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A new 5-hydroxy-indole derivative with preferential affinity for 5-HT_{1B} binding sites

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The affinities of several 5-hydroxy-indole derivatives for serotonin-1 (5-HT_1) binding site subtypes, labeled with 2 nM [^{3}H]5-HT, were assessed by quantitative autoradiography on rat brain sections. The results obtained with known ligands, namely 5-hydroxytryptamine (5-HT), 5-methoxytryptamine (5-Me-OT), 5-methoxy-N,N-dimethyl-tryptamine (5-Me-ODMT), 5-hydroxy-N,N-dimethyl-tryptamine (bufotenine) and 8-hydroxy-2-[di-N-propylamino]tetralin (8-OH-DPAT) demonstrate the reliability and the advantages of this technique for pharmacological studies. Novel serotonin derivatives were synthesized by carboxymethylation of the hydroxyl group. One of those new ligands, serotonin-O-carboxy-methyl-glycyl-tyrosinamide (S-CM-$GTNH_2$), inhibited 2 nM [^{3}H]5-HT binding to the substantia nigra with an IC_{50} of 22.4 nM, a value which is 22 times lower than that found in the dentate gyrus and choroïd plexus. This demonstrates the preferential affinity of S-CM-$GTNH_2$ for 5-HT_{1B} versus 5-HT_{1A} and 5-HT_{1C} binding sites. S-CM-$GTNH_2$ contains a tyrosine residue, which may be useful for the synthesis of a radioactive iodinated molecule and for the preparation of 'long-lasting ligands' linked through peptide bonds with a protein. These derivatives could be of great interest for ultrastructural and behavioral studies relevant to 5-HT_{1B} sites.

Quantitative autoradiography; Indole derivatives; 5-HT_1 binding sites; (Rat)

1. Introduction

It is known since the work of Fuxe (1965) that the majority of serotonergic (5-HT) neuronal cell bodies are clustered in the raphe nuclei and that the processes of these neurons are highly branched. The 5-HT neurons of the raphe innervate all brain structures. The proportion of axon terminals involved in synapse formation varies with the particular location, but can be quite low (Beaudet and Descarries, 1978). This wide axon terminal distribution, associated with the non-synaptic liberation of 5-HT, means that neuromodulation can be exerted over widespread cerebral areas, with the specificity of the effect depending on the type of receptor involved.

Four main types of central nervous system 5-HT binding sites have been distinguished (Schmidt and Peroutka, 1989), i.e. the 5-HT_1, 5-HT_2, 5-HT_3 and 5-HT_4 (Dumuis et al., 1988) sites. The high-affinity binding site for serotonin (5-HT_1) consists, in fact, of four subtypes: 5-HT_{1A} and 5-HT_{1B} sites were demonstrated on the basis of their different affinities for spiperone and 8-hydroxy-2-[di-N-propylamino]tetralin (8-OH-DPAT) (Pedigo et al., 1981; Middlemiss and Fozard, 1983); 5-HT_{1C} sites, found primarily in the choroid plexus and characterized by high affinity for mesulergine (Pazos et al., 1984; Pazos and Palacios, 1985), and, finally, 5-HT_{1D} sites, which seem to take the place of 5-HT_{1B} sites which do not exist in guinea pig, cattle or man (Heuring and Peroutka, 1987; Hoyer et al., 1988; Hoyer and Middlemiss, 1989). Quantitative autoradiography, which combines great sensitivity with a high degree of spatial resolution, has provided evidence for a differential regional distribution of the subtypes of the 5-HT_1 sites (Pazos and Palacios, 1985). The 5-HT_1 binding site subtypes seem to exert different functions at both cellular and behavioral levels (Kennett et al., 1987; Hamon et al., 1988; Peroutka, 1988; King et al., 1989; Schoeffter and Hoyer, 1989). However, the delineation of the respective roles of the 5-HT_{1B} and 5-HT_{1D} sites is difficult in view of the low specificity and/or the weak affinity of the existing ligands.

Using quantitative autoradiography, we initiated a study on the affinity of 5-HT_{1A} and 5-HT_{1B} binding sites for several, commercially available, indolic ligands including 5-hydroxytryptamine (5-HT), 5-methoxytryptamine (5-Me-OT), 5-methoxy-N,N-dimethyl-

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tryptamine (5-Me-ODMT), 5-hydroxy-N,N-dimethyl-tryptamine (bufotenine), as well as for 8-OH-DPAT, which was used as reference standard for the characterization of the 5-HT_{1A} binding sites. We then used the same method to screen new 5-HT derivatives obtained by carboxymethylation of the hydroxyl group and to compare their affinities for 5-HT_1 binding sites with those of previously tested indolic ligands.

2. Materials and methods

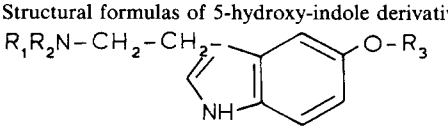
2.1. Quantitative autoradiography

Adult male Sprague-Dawley rats (200-300 g body weight) were decapitated under chloralhydrate anesthesia (0.12 ml of a 35% solution per 100 g i.p.). The brains were rapidly extracted from the skull and frozen by immersion in isopentane refrigerated with liquid nitrogen. Coronal brain sections (20 μm thick), obtained with a cryostat at $-20\,^{\circ}$C, were thaw-mounted on gelatin-coated slides and stored at $-20\,^{\circ}$C until used. Sections were preincubated for 1 h at 4°C in Krebs solution (mM: 118 NaCl; 4.8 KCl; 1.2 $CaCl_2$; 1.2 $MgCl_2$; 15 Tris; pH 7.40) to eliminate endogenous ligands. Incubations were carried out for 45 min at 20°C in Krebs solution to which were added pargyline (final concentration 10 μM), ascorbic acid (final concentration 0.57 mM) and [^{3}H]5-HT (final concentration 2 nM) in order to determine the total binding (TB) to 5-HT_1 sites (Segu et al., 1986). Non-specific binding (NSB) was determined with 10 μM 5-HT. Binding of the radioligand was inhibited with 15 increasing concentrations of 10 non-radioactive competitors: 5-HT, 5-Me-OT, 5-Me-ODMT, bufotenine, serotonin-O-carboxy-methyl-free acid (S-CMOH), serotonin-O-carboxy-methyl-amide (S-$CMNH_2$), bufotenine-CMOH, bufotenine-$CMNH_2$, serotonin-O-carboxy-methyl-glycyl-tyrosinamide (S-CM-$GTNH_2$), serotonin-O-carboxy-methyl-tyrosyl-glycinamide (S-CM-$TGNH_2$), which are 5-hydroxy-indole derivatives (table 1), and 8-OH-DPAT, which was used as reference to characterize 5-HT_{1A} binding sites. Inhibition of 2 nM [^{3}H]5-HT binding by S-CM-$GTNH_2$ was carried out in the presence of 100 nM 8-OH-DPAT and 100 nM mesulergine, which mask the labeling of 5-HT_{1A} and 5-HT_{1C} sites, respectively. After incubation, the sections were rinsed (3 × 20 s) in cold distilled water and dried under a stream of warm air.

Two methods were used to measure bound radioactivity: two sections per trial were dissolved in 0.3 ml of soluen (NCS, Amersham) and radioactivity was measured with a liquid scintillation counter; alternate sections were apposed in the dark to a tritium-sensitive film (LKB Ultrofilm) for two months, in the presence of tritium standard strips ([^{3}H]Microscale 1.3-33 nCi/mg tissue, Amersham). Films were then developed for 6 min with D19 (Kodak), rinsed and fixed for twenty min in AL4 (Kodak). Quantitative analysis of the autoradiograms was carried out for the different anatomical structures with a computer device for image analysis (BIOLAB, Macintosh II; Segu et al., 1990). The method makes possible the transformation of different intensities of grey into fmol of labeled molecule/mg of tissue-equivalent. Inhibition of 2 nM [^{3}H]5-HT binding is expressed as the ratio between the specific binding (SB = TB − NSB) obtained for a given concentration of ligand and the specific binding obtained without competitor. Values were obtained for sections coming from six animals processed in two separate experimental series for each ligand, and 15 anatomical structures (Paxinos and Watson, 1986) were analyzed in the whole brain. All the sigmoid inhibition curves were fitted as monophasic curves with a computer program (Cigale) even if they were apparently biphasic, so that we obtained only one IC_{50} value for each anatomical structure, this value being related to the 5-HT_{1A}/5-HT_{1B} density ratios in mixed structures. The IC_{50} values (Weiland and Molinoff, 1981) were calculated with the equations of the fitted curves, and the mean IC_{50} was calculated

TABLE 1

Structural formulas of 5-hydroxy-indole derivatives.



	Serotonin (5-HT,S)	S-CMOH	S-$CMNH_2$	Bufotenine	Bufotenine-CMOH	Bufotenine-$CMNH_2$	S-CM-$GTNH_2$	S-CM-$TGNH_2$
$R_1 = R_2$	H	H	H	CH_3	CH_3	CH_3	H	H
R_3	H	CH_2-COOH	CH_2-$CONH_2$	H	CH_2-COOH	CH_2-$CONH_2$	CH_2-CO-NH glycyl-tyrosinamide	CH_2-CO-NH tyrosyl-glycinamide

specifically for each of the seven characteristic anatomical structures of the mesencephalon. Student's t-test was used to compare mean IC_{50} values obtained with the different ligands. Comparison of the efficacy of ligands was done by linear regression analysis between log IC_{50} values calculated for the seven regions. The [^{3}H]5-HT binding values resulting from inhibition by S-CM-$GTNH_2$ in the presence of 100 nM 8-OH-DPAT and mesulergine were analyzed by using the Hill plot to obtain IC_{50} and Hill coefficient (nH) values.

2.2. Chemicals

[^{3}H]5-HT (26-30 Ci/mmol of specific activity) came from New England Nuclear, U.S.A.; 5-HT creatinine sulfate, 5-Me-OT, 5-Me-ODMT, bufotenine monooxalate hydrate, (N-Methyl-N-benzyl-2-propynylamine) hydrochloride (pargyline), Trizma Base and Trizma HCl were obtained from Sigma, U.S.A.; 8-OH-DPAT HBr came from R.B.I., U.S.A., and all other chemicals were purchased from Prolabo, France. Other 5-OH-indoles used in this work were synthesized in our laboratory (Segu et al., submitted). Briefly, the amino group of 5-HT was protected by diterbutyldicarbonate (BOC). In a first step, carboxymethylation of the hydroxyl group of BOC-5-HT or of bufotenine was carried out with bromoacetic acid at alkaline pH. When the amino group was unmasked by removing the BOC with trifluoroacetic acid, we obtained S-CMOH or bufotenine-CMOH, respectively. When the carboxyl group of carboxymethylated BOC-5-HT or bufotenine was converted into the amido derivative and the amino group unmasked, we obtained S-$CMNH_2$ and bufotenine-$CMNH_2$. Finally, activation of the carboxyl group of carboxymethylated BOC-5-HT by ethylchloroformate allowed an amide bond to be made with glycyl-L-tyrosinamide ($GTNH_2$). After unmasking of the amino group, we obtained either S-CM-$GTNH_2$ or S-CM-$TGNH_2$ if $TGNH_2$ was used instead of $GTNH_2$ (table 1).

3. Results

3.1. Liquid scintillation counting

[^{3}H]5-HT binding inhibition curves established by liquid scintillation counting on whole coronal rat brain sections were fitted as monophasic. On the basis of the IC_{50} values calculated from these curves, ligands were classified as follows, in decreasing order of affinity for total 5-HT_1 sites: 5-HT (1.4 nM) > 5-Me-OT (19.6 nM) > bufotenine (39.5 nM) $\geqslant$ 5-Me-ODMT (67.8 nM) $\geqslant$ S-CM-$GTNH_2$ (75 nM) = S-CM-$TGNH_2$ (82 nM) > S-$CMNH_2$ (1 000 nM) $\geqslant$ bufotenine-$CMNH_2$ (2 300 nM) > 8-OH-DPAT (12 000 nM) > S-CMOH (27 000 nM) > bufotenine-CMOH (43 000 nM).

3.2. Autoradiography

The regional distribution of specific [^{3}H]5-HT binding to 5-HT_1 receptors was identical in all brains studied. Under our experimental conditions, we found the highest density of 5-HT_1 sites in the choroïd plexus and dorsal raphe. Very high densities were found in the dentate gyrus, dorsal subiculum and substantia nigra. The globus pallidus, lateral septum, the hippocampal CA_1 area and ventral subiculum had a high density; superficial gray layers of the superior colliculus, central gray, caudate nucleus, as well as anterior, visual and entorhinal cortex had a density four times lower than that of the choroid plexus (figs. 1A, B). NSB was 10% of the total binding.

In all the anatomical structures studied, the binding of 2 nM [^{3}H]5-HT was entirely inhibited by low concentrations of 5-HT (4×10^{-8} M), higher concentrations of 5-Me-OT (10^{-6} M) and very high concentrations of S-$CMNH_2$, S-CMOH, bufotenine-$CMNH_2$ and bufotenine-CMOH ($\geqslant 10^{-5}$ M).

The characteristics of the inhibition of [^{3}H]5-HT binding by the other competitors were not the same in all structures. In the dentate gyrus, hippocampal CA_1 area and dorsal raphe, radioligand binding was largely inhibited by low concentrations of 8-OH-DPAT (10^{-7} M), higher concentrations of bufotenine and 5-Me-ODMT (10^{-6} M) and very high concentrations of S-CM-$GTNH_2$ ($> 10^{-5}$ M). In contrast, in the globus pallidus, caudate nucleus, dorsal subiculum, substantia nigra, central gray, superficial gray layers of the superior colliculus, anterior cortex, visual cortex, entorhinal cortex, thalamus and hypothalamus, only very high concentrations of 8-OH-DPAT ($> 10^{-4}$ M), relatively high concentrations of bufotenine and 5-Me-ODMT (10^{-5} M) and lower concentrations of S-CM-$GTNH_2$ (10^{-6} M) inhibited [^{3}H]5-HT binding completely (fig. 1).

3.3. Quantitative analysis

Quantification of autoradiograms obtained after incubation of the sections with increasing concentrations of cold ligands allowed the construction of 2 nM [^{3}H]5-HT binding inhibition plots (fig. 2). The mean interindividual variability was 20%.

Ligands were subdivided into three groups according to their efficiency (table 2).

(1) For 5-HT and 5-Me-OT, the IC_{50} values were nearly the same in all the anatomical regions tested, but these values were 10 times higher with 5-Me-OT, except in the choroid plexus, than with 5-HT. Similar results were obtained with S-$CMNH_2$, S-CMOH, bufotenine-$CMNH_2$ and bufotenine-CMOH, but the IC_{50} values

were much higher ($\geq 10^{-6}$ M). The last four ligands will not be taken into account in the subsequent analysis because of their quite low affinity for all 5-HT_1 subtypes.

(2) As expected, IC_{50} values for the inhibition of [^{3}H]5-HT binding by 8-OH-DPAT were significantly different in the 15 individual regions studied. The structures can be classified in the order of increasing IC_{50}

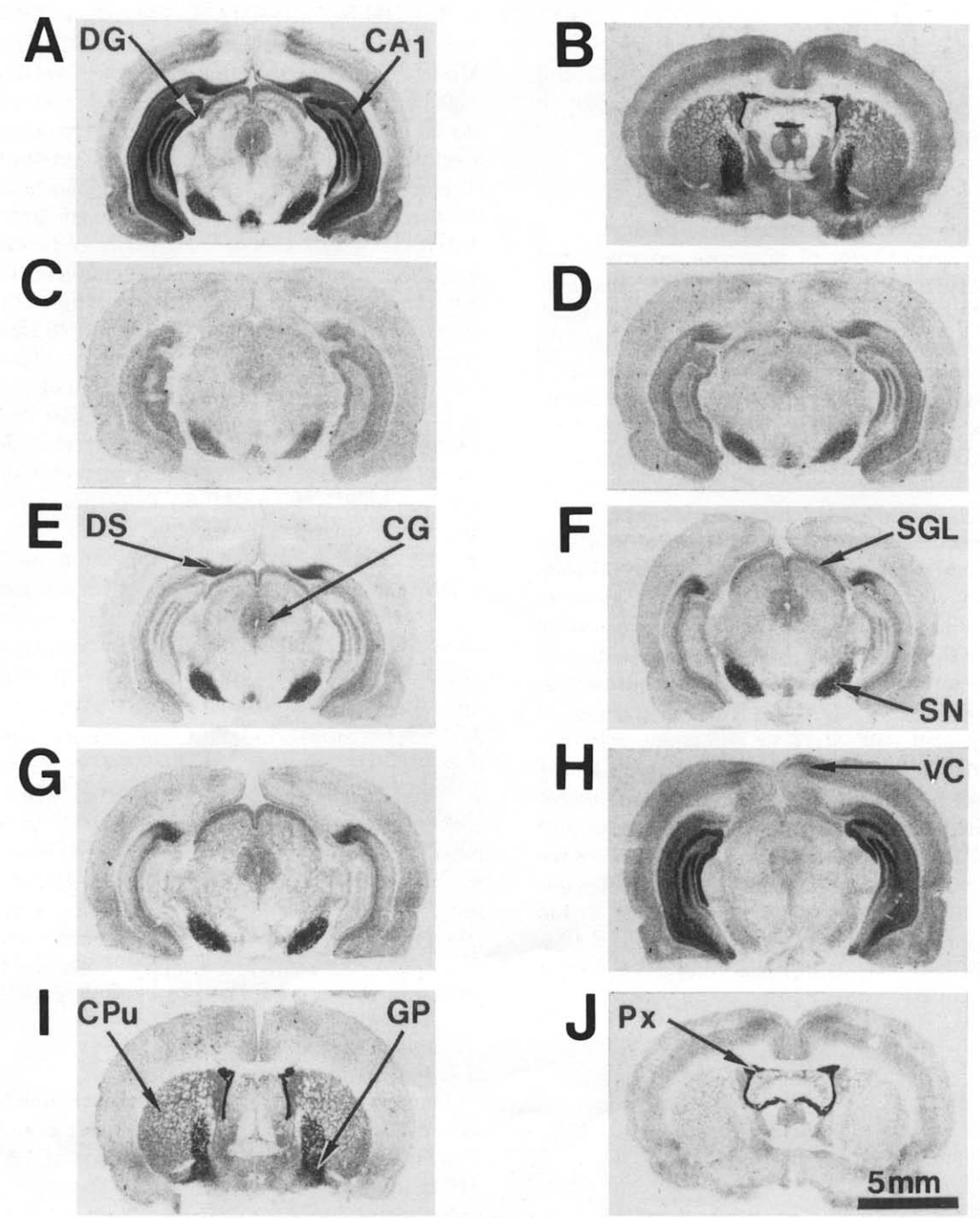


Fig. 1. Autoradiograms of mesencephalic (A and C-H) and anterior (B, I, J) brain sections. (A-B) Total binding of 2 nM [^{3}H]5-HT to 5-HT_1 sites at the mesencephalic (A) and anterior (B) levels. Labeled anatomical structures are the hippocampus (particularly dentate gyrus (DG) and CA_1 area), substantia nigra (SN), dorsal subiculum (DS), superficial gray layers of the superior colliculus (SGL), caudate-putamen (C-Pu), globus pallidus (GP) and choroid plexus (Px). (C-J) Inhibition of 2 nM [^{3}H]5-HT binding by 4×10^{-9} M 5-HT (C) and 5-Me-OT (D), and by 10^{-7} M 5-Me-ODMT (E), bufotenine (F), 8-OH-DPAT (G,I) and S-CM-$GTNH_2$ (H,J). 5-HT and 5-Me-OT inhibited the binding of [^{3}H]5-HT with the same affinity for all anatomical structures; 5-HT affinities were slightly higher than those of 5-Me-OT. 5-Me-ODMT, bufotenine and 8-OH-DPAT inhibited the binding to SN sites at a higher concentration than at hippocampal sites and the opposite effect was found with S-CM-$GTNH_2$. [^{3}H]5-HT, 2 nM, binding in the caudate-putamen and globus pallidus was inhibited by S-CM-$GTNH_2$ but not by 8-OH-DPAT. Radioligand binding to the choroid plexus (Px) was not affected by 10^{-7} M 8-OH-DPAT or 10^{-7} M S-CM-$GTNH_2$.

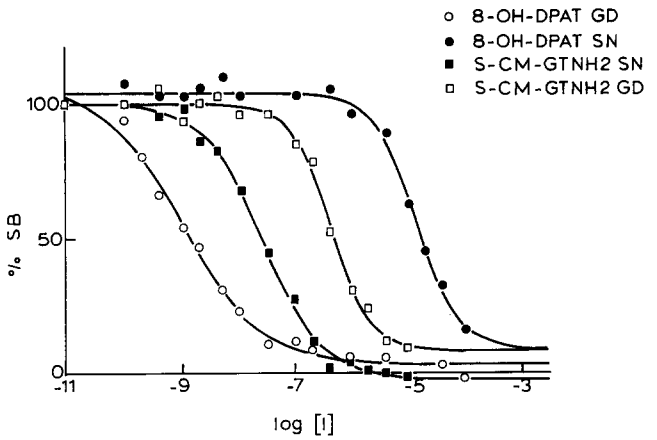


Fig. 2. Inhibition curves of 2 nM [^{3}H]5-HT binding with 8-OH-DPAT and S-CM-GTNH$_2$ in the rat dentate gyrus and substantia nigra. The percent of specific binding (% SB) was plotted versus the log of the inhibitor concentration (log [I]). The concentration of 8-OH-DPAT needed to inhibit radioligand binding in the dentate gyrus (mostly 5-HT$_{1A}$ sites) was far lower than in the substantia nigra (almost exclusively 5-HT$_{1B}$ sites). Conversely, the concentration of S-CM-GTNH$_2$ needed to inhibit radioligand binding was higher in the dentate gyrus than in the substantia nigra.

values for 8-OH-DPAT: dentate gyrus < hippocampic CA1 area < dorsal raphe < visual cortex < ventral subiculum ⩽ interpeduncular nucleus < anterior cortex = choroid plexus ⩽ superficial gray layers of the superior colliculus ⩽ entorhinal cortex < caudate nucleus ⩽ dorsal subiculum ⩽ central gray = globus pallidus ⩽ substantia nigra.

(3) The last four indolic derivatives bound with different efficiencies. Bufotenine and 5-Me-ODMT reacted in the same way as 8-OH-DPAT, but the difference between IC_{50} values obtained in the dentate gyrus and substantia nigra was less pronounced. The results were opposite to those obtained with S-CM-GTNH$_2$ and S-CM-TGNH$_2$, the IC_{50} values being higher in the dentate gyrus than in the substantia nigra. The IC_{50} values for the four ligands in the choroid plexus were not different from those found in the dentate gyrus.

The mean IC_{50} values for each ligand in the seven structures (table 2) were calculated. Paired comparison of the mean values using Student's t-test showed the existence of significant differences ($P < 0.05$) between the seven competitors, except between bufotenine and 5-Me-ODMT, S-CM-GTNH$_2$ (or S-CM-TGNH$_2$) and bufotenine, S-CM-GTNH$_2$ (or S-CM-TGNH$_2$) and 5-Me-ODMT, S-CM-GTNH$_2$ and S-CM-TGNH$_2$.

Linear regression analysis showed that the seven log IC_{50} values obtained for one competitor and the seven values obtained with another competitor were not correlated (correlation coefficient [R] < 0.7545, $\alpha < 5\%$) (table 2), except for 5-Me-ODMT and 8-OH-DPAT (0.97), bufotenine and 8-OH-DPAT (0.98), bufotenine and 5-Me-ODMT (0.98). We found good correlation coefficients when we compared S-CM-GTNH$_2$ (or S-CM-TGNH$_2$) with 8-OH-DPAT (0.89), bufotenine (0.94) and 5-Me-ODMT (0.96), but the slopes of the linear regression plots were negative (fig. 3).

S-CM-GTNH$_2$ in the presence of 100 nM 8-OH-DPAT and 100 nM mesulergine inhibited 2 nM [^{3}H]5-HT binding to the substantia nigra with an IC_{50} value of 24.9 ± 8.9 nM (nH = 0.91 ± 0.03).

4. Discussion

The regional distribution of 5-HT$_1$ sites in rat brain sections described here was similar to that presented by other authors (Biegon et al., 1982; Pazos and Palacios, 1985). The interpretation of the results obtained by both pharmacological and autoradiographic techniques is easy in view of the specific anatomical localizations of each receptor type or subtype. The IC_{50} values calculated from autoradiograms for all ligands tested (except the newly syntethized ones) were in agreement with those found with rat brain membrane preparations (Hamon et al., 1986). With a good image analysis device, quantitative autoradiography allows one to obtain pertinent pharmacological results with good accuracy

TABLE 2

Inhibition of 2 nM [^{3}H]5-HT binding to rat brain sections by 5-HT, five indolic derivatives and 8-OH-DPAT in seven characteristic anatomical regions of the mesencephalon and in the choroid plexus. Mean IC_{50} values (nM) ± S.E.M. were calculated from two independent experiments with six different rat brains. SGL = superficial gray layers of the superior colliculus.

	5-HT	5-Me-OT	Bufotenine	5-Me-ODMT	8-OH-DPAT	S-CM-GTNH$_2$	S-CM-TGNH$_2$
Dentate gyrus	2.0±0.3	11.9±0.4	13.0± 5.4	27.4± 7.5	5.8± 2.6	503.1±142.0	517.5±50.5
Hippocampal CA1	1.7±0.3	14.3±1.8	19.0± 6.6	29.5± 7.6	7.0± 2.4	512.5± 47.7	534.3±19.0
Visual cortex	1.8±0.6	14.6±3.5	74.0±36.7	38.9± 5.0	4271 ±2103	457.7±100.9	211.3±23.2
SGL	1.4±0.3	11.9±1.9	200.2±14.8	295.2± 51.4	13263 ±2632	54.3± 10.3	57.7± 7.1
Dorsal subiculum	1.5±0.3	12.6±1.2	289.3±50.1	443.4±128.4	25098 ±3134	33.8± 3.7	34.7± 3.5
Central gray	2.4±0.3	21.1±3.7	273.4±50.6	416.1± 36.2	30736 ±5446	57.2± 9.7	50.1± 7.2
Substantia nigra	3.6±0.6	19.6±3.9	385.8±96.2	510.7± 77.2	37147 ±5674	22.5± 3.6	25.7± 2.3
Choroid plexus	1.2±0.04	4.9±0.4	13.0± 7.2	63.8± 28.2	8559 ±2940	512.9± 85.4	-

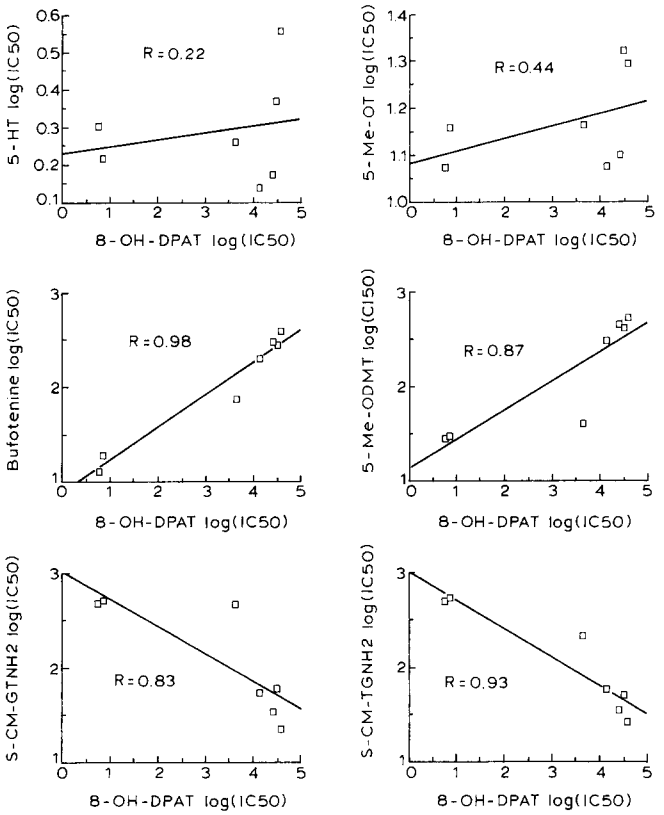


Fig. 3. Comparison between affinities of 5-hydroxy-indole derivatives and 8-OH-DPAT for 5-HT_1 binding sites. The log IC_{50} values for each of the six 5-hydroxy-indole derivatives (table 2) were plotted as a function of the log IC_{50} values obtained with 8-OH-DPAT for the seven mesencephalic regions analyzed. From left to right: dentate gyrus, hippocampal CA_1, visual cortex, superficial gray layers of the superior colliculus, dorsal subiculum, central gray and substantia nigra. IC_{50} in nM. Linear regression analysis allowed calculation of the correlation coefficients (R) comparing the potency of the drugs to inhibit [^{3}H]5-HT binding in the various regions.

and great anatomical resolution. Thus, we can determine possible differences in the pharmacological reactions of the binding sites of many small-sized brain structures which do not lend themselves to dissection for analysis of binding to membrane preparations.

The ligand 8-OH-DPAT is known to bind exclusively to 5-HT_{1A} sites and not to other subtypes at concentrations below 10^{-7} M (Emerit et al., 1985). 8-OH-DPAT was used to study the localization of 5-HT_{1A} and 'non-A' sites (which are principally 1B, except in the choroid plexus). It was found that the hippocampal dentate gyrus and CA_1 area contained mostly 5-HT_{1A} sites, while in the substantia nigra the sites were almost exclusively of the 5-HT_{1B} subtype (Marcinkiewicz et al., 1984; Pazos and Palacios, 1985). Linear regression analysis showed that the indole derivatives fell into three classes when 8-OH-DPAT was used as a reference compound. (1) For the two compounds 5-HT and 5-Me-OT, the seven individual IC_{50} values for the anatomical regions examined showed poor correlation with the 8-OH-DPAT values because the affinity of the two compounds was the same for all the structures analyzed. (2) In contrast, 5-Me-ODMT and bufotenine showed very good positive correlation coefficients with 8-OH-DPAT: the increasing order of the IC_{50} values found in seven characteristic regions was the same as that for 8-OH-DPAT; the highest affinity was found in the dentate gyrus (5-HT_{1A}) and the lowest in the substantia nigra (5-HT_{1B}). (3) The last group of indolic derivatives examined consisted of S-CM-$GTNH_2$ and S-CM-$TGNH_2$. Regional IC_{50} values defined with these two ligands in the seven anatomical structures showed good negative correlation coefficients with those obtained with 8-OH-DPAT. This means that the classification of the IC_{50} values for S-CM-$GTNH_2$ was the reverse of that for 8-OH-DPAT. Contrary to 8-OH-DPAT, S-CM-$GTNH_2$ showed a preferential affinity for 5-HT_{1B} versus 5-HT_{1A} sites. It was interesting to calculate the ratio between the IC_{50} values in the substantia nigra and in the dentate gyrus (IC_{50} SN/IC_{50} DG), which gives an index of the 5-HT_{1B} site versus 5-HT_{1A} site affinities of each ligand tested. This ratio was about 1 for 5-HT, 5-Me-OT, S-$CMNH_2$, S-CMOH, bufotenine-$CMNH_2$ and bufotenine-CMOH, i.e. none of these compounds discriminated between the two receptor subtypes. The ratio was 19 for 5-Me-ODMT, 31 for bufotenine and 6450 for 8-OH-DPAT, which is the best known ligand for discrimination between 5-HT_{1A} and 5-HT_1 non-A

sites. These values for the ratios are in accordance with those published previously (Sills et al., 1984; Hamon et al., 1986). The newly synthesized indolic derivatives showed a ratio IC_{50} SN/IC_{50} DG = 1/22 for S-CM-$GTNH_2$ and 1/20 for S-CM-$TGNH_2$; this means that the affinity of these compounds for SN binding sites (almost exclusively 1B) is 22 and 20 times greater, respectively, than for DG sites (mostly 1A). The IC_{50} value for 5-HT_{1B} sites obtained with S-CM-$GTNH_2$ after saturation of 5-HT_{1A} and 5-HT_{1C} sites was equivalent to the value obtained without 8-OH-DPAT and mesulergine in the substantia nigra. The Hill coefficient (nH) nearly equalled 1, thus there was no cooperativity between S-CM-$GTNH_2$ and 5-HT. Furthermore, S-CM-$GTNH_2$ had the same affinity for DG as for the sites of the choroid plexus (IC_{50} = 512 nM), an area known to contain essentially 5-HT_{1C} subtype sites (Pazos and Palacios, 1985).

There is a relationship between the chemical structure of several 5-HT derivatives and their activity on 5-HT sites. Previous studies dealt with this structure-affinity relationship (McKenna et al., 1990). Several findings emerged from our present work: 5-Me-OT, which has a methyl substitution on the hydroxyl group of 5-HT had a lower affinity for 5-HT_{1A}, 5-HT_{1B} and, to a lesser extent, for 5-HT_{1C} sites compared to 5-HT. The presence of a tertiary amine function on the bufotenine molecule led to a lower affinity for 5-HT_1 sites, but this change was greater for 5-HT_{1B} than for 5-HT_{1A} sites, so that the two sites could be discriminated from one another. The combination of these two substitutions in 5-Me-ODMT led to the compound having a lower affinity for 5-HT_{1A} sites than bufotenine, but the affinity change for the 5-HT_{1B} sites was nearly the same for the two derivatives. It appeared that substitution on the amino group made possible the discrimination between 5-HT_{1A} and 5-HT_{1B} sites, essentially by a loss of affinity for the 5-HT_{1B} site, but these substituted derivatives did not discriminate between 5-HT_{1A} and 5-HT_{1C} sites. The substitution on the hydroxyl group tended to slightly decrease the affinity for 5-HT_1 sites. When the hydroxyl was substituted with CH_2CONH_2 or CH_2COOH, a dramatic decrease in the affinity for 5-HT_1 sites occurred together with the disappearance of the ability of bufotenine to discriminate between 1A and 1B subtypes. In contrast, when a dipeptide was linked to the carboxyl group, there was a smaller decrease in affinity and this new type of ligand showed a preferential affinity for 5-HT_{1B} sites compared with 5-HT_{1A} and 5-HT_{1C} sites (without discriminating 5-HT_{1A} from 5-HT_{1C} sites). Replacement of the hydroxyl group with a carboxyamido group (5-carboxyamidotryptamine, 5-CT) increased the affinity for 5-HT_1 sites but did not allow discrimination between the subtypes (Peroutka, 1988). Many of the existing ligands, which recognize 5-HT_{1B} sites (with a very weak preference of affinity for 1B versus 1A), like cyanopindolol (Hoyer et al., 1985) and sumatriptan (Humphrey et al., 1988; Peroutka and McCarthy, 1989), are indolic compounds.

Hydroxy-indole derivatives showed only a weak difference in affinity between 5-HT_{1A} and 5-HT_{1B} subtypes as compared with 8-OH-DPAT. They could nevertheless be used to synthesize new types of ligands such as S-CM-$GTNH_2$, which is characterized by greater specificity for 5-HT_{1B} subtype sites than all other existing ligands (Hamon et al., 1986; Neale et al., 1987).

As 5-HT_{1D} sites have the same affinity for 5-Me-OT and 5-HT (Heuring and Peroutka, 1987) and very poor affinity for cyanopindolol (Hoyer et al., 1988), it might be interesting to test their affinity for the new derivative, S-CM-$GTNH_2$. Moreover, the tyrosine group of the ligand could be coupled to ^{125}Iodine to permit direct autoradiography, or the tyrosine could serve as linker to a protein to yield a long-lasting ligand (Garrigues et al., 1985). These new ligands could be of great interest for the study of both the localization of the subtypes at the electron microscopic level and of the behavioral implications of 5-HT.

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