Distribution of D_1- and D_2-Dopamine Receptors, and Dopamine and Its Metabolites in the Human Brain

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Densities and distribution of D_1-dopamine and D_2-dopamine receptors were investigated in vitro using [^{3}H]SCH 23390 and [^{3}H]raclopride in receptor binding assays and autoradiography on human post mortem whole hemisphere slices to serve as anatomical correlates to PET studies using [^{11}C]SCH 23390 and [^{11}C]raclopride. In addition, the levels of dopamine and its metabolites were determined by HPLC in various brain regions. Both dopamine receptor subtypes, as well as dopamine, HVA and DOPAC, were primarily found in the basal ganglia. Very high densities of D_1-dopamine receptors were found particularly in the medial caudate nucleus, whereas D_2-dopamine receptors were evenly distributed throughout the caudate. The densities of D_1- and D_2-dopamine receptors were similar in the caudate nucleus and the putamen, whereas there were 4 to 7 times higher densities of the D_1- than of the D_2-dopamine receptors in several limbic and neocortical regions. The receptor distribution in the autoradiographic study was consistent with that demonstrated in the living human brain using [^{11}C]SCH 23390 and [^{11}C]raclopride. ***[Neuropsychopharmacology 11:245–256, 1994]***

KEY WORDS: *Autoradiography; Dopamine; Dopamine receptors; HPLC analyses; Human brain; In vitro binding; [^{3}H]raclopride; [^{3}H]SCH 23390*

In the human brain, the two major dopamine receptor subtypes D_1 and D_2 (Kebabian and Calne 1979; Stoof and Kebabian 1984) are found mainly in the terminal regions of the dopaminergic systems. High receptor densities are found in the caudate nucleus and putamen, and lower amounts are found in the nucleus accumbens. Whereas D_1-dopamine receptors are also found in the cerebral cortex (Hall et al. 1988; Lidow et al. 1991), there are only minute amounts of D_2-dopamine receptors in cortical regions, such as in the entorhinal cortex (Lidow et al. 1989; Hall et al. 1991). In autoradiographical studies (Camus et al. 1986; Camps et al. 1989; Cortés et al. 1989; Lidow et al. 1991; Palacios and Mengod 1992), the distribution of D_1-dopamine and D_2-dopamine receptors has been described in post mortem human brains. Only few quantitative studies on the distribution of the dopamine receptors in the human brain have been reported (Camus et al. 1986; Hall et al. 1988).

Similar to the distribution of the receptors, the levels of dopamine and its metabolites are highest in the caudate nucleus and putamen (Adolfsson et al. 1979; Ebinger et al. 1987a; Hardy et al. 1987; Wester et al. 1990). No studies comparing dopamine receptor densities and transmitter levels in the same brain material have hitherto been published.

There are three main types of receptor binding techniques, binding in tissue homogenates, autoradiogra-

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Received February 17, 1994; revised June 21, 1994; accepted July 25, 1994.

Published by Elsevier Science Inc.
655 Avenue of the Americas, New York, NY 10010

0893-133X/94/$7.00

phy, and positron emission tomography (PET). The latter technique, PET, is used to study receptor densities and affinities in the living human brain (Wagner et al. 1983; Sedvall et al. 1986a; Sedvall 1992). Quantitative PET determinations of D_1-dopamine receptors have been performed during the last decade using mainly the benzazepine [^{11}C]SCH 23390 (Halldin et al. 1986; Sedvall et al. 1986b; Farde et al. 1987, 1992). The substituted benzamide [^{11}C]raclopride is one of the most commonly used radioligands for the study of the D_2-dopamine receptor by PET (Ehrin et al. 1985; Farde et al. 1985, 1986). In PET studies, the dopamine receptors can be studied quantitatively in structurally well defined central nuclei with high densities, such as in the basal ganglia (caudate nucleus and putamen).

In vitro receptor autoradiography provides images with high resolution that can serve as anatomical correlates to the PET studies. These studies also allow an examination of the validity of reference regions in PET, such as the cerebellum, used for the determination of nonspecific binding and free radioligand in the brain (Litton et al. 1994).

The aim of the present study was to map the distribution of D_1-dopamine and D_2-dopamine receptors using human post mortem whole hemisphere autoradiography and receptor binding in human brain homogenate with the two radioligands [^{3}H]SCH 23390 (Hyttel 1983) and [^{3}H]raclopride (Köhler et al. 1985). The anatomical distribution of dopamine and its metabolites was also measured in a number of regions of the same human brain material using HPLC (High Performance Liquid Chromatography) (Magnusson et al. 1980). The preparation of the whole hemisphere cryosections of post mortem human brains was performed as described before (Persson et al. 1991) to obtain slices in the same planes as obtained in previous PET studies. The same ligands were used as in the PET studies, where [^{11}C]SCH 23390 and [^{11}C]raclopride were used, to obtain comparative data. These two ligands are not totally selective, because [^{3}H]SCH 23390 binds to both D_1- and D_5-dopamine receptors, and [^{3}H]raclopride binds to both D_2- and D_3-dopamine receptors. The possible binding of [^{3}H]SCH 23390 to 5-HT_2 receptors was blocked by the addition of ketanserin.

MATERIALS AND METHODS

Human Post Mortem Brain

The human brains used were obtained from clinical autopsy at the National Institute of Forensic Medicine, Karolinska Institutet, Stockholm, Sweden. The study was approved by the Ethics Committee at Karolinska Institutet and the Swedish Board of Social Welfare. Brains removed at clinical autopsy (see Table 1 for details) were handled similarly to what has been described earlier (Hall et al. 1988). After dividing the brain along the midsagittal line, each hemisphere was placed with the sagittal plane on a glass-plate and was then put in a plastic bag before freezing (−85°C) to avoid freeze-drying during storage.

The cryosectioning and autoradiography on whole hemisphere sections were performed essentially as described previously (Persson et al. 1991). To prepare for cryosectioning, the glass-plate was removed from the frozen hemisphere, and steel needles were inserted through the anterior and posterior commissures perpendicularly to the horizontal plane. The two needles were used to define the horizontal plane and to fixate the hemisphere in a cooled (−70°C) metal frame. A semi-liquid gel (+4°C) of carboxymethyl-cellulose (CMC) was added to obtain a brain-CMC block, which was frozen to −85°C. Serial sectioning of the block was performed with a heavy-duty cryomicrotome (LKB 2250, LKB, Stockholm, Sweden).

For HPLC determinations and receptor binding in tissue homogenates, transparent tape (3M type 800) was fastened to the block by gentle rubbing, and 400 μm tissue sections were cut with the microtome. The sections were transferred to a cryotable with a surface temperature of approximately −12°C, where regions were punched out using stainless steel needles. After punching, the still-frozen tissue was expelled into test tubes (2 ml) in a cryobath filled with methanol (−40°C). The

Table 1. Human Subjects

No.	Age (years)	Sex	Postmortem Time (hours)	Cause of Death	Used for
1	59	Male	8.2	Heart failure	Receptor binding
2	64	Male	4.3	Heart failure	Receptor binding
3	60	Male	4.3	Heart failure	Receptor binding
4	54	Male	23.5	Subarachnoidal bleeding	Autoradiography
5	51	Male	10	Heart failure	Autoradiography
6	58	Male	13	Heart failure	Autoradiography

None of the brains exhibited damage, abnormalities, or neurological features.

weight of the tissue was calculated from the area and the slice thickness of the punches, assuming a specific weight of the tissue = 1 g/cm^3. The tissue was stored in a freezer (−85°C) until used in the HPLC or binding assays.

For autoradiography the brain was cryosectioned using the heavy-duty cryomicrotome into 100-μm horizontal (canto-meatal) tissue sections parallel to the 400-μm sections used for HPLC and receptor binding. A thin tissue paper (Kleenex) was put on the section, after which a transparent type (3M type 800) was fastened to the block by gentle rubbing. Tissue sections, 100 μm thick, were cut and transferred to cooled, gelatinized treated glass plates (15 × 20 cm). The tape and the paper were carefully taken away, with the sections thawed onto the glass plates. The sections were allowed to dry at room temperature and were then stored in a freezer (−85°C) until they were used in the autoradiography studies.

Radioligands

[Methoxy-^{3}H]SCH 23390 (lots no. 2476-193 and 2476-257, specific radioactivities 60.4 and 70.7 Ci/mmol, respectively) and [methoxy-^{3}H]raclopride (lots no. 2475-272 and 2553-237, specific radioactivities 67.6 and 74.5 Ci/mmol, respectively) were obtained from New England Nuclear, Boston, MA, USA. One batch of [methoxy-^{3}H]SCH 23390 (specific radioactivity 32.5 Ci/mmol) was synthesized according to the following procedure. [^{3}H]Methyl iodide (100 μl, in toluene solution from Amersham) was added to a reaction vial (1 ml) containing desmethyl-SCH 23390 (1 mg) in acetone (400 μl). The vessel was sealed and heated at 90°C for 5 minutes. Mobile phase (600 μl) was added before injection (in 100 to 200 μl portions) on to the semi-preparative HPLC column (μ-Poracil Waters 300 × 7.8 mm, 10 μm: mobile phase = methylene chloride/methanol/triethylamine = 94/6/0.6 [methanol/triethylamine preadjusted to pH = 8 with concentrated acetic acid], flow = 2.0 ml/min, wavelength = 280 nm). [^{3}H]SCH 23390 eluted after 7 to 8 minutes with a retention time identical to a standard reference sample. After evaporation of the HPLC fraction collected, the residue was diluted in 75% ethanol.

Compounds

(+)-Butaclamol was obtained from Research Biochemicals International, Natick, MA, USA; cis-flupentixol from Lundbeck A/S, Copenhagen, Denmark; ketanserin from Janssen Pharmaceutica, Beerse, Belgium. Desmethyl-SCH 23390 was a gift from Dr. A. Barnett, Schering-Plough. Other chemicals were obtained from commercial sources and were of analytical grade wherever possible.

Receptor Binding Assay

The brain tissue was thawed before preparation of the tissue homogenate. The assays were performed essentially as described earlier (Köhler et al. 1985; Hall et al. 1986; Hall and Wedel 1986). The tissue was homogenized at 0°C in 20 ml Tris-HCl buffer (pH 7.7, 0.05 mol/L) using a Branson B30 sonifier. After homogenization, the homogenate was centrifuged at 6°C for ten minutes at 48,000g. The pellet was resuspended and recentrifuged. The final pellet was resuspended in Tris-HCl buffer (pH 7.7, 0.05 mol/L, containing 0.1% (weight/vol) ascorbic acid, 120 mmol/L NaCl, 5 mmol/L KCl, 2 mmol/L $CaCl_2$, and 1 mmol/L $MgCl_2$) to a final concentration corresponding to 5 mg original wet weight/ml.

The incubations were performed at room temperature (approximately 23°C) for 60 minutes ([^{3}H]SCH 23390) and 90 minutes ([^{3}H]raclopride). The binding of each concentration of radioligand was determined in duplicate, and all values obtained were used in the calculations. Saturation analysis with nine to ten concentrations of [^{3}H]SCH 23390 (0.50 to 6 nmol/L) and of [^{3}H]raclopride (0.50 to 10 nmol/L) were used to determine K_D and B_{max} for each region. One μmol/L (+)-butaclamol was added for the determination of the nonspecific binding of both [^{3}H]SCH 23390 and [^{3}H]raclopride. The total incubation volume was 1.0 ml. The incubation was terminated, and bound radioligand was separated from free by filtration and subsequent washing on glass fiber filters (Whatman GF/B) using a cell harvester (Hall and Thor 1979). The radioactivity of the filters was determined with a scintillation counter at an efficiency of approximately 50%. Binding of [^{3}H]SCH 23390 to 5-HT_2 receptors was blocked by the addition of 40 nmol/L ketanserin (Leff et al. 1984).

The results presented were obtained from total binding values using the iterative nonlinear least squares curve-fitting program LIGAND (Munson and Rodbard 1980). For the saturation analyses, the nonspecific binding was calculated from the binding in the presence of (+)-butaclamol, and was used as a constant parameter. The estimates of K_D and B_{max}, as well as standard errors of the estimates as calculated by LIGAND, are presented in the tables.

Autoradiography

The autoradiographic procedure was essentially as described previously (Persson et al. 1991). The sections (placed horizontally) were surrounded by a rim of plasticine before the radioligand solution (approximately 10 ml, in Tris-HCl buffer pH 7.7, 0.05 mol/L, containing 0.1% [weight/vol] ascorbic acid, 120 mmol/L NaCl, 5 mmol/L KCl, 2 mmol/L $CaCl_2$ and 1 mmol/L $MgCl_2$)

was added. Radioligand concentrations and, when applicable, the competing substance are indicated in the figure legends. The incubations lasted 60 minutes at room temperature, followed by washing (2 × 2 minutes in approximately 10 ml cold buffer and by a brief cold wash by dipping the sections into distilled water) and drying on a warm plate with a gentle stream of warm air. The sections were then exposed to tritium sensitive film (Amersham, ^{3}H-Hyperfilm) for 4 weeks before development. The autoradiograms were analyzed using computerized densitometry using a MTI CCD72 high resolution video camera connected to a Macintosh IIcx (Image 1.52, NIH, USA).

Dopamine and Metabolite Analyses

Concentrations of dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), and 3-methoxytyramine (3-MT) were determined by reversed phase HPLC with coulometric detection essentially as described elsewhere (Magnusson et al. 1980; Mohringe et al. 1986). One to two tissue samples of each region per hemisphere and brain were studied, and the median values were calculated. Briefly, brain tissue was homogenized by sonication in 0.1 mol/L perchloric acid containing Na_2EDTA (100 mg/1000 ml) and sodium bisulphite (60 mg/1000 ml). Following centrifugation, samples of the supernatant were injected into the chromatographic system. The chromatographic equipment consisted of an LKB 2150 HPLC pump (LKB, Bromma, Sweden), a Waters Associates WISP 710 B autoinjector (Milford, MA, USA), a model 5100A coulometric detector including a model 5011 analytical cell (ESA, Bedford, MA, USA) and a Hewlett-Packard 3390A integrator (Avondale, PA, USA). The stationary phase consisted of an Altex Ultrasphere-ODS 5 μm chromatographic column 4.6 × 150 mm and the mobile phase essentially of 0.55 mmol/L citrate buffer pH 3.80, 0.55 mmol/L octylsulphate and methanol (10% to 15%). The column temperature was kept at +30°C by means of a plastic jacket coupled to a water thermostat, and the ESA settings were −0.05 V for cell 1 and +0.45 V for cell 2.

RESULTS

[^{3}H]SCH 23390 Binding to Brain Homogenates

Specific [^{3}H]SCH 23390 binding was primarily found in the basal ganglia with the highest densities of binding sites in lateral putamen and the caput and corpus of the caudate nucleus (Figure 1). Significantly lower binding was obtained in medial putamen and in nucleus accumbens. In other regions of the mesencephalon (globus pallidus and amygdala), the levels of specific [^{3}H]SCH 23390 binding were 10% to 20% of that in the caudate nucleus and similar to the density found in the cortical regions. Specific binding was also found in the two parts of the substantia nigra. Very low density of binding sites was found in the grey matter of cerebellum and in corpus callosum, whereas no specific binding was seen in cerebellar white matter. No significant differences between the left and right hemispheres on the density of D_1-dopamine receptors could be observed (data not shown). The K_D values for [^{3}H]SCH 23390 binding were similar in all regions (mean ± SEM: 1.37 ± 0.24 nmol/L) (Figure 2).

[^{3}H]Raclopride Binding to Brain Homogenates

[^{3}H]Raclopride binding was found primarily in the basal ganglia with the highest levels in the lateral putamen (Figure 1). Somewhat lower densities were obtained in the medial putamen, in the caput and corpus of the caudatus, and in the nucleus accumbens (Figure 1B). In cortical regions including the hippocampus, the levels of specific [^{3}H]raclopride binding were 5% to 10% of that in the caudate nucleus. A low density of D_2-dopamine receptors was found in the two parts of the substantia nigra. Virtually no specific binding was obtained in the cerebellum (white and grey matter), whereas low but significant levels of binding sites were seen in the corpus callosum. No significant differences between the left and right hemispheres on the density of D_2-dopamine receptors were found (data not shown). The K_D values for [^{3}H]raclopride binding were similar in all regions (mean ± SEM: 1.25 ± 0.14 nmol/L) (Figure 2).

D_1-/D_2-Dopamine Receptor Ratios in Brain Homogenates

There was a more than 10-fold difference in the relative proportion of D_1- to D_2-dopamine receptors in different brain regions. The densities of D_1-dopamine and D_2-dopamine receptors were roughly similar in the basal ganglia, giving a ratio of approximately 1 (Figure 1C, 1D). The D_1-dopamine receptors predominated in most cortical areas including the cingulum and parahippocampus, with the highest proportion D_1-dopamine receptors in the entorhinal cortex. However, in the frontal superior cortex, the densities of the two dopamine receptor subtypes were similar. Both parts of substantia nigra studied had higher densities of D_1-dopamine receptors than of D_2-dopamine receptors.

Autoradiography: D_1-Dopamine Receptors

The whole hemisphere autoradiograms showed that [^{3}H]SCH 23390 bound primarily to D_1-dopamine receptors in the putamen and the caudate nucleus (Fig-

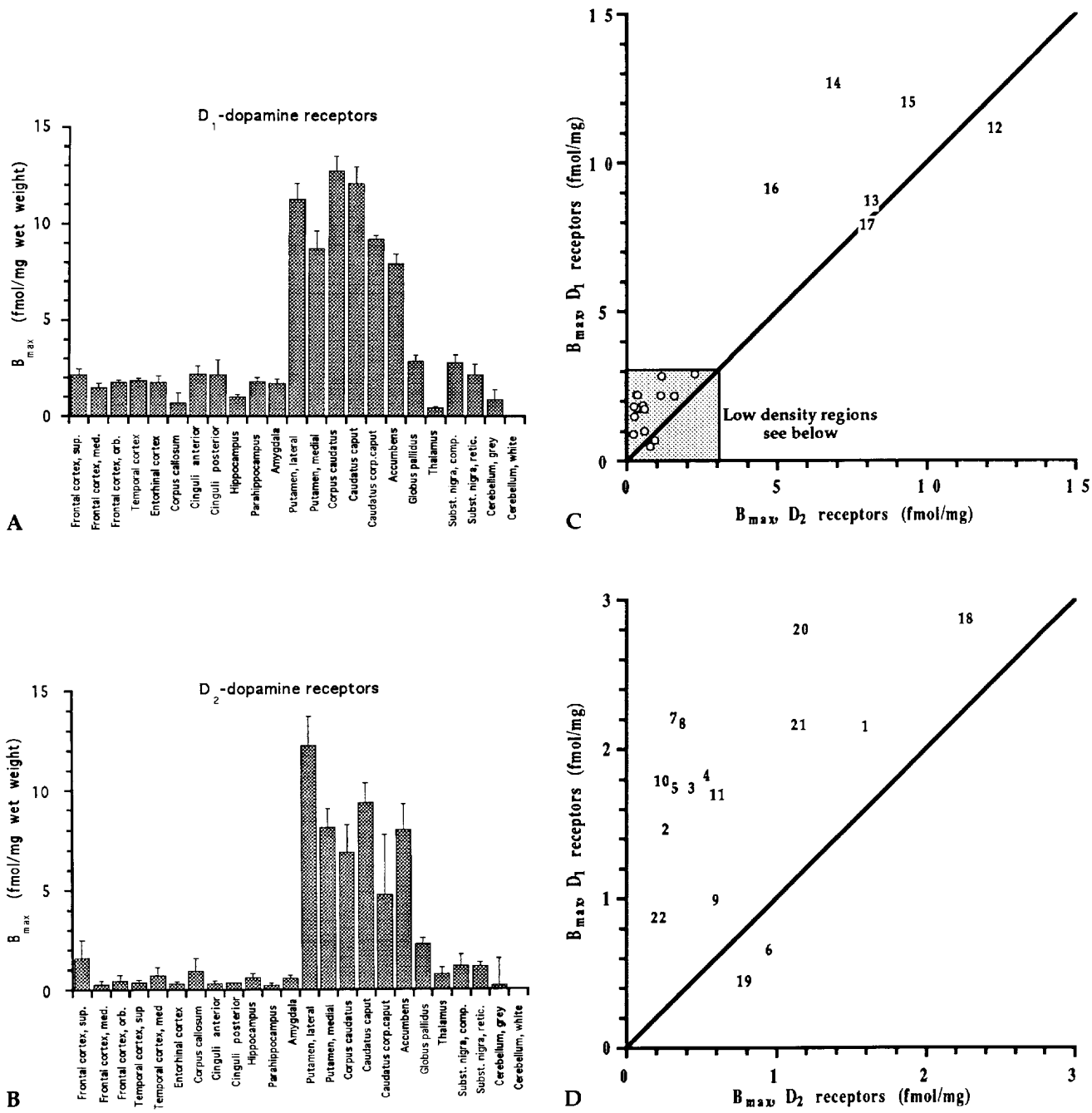


Figure 1. Densities (B_{max}-values, mean ± SEM for 4 to 6 determinations) of (**A**) D_1-dopamine; (**B**) D_2-dopamine; and (**C** and **D**) D_1-dopamine versus D_2-dopamine receptors in various human brain regions as determined using in vitro receptor binding assay. The numbers in panels **C** and **D** refer to the following regions: (1) Frontal cortex, sup.; (2) Frontal cortex, med.; (3) Frontal cortex, orb.; (4) Temporal cortex, sup.; (5) Entorhinal cortex; (6) Corpus callosum; (7) Cinguili anterior; (8) Putamen, medial; (9) Corpus caudatus; (10) Caudatus caput; (11) Caudatus corp. caput.; (12) Accumbens; (13) Globus pallidus; (14) Thalamus; (15) Subst. nigra, p. comp.; (16) Subst. nigra, p. retic.; (17) Cerebellum, grey.

ure 3A). Low but distinct levels of specific [^{3}H]SCH 23390 binding were seen in most parts of the cerebral cortex. There was only a faint nonspecific labeling of the grey matter. Virtually no binding was seen in white matters. The addition of cis-flupentixol (10 μmol/L) abolished all binding of [^{3}H]SCH 23390 to basal ganglia and cerebral cortex. The magnifications of the basal ganglia region (Figure 4A) showed that the density of binding sites was very high in the medial parts of the caudate nucleus, whereas the binding was more evenly

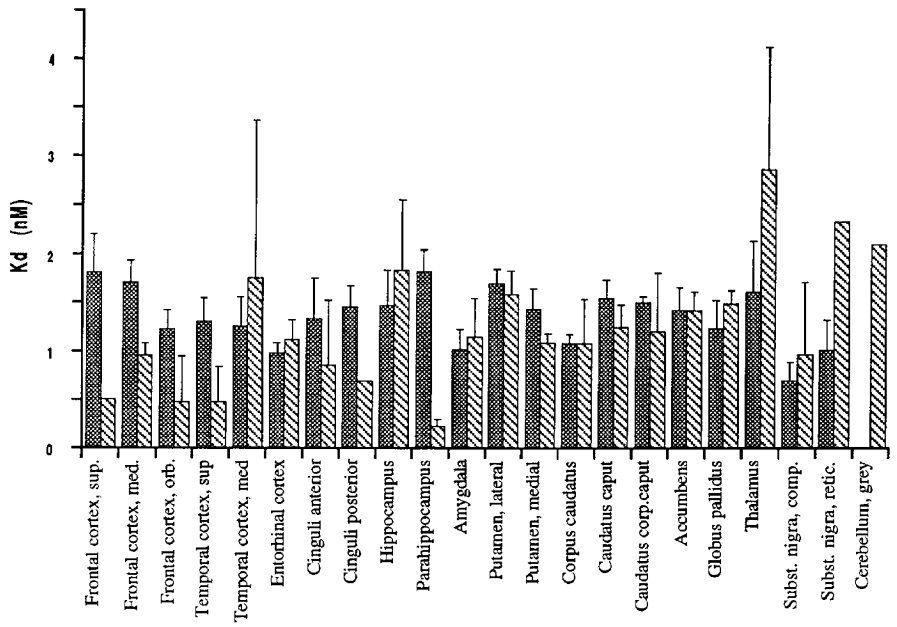


Figure 2. Affinity (K_D-values, mean ± SEM of 4 to 6 determinations) of [^{3}H]SCH 23390 binding for D_1-dopamine receptors (*shaded bars*) and [^{3}H]raclopride binding for D_2-dopamine receptors (*hatched bars*) in various human brain regions as determined using in vitro receptor binding assay. Mean ± SEM for D_1-dopamine: 1.37 ± 0.24 nmol/L, for D_2-dopamine: 1.25 ± 0.14 nmol/L.

distributed in the putamen. Low but significant binding of [^{3}H]SCH 23390 could be seen in the substantia nigra (Figure 4C).

Autoradiography: D_2-Dopamine Receptors

[^{3}H]Raclopride binding was found predominantly in the putamen and the caudate nucleus, as visualized in the whole hemisphere autoradiograms (Figure 3B). No, or very low, specific [^{3}H]raclopride binding was obtained in the cerebral cortex, and no binding was seen in the cerebellum. The addition of (+)-butaclamol (10 μmol/L) abolished all binding of [^{3}H]raclopride to the basal ganglia leaving only a faint nonspecific labeling of the grey matter. The close up of the basal ganglia region (Figure 4B) shows that the binding was rather evenly distributed in both the caudate nucleus and the putamen, thus contrasting to the uneven labelling in the caudate seen with [^{3}H]SCH 23390. Only very low densities of D_2-dopamine receptors could be seen in the two parts of substantia nigra (Figure 4D).

Levels of Dopamine and Dopamine Metabolites

High concentrations of dopamine and its metabolites DOPAC, HVA, and 3-MT were predominantly found in the basal ganglia with the highest dopamine level in the lateral putamen (approximately 30 pmol/mg, Figure 5). Dopamine concentrations in the range of 10 to 20 pmol/mg were found in putamen, caudate nucleus and, at a somewhat lower concentration, in the nucleus accumbens. Generally DOPAC, HVA, and 3-MT were distributed similarly to dopamine, though the relative magnitude of the concentrations varied markedly. In the cortical areas, significant concentrations of HVA (0.5 to 1 pmol/mg), but not of the other two dopamine metabolites studied were found (Figure 5). In contrast to dopamine, DOPAC, and 3-MT, HVA was found in approximately as high concentrations in the substantia nigra as in the caudate areas. The levels of dopamine or its metabolites were not significantly different in any regions of the left and right hemispheres (data not shown).

DISCUSSION

In this article the dopamine receptor subtypes and the levels of dopamine and its metabolites have been measured in the same brain material from a number of regions of both human brain hemispheres to serve as an anatomical correlate to PET studies on the D_1- and D_2-dopamine receptors. In agreement with the literature (Mackay et al. 1978; Adolfsson et al. 1979; Ebinger et al. 1987a; Hardy et al. 1987; Wester et al. 1990), the highest contents of dopamine and its main metabolites were found in the basal ganglia and in the substantia nigra. The concentrations agreed on the whole with previous studies, though large deviations were found that may be a result of significant differences within a limited area. In comparison with our data, Ebinger and coworkers (Ebinger et al. 1987a, b) reported similar HVA values, somewhat lower dopamine concentrations, and markedly higher DOPAC concentrations.

The HVA/dopamine ratio (and DOPAC/dopamine ratio) has been considered as a reflection of dopamine

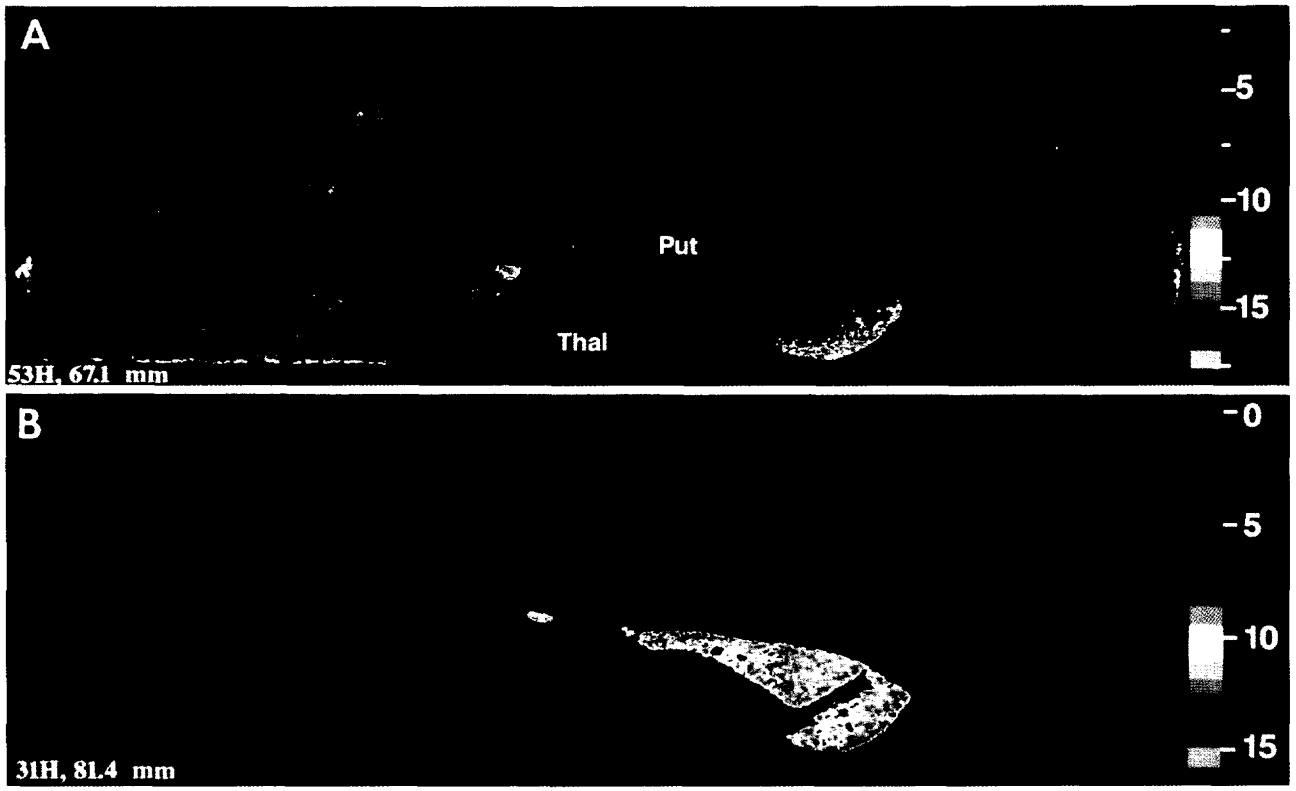


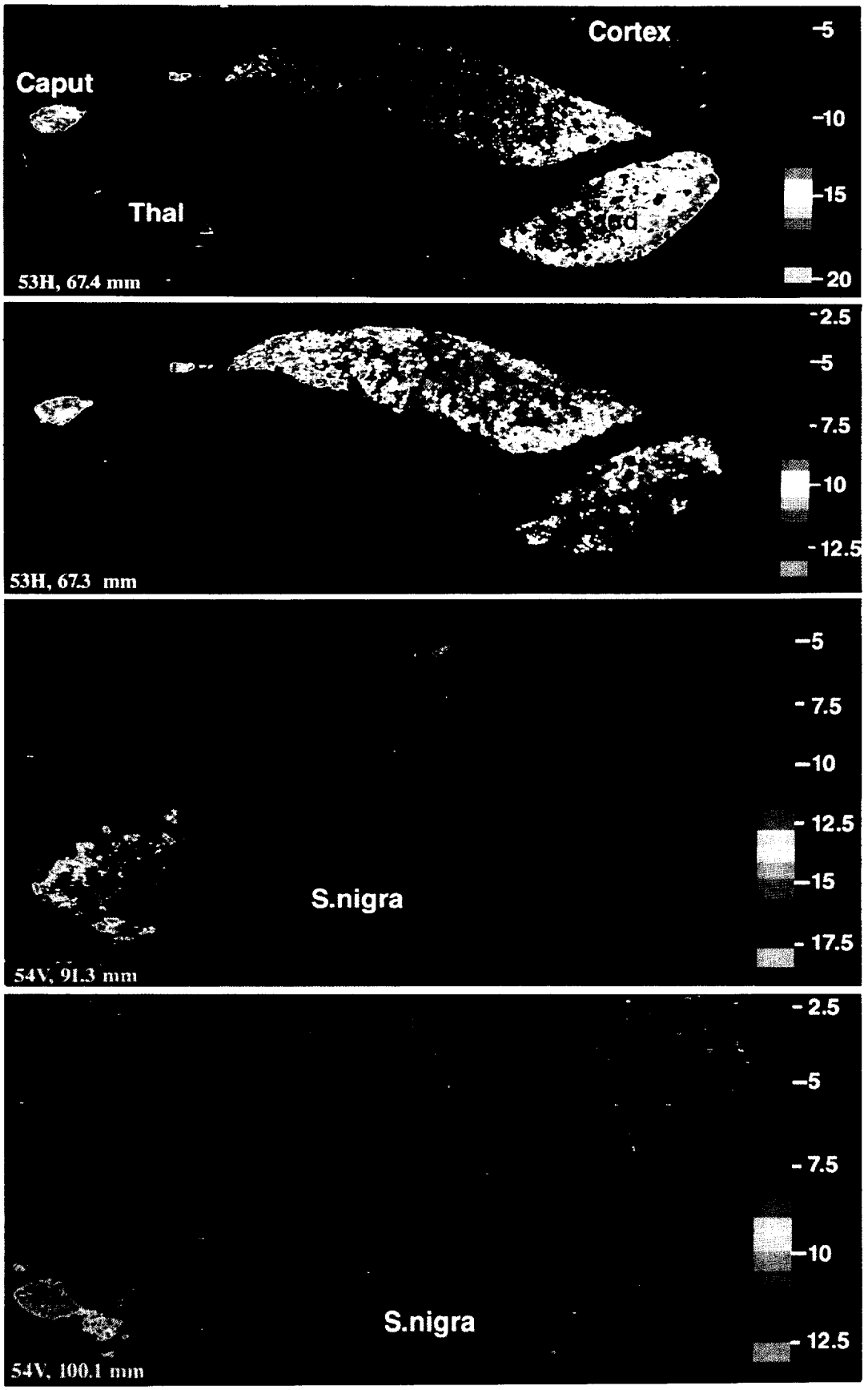
Figure 3. Color coded autoradiograms on human post-mortem brain sections using 2.2 nmol/L [^{3}H]SCH 23390 (**A**) and using 2.9 nmol/L [^{3}H]raclopride (**B**). Ketanserin (40 nmol/L) was added to the [^{3}H]SCH 23390 autoradiography. The autoradiograms represent the amount radioligand totally bound. The color scale is graded in pmol/g tissue wet weight. The internal brain number and the level of the section from vertex is indicated on the panels.

turnover. The results obtained in the present study may accordingly indicate large differences in the dopamine turnover between various areas of the basal ganglia, with the HVA/dopamine ratio varying from 1 (in the lateral putamen) to 13 (globus pallidus) (Table 2). Ratios calculated from the literature give similar variations (Ebinger et al. 1987a; Hardy et al. 1987; Wester et al. 1990). A prerequisite that the regional variations in the HVA/dopamine ratios do reflect differences in dopamine turnover is that dopamine is metabolized and HVA accumulated and removed at similar rates in the brain regions studied. Differences in dopamine reuptake and storage may also contribute to the differences found. It is plausible that certain regional HVA/dopamine ratios may be preferentially affected in psychiatric and neurologic diseases. For example, the calculated

Table 2. Dopamine Metabolite/Dopamine Ratios in Discrete Areas of the Basal Ganglia

	HVA/Dopamine		DOPAC/ Dopamine		3MT/Dopamine	
Region	**Median**	**Mean**	**Median**	**Mean**	**Median**	**Mean**
Putamen lateral	1.06	1.37	0.05	0.05	0.45	0.54
Putamen medial	2.96	2.84	0.06	0.06	0.77	0.74
Caudatus, corpus	2.25	2.25	0.10	0.10	1.02	1.02
Caudatus, caput	2.01	1.77	0.09	0.09	0.80	0.79
Caudatus, corpus-caput	1.92	1.92	0.14	0.14	0.66	0.66
Caudatus, cauda	2.76	2.76	0.48	0.48	1.04	1.04
Nucleus accumbens	2.16	3.20	0.13	0.13	1.02	1.11
Globus pallidus	13	14	0.10	0.10	1.92	1.87

The mean and median ratio values have been calculated from the mean and median levels of the transmitter levels (number of determinations = 3 to 6 per region).


Cortex
Caput
Thal
53H, 67.4 mm
-5
-10
-15
-20
A
-2.5
-5
-7.5
-10
-12.5
B
53H, 67.3 mm
-5
-7.5
-10
-12.5
-15
-17.5
S.nigra
C
54V, 91.3 mm
-2.5
-5
-7.5
-10
-12.5
S.nigra
D
54V, 100.1 mm

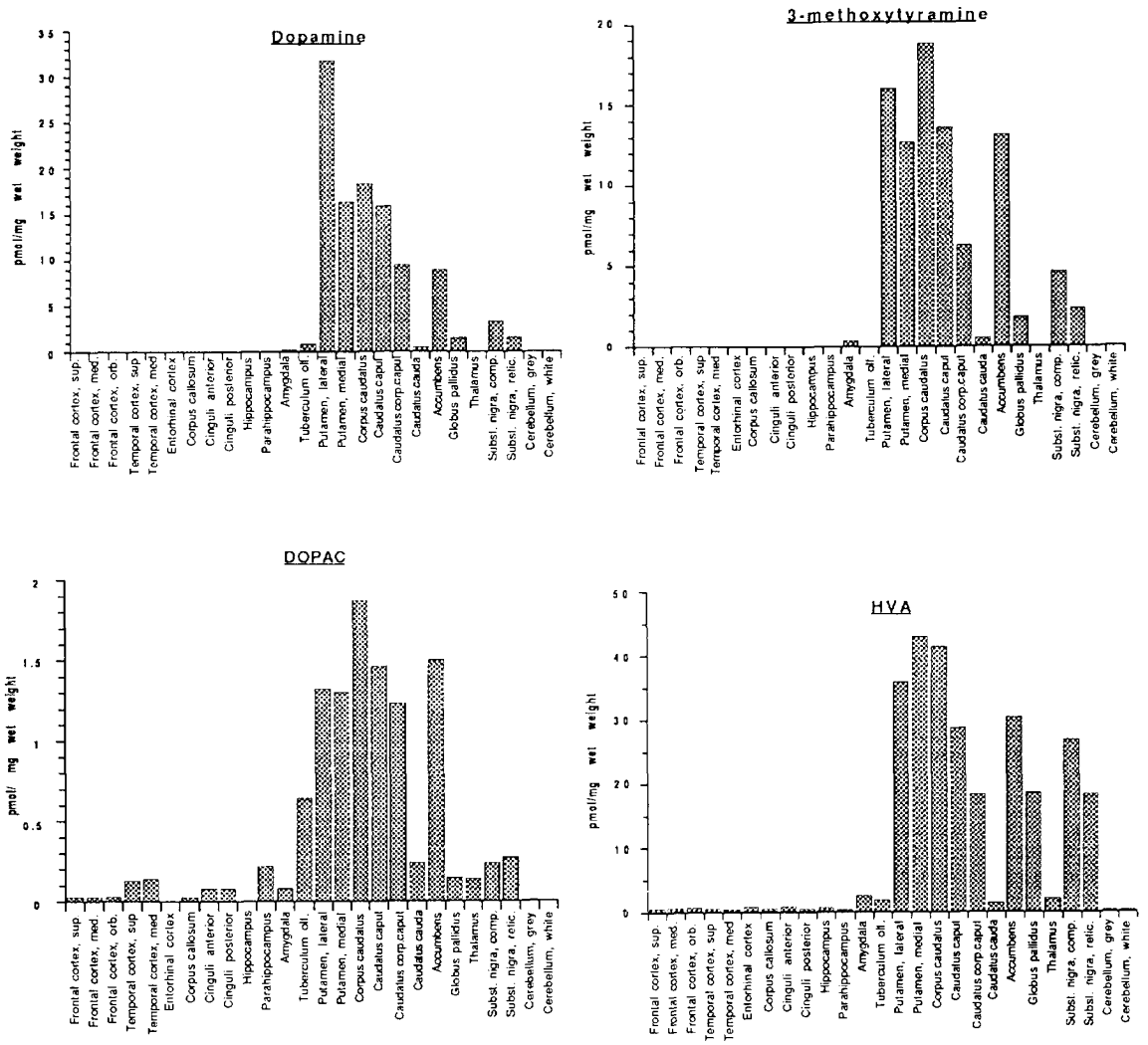


Figure 5. Median concentrations (pmol/mg wet weight, n = 3 to 6) of dopamine, DOPAC, HVA, and 3-methoxytyramine in various brain regions of the human brain as studied using HPLC.

caudate and putamen HVA/dopamine ratios were shown to be lower in brains from schizophrenic patients than from control brains (Crow et al. 1979). In another study, the HVA/dopamine ratios (calculated from given data) were markedly lower in the globus pallidum of patients with Alzheimer's disease as compared to those of the controls (Ebinger et al. 1987a).

The levels of 3-MT, which is suggested to be a predominantly post mortem metabolite of dopamine (Sparks et al. 1986, 1989), were of similar magnitude as earlier reported (Hardy et al. 1987) but higher than those found more recently (Wester et al. 1990). The lower 3-MT levels reported by Wester et al. (1990) may result from the cause of death (i.e., heart failure), and a longer post mortem interval than in our study. Thus, the content of 3-MT in the putamen has been found to decrease with increasing post mortem interval in cases where the cause of death was a result of organic heart failure.

The distributions of D_1- and D_2-dopamine recep-

Figure 4. Close ups of color coded autoradiograms on human post-mortem brain sections using [^{3}H]SCH 23390 (2.0 nmol/L and 2.3 nmol/L, respectively in panels **A** and **C**) and using [^{3}H]raclopride (3.1 nmol/L in panels **B** and **D**). Panels **A** and **B** show an enlargement of the caudate and putamen, whereas panels **C** and **D** show a section including the substantia nigra. Ketanserin (40 nmol/L) was added to the [^{3}H]SCH 23390 autoradiography. The autoradiograms represent the amount radioligand totally bound. The color scale is graded in pmol/g tissue wet weight. The internal brain number and the level of the section from vertex are indicated on the panels.

tor as investigated both by in vitro homogenate binding as well as with autoradiography on human whole hemisphere cryosections were, as expected, mostly confined to the basal ganglia. This parallels a voluminous literature on studies in animals and in humans obtained with dopaminergic radioligands of different chemical classes. It is also a confirmation of the distribution pattern obtained in PET with analogous ligands, [^{11}C]SCH 23390 (Halldin et al. 1986; Sedvall et al. 1986a, 1986b; Farde et al. 1987) and [^{11}C]raclopride (Ehrin et al. 1985; Farde et al. 1985, 1986), where, however, the image resolution is approximately two magnitudes lower than in the present autoradiographical images.

The D_2-dopamine receptor was found to have an even distribution in both the caudate nucleus and the putamen. This was in contrast to the distribution of the D_1-dopamine receptor that had a higher density in the medial parts of the caudate nucleus but a rather even distribution in the putamen. The localization of the very high density of D_1-dopamine receptors in the medial caudatus has been observed in previous autoradiographic studies with [^{3}H]SCH 23390 and its analogue [^{3}H]SCH 39166 (Hall et al. 1993) as well as with some other D_1-dopamine receptor antagonists such as NNC 756 (Andersen et al. 1992; Halldin et al. 1993; Hall, unpublished observations). The specific distribution pattern of the D_1-dopamine receptors in the medial basal ganglia is not seen in the PET studies with currently available systems. The reasons for this medial distribution are, however, unclear.

There was a marked labeling of the D_1-dopamine receptor in the cortical areas by [^{3}H]SCH 23390 with the 5-HT_2 antagonist ketanserin added to abolish the binding to cortical 5-HT_2 receptors. In PET [^{11}C]SCH 23390 has been shown to bind to receptors in the cerebral cortex, and this binding could not be displaced in the monkey brain in vivo by a high dose of ketanserin (Farde et al., unpublished observations). The present in vitro study thus confirms the presence of considerable densities of D_1-dopamine receptors in the human neocortex. The densities of the two dopamine receptor subtypes paralleled each other roughly but with some notable exceptions: the D_1-dopamine receptor is the dominating receptor subtype in the substantia nigra and in most cortical regions. This was especially in the entorhinal and parahippocampal cortex, where there were 4 to 7 times more D_1- than D_2-dopamine receptors. The finding of a low but distinct density of D_2-dopamine receptors in the corpus callosum, but not in the cerebellar white matter, is interesting in the view of the previous demonstration of cells containing tyrosine hydroxylase immunoreactivity in rat white matter (Pearson et al. 1990).

Recently, new subtypes of the dopamine receptors have been described (Sokoloff et al. 1990; Sunahara et al. 1991; Van Tol et al. 1991). The D_3- and D_4-dopamine receptors belong to the same group as the D_2-dopamine receptor, and the structure of the D_5-dopamine receptor is similar to that of the D_1-dopamine receptor. There are as yet no selective radioligands for these new receptor subtypes, with the possible exception of the D_3-dopamine receptor (Lévesque et al. 1992). Similar to other D_1-dopamine receptor radioligands, the radioligand [^{3}H]SCH 23390 binds to the D_5-dopamine receptor in addition to the D_1-dopamine receptor (Sunahara et al. 1990), and the receptor distribution obtained using [^{3}H]SCH 23390 autoradiography is thus a composite distribution of the two receptors. As judged from the distribution of mRNA in the rat, visualized using in situ hybridization studies, the D_5-dopamine receptor is not localized to the caudate nucleus and the putamen, but to hippocampus, lateral mammillary bodies, and thalamic nuclei (Tiberi et al. 1991). The labeling of caudate nucleus and putamen, as well as of the cerebral cortex in the presence of ketanserin, by [^{3}H]SCH 23390 is thus mainly to the D_1-dopamine receptor.

[^{3}H]raclopride, like most other radioligands for the D_2-dopamine receptors, has an affinity for the D_3-dopamine receptors of the same magnitude as for D_2-dopamine receptors (Sokoloff et al. 1990), and the labeling could thus be to both these receptor types. In situ hybridization studies of rat distribution of mRNA encoding for the D_3-dopamine receptor have indicated a very limited distribution mostly in extrastriatal areas, such as islands of Calleja and nucleus accumbens. Therefore, the labeling of caudate nucleus and putamen by [^{3}H]raclopride should predominantly reflect D_2-dopamine receptors. However, more selective and potent tools are needed for the specific evaluation of the localization and density of these other receptor subtypes in striatal and extrastriatal regions.

The cerebellum is often used as a reference region to determine free and nonspecific binding in dopamine receptor studies because of its low number of dopamine receptors. Specific binding of neither [^{3}H]SCH 23390 nor [^{3}H]raclopride was observed in the cerebellum in the autoradiographic images. However, the quantification of the binding in the homogenate receptor assays revealed a low but significant binding of both ligands. Especially [^{3}H]SCH 23390 showed labeling of receptors in cerebellar grey, but not white, matter. However, the cerebellar density of D_1-dopamine receptors was less than 10% of that in the basal ganglia, whereas the density of the D_2-dopamine receptors was approximately 1% to 2% of the highest densities. These data confirm the view that the cerebellum is a suitable reference region for the study of D_2-dopamine receptors in vivo, whereas further studies are required to justify the use of cerebellum as a reference region for D_1-dopamine receptor studies.

ACKNOWLEDGMENTS

We are grateful to Dr. Allen Barnett, Schering-Plough Co., USA, for providing us with SCH 23390 and desmethyl-SCH 23390 used for radiolabeling. The excellent technical assistance of Mrs. Barbro Berthelsson and Mrs. Siv Eriksson is greatly acknowledged. This work was supported by grants from the Swedish Medical Research Council (03560 and 10360), the Swedish Natural Science Research Council (K-KU 9973-300), the Bank of Sweden Tercentenary Fund (88/427), the National Institute of Mental Health, USA (NIMH, Grant No. MH44814-05), Söderström-Königska stiftelsen and Karolinska Institutet.

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