Dopamine D₂ Receptors in the Rat Brain: Autoradiographic Visualization Using a High-Affinity Selective Agonist Ligand

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The non-catechol, selective dopamine D₂-agonist compound ³H-205-502 was used to localize dopamine D₂ receptors by autoradiography after *in vitro* labeling of brain sections. The characteristics of the binding of this ligand to tissue sections were those expected from the labeling of dopamine D₂ receptors. The binding of ³H-205-502 was inhibited selectively and stereospecifically by dopamine D₂ agents but not by dopamine D₁ compounds.

The autoradiographic localization of ³H-205-502 binding sites showed high densities of dopamine D₂ receptors in areas such as the glomerular layer of the olfactory bulb, the nucleus accumbens, caudate-putamen, olfactory tubercle, the lateral septum, and the islands of Calleja. Besides these dopamine-innervated areas the substantia nigra and the ventral tegmental area also showed important receptor densities. Other areas where dopamine D₂ receptor binding was found were the stratum lacunosum-moleculare of the hippocampus, bands of labeling in the molecular layer of the 9th and 10th lobules of the cerebellum, and several components of the visual system.

This distribution presents similarities and differences with previously reported distributions of dopamine D₂ receptors visualized autoradiographically using ³H-labeled agonists and antagonists. In view of the high affinity, guanine nucleotide insensitivity, and dopamine D₂ selectivity of this agonist ligand, it is suggested that dopamine D₂ receptors exist in different states in different areas. ³H-205-502 appears to be a new and useful tool for the study of dopamine D₂ receptors.

Many different radiolabeled molecules have been used to study dopamine D₂ receptors in the mammalian nervous system (Seeman, 1981; Creese et al., 1983). The development of these different radioligands has been justified by their lack of pharmacological selectivity for dopamine receptors or their subtypes—D₁ and D₂ (Kebabian and Calne, 1979)—and because of the poor technical properties of some of these ligands (i.e., high nonspecific binding or poor chemical stability). The use of radiolabeled dopamine agonists has presented special problems because of the postulated existence of different affinity states of the dopamine receptors (Creese et al., 1984). ³H-Agonists appear to label predominantly a high-affinity state of the dopamine D₂ receptor characterized by its high affinity for a third membrane

component, one of the guanine-nucleotide binding proteins, a G protein, to form what is called the “ternary complex” (Wreggett and Seeman, 1983). This complex has been particularly well characterized for dopamine D₂ receptors (Wreggett and de Léan, 1984) in the pituitary.

Some of these ligands have been used for the autoradiographic localization of dopamine receptors after *in vivo* or *in vitro* labeling (Palacios and Wamsley, 1984; Kuhar et al., 1986; Palacios and Pazos, 1987). The most widely used ligand is ³H-spiperone, a ligand with limited receptor selectivity that, besides dopamine D₂ and 5-HT₂ receptors, also binds with high affinity to a recognition site without a known pharmacological role, the “spirodecane binding site” (Palacios et al., 1981; Altar et al., 1985a, b). More recently, several ³H- and ¹²⁵I-labeled benzamides have been introduced as selective D₂ ligands and utilized in receptor localization. These include ³H-sulpiride (Jastrow et al., 1984; Gehlert and Wamsley, 1985), ¹²⁵I-sulpride (Martres et al., 1985a, b), ³H-(−)-DO 710 (Sokoloff et al., 1985), and ³H-eticlopride (Köhler et al., 1986). A novel pyrroloisoquinoline, ³H-RO 22-1319 (piquindone; Nakajima and Iwata, 1984), has also been used in autoradiographic experiments (Nock et al., 1986). Finally, autoradiographic localization of dopamine receptors has been investigated with some ³H-labeled agonists, although satisfactory results have been obtained only with ³H-*N*-*n*-propylnorapomorphine (Fuxe et al., 1983; Scatton et al., 1984; Dubois and Scatton, 1985; Dubois et al., 1986), ³H-apomorphine (Bouthenet et al., 1985) being a poor ligand. Recently, 2 new non-catechol dopaminergic agonists with high affinity and selectivity for dopamine D₂ receptors have been described (Closse et al., 1985; A. Closse, unpublished observations). These ligands (±)205-501 [= (±)-*N,N*-diethyl-*N'*-[(3α,4α,10β)-1,2,3,4,4a,5,10,10a-octahydro-7-hydroxy-1-propyl-3-benzog]quinolinyl]sulfamide] and the 6-hydroxy counterpart analog, compound 205-502, are members of a new family of recently synthesized dopamine agonists structurally related to apomorphine and to dopaminomimetic ergolines (Nordmann and Petcher, 1985). They exceed the receptor specificity of apomorphine, being selective D₂ agonists, and, as the ergolines, are long-lasting potent agonists. We have now used one of these ligands, ³H-205-502, to visualize dopamine D₂ receptors in the rat brain.

Materials and Methods

Rats (male Wistar, 180–250 g) were sacrificed by intracardial perfusion with phosphate-buffered saline containing 0.1% formalin, after pentobarbital anesthesia (50 mg/kg). The brains were removed and frozen in liquid nitrogen. Tissue sections, 10 μm thick, were cut using a microtome-cryostat (Leitz 1720, from Leitz, Wetzlar, F.R.G.), mounted onto gelatin-coated microscope slides and stored at −20°C until used.

Slide-mounted rat brain sections were preincubated for 30 min in

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Figure 1. Concentration dependence of the binding of ^3H -205-502 to rat brain striatal sections.

170 mM Tris-HCl buffer, pH 7.5, at room temperature, then incubated for 90 min at room temperature in 170 mM Tris-HCl buffer, pH 7.5, with various concentrations (for saturation experiments) or 1 nM ^3H -205-502 and drugs as indicated. At the end of the incubation the slides were washed twice (1 min each) in 170 mM Tris-HCl buffer at 4°C. For biochemical studies of ^3H -205-502 binding, tissue was wiped from the slide using a Whatman GF/B glass filter fiber and radioactivity determined by liquid scintillation counting. Specific binding was calculated as the difference in radioactivity bound in the presence and absence of 1 μM (+)butaclamol.

After the washing period, tissues were dried with cold air, and autoradiograms were generated by apposing the labeled tissue to ^3H -Ultrafilm (LKB, Sweden) as described by Unnerstall et al. (1982). Exposure period was 60 d at 4°C. Brain gray and white matter standards containing known amounts of a nonvolatile ^3H -labeled compound were exposed to the film along with the labeled tissue sections. Films were analyzed using a computerized image-analysis system that provides values of receptor densities expressed in fmol/mg protein. Four to six bilateral measurements were made per nucleus at the level described in each animal, and the mean value was calculated. Values of "specific binding" for each rat were obtained by subtracting the nonspecific binding from the total value. The mean value from equivalent areas in different animals was calculated. Results reported in this study are from at least 2 separate experiments, involving tissues from 3 animals per experiment. Brain nuclei were identified and named using the rat brain atlas of Paxinos and Watson (1982).

^3H -205-502 (107 Ci/mmol) was synthesized and labeled by Dr. R. Voges of the Biopharmaceutical Department, Sandoz Ltd. (Basle, Switzerland). Other chemicals or drugs were of commercial origin or kindly provided by the original manufacturers.

Results

Characteristics of the binding of ^3H -205-502 to slide-mounted rat brain sections

Initially, the incubation conditions described by Closse and collaborators (1985) were used to establish optimal washing parameters for slide-mounted tissues. While the specific binding of ^3H -205-502 was stable at 4°C for a relatively long period, nonspecific binding decreased rapidly after 10–15 min at 4°C, declining more slowly afterwards. A double 1 min washing protocol was selected for further experiments. Association of specific ^3H -205-502 binding was rapid at room temperature, reaching equilibrium at 60 min incubation. An incubation time of 90 min was selected.

Under the selected conditions, ^3H -205-502 binding was found to be saturable and of high affinity (Fig. 1). The calculated K_d and B_{max} values were 0.6 ± 0.1 nM and 67.1 ± 5.6 fmol/mg protein in rat striatal sections.

Table 1. Pharmacology of ^3H -205-502 binding to sections of the rat striatum

Compounds	IC_{50}^a (nM)	(n)
Dopamine agonists		
Dopamine	804.3 ± 86.7	(3)
Apomorphine	38.1 ± 6.6	(3)
Bromocriptine	28.1 ± 2.2	(3)
LY 171 555	333.0 ± 59.9	(3)
(+)-N-Propylnorapomorphine	1493.0 ± 370.0	(3)
(+)-3-PPP	4485.0 ± 913.0	(3)
(-)-3-PPP	386.3 ± 61.5	(3)
205-501	50.9 ± 1.8	(4)
205-502	5.5 ± 0.6	(6)
SKF 38393	6170.0 ± 251.0	(3)
Dopamine antagonists		
Spiperone	0.38 ± 0.06	(3)
(+)-Butaclamol	7.0 ± 0.7	(3)
(-)-Butaclamol	$15,000.0 \pm 1899.0$	(3)
YM-09151-2	0.47 ± 0.02	(3)
SCH 23390	1977.0 ± 700.0	(3)

^a Means $\pm$ SEM.

The specificity and selectivity of ^3H -205-502 for dopamine D_2 receptors were then examined. The binding of ^3H -205-502 to rat striatal sections was inhibited with high affinity by several dopamine agonists (Table 1), including dopamine itself, apomorphine, bromocriptine, and the selective dopamine D_2 agonist 205-501, unlabeled 205-502, and quinpirole (compound LY 171555; Tsuruta et al., 1981; Hahn et al., 1983). In contrast, the selective dopamine D_1 agonist SKF 38393 (Molloy and Waddington, 1984) showed only low affinity for the sites labeled by ^3H -205-502. The inhibition of ^3H -205-502 by dopamine agonists was stereospecific, with (+)-N-propylnorapomorphine and (+)-3PPP showing affinities in the micromolar range (Table 1). Dopamine D_2 receptor antagonists were also potent displacers of ^3H -205-502 binding. Again, the displacement was stereoselective, as shown by the differences in affinities exhibited by the *d*- and *l*-stereoisomers of sulpiride and by (+) and (-) butaclamol. Selective D_2 antagonists such as YM-09151-2 (Grewe et al., 1982; Usuda et al., 1982) were potent blockers, while the selective dopamine D_1 antagonist SCH 23390 (Hyttel, 1983; Iorio et al., 1983) was very weak (Table 1).

The inhibition of ^3H -205-502 binding to rat striatal sections by a number of benzamides was found to depend on the concentration of Na^+ ions in the incubation medium (Table 2). In the absence of Na^+ ions the potencies of sulpiride, remoxipride,

Table 2. Effect of Na^+ ions on modifying the displacement of ^3H -205-502 binding to rat striatum sections by substituted benzamides

Compounds	IC_{50}^a (nM)	
	120 mM NaCl in 50 mM Tris-HCl	170 mM Tris-HCl
<i>l</i> -Sulpiride	47.3 ± 1.1 (3)	5020 ± 413.0 (3)
<i>d</i> -Sulpiride	964.0 ± 42.0 (3)	$23,000.0 \pm 7200.0$ (3)
Remoxipride	276.5 ± 56.0 (3)	$32,450.0 \pm 1040.0$ (3)
Raclopride	15.7 ± 1.3 (3)	398.0 ± 10.0 (3)

^a Means $\pm$ SEM. Number of experiments given in parentheses.

Figure 2. Distribution of dopamine D₂ receptors, as labeled with ³H-205-502, in coronal sections of the rat brain. *A–K*, Photographs from autoradiograms obtained by incubating rat coronal sections with ³H-205-502 as described in the text. Dark areas indicate relevant levels of binding. The photographs shown here were printed individually to optimize the illustration of receptor distribution, but do not provide information on the quantitative distribution of sites (see Table 2 for these data). Nonspecific binding as defined using 1 μ M (+)butaclamol was homogeneously distributed (see Fig. 4*B*). Sections were cut approximately in the plane of the atlas of Paxinos and Watson (1982). *A*, Section at about the interaural (i.a.) level 14.2 mm illustrating a high density of receptors in the glomerular layer (*Gl*) of the olfactory bulb. *B* (i.a. level, 11.2 mm), High binding in the head of the nucleus caudatus-putamen (*CPu*), nucleus accumbens (*Acb*), and olfactory tubercle (*Tu*). *C* (i.a. level, 10.7 mm), Also illustrates the high density of D₂ sites in *CPu*, *Acb*, and *Tu*; printed with a lower contrast to show the high density of D₂ sites in the islands of Calleja (*ICj*). *D* (i.a. level, about 8.7 mm), Besides high levels of D₂ binding in *CPu* and *Tu* and the fundus striati (*FStr*), moderate concentrations of these sites are

visible in the lateral septum (*LS*) and the bed nucleus of the stria terminalis (*BST*). *E* (i.a. level, 8.2 mm), A more caudal level, where, besides the areas mentioned in *D*, low binding densities are seen in the globus pallidus (*GP*). *F* (i.a. level, 7.2 mm), Receptors at a caudal level of the CPu. *G* (i.a. level, 6.7 mm), Absence of receptor binding in the thalamus (*Th*) and a somewhat higher, uniform distribution of sites in the hypothalamus (*Hy*) and central amygdaloid nucleus (*Ce*). *H* (i.a. level, 4.2 mm), Band of moderate density of binding in the stratum lacunosum (*stl*) moleculare of the CA1 field of the hippocampus and binding to the olivary pretectal nuclei (*OPT*) and the lateral mammillary nuclei (*LM*). *I*, Midbrain section (i.a. level, ~3.2 mm), showing the presence of D₂ receptor binding in the superficial gray layer of the superior colliculus (*SuG*), central gray (*CG*), substantia nigra compacta (*SNC*) and reticulata (*SNR*), nucleus paranigralis (*PN*), and nucleus interpeduncularis (*IPC*). *J*, Adjacent level illustrating D₂ binding to the ventral tegmental area (*VTA*). *K*, Section at about an i.a. level -4.3 mm showing the presence of dopamine D₂ sites in the nucleus tractus solitarius (*sol*) and molecular layer of the cerebellar lobules 9 and 10 (*mol*), where these sites present a columnar organization. Bar, 5 mm.

Figure 3. Dopamine D₂ receptor localization, as visualized with ³H-205-502, in 4 horizontal rat brain sections corresponding to the following interaural (i.a.) levels (Paxinos and Watson, 1982): *A*, 5.9 mm; *B*, 4.4 mm; *C*, 3.4 mm; and *D*, 1.9 mm. See Figure 1 for abbreviations. Bar, 5 mm.

Figure 4. ^3H -205-502 binding site distribution in a sagittal section of the rat brain. *A*, Total binding; *B*, ^3H -205-502 binding in the presence of $1\ \mu\text{M}$ (+)butaclamol (nonspecific binding). See Figure 1 for abbreviations. Bar, 5 mm.

and raclopride were 20–100 times lower than in the presence of 120 mM NaCl. The presence of Na^+ ions was without influence on the binding of $1\ \mu\text{M}$ ^3H -205-502, as was the addition of 100 μM GTP to the incubation medium.

The regional sensitivity of ^3H -205-502 binding to drugs was examined by autoradiography using single concentrations of displacers and sagittal and coronal brain sections. The binding of ^3H -205-502 was equally sensitive to $1\ \mu\text{M}$ butaclamol in all the brain regions and insensitive to incubations with $1\ \mu\text{M}$ SCH 23390, $1\ \mu\text{M}$ SKF 38393, $1\ \mu\text{M}$ clonidine, $1\ \mu\text{M}$ propranolol, and 1 mM GppNHp.

Regional distribution of ^3H -205-502 binding sites (DA-D_2 receptors) in the rat brain

The distribution of dopamine D_2 receptors as labeled by ^3H -205-502 in the rat brain is illustrated in Figures 2–4. The densities (expressed in fmol/mg protein) of ^3H -205-502 binding sites determined microdensitometrically are given in Table 3. The highest densities of dopamine D_2 sites labeled by ^3H -205-502 were found in areas of the basal ganglia and associated structures. The caudate-putamen contained the highest densities. The distribution of binding sites in this region was, however, very heterogeneous, with the ventrolateral and lateral areas showing the highest densities and the medial levels the lowest. The islands of Calleja were also very rich in receptor sites. The nucleus accumbens and olfactory tubercles also showed high densities, while the globus pallidus was an area poor in D_2 sites. In the

midbrain, the substantia nigra compacta and the ventral tegmental area showed high densities of ^3H -205-502 binding sites, while the substantia nigra reticulata showed only low levels of binding. In addition, the superficial gray layer of the superior colliculus also exhibited high-density binding. Interestingly, another component of the visual pathway, the olivary pretectal nucleus (OPT) was also labeled (Fig. 2*H*). Significant numbers of dopamine D_2 receptors were labeled by ^3H -205-502 in other brain areas, outside the nigrostriatal and mesolimbic pathways. These included the glomerular layer of the olfactory bulb (Figs. 2*A*, 3, and 4), the lateral septum, and the lateral mammillary bodies. In the hippocampal formation, a band of high-density binding was found that corresponded to the stratum lacunosum moleculare of the dorsal hippocampus, densities of ^3H -205-502 sites being lower in the rest of the hippocampus and dentate gyrus. The entorhinal cortex showed a laminar distribution of sites, with the external laminae being richer than the deeper ones (Fig. 3*B*).

Low densities of receptors were seen in many other brain areas, including the cortex (particularly the deepest laminae of the frontal cortex), stria terminalis, hypothalamus, forebrain, periventricular gray, inferior colliculi, the nucleus interpeduncularis in the midbrain, and the nucleus tractus solitarius in the medulla oblongata.

The cerebellum contained only very low, uniformly distributed numbers of binding sites with the remarkable exception of the molecular layer of the 9th and 10th lobules, where high-density bands of ^3H -205-502 binding sites were visualized.

Table 3. Quantitative autoradiographic analysis of ³H-205-502 binding in the rat brain

Brain regions	³ H-205-502 ^a (fmol/mg protein)
Caudate putamen	
Head	283.08 ± 12.64
Body	218.22 ± 1.84
Tail	224.67 ± 3.23
Medial	127.39 ± 8.66
Dorsomedial	249.79 ± 7.10
Lateral	346.68 ± 7.82
Ventral	223.84 ± 12.21
Middle	218.89 ± 9.56
Ventrolateral	410.12 ± 19.90
Globus pallidus	55.71 ± 3.64
Nucleus accumbens	223.76 ± 0.47
Olfactory tubercles	152.69 ± 5.68
Glomerular layer of the olfactory bulb	184.49 ± 6.17
Islands of Calleja	372.63 ± 52.24
Cortex	47.44 ± 2.55
Entorhinal cortex	138.30 ± 8.26
Hippocampus	
Dentate gyrus	75.86 ± 10.75
Molecular layer	114.13 ± 16.72
Pyramidal cell layer	46.63 ± 2.86
Septum lateralis	121.84 ± 13.37
Medial habenular nucleus	41.92 ± 2.12
Lateral habenular nucleus	60.77 ± 6.47
Laterodorsal thalamic nucleus	16.76 ± 4.80
Lateral postthalamic nucleus	21.84 ± 9.11
Zona incerta	19.39 ± 0.49
Stria terminalis	48.91 ± 4.09
Ventromedial hypothalamic nucleus	77.06 ± 16.55
Dorsomedial hypothalamic nucleus	67.71 ± 0.20
Cerebellum	
Granular & molecular layer	45.07 ± 6.16
Lobule 9 or 10	129.59 ± 9.81
Superior colliculus	156.04 ± 18.78
Inferior colliculus	94.85 ± 7.08
Substantia nigra	
Pars compacta	227.80 ± 10.22
Pars reticulata	54.71 ± 16.34
Ventral tegmental area	180.38 ± 5.94
Tractus solitarius	31.66 ± 1.17
Substantia gelatinosa	61.97 ± 10.17
Locus coeruleus	88.65 ± 6.99

^a Means ± SEM. The data represent the specific ³H-205-502 binding at a concentration of 1 nM (occupancy: 62%).

Discussion

The main findings of these investigations could be summarized as follows: (1) The non-catechol dopamine agonist ³H-205-502 labels sites with the characteristics of dopamine D₂ receptors in mounted tissue sections of rat brain. (2) The autoradiographic distribution of these sites reveals similarities and differences with previously reported distributions of dopamine D₂ sites studied with other ligands.

Evidence for the binding of ³H-205-502 to D₂ sites is provided by the selective blockade of the binding of this ligand by do-

pamine D₂ compounds such as quinpirole (LY 171555; Tsuruta et al., 1981; Hahn et al., 1983), 205-501 (Closse et al., 1985), sulpiride, YM-09151-2, and other benzamides (Grewe et al., 1982; Usuda et al., 1982) but not by D₁ compounds such as SKF 38393 and SCH 23390 (Hyttel, 1983; Iorio et al., 1983; Molloy and Waddington, 1984). The binding of ³H-205-502 was stereospecifically blocked by the isomers of butaclamol and sulpiride. In addition, the inhibition of ³H-205-502 by the benzamide neuroleptics was found to be dependent on the presence of Na⁺ ions, while the presence or absence of Na⁺ ions was without influence on the binding of the ligand (Stefanini et al., 1980). Under our incubation conditions the addition of the guanylnucleotide GTP to the incubation medium was also without significant effect on ³H-205-502 binding. These characteristics are similar to data obtained in bovine caudate membrane preparations with this ligand (A. Closse, unpublished observations) and with ³H-205-501, a closely related molecule (Closse et al., 1985), contrasting with results obtained with other ³H-labeled dopamine analogs, such as ³H-*N*-*n*-propylnorapomorphine (Creese et al., 1984).

Dopamine D₂ receptors have been visualized autoradiographically using several ligands differing in their receptor selectivity, agonist or antagonist character, and technical characteristics (see introduction). The results obtained with most of these ligands are in close agreement regarding the labeling of a number of brain areas where dopaminergic innervation is well documented. These areas include first the striatonigral pathway, where the highest densities of dopamine D₂ receptors in the rat brain have been localized in the nucleus caudate putamen, while little binding has been seen in the globus pallidus (Palacios et al., 1981; Jastrow et al., 1984; Altar et al., 1985a, b; Dubois and Scatton, 1985; Gehlert and Wamsley, 1985; Joyce et al., 1985; Martres et al., 1985a, b; Sokoloff et al., 1985; Köhler et al., 1986; Nock et al., 1986). Furthermore, a heterogeneous distribution of sites has been observed, with highest densities being localized with both ³H-spiperone and ³H-*N*-*n*-propylnorapomorphine in the ventral lateral aspects of the nucleus and lower densities in the medial and lateral dorsal striatum (Dubois and Scatton, 1985; Joyce et al., 1985). High densities of D₂ receptors are also labeled by the different ligands used until now in the nucleus accumbens and olfactory tubercle (references as above). Although not always reported, it appears from the published autoradiograms as if the islands of Calleja were also highly labeled by all the dopamine D₂ radioligands used in autoradiography. Relatively high to moderate densities of dopamine D₂ receptors have also been localized to dopaminergic cell bodies in the substantia nigra pars compacta and ventral tegmental area, while lower concentrations were generally observed in the substantia nigra zona reticulata. High densities of dopamine D₂ receptors have also been visualized in all previous studies with different ligands in the glomerular layer of the olfactory bulb (Palacios et al., 1981). These studies also agree on the presence of moderate concentrations of D₂ sites in areas such as the superficial gray layer of the superior colliculi, the central gray, the lateral mammillary nucleus, and the lateral septum. The distribution of ³H-205-502 binding sites in these brain areas reported here is also in good qualitative agreement with the results obtained with other D₂ dopaminergic ligands.

However, outside of these well-characterized dopaminergic areas, the diverse autoradiographic studies present many discrepancies regarding the labeling of D₂ receptors. Thus, Martres

and colleagues (1985a, b) have reported a widespread distribution of dopamine D₂ receptors in the rat brain visualized by the use of ¹²⁵I-iodosulpride, a ligand with 20–100 times higher specific activity than that of previously used probes. Using ¹²⁵I-iodosulpride, sites with the properties of dopamine D₂ receptors were visualized in the neocortex, the cerebellar cortex, and the stratum lacunosum moleculare of the hippocampus. These sites were not labeled in previous studies, in particular, in those using ³H-spiperone. The reasons for this could be related to the fact that the densities in neocortex and cerebellum are on the order of 1–5% of those of the striatum and to the presence of high densities of the so-called spirodecane binding site in the hippocampus.

Gehlert and Wamsley (1985), using another benzamide ligand, ³H-sulpiride, reported the presence of D₂ receptors in the stratum lacunosum moleculare and in the cerebellum, although in this structure the binding was limited to the nodulus. However, other investigators, using ³H-sulpiride (Jastrow et al., 1984), other ³H-labeled benzamide ligands such as ³H-(–)-DO 710 (Sokoloff et al., 1985) or ³H-eticlopride (Köhler et al., 1986), or the rigid pyrroloisquinoline ³H-piquindone (Nock et al., 1986) have failed to visualize any binding in these regions. Dubois et al. (1986), who used the agonist ligand ³H-*N*-*n*-propylnorapomorphine, failed to localize D₂ sites in neocortical areas (first reported after *in vivo* labeling with ³H-spiperone by Klemm et al., 1979) or the cerebellar cortex, although a low level of binding was seen in the hippocampus.

³H-205-502 clearly labels sites with the properties of dopamine D₂ receptors in areas where ¹²⁵I-iodosulpride and, in some cases, ³H-sulpiride have also shown the presence of these sites, namely, the neocortex, the stratum lacunosum moleculare of the hippocampus, the entorhinal cortex, and the cerebellum (Gehlert and Wamsley, 1985; Martres et al., 1985a, b). However, clear differences exist between the distribution of ¹²⁵I-iodosulpride sites and those labeled in the neocortex and cerebellum by ³H-205-502. Thus, the sites labeled by the non-catechol agonist were localized in the neocortex to the deep (V and VI) laminae. In the cerebellum they were organized in bandlike structures in the molecular layers of lobules 9 and 10 in the cerebellum, probably corresponding to the organization of the climbing fibers (Beyerl et al., 1982). In contrast, those labeled by the benzamide were seen in laminae I–III and V of the neocortex and in the molecular layer of the entire cerebellum (Martres et al., 1985b).

In addition to the above-mentioned differences in the distribution of putative dopamine D₂ receptors visualized with different ligands, it should also be mentioned that the entorhinal cortex and some amygdaloid nuclei appear to be differentially labeled by these ligands. Thus, binding to the entorhinal cortex has been seen with ³H-spiperone (Altar et al., 1985a, b; Palacios and Pazos, 1987), ³H-sulpiride (Gehlert and Wamsley, 1985), ¹²⁵I-iodosulpride (Martres et al., 1985a, b), ³H-eticlopride (Köhler et al., 1986), and ³H-205-502 (this study) but not with ³H-*N*-*n*-propylnorapomorphine (Dubois et al., 1986) or ³H-sulpiride (Jastrow et al., 1984) and is not mentioned in the studies with ³H-(–)-DO 710 (Sokoloff et al., 1985) and ³H-piquindone (Nock et al., 1986). The central amygdaloid nucleus is highly labeled by ³H-spiperone but not so strongly by other ligands, including ³H-sulpiride (Gehlert and Wamsley, 1985), ¹²⁵I-sulpride (Martres et al., 1985a), or ³H-205-502 (this study).

An additional complication encountered when comparing the

results obtained in the autoradiographic studies on dopamine D₂ receptors in the rat brain is the marked difference in the number of sites and in the relative proportions of these sites labeled by the different ligands. In part, these differences are due to differing degrees of receptor occupancy. Thus, values of about 2700 and 4600 fmol/mg protein ³H-spiperone binding in the lateral striatum are reported by Altar et al. (1985a, b), while in the same region we found, with the same ligand, densities of about 1500 fmol/mg protein. Results with labeled benzamides vary from 17.3 fmol/mg tissue (Gehlert and Wamsley, 1985) to 300 fmol/mg protein (Jastrow et al., 1984) for ³H-sulpiride, while ¹²⁵I-iodosulpride (Martres et al., 1985b) shows a maximal capacity of 33.7 fmol/mg protein in striatal membrane preparations. More interestingly, differences are also found in the relative labeling of different regions by the diverse ligands, where differences in occupancy should not have an influence. Thus, ratios lateral striatum/substantia nigra compacta of 3.8 (Palacios and Pazos, 1987), 8.3 (Jastrow et al., 1984), 6.2 (Gehlert and Wamsley, 1985), 9.2 (Dubois et al., 1986), 8.4 (Nock et al., 1986), and 1.5 (present results) have been reported.

Since all these ligands appear to label dopamine D₂ receptors, as defined by the affinity and selectivity of reputed selective agonists and antagonists, the differential labeling of subclasses of the D₂ receptor or of different affinity states or forms of the same receptor have to be postulated in order to reconcile the diverse results, unless unknown technical factors determine the differences in distribution and density of sites labeled by the different ligands. In fact, differences exist in, for example, the dependency on the presence of Na⁺ ions for the binding of some antagonists and the sensitivity to guanylnucleotides of some agonists and antagonists. Thus, while the binding of ³H-sulpiride and ³H-piquindone are Na⁺ sensitive (Stefanini et al., 1980; Nakajima and Iwata, 1984), ³H-spiperone binding is not. On the other hand, a decrease in ³H-*N*-*n*-propylnorapomorphine binding and an increase in ¹²⁵I-iodosulpride binding have been found when GTP is added to the incubation medium, whereas in our studies ³H-205-502 was found to be insensitive to the nucleotide.

In conclusion, the use of ³H-205-502 has revealed the presence of dopamine D₂ receptors in significant densities in areas where other dopaminergic ligands did not exhibit relevant binding, as well as in other well-characterized brain areas for which agreement already exists about the presence of dopamine D₂ receptors. Further studies are, however, still necessary to clarify the exact characteristics of some of these binding sites, the existence of different forms or states of the receptor in different brain areas, and the functional significance, if any, of these sites.

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