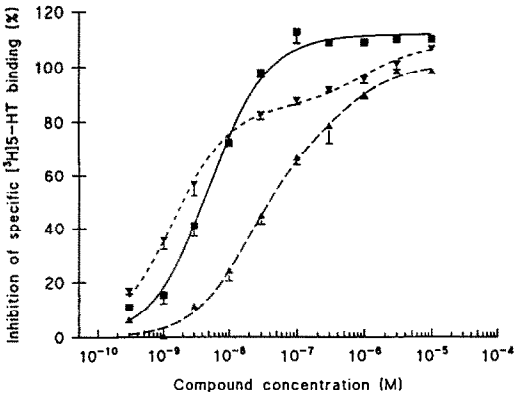

Fig. 1. Inhibition of [^{3}H]5-HT binding to sheep caudate membranes by 5-HT, 5-CT and sumatriptan. Inhibition of 3 nM [^{3}H]5-HT binding was performed in the presence of 1 μM pindolol and 0.1 μM mesulergine, and measured as described in Materials and Methods. Each curve represents the mean ± SD of five independent experiments. The 5-HT (■—■) inhibition curve fitted best to a one-site model yielding a K_i value of 3.3 nM. 5-CT (▼--▼) and sumatriptan (▲--▲) gave a biphasic inhibition curve yielding high affinity values of 0.8 and 10.1 nM and low affinity values of 1000 and 206 nM.

Table 2 lists the affinity values of the tested compounds on the inhibition of [^{3}H]5-HT binding to sheep caudate, rat cortex, human caudate and human cortex membranes. In sheep caudate membranes in the presence of 1 μM pindolol and 0.1 μM mesulergine, the compounds displayed a rank order of binding affinity different from that obtained with inhibition of [^{3}H]5-HT binding in rat cortex in the presence of 0.1 μM 8-OH-DPAT and 0.1 μM mesulergine. 8-OH-DPAT, yohimbine and rauwolscine were between 9- and 30-times less potent on inhibition of [^{3}H]5-HT binding in rat cortex membranes. In contrast *m*-trifluoro-methyl-phenyl-piperazine (TFMPP), 5-methoxy-3-1,2,3,6-tetrahydro-4-pyridindyl-1*H*-indole (RU 24,969) and 3-(1,2,5,6)-tetrahydro-4-pyridyl-5-pyrrolo(3,2-*b*)pyril-5-one (CP 93,129) were 15–109-times less potent on inhibition of [^{3}H]5-HT binding in sheep caudate membranes. *m*-Chlorophenylpiperazine (mCPP) was also 7-times less potent. The rank order of affinity values obtained with sheep membranes was in good agreement with that determined previously in binding studies performed with [^{3}H]5-HT in human caudate [3] and cortex [12]. Figure 1 compares the inhibition curves of 5-HT, 5-CT and sumatriptan in sheep caudate nucleus. 5-HT yielded a monophasic inhibition curve with a K_i value of 3.6 nM. In contrast, a biphasic curve for 5-CT and sumatriptan was consistently observed with high (0.8 and 10.1 nM) and low (1000 and 206 nM) affinity components. The proportion of the high affinity 5-CT and sumatriptan component was 79 and 72%, respectively.

Discussion

The present data show that 5-HT$_1$ binding sites in sheep caudate nucleus membranes are mainly of the 5-HT$_{1D}$ subtype. 5-HT$_{1A}$ binding sites are present though they represent only 9% of total 5-HT$_1$ binding sites. The binding sites do not appear to be of the 5-HT$_{1B}$ type since the binding affinity profile of the tested compounds did not match that of 5-HT$_{1B}$ receptors in rat cortex [2] (Table 1). The selective 5-HT$_{1B}$ agonist, CP 93,129, displayed micromolar affinity for inhibition of [^{3}H]5-HT binding to sheep caudate in comparison to its nanomolar affinity for 5-HT$_{1B}$ sites in the rat. Two other compounds with high 5-HT$_{1B}$ affinity, TFMPP and RU 24,969, were also 15- and 17-times less potent in inhibiting [^{3}H]5-HT binding in sheep caudate as compared to rat cortex. 5-HT$_{1C}$ receptors are probably absent since no specific binding could be observed with [^{3}H]mesulergine. The binding affinity profile of the tested compounds is very similar with that reported for 5-HT$_{1D}$ binding sites in human caudate [3, 13] and cortex [12]. With the exception of rauwolscine, both the rank order of affinity and the individual affinity values of the tested compounds were very close in these membrane preparations (Table 1). Rauwolscine displayed a 17-times higher affinity for 5-HT$_{1D}$ binding sites in sheep caudate than human caudate membranes [3]. The affinity for rauwolscine does, however, fit very well with its reported affinity for 5-HT$_{1D}$ receptors in calf caudate [3]. The proportion of 5-HT$_{1D}$ to 5-HT$_{1E}$ binding sites in the sheep caudate preparation determined by the inhibition of binding by 5-CT was 79%. This finding is of interest since most preparations studied, such as dog cortex, guinea-pig cortex and caudate, rabbit cortex, pig cortex and caudate, human cortex, hippocampus and amygdala show approximately equal numbers of 5-HT$_{1D}$ and 5-HT$_{1E}$ binding sites [7, 14, 15]. Whereas in human caudate and putamen only 20% of the combined 5-HT$_{1D}$/5-HT$_{1E}$ sites are of the 5-HT$_{1D}$ subtype, human globus pallidus possesses 70% 5-HT$_{1D}$ sites [14, 15]. This latter value is similar to that obtained in calf caudate [7]. In conclusion, sheep caudate nucleus represents a particularly useful brain tissue to study the interaction of compounds with 5-HT$_{1D}$ binding sites. These sites can be labeled with [^{3}H]5-HT almost without interference of other 5-HT$_1$ receptor subtypes. The binding affinity profile of these sites correlates very well with that of human brain tissue.

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