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Imaging Dopamine D_4 Receptors in the Living Primate Brain: A Positron Emission Tomography Study Using the Novel D_1/D_4 Antagonist [^{11}C]SDZ GLC 756

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ABSTRACT The dopamine D_4 receptor has lately attracted interest since it has been hypothesized to be involved in the pathogenesis and pharmacotherapy of neuropsychiatric diseases. The present study provides first in vivo evidence of dopamine D_4 receptors in primate brain using a [^{11}C]benzo[g]quinoline, the novel radioligand [^{11}C]SDZ GLC 756 ([^{11}C]GLC: in vitro dissociation constants at human receptor clones [nM]: 1.10 at D_1; 0.40 at D_2; 25 at D_3; 0.18 at $D_{4.2}$; 6.03 at D_5). Dynamic positron emission tomography scans were performed on healthy baboons (*Papio hamadryas*, n = 3). Specific receptor binding (SB) was calculated for striatum and neocortex (frontal, temporal, parietal, and occipital) based on the differences between the regional and the cerebellar concentration of [^{11}C]. Blockade of D_1 and D_5 receptors by SCH23390 (1.7 μmol/kg) diminished SB in the striatum by 55 ± 4% (mean ± standard deviation, $P < 0.05$) and in the frontal cortex by 13 ± 8% ($P < 0.05$) when compared to SB in the unblocked state ($SB_{D1–D5}$). In the presence of the dopamine antagonists SCH23390 (1.7 μmol/kg) and raclopride (5.7 μmol/kg)—which mask the D_1, D_2, D_3, and D_5 subtypes—SB of [^{11}C]GLC to D_4 receptors (SB_{D4}) was demonstrated in the striatum and all cortical regions of interest. In the striatum, the ratio of $SB_{D4}/SB_{D1–D5}$ was 0.13 ± 0.07. In the neocortex, $SB_{D4}/SB_{D1–D5}$ was notably higher (0.77 ±0.29; mean of all cortical regions of interest). The widespread distribution of dopamine D_4 receptors suggests a basic functional role of this receptor subtype in the modulation of cortical and subcortical neuronal activity. **Synapse 30:341–350, 1998.**

INTRODUCTION

Dopamine receptors are involved in the regulation of human movement, motivation, and cognitive performance, as well as in the pathophysiology of neuropsychiatric diseases (Jackson and Westlind-Danielsson, 1994; Nestler, 1997; Seeman et al., 1993; Seeman, 1995; Volkow et al., 1997; Williams and Goldman-Rakic, 1995). The physiological effects of dopamine on neurotransmission in mammalian brain are mediated by a family of guanine nucleotide binding protein coupled receptors (Dohlmann et al., 1991). Molecular cloning has revealed the existence of five mammalian dopamine receptor genes (D_1–D_5) which encode pharmacologically distinct dopamine receptor subtypes (Civelli et al., 1993; Gingrich and Caron, 1993; Jackson and Westlind-Danielsson, 1994; Nestler, 1994; Seeman et al., 1993; Seeman, 1995). Based on pharmacological and structural criteria, these five subtypes have been classified into two subfamilies of D_1-like (D_1, D_5) and D_2-like (D_2, D_3, D_4) dopamine receptors. D_1-like and D_2-like receptors exert synergistic as well as opposite effects at the molecular, cellular, and systems levels (Civelli et al., 1993; Gingrich and Caron, 1993; Jackson and Westlind-Danielsson, 1994; Nestler, 1994; Seeman, 1995). D_1-like receptors stimulate adenylate cyclase

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and D_2-like receptors act inhibitorily on the adenylate cyclase and the cAMP pathway (Jackson and Westlind-Danielsson, 1994; Nestler, 1994). The distribution of cerebral D_1-like and D_2-like dopamine receptors has been studied in primates and man in vitro (Lidow et al., 1991; Hall et al., 1994) and—irrespective of their corresponding subtypes—in vivo (Farde et al., 1985, 1997; Haldin et al., 1986; Volkow et al., 1997; et al., 1998). Previous attempts to quantify the D_4 receptor density in vitro in postmortem human brains have led to conflicting results (Kramer et al., 1997; Lahti et al., 1996; Murray et al., 1995; Mrzljak et al., 1996; Reynolds and Mason, 1995; Seeman et al., 1993, 1995).

One basic reason why the dopamine D_4 receptor has recently attracted such particular interest is because it has been localized immunohistochemically on pyramidal cells and on GABAergic nonpyramidal neurons in primate brain (Mrzljak et al., 1996). The presence of D_4 receptors in pyramidal cells has suggested a direct inhibitory effect of dopamine on pyramidal cell activity, while the stimulation of D_4 receptors on nonpyramidal GABAergic neurons has been related to a disruption of the feedforward inhibition of GABAergic neurons on pyramidal cells (Mrzljak et al., 1996). A functional role of the D_4 receptor in the balance of excitatory and inhibitory factors on pyramidal neurons in the primate neocortex has thus been proposed. Moreover, the recent finding that dopamine, epinephrine, and norepinephrine all show binding to recombinant human D_4 receptors in the nanomolar range, and that they activate these receptors in vitro, has suggested a novel mechanism for the integration of catecholamine signaling in the brain (Lanau et al., 1997).

From the clinical point of view, the D_4 receptor has been attributed to involvement in the pathogenesis of schizophrenia, since a group of atypical neuroleptics, such as clozapine, has a high affinity for this dopamine receptor subtype (Nestler, 1997; Seeman et al., 1997a; Van Tol et al., 1992). It has been hypothesized that the therapeutic properties of this drug are due to its action on D_4 receptors. When subtraction techniques with ligands of different subtype binding profiles are used, an increase in the density of D_4 receptors is discovered in the postmortem striatal tissue of patients suffering from schizophrenia (Murray et al., 1995; Seeman et al., 1993). Recently, a postmortem study using schizophrenia brain striata and [^{3}H]GLC found increased levels of a D_2-like binding site not detected in control human brains or Alzheimer's, Huntington's, or Parkinson's disease brains (Seeman et al., 1997b). This D_2-like binding site showed a receptor density (B_{max}) of 6.6 pmol/g wet weight measured in the presence of drugs which occluded the D_1, D_2, D_3, and D_5 receptors (Seeman et al., 1997b). However, the hypothesis that a single dopamine receptor subtype such as the D_4 receptor may be a principal constituent in the pathogensis of schizophrenia has been challenged by recent clinical postmortem data (Helmeste et al., 1996; Kramer et al., 1997; Lahti et al., 1996; Reynolds and Mason, 1995).

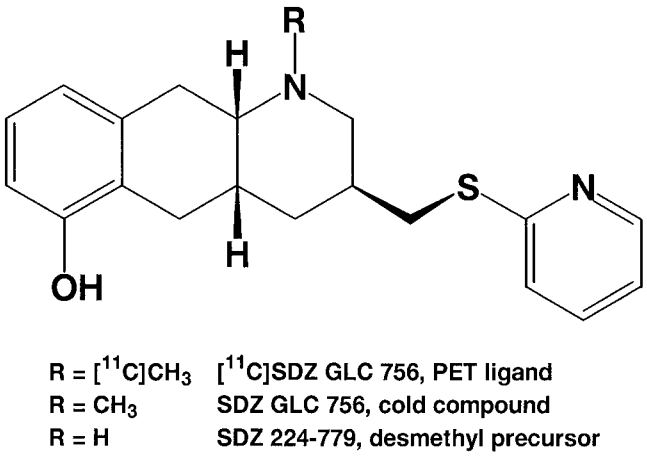

Fig. 1. Chemical structure of SDZ GLC 756 (−)[(3R,4aR,10aR)-1,2,3,4,4a,5,10,10a-octahydro-6-hydroxy-1-methyl-3-[(2-pyridyl-thio)-methyl]-benzo[g]quinoline].

TABLE I. In vitro binding affinities (agonist or antagonist) dissociation constants of different ligands to dopamine receptor clones [nM]

	D_1	D_5	D_2	D_3	D_4
SDZ GLC 756	1.10[a]	6.03[a]	0.40[b]	25[b]	0.18[a]
SCH23390	0.37[a]	0.30[a]	634[a]	780[a]	3,000[a]
Raclopride	18,000[a]	n.d.	1.80[a]	3.50[a]	1,500[a]
Spiperone	350[a]	3,500[a]	0.07[a]	0.60[a]	0.08[a]

n.d. = not determined.
[a]Antagonist binding.
[b]Agonist binding.

The present study investigated the distribution of the D_4 receptor under in vivo conditions in the nonhuman primate brain on a macroscopic scale using the novel dopaminergic radioligand [^{11}C]GLC (Markstein et al., 1996, Seeman et al., 1997b) and positron emission tomography (PET). [^{11}C]GLC, the chemical structure of which is shown in Figure 1, has among all known subtypes the highest affinity for D_4-receptors (Table I). The strategy was to quantify D_4 receptors in the presence of drugs that mask the D_1, D_2, D_3, and D_5 subtypes.

MATERIALS AND METHODS

Animal studies

PET studies were performed using healthy laboratory-bred baboons (*Papio hamadryas*, one female and two males, body weight 5–7 kg) obtained from the German Primate Research Center in Göttingen, Germany. All study protocols were government-approved and corresponded to the ethical codes and German legislation on animal experiments. General anesthesia was induced by an intramuscular injection of ketamine (10 mg/kg) and maintained by a continuous infusion of methohexital (30–40 mg/kg/h). The intubated animals were venti-

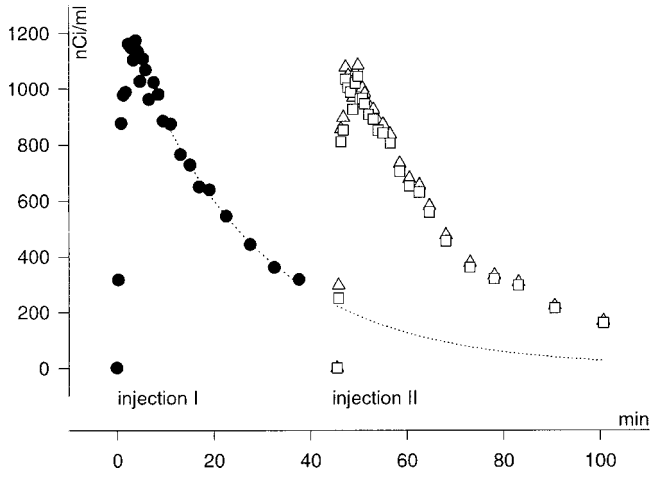

Fig. 2. A representative PET experiment showing the concentration of [^{11}C] in primate neocortex following two injections of [^{11}C]GLC (injection I: 4.75 mCi; injection II: 5.07 mCi). The present experimental protocol (exp. 1) reflects time–activity curves in the absence of cold dopamine antagonists: (●) following injection I, raw data; (△) following injection II, raw data; (□) following injection II, data corrected by the subtraction of the residual radioactivity due to injection I. The dotted lines depict the corresponding monoexponential fit (. . . . . .) and its extrapolation (.....).

lated with 100% O_2 at an end tidal CO_2 content of 35–38%. The noncentrally acting anticholinergic drug glycopyrrolate (0.02 mg/kg) was given intravenously to minimize salivation. A 20 gauge arterial catheter inserted into the femoral artery served for blood sampling procedures. Another 20 gauge venous catheter placed in the femoral vein of the opposite leg was used for the application of the radiotracer, drugs, and fluids. In 1 of 12 experiments, two venous catheters were inserted instead. The head of each baboon was positioned using a specially designed head holder with an individual polymer bed. Body temperature was kept constant at 37°C using a heating pad during the entire experiment. Vital signs (heart rate, blood pressure, respiration rate, oxygen saturation, expired carbon dioxide, and body temperature) were continuously monitored.

Each baboon was subjected to four experimental protocols (exp. 1 to exp. 4) at intervals of 7 days. During each PET study, two intravenous slow bolus injections (0.8 mCi/kg; injection time: 20–25 s) of [^{11}C]GLC were applied at an interval of 40–45 min, i.e., injection I and injection II (Fig. 2). The first experiment was performed without any receptor blockade. During the following experiments (exp. 2 to exp. 4), dopamine receptor antagonists were given intravenously 20 min prior to injection II in doses sufficient to block the respective receptors. SCH23390 (1.7 μmol/kg) was used to mask D_1 and D_5 receptors (exp. 2). Blockade of D_1, D_2, D_3, and D_5 receptors (exp. 3) was enabled using SCH23390 (1.7 μmol/kg) + raclopride (5.7 μmol/kg). A total blockade of dopamine receptors, including the D_4 subtype (exp. 4), was achieved by the application of SCH23390 (1.7 μmol/kg) + raclopride (5.7 μmol/kg) + spiperone (2.5 μmol/kg). Raclopride tartrate was from Astra Arcus, Södertällje, Sweden. Spiperone and SCH23390 were purchased from Research Biochemicals International, Biotrend, Cologne, Germany. Dynamic PET scans started with the injection of [^{11}C]GLC using a GE PC 4096 PLUS whole body PET camera (Rota Kops et al., 1990). In the wobbled and interleaved mode, the resolution was 5.5 mm in the center field of view (interplane distance of 3.2 mm). Iterative reconstruction resulted in 30 slices and included corrections for attenuation, scatter, random events, and deadtime. Images obtained without previous receptor blockade were employed to identify regions of interest (ROI). Orbito-meatal slices were matched to a baboon brain atlas (Riche et al., 1988) and square ROIs (9 × 9 mm) were placed to encompass striatum, neocortex (frontal, temporal, parietal, and occiptal), and cerebellum. In the double scan protocol, the images from the first and second scan sequences were in spatial register. Thus, identical ROIs were placed on the first and second image sets from that protocol.

Data analysis

For the two PET scans following injections I and II of [^{11}C]GLC, decay corrected time–activity curves were constructed by plotting the percent injected dose per ml of the tissue [%ID/ml] versus time p.i. The tissue time–activity curves of the second PET scan following injection II of [^{11}C]GLC were corrected by the subtraction of the residual activity due to injection I using a standardized curve-fitting procedure (Fig. 2). The procedures for making such a correction were defined from the extrapolation of the first scan sequences extended to the end of the second scan sequence. It was found that image intensity values during the period of interest decreased time dependently with a good approximation to a monoexponential function (Fig. 2). Since striatal, cortical, and cerebellar intensities decline with different decay constants, a procedure was adopted in which the respective decreasing regional intensity values observed in the first scan sequence were fitted by least-square fitting to a monoexponential decay function of time (t):

$$f(t) = a^{(bt+c)} \quad (1)$$

The parameters a, b, and c were used to calculate the regional intensity value from the first GLC injection that would have been present at the given time during the second scan sequence. This intensity value was then reapplied as a subtractive correction to yield the image intensities due to the second injection of [^{11}C]GLC (Fig. 2).

Specific binding (SB) of [^{11}C]GLC[%ID/l] was defined as the difference between cortical or striatal and cerebellar [^{11}C] concentration integrated between 10 and 60

min p.i.: no receptor blockade (SB_{D1-D5}); receptor blockade by SCH23390 (SB_{D2-D4}); blockade of all non-D_4 receptors by SCH23390 and raclopride (SB_{D4}); blockade of all known dopamine receptors by SCH23390, raclopride, and spiperone, i.e., nonspecific binding of [^{11}C]GLC (NSB). Statistical analysis evaluating specific and nonspecific binding of [^{11}C]GLC during the various blocking protocols was performed using the one-tailed paired *t*-test. The significance level was set at $P = 0.05$. All sample data are presented as mean ± standard deviation (n = 3).

Blood sampling and metabolite analysis

Arterial blood samples (0.5–1 ml) were collected manually at intervals from 15 sec to 10 min up to 60 min p.i., diluted with the same amount of isopropanol, and centrifuged at 2,000*g* for 3 min. Plasma radioactivity was measured using aliquots (50 µl) and a calibrated automatic gamma counter (Wallac, 1480 Wizard 3). Blood samples (taken from 15 sec to 20 min p.i.) prepared for metabolite analysis by optimized radio HPLC were taken using a Merck-Hitachi (L-6000) pump and a Knauer UV/VIS filter photometer (9700) with a detector wavelength of 254 nm. A sample injection was accomplished with a Rheodyne 7125 Injector Block. To facilitate continuous measurement of radioactivity, the UV detector outlet was connected to an NaI(T1) well-type scintillation detector and the recorded data were processed by a software system (Nuclear Interface, Datentechnik für Strahlungsmeßgeräte GmbH). For purposes of HPLC analysis, aliquots of the supernatant blood plasma (500 µl) were injected into the HPLC column (Kromasil C-18, 5 µm, 250 ∗ 4 mm, equipped with a precolumn RP-18, 10 µm, 20 ∗ 4 mm) and eluted with acetronitrile/0.025 M aqueous diammonium hydrogen phosphate (55/45, v/v) at a flow of 1 ml/min.

Synthesis of the radioligand

For intravenous application, [^{11}C]GLC was synthesized with a chemical and radiochemical purity of >99% and a maximum specific activity of >29 TBq/mmol: no carrier-added [^{11}C]$CF_3SO_3CH_3$ was produced, according to a standard procedure (Holschbach and Schüller, 1993). The [^{11}C]methyltriflate was carried by helium into a reaction vial containing the desmethyl precursor (0.2 mg, 0.6 µmol) in dry 1,2-dichloroethane (500 µl). After 5 min reaction time at room temperature, ethanol (2 ml) was added and the solution was injected into a semipreparative HPLC. In order to circumvent rotary evaporation of the eluent and in keeping with a straightforward pharmaceutical formulation procedure, the HPLC fraction containing the product was cut out of the eluent flow and passed over a cation exchange cartridge (Adsorbex SCX), conditioned before use with HCl (0.1 N, 15 ml) and washed with water (5 ml), $NaHCO_3$ (0.1 N, 10 ml), and water (5 ml)). Elution of the product through a 0.22 µm sterile filter into a sterile vial was performed with ethanol (1 ml) followed by physiological saline (9 ml). This procedure resulted in a recovery of about 95% of the trapped radioactivity. The specific activity of [^{11}C]GLC was determined by HPLC assay of an aliquot of the labeled product. The corresponding signal was compared with a standard curve of the UV absorption spectrum of the cold compound recorded from a solution of a known concentration. [^{11}C]GLC and the desmethyl compound were provided by Novartis Pharma AG, Switzerland.

RESULTS

The results depicted in Figure 3A reflect [^{11}C]GLC binding to all dopamine receptor subtypes as well as nonspecific [^{11}C]GLC binding. [^{11}C]GLC binding was high in striatum and moderate in neocortical regions. Due to the blockade of D_1 and D_5 receptors by SCH23390 (Fig. 3B), the accumulation of [^{11}C]GLC was markedly reduced in the striatum, while binding in the neocortex declined only slightly. Blockade of all non-D_4 receptors by SCH23390 and raclopride resulted in a homogeneous and low accumulation of [^{11}C]GLC in striatum and in frontal, temporal, parietal, and occipital cortex (Fig. 3C). Following the combined use of SCH23390, raclopride, and spiperone, the binding of [^{11}C]GLC was reduced to the nonspecific level (Fig. 3D).

Time–activity curves (Fig. 4) showed a high accumulation and a relatively slow washout of [^{11}C]GLC in the striatum, a region of brain with a dense dopaminergic innervation. In the striatum, the concentration of [^{11}C] reached a peak at 35% ID/l within the first 5 min p.i. During this time, the radioactivity concentration of the cerebellum reached a peak of 30% ID/l, which slightly exceeded that of the neocortex (displayed as mean of frontal, temporal, parietal, and occipital ROIs). In the striatum and neocortex, the time–activity curve decreases reflected the blockade of distinct receptor populations due to the application of cold dopamine antagonists (exp. 2 to exp. 4). The cerebellar concentration of [^{11}C] used here as a region of nonspecific binding was not influenced by the different blocking procedures. The curves showing the difference of regional striatal/neocortical and cerebellar radioactivity (Fig. 5) reached a peak at 15 to 20 min p.i. Differences between the

Fig. 3. Example of differential dopamine-receptor-subtype mapping in a baboon brain with [^{11}C]GLC. Coronal and saggital planes are cut through the anterior portion of the left striatum (slice thickness: 3.2 mm; integration time: 10 to 60 min after injection). The [^{11}C]GLC concentration is normalized to the injected dose and to the highest regional uptake in the unblocked state (set to 1.0, linear scale) observed in the striatum. Binding of [^{11}C]GLC in the unblocked state (**A**) and following the intravenous application of dopamine receptor antagonists 20 min prior to the injection of [^{11}C]GLC, i.e., SCH23390 (**B**), SCH23390 + raclopride (**C**) and SCH23390 + raclopride + spiperone (**D**).

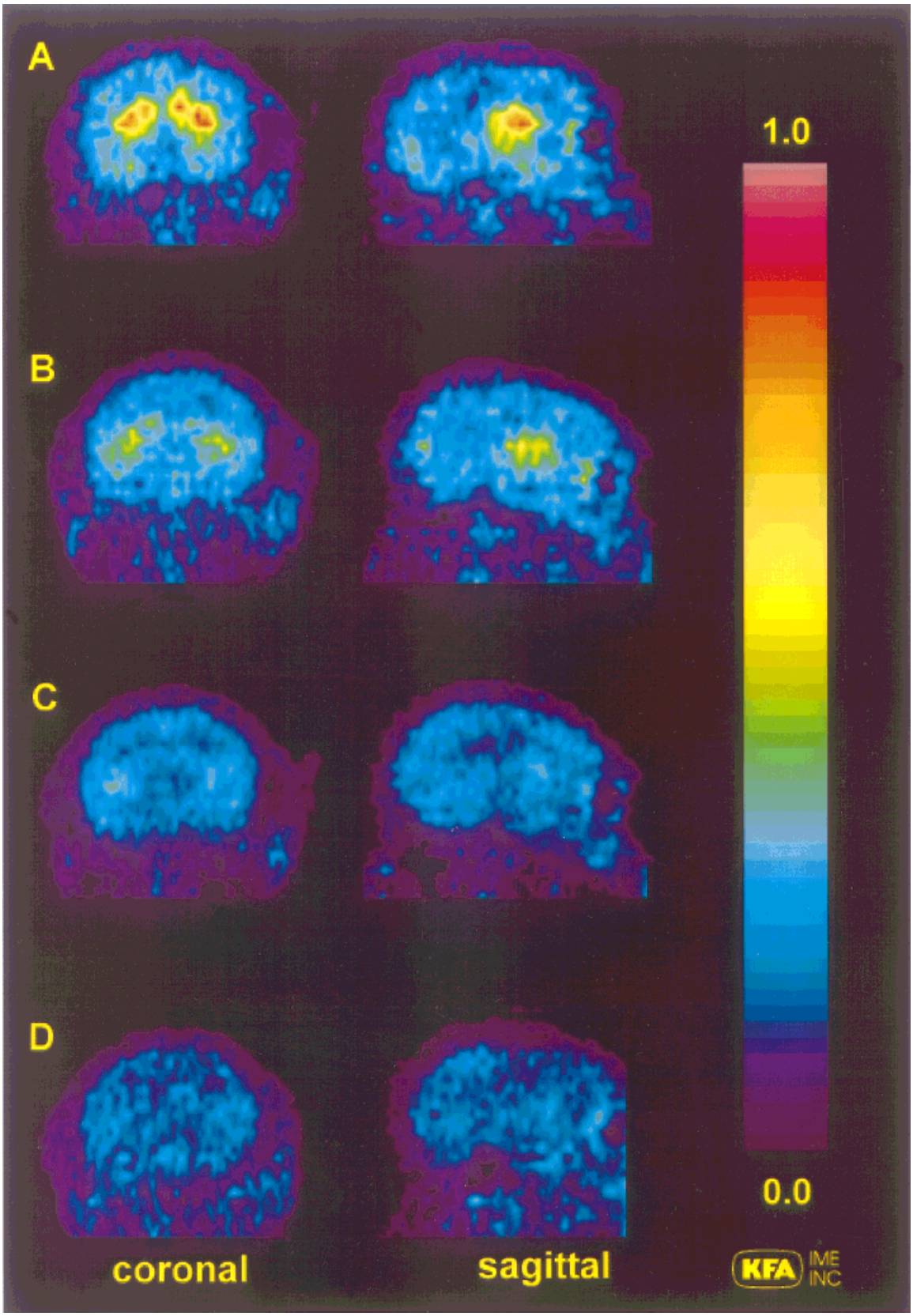

Fig. 3

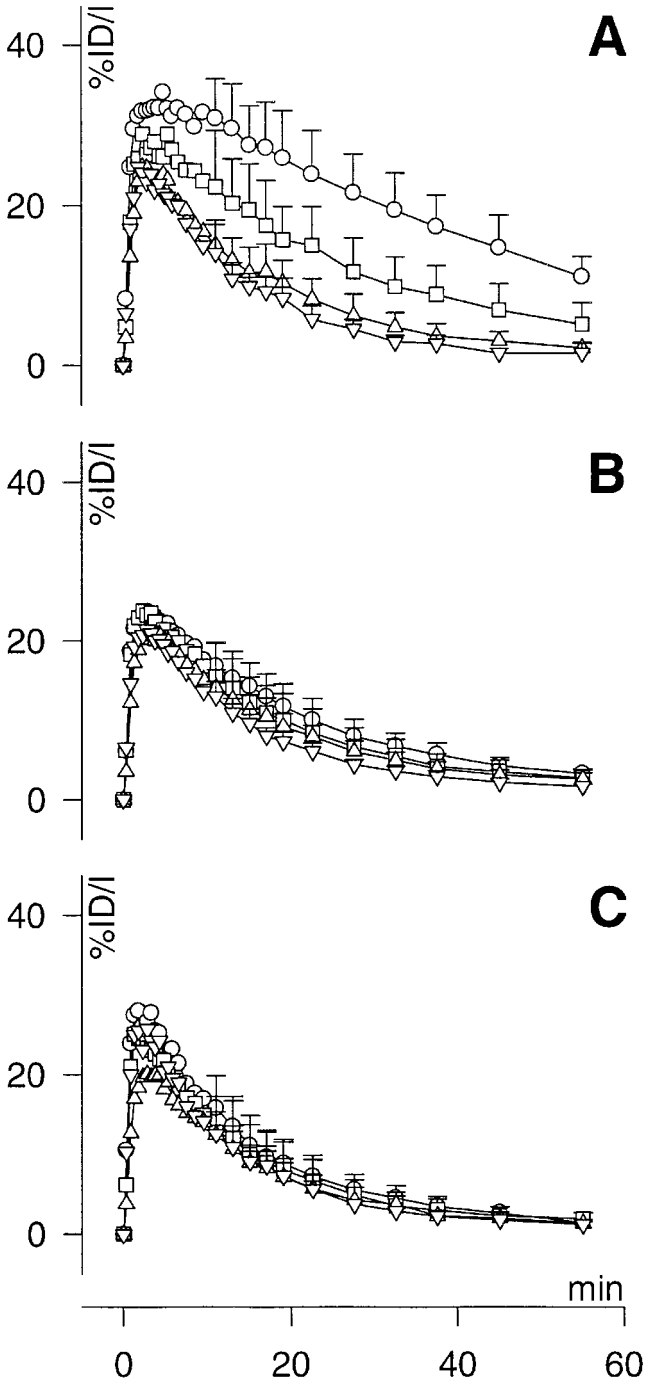

Fig. 4. Time course of [^{11}C] concentration in different regions of primate brain after the i.v. injection of [^{11}C]GLC (0.8 mCi/kg). Curves reflect the unblocked state (○) and the reductions of regional [^{11}C] concentration due to the i.v. application of SCH23390 (□), SCH23390 and raclopride (△), SCH23390, raclopride, and spiperone (▽). For the interval of 10–60 min p.i., data normalized to the injected dose per volume [%ID/l] are presented as mean ± standard deviation, n = 3; (**A**) striatum; (**B**) neocortex, mean of frontal, temporal; parietal, and occipital; (**C**) cerebellum.

curves reflected the reductions of specific binding caused by the fractionated blockade of dopamine receptor subtypes.

Figure 6 summarizes the regional specific binding (SB) of [^{11}C]GLC in the course of the differential dopamine receptor mapping approach. The statistical evaluation of differences of means between the different experiments is given in Table II. In the unblocked state (exp. 1), $SB_{D1–D5}$ [%ID/l] in striatum (150.2 ± 27.9) was about six times higher than in frontal (25.0 ± 3.1), temporal (24.7 ± 3.1), parietal (24.8 ± 3.3), and occipital neocortex (26.6 ± 2.2). Following the application of SCH23390, SB was reduced by 55 ± 4% in the striatum ($SB_{D2–D4}$ vs. $SB_{D1–D5}$, $P < 0.05$). In the neocortex, the reduction of SB reached statistical significance in the frontal ROIs (−13 ± 8%, $SB_{D2–D4}$ vs. $SB_{D1–D5}$, $P < 0.05$). The binding difference of [^{11}C]GLC between exp. 2 and exp. 3 due to the application of SCH23390 and raclopride mirrored the presence of D_2 and D_3 dopamine receptors in the striatum (SB_{D4} vs. $SB_{D2–D4}$, $P < 0.05$). SB of [^{11}C]GLC to D_4 receptors [%ID/l] was found in frontal (18.4 ± 5.5), temporal (19.2 ± 5.3), parietal (18.4 ± 6.0), and occipital neocortex (19.3 ± 5.5), as well as within the striatum (20.0 ± 11.0). The differences between exp. 3 and exp. 4 (SB_{D4} vs. NSB) revealed the presence of D_4 receptors since [^{11}C]GLC binding decreased significantly in striatal and in all neocortical regions. In the striatum, $SB_{D4}/SB_{D1–5}$, the ratio of SB of [^{11}C]GLC to D_4 receptors (exp. 3) and the SB to all dopamine receptor subtypes (exp. 1) was 0.13 ± 0.07. In the neocortex, $SB_{D4}/SB_{D1–5}$ was notably higher (0.77 ± 0.29; mean of all cortical ROIs).

There was a rapid clearance of [^{11}C]GLC from the blood, reaching values of <0.015% ID/ml at 10 min p.i. (Fig. 7). Figure 8 displays the chromatogram of a representative arterial blood sample obtained 2.25 min after the injection of [^{11}C]GLC. Metabolites of [^{11}C]GLC generated two major peaks and were not expected to pass the blood–brain barrier because of abundant polar groups. The fraction of nonmetabolized [^{11}C]GLC in the arterial plasma compartment (Fig. 9) at 1, 2, 5, and 10 min after injection was 42.3, 24.9, 15.5, and 9.5%, respectively. The HPLC results using probes obtained after the application of SCH23390 and raclopride (exp. 3) or using venous blood sampling were in the same range (Fig. 9).

DISCUSSION

For five years following the cloning of D_4 receptor (Van Tol et al., 1992), the low level of D_4 expression in brain tissue together with the lack of a D_4 receptor-specific ligand made the localization and physiological characterization of this dopamine receptor particularly difficult. For example, in postmortem striatum from healthy human subjects the density of D_4 receptors was reported in the range of 2.1 to 5.2 pmol/g wet weight (Seeman et al., 1993, 1995) or about 40 pmol/g protein weight (Murray et al., 1995). In the basal ganglia of the healthy rat, a B_{max} of 6.4 pmol/g wet weight was

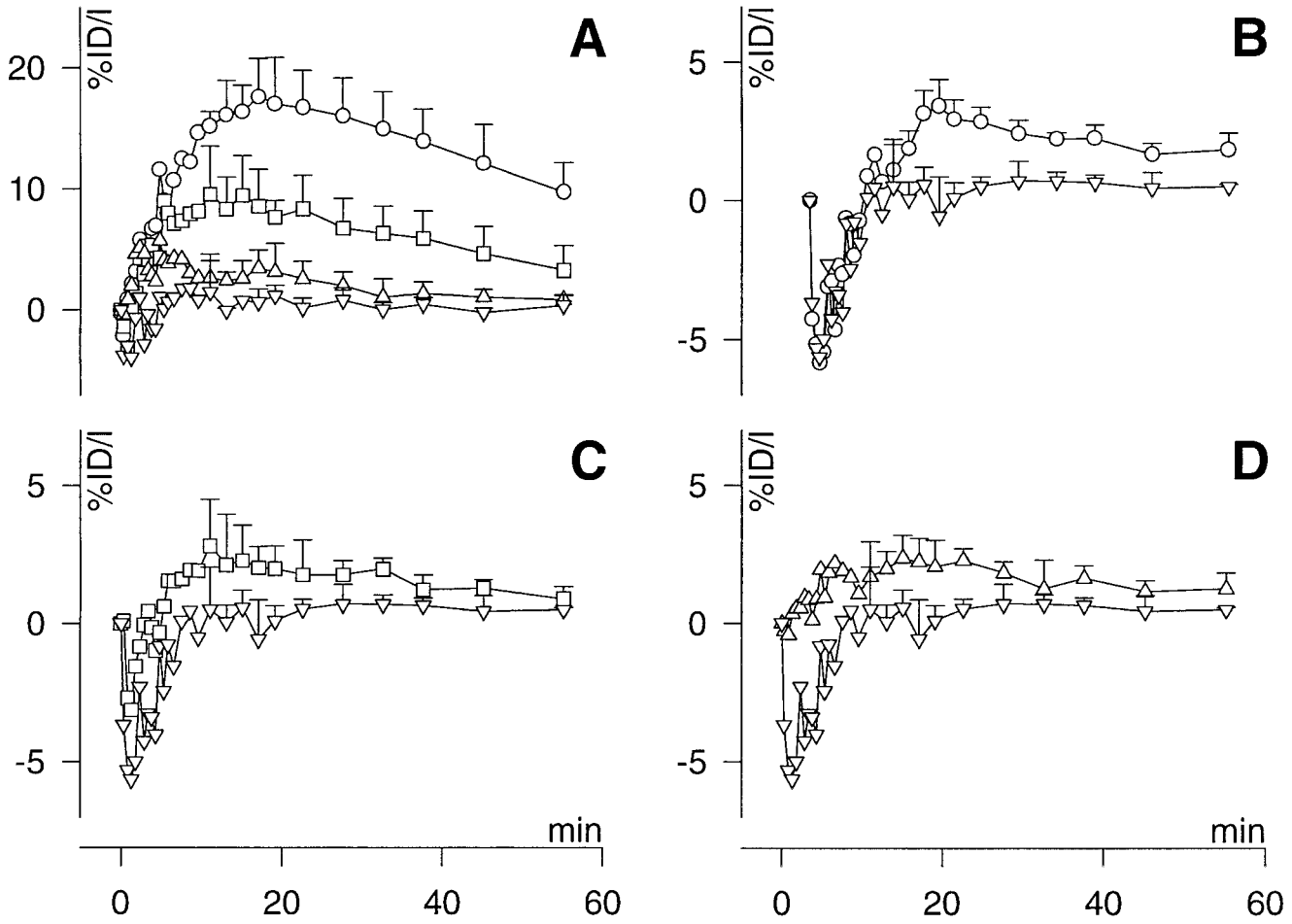

Fig. 5. Difference of striatal or neocortical and cerebellar [^{11}C] concentration as a function of time after the injection of [^{11}C]GLC in the unblocked state (○) and following the application of SCH23390 (▭), SCH23390 and raclopride (△), or SCH23390, raclopride, and spiperone (▽). For the interval of 10–60 min p.i., data normalized to the injected dose per volume [%ID/l] are presented as mean ± standard deviation, n = 3. (**A**) striatum; (**B**–**D**) neocortex: mean of frontal, temporal, parietal, and occipital.

detected (Schoots et al., 1995). Our investigation, which used [^{11}C]GLC and PET, is providing the first in vivo evidence of a widespread distribution of dopamine D_4 receptors in striatum and neocortex in the brain of the healthy primate.

The mapping approach of D_4 receptors taken in the present study is unlikely to be significantly influenced by [^{11}C]GLC binding to non-D_4 dopamine- or non-dopamine receptors for a number of reasons:

First, the blockades of dopamine receptors by SCH23390, raclopride, and spiperone are highly specific. These receptor antagonists bind with nanomolar affinities to the dopamine receptor subtypes (Table I).

Second, the blockade of non-D_4 dopamine receptors was achieved using SCH23390 and raclopride in doses of up to two orders of magnitude above those shown to block the respective receptors (Farde et al., 1992). According to PET studies on healthy human subjects, a single intravenous dose of 0.42 to 0.81 mg SCH23390 per patient could be related to a D_1 receptor occupancy of up to 59%. The injection of 0.2 to 0.5 mg raclopride per patient gave a brain D_2 receptor occupancy of up to 73%. Such data, therefore, suggest that an adequate occupancy of the receptors on the basis of the blocking procedures used can be assumed.

Third, binding of [^{11}C]GLC to non-dopamine receptors, which would bias the measurement of D_4 receptors, is not likely since spiperone—under the experimental protocol invoked here to block D_4 receptors—does not bind with high affinity to receptors such as 5HT, adrenergic, cholinergic, or sigma receptors (Waeber et al., 1988; Hoyer, 1991; Goffinet et al., 1990; Ford et al., 1994; Roth et al., 1995).

In the present investigation, dopamine D_4 receptors were found in the cortical and subcortical regions known to receive dopaminergic input: dopamine neurons of the ventral mesencephalon designated as A8, A9, and A10 cell groups project into the striatum, the limbic system, and also extensively into the cortex—

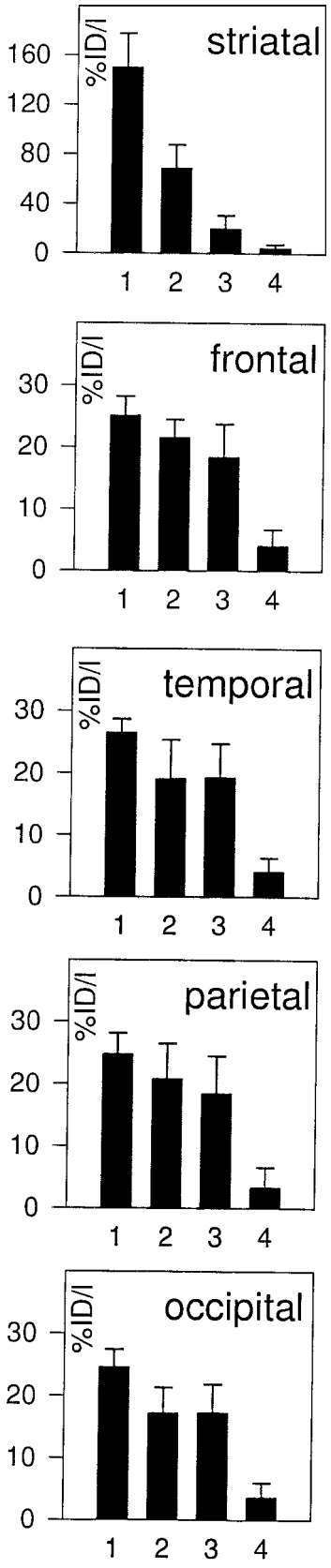

Fig. 6. Specific binding (SB) of [^{11}C]GLC defined as the difference regional and cerebellar uptake integrated between 10 and 60 min after injection [%ID/l]. Columns indicate SB during progressive blockade of dopamine receptors 20 min before the injection of [^{11}C]GLC: (1) $SB_{D1\text{-}D5}$, no receptor blockade; (2) $SB_{D2\text{-}D4}$, receptor blockade by SCH23390; (3) SB_{D4}, blockade of all non-D_4-receptors by SCH23390 and raclopride; (4) NSB, blockade of all known dopamine receptors by SCH23390, raclopride, and spiperone (mean ± standard deviation, n = 3).

TABLE II. Statistical analysis evaluating the differences of the specific binding of [^{11}C]GLC due to the application of different dopamine receptor antagonists

	Exp. 2 vs. exp. 1	Exp. 3 vs. exp. 2	Exp. 4 vs. exp. 3
Striatum	0.01	0.01	0.04
Frontal ctx	0.05	0.29	0.01
Temporal ctx	0.13	0.46	0.01
Parietal ctx	0.18	0.38	0.01
Occipital ctx	0.18	0.38	0.01

As displayed in Figure 6 with *P*-values of obtained from the one-tailed paired *t*-test; n = 3. Exp. = experiment; ctx = cortex.

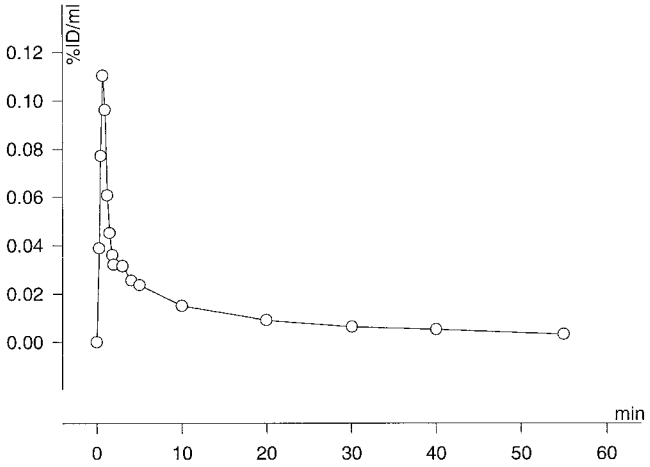

Fig. 7. Representative arterial plasma concentration of [^{11}C] following the injection of 0.8 mCi [^{11}C]GLC per kg body weight [%ID/l] (mean ± standard deviation, n = 3).

especially the prefrontal and cingulate cortices (Bjorklund and Lindvall, 1984; Berger et al., 1991). The present D_4 receptor distribution revealed by [^{11}C]GLC is consistent with both the distribution pattern of D_4 receptor-coding mRNA and the distribution of the encoded receptor protein in the brains of nonhuman primates. First, high levels of D_4-coding mRNA have been detected in frontal cortex, midbrain, medulla, and amygdala and lower concentrations have been reported for striatum and hippocampus, whereas none was found within the cerebellum (Van Tol et al., 1992). Second, D_4-immunoreactive neurons have been localized in frontal, temporal, and parietal neocortex, in the globus pallidus and—showing low staining—in caudate nucleus and putamen (Mrzljak et al., 1996).

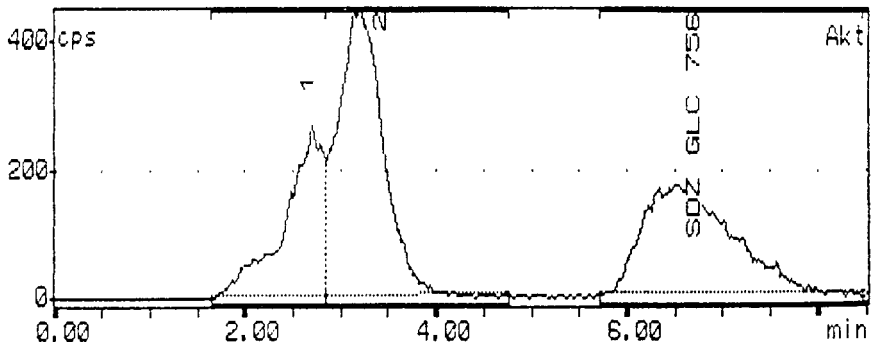

Fig. 8. Metabolism studies of [^{11}C]GLC: representative chromatogram of an arterial blood sample obtained 2.25 min p.i. showing the nonmetabolized radioligand (SDZ GLC 756) and two peaks of stable polar metabolites. In HPLC, the measured activity (Akt) is given as counts per seconds (cps). (SDZ GLC 756) nonmetabolized radioligand (1, 2) metabolites.

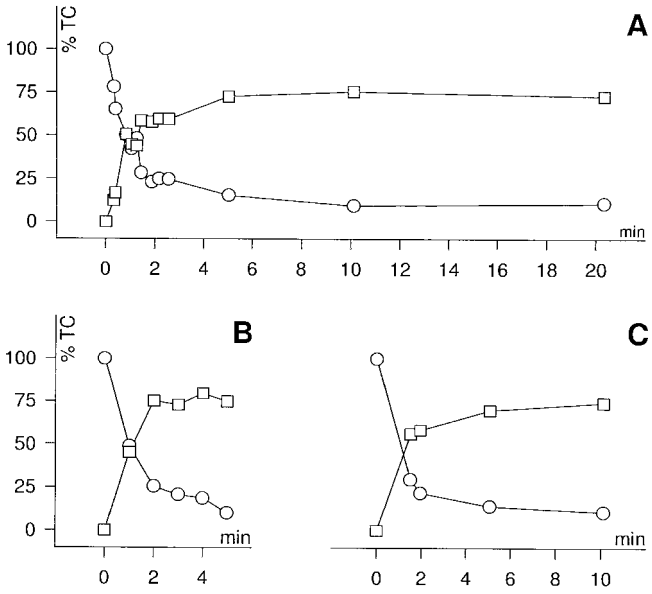

Fig. 9. HPLC analysis of blood samples following i.v. injection of [^{11}C]GLC (0.8 mCi/kg) under different experimental conditions. Curves reflect the percent of nonmetabolized [^{11}C]GLC (○) and its polar metabolites (□) as a fraction of the total counts (TC): (**A**) Arterial blood samples, data of two baboons, exp. 1, i.e., no pretreatment with cold dopamine receptor antagonists; (**B**) venous blood samples, exp. 1; (**C**) arterial blood samples, exp. 3, i.e., following the intravenous application of SCH23390 and raclopride.

In conclusion, [^{11}C]GLC is a candidate PET ligand for the in vivo investigation of D_4 receptors or D_4-like binding sites in basic science and clinical protocols. The widespread distribution of D_4 receptors in primate neocortex suggests that among the relatively few cortical dopamine receptors, this receptor subtype constitutes a significant proportion and may play a major role in catecholaminergic modulation of neuronal activity in primate neocortex.

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