



## Effects of Dopamine on the *in Vivo* Binding of Dopamine D<sub>2</sub> Receptor Radioligands in Rat Striatum

Rosa-Maria Moresco,<sup>3</sup> Christian Loc'h,<sup>1</sup> Michelle Ottaviani,<sup>1</sup> Bernard Guibert,<sup>2</sup>  
Vincent Leviel,<sup>2</sup> Mariannick Maziere,<sup>1</sup> Ferruccio Fazio<sup>3</sup> and Bernard Maziere<sup>1</sup>

<sup>1</sup>SERVICE HOSPITALIER FREDERIC JOLIOT, DRM/CEA, F-91406 ORSAY, FRANCE; <sup>2</sup>INSTITUT A. FESSARD, CNRS, F-91198 GIF SUR YVETTE, FRANCE; AND <sup>3</sup>INB-CNR, SCIENTIFIC INSTITUTE H SAN RAFFAELE, DEPARTMENT OF NUCLEAR MEDICINE, UNIVERSITY OF MILAN, MILAN, ITALY

**ABSTRACT.** The effects of moderate changes in extracellular dopamine concentrations on the *in vivo* binding of specific dopaminergic D<sub>2</sub> radioligands with different affinities and kinetics were investigated in rats. Either [<sup>125</sup>I]NCQ298 (K<sub>d</sub> = 19 pM), or [<sup>125</sup>I]iodolisuride (K<sub>d</sub> = 0.27 nM) or [<sup>3</sup>H]raclopride (K<sub>d</sub> = 1.5 nM) were administered intravenously (IV) to animals 1 h after the intraperitoneal (IP) injection of either  $\alpha$ -methyl-*p*-tyrosine (AMPT) (250 mg/kg) or nomifensine (15 mg/kg), or saline. The kinetics of radioactivity concentration in the striatum, cerebellum, and plasma were measured for up to 4 h after [<sup>125</sup>I]NCQ298 or [<sup>125</sup>I]iodolisuride injection and up to 1.5 h after [<sup>3</sup>H]raclopride injection. For each tracer, the striatum-to-cerebellum radioactivity concentration ratios (S/C) and the binding potential (BP), calculated as the association to dissociation binding rate constant ratios (k<sub>3</sub>/k<sub>4</sub>), were assessed and related to the changes in extracellular dopamine concentration induced by drug treatments. Results show that S/C and BP of [<sup>3</sup>H]raclopride were significantly diminished by pretreatment with nomifensine, a drug that increases extracellular dopamine concentration. Nomifensine pretreatment induced no changes in the *in vivo* binding indexes of the high affinity [<sup>125</sup>I]NCQ298 and a slight but not significant decrease of the binding indexes of [<sup>125</sup>I]iodolisuride. Treatment with AMPT, which induced a 40% reduction in dopamine concentration, did not change [<sup>125</sup>I]NCQ298 binding indexes but slightly increased those of [<sup>3</sup>H]raclopride and [<sup>125</sup>I]iodolisuride. In conclusion, the change of dopamine concentration induces modification of radiotracer kinetics. Thus, the combined use of tracers with high and low affinities could allow us to obtain information both on receptor density and neurotransmitter release *in vivo*. However, as indicated by the [<sup>3</sup>H]raclopride study with AMPT, small changes in the concentration of intrasynaptic dopamine cannot be easily detected. NUCL MED BIOL 26;1:91–98, 1999. © 1998 Elsevier Science Inc.

**KEY WORDS.** Dopamine receptors, Emission tomography, Raclopride, NCQ298, Iodolisuride

### INTRODUCTION

An impairment of the dopaminergic system, in particular of D<sub>2</sub> receptors, has been postulated in several neurodegenerative and psychiatric diseases. The development of emission tomography techniques such as positron emission tomography (PET) and single photon emission tomography (SPET) has allowed the *in vivo* assessment of receptors availability in human subjects. However, PET studies of D<sub>2</sub> dopamine receptor in patients with Parkinson's disease or schizophrenia have provided conflicting results. In particular, studies with the high affinity tracer [<sup>11</sup>C]N-methylspiperone have demonstrated an increase in D<sub>2</sub> dopamine receptors in drug-naive schizophrenic patients, whereas no differences were found when the low affinity tracers [<sup>11</sup>C]raclopride or [<sup>76</sup>Br]lisuride were used (14, 37, 50). Similar discrepancies between high affinity spiperone derivatives and low affinity benzamides, such as raclopride and IBZM, were found in studies in early Parkinson's disease (16, 17, 40, 41). The effect of endogenous neurotransmitters on radioligand binding is a relevant issue for the *in vivo* measurement of receptor density; in fact, the sensitivity of the tracers used as

probes to the levels of synaptic dopamine concentration might in part account for the above discrepancies (36, 45). Several studies have demonstrated that the *in vitro* and *in vivo* binding of radioligands with a K<sub>d</sub> value for D<sub>2</sub> receptors in the nanomolar range, such as raclopride or *N*-propylapomorphine, is inhibited by increasing synaptic dopamine concentration, whereas it is enhanced by pharmacological treatments that reduce dopamine synthesis or release (22, 27, 42, 43, 45, 51). Similar results have been reported on superfused rat striatal slices (15). Recently, the sensitivity to changes in the concentration of intrasynaptic dopamine of low affinity radioligands, such as raclopride or IBZM, has been proposed and used as a tool for the *in vivo* imaging of dopamine release in nonhuman primates and human subjects (8, 9, 11, 13, 29, 48). Whereas a consistent although moderate effect of synaptic dopamine on the *in vivo* binding of raclopride or IBZM was reported in rats and primates, results concerning tracers with higher affinity are not conclusive and the issue needs further investigation. Results of these studies indicated that the *in vivo* binding of spiperone, methyl-spiperone, or pimozone is either unaffected by endogenous dopamine concentration (1, 7, 30, 51) or is increased/decreased in a opposite direction to that expected according to the interaction between dopamine and radioligand (2, 6, 27, 28).

In *in vivo* experiments the competition between radioligand and neurotransmitter depends on the relative affinity for the receptor, on the concentration of neurotransmitter in the synaptic cleft, and

Address correspondence to: Dr. Rosa Maria Moresco, Department of Nuclear Medicine, University of Milan, c/o H San Raffaele, Via Olgettina 60, 20132 Milano, Italy; e-mail: rosam@mednuc.hsr.it.

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also on the tissue clearance of the radioligand, which is related to tissue-to-plasma and plasma-to-tissue transport constants (35).

The aim of the present study was to investigate in rats, the effect of moderate changes in extracellular dopamine concentration on the *in vivo* binding of two tracers used for the *in vivo* measurement of D<sub>2</sub> dopamine receptor by emission tomography, that differ for *in vitro* binding affinity, chemical class, and kinetics behavior: [<sup>125</sup>I]NCQ298: K<sub>d</sub> = 0.019 nM (19), [<sup>125</sup>I]iodolisuride: K<sub>d</sub> = 0.27 nM (3, 4, 35), and [<sup>3</sup>H]raclopride: K<sub>d</sub> = 1.2 nM (24). The synaptic dopamine concentration was modified by pharmacological treatments. It was reduced by pretreatment with  $\alpha$ -methyl-*p*-tyrosine (AMPT), a tyrosine hydroxylase inhibitor (46), and increased by the dopamine reuptake site blocker nomifensine. Measuring with high performance liquid chromatography (HPLC) the concentration of dopamine, dihydroxyphenylacetic (DOPAC), and homovanilic acid (HVA) in one striatum of each rat allowed us to monitor the effect of drug pretreatment. The kinetics of the radioactivity concentration in the contralateral striatum, the cerebellum, and plasma were measured up to 4 h after [<sup>125</sup>I]NCQ298 and [<sup>125</sup>I]iodolisuride injection and up to 1.5 h after [<sup>3</sup>H]raclopride injection. For each tracer, the striatum-to-cerebellum radioactive concentration ratios (S/C) and the binding potential calculated as the association to dissociation binding rate constant ratios (k<sub>3</sub>/k<sub>4</sub>) were measured and related to the changes in extracellular dopamine concentration following by the drug treatments.

## MATERIALS AND METHODS

Male Wistar rats (200–250 g) were obtained from Charles River; experiments were carried out in strict accordance with the recommendations of the European Economic Community and the French National Committee for the care and use of laboratory animals. [<sup>3</sup>H]Raclopride (3.2 GBq/ $\mu$ mol) was provided from New England Nuclear, [<sup>125</sup>I]iodolisuride and [<sup>125</sup>I]NCQ298 (81 GBq/ $\mu$ mol) were prepared in our facilities as previously described (20, 32). The radiotracers were diluted with saline to obtain a 2.5% ethanol solution.  $\alpha$ -Methyl-*d,l*-paratyrosine methylester hydrochloride (AMPT) was provided from Sigma (St. Louis, MO) and nomifensine from Hoechst Laboratories. All other chemicals were of analytical grade.

### Drug Treatment

Nomifensine, and AMPT were administered intraperitoneally (IP). The dose, route of administration, and time of pretreatment were chosen on the basis of the known effect of AMPT and nomifensine on intrasynaptic dopamine concentration (5, 19, 31, 39, 47). Treatment protocol was set up to induce moderate changes in the extracellular concentration of dopamine that remains stable for the whole experimental time. Synaptic dopamine concentration was either increased by nomifensine, a dopamine reuptake sites blocker (15 mg/kg 1 h before tracer injection) or reduced by the tyrosine hydroxylase inhibitor AMPT (250 mg/kg 1 h before tracer injection). AMPT was chosen because of its moderate reduction of extracellular dopamine concentration when given alone and the rapid onset of its effect.

### Dopamine, DOPAC, and HMV Assay

The concentration of dopamine and its major metabolites, DOPAC and homovanilic acid (HVA), were measured in the striatal homogenates from control and AMPT- and nomifensine-treated

rats, using reverse phase HPLC and electrochemical detection according to a procedure previously described with minor modifications (31, 38). Briefly, frozen striata were homogenized and deproteinized by sonication in 0.1 M HClO<sub>4</sub> containing edetate disodium salt (EDTANa<sub>2</sub>, 2 mM) and Epinine as internal standard. Samples were then centrifuged (15000 g) for 15 min at 5°C. The supernatants were used for catechols and indols analysis by HPLC on an octadecyl reverse phase Hypersil BDS column (100 mm  $\times$  4.6 mm, Shandon), followed by electrochemical detection (Waters M460) set at 750 mV. The mobile phase (1 mL/min) was composed of 50 mM KH<sub>2</sub>PO<sub>4</sub>, 0.1 mM EDTANa<sub>2</sub>, 0.8 mM sodium octylsulfonate, 7% methanol (v/v) adjusted to pH 4.0 and maintained at 34°C. The precipitates were used for the protein analysis according to the method of Lowry using bovine serum albumin as a standard. The results were expressed in ng/mg of protein.

### In Vivo Binding Assay

[<sup>3</sup>H]Raclopride (170 kBq, 53 pmol), [<sup>125</sup>I]iodolisuride (330 kBq, 4 pmol), or [<sup>125</sup>I]NCQ-298 (330 kBq, 4 pmol) were injected into a tail vein. Five hundred milliliters of AMPT (250 mg/kg), nomifensine (15 mg/kg), or saline were administered IP 1 h before tracer injection. Animals (*n* = 2 at each time point) were sacrificed at various time intervals after the radioligand injection: 15, 30, 45, 60, and 90 min for [<sup>3</sup>H]raclopride studies, and 15, 30, 60, 120, and 240 min for [<sup>125</sup>I]iodolisuride and [<sup>125</sup>I]NCQ-298 studies. At each time point a blood sample was also collected and centrifuged for the evaluation of the radioactivity concentration in plasma. Immediately after killing, the brains were removed, and striatum and cerebellum dissected out and weighed. One striatum was frozen and kept at -80°C for dopamine, DOPAC, and HVA HPLC assay. Radioactivity in the contralateral striatum, the cerebellum, and in plasma was determined by liquid scintillation counting for [<sup>3</sup>H] and by gamma counting for [<sup>125</sup>I] and expressed as percent of injected dose per gram of tissue (% ID/g).

For both tracers the half-live of free plus nonspecifically bound radioactivity concentration in tissue were estimated in cerebellum and calculated by fitting a biexponential function to the experimental data.

The effect of drug treatments on striatal to cerebellar radioactive concentration ratios (S/C) of [<sup>125</sup>I]NCQ298 was evaluated at 4 h after tracer injection, whereas the effect of drug treatment on [<sup>3</sup>H]raclopride and [<sup>125</sup>I]iodolisuride S/C was evaluated at 1 h and 2 h after tracer injection, respectively. Experimental times were chosen according to the kinetic behavior of tracers in control rats.

In order to calculate the effect of drug treatment on the *in vivo* binding potential (k<sub>3</sub>/k<sub>4</sub>) of radioligands to D<sub>2</sub> receptor, a graphical analysis (34) was applied to experimental data.

The graphical analysis, designed specifically for reversible systems, allows a direct calculation of the steady state distribution volume ratios between a region containing receptor and a reference region devoid or with negligible receptor concentration. For reversible tracers the plot of  $\int_0^T Ct(t)dt/Ct(T)$  vs.  $\int_0^T Cp(t)dt/Cp(T)$  at any time, *T*, following the administration of the tracer (where *Ct* and *Cp* are the concentration of the tracer in tissue and plasma, respectively) become linear after some time, *t'*, with a slope equal to the steady-state distribution volume of the tracer. In region devoid of receptors, *DV* is equal to:

$$DV_{NSR} = K1/k2 \times (1 + NS)$$

and in region containing receptor, *DV* becomes:

$$DV_{TR} = K1/k2 \times [1 + NS + (k3/k4)]$$

where NSR is the region with nonspecific binding, TR is the target region, K1 and k2 are the plasma-to-tissue and tissue-to-plasma transport rate constants, respectively, and NS is the ratio between the association and dissociation rate constants for nonspecific binding. Assuming that the ratios between the plasma-to-tissue and tissue-to-plasma transfer constant (K1/k2) are equivalent in both regions and that the nonspecific binding is negligible or rapidly equilibrates, then

$$DV_{TR}/DV_{NRS} = (k3/k4) - 1$$

Moreover, the DV ratio is not sensitive to changes in tracer delivery due to changes in the input function or regional cerebral blood flow (21, 35). Kinetic analysis was carried out using the average time course of radioactivity concentration in the striatum, cerebellum, or plasma of all the animals.

Results from drug-treated and control rats were then compared by a two-tailed, unpaired *t*-test.

## RESULTS

### Dopamine, DOPAC, and HVA Assay

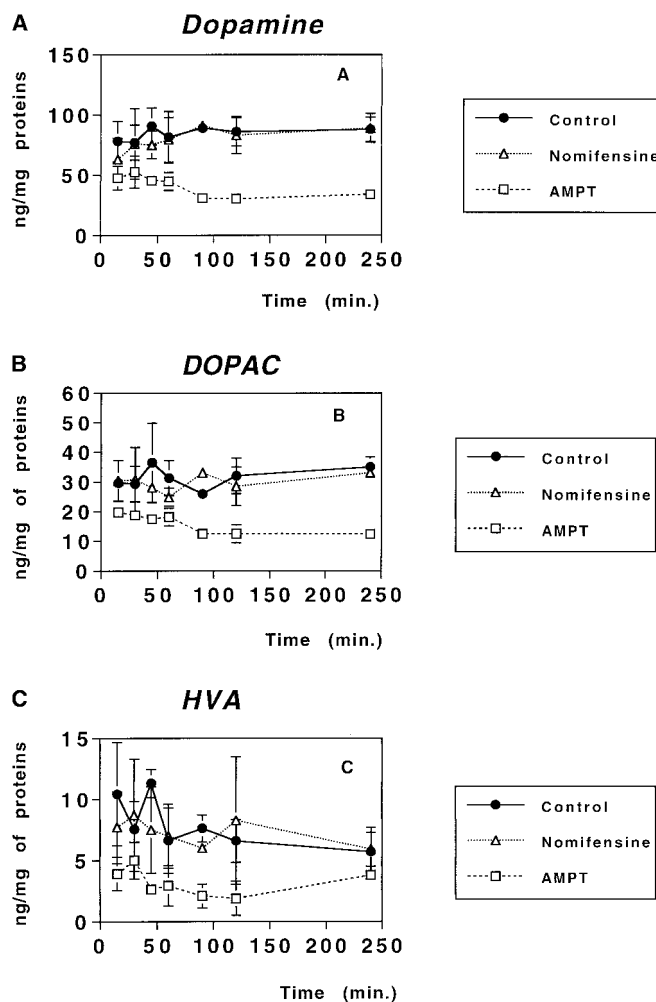
The effects of nomifensine and AMPT on total striatal dopamine, DOPAC, and HVA concentration are shown in Figure 1. Inhibition of tyrosine hydroxylase induced by AMPT treatment produced a significant reduction in the concentration of dopamine, DOPAC, and HVA in the striatal tissue for the whole experiment. At 1, 2, and 3 h after AMPT treatment, the dopamine concentrations were  $60 \pm 13\%$ ,  $53 \pm 8\%$ , and  $37 \pm 5\%$  of the control values, respectively (control value =  $87 \pm 11$  ng/mg of tissue proteins). The effect of AMPT on dopamine metabolites concentration were approximately of the same magnitude. As expected, when the dopamine uptake sites were blocked by nomifensine no apparent increase in the whole concentration of dopamine and its metabolites were observed.

### In Vivo Binding

Figure 2 shows the kinetics of radioactivity concentration in the cerebellum and in the striatum after the IV injection of [<sup>125</sup>I]NCQ298, [<sup>125</sup>I]iodolisuride, or [<sup>3</sup>H]raclopride in control animals. At the injected dose, based on the known density of D<sub>2</sub> receptor and of the concentration of radioactivity in striatum, we calculated that less than 3% of the D<sub>2</sub> receptors were occupied by [<sup>3</sup>H]raclopride and less than 0.1% by [<sup>125</sup>I]NCQ298 and [<sup>125</sup>I]iodolisuride.

After injection of [<sup>125</sup>I]NCQ298, radioactivity progressively accumulated in rat striatum and reached a plateau at 60 min after tracer injection that remained fairly stable for the following 3 h. Four hours after tracer injection, the striatum-to-cerebellum radioactivity concentration ratio was  $59 \pm 7$ . After [<sup>3</sup>H]raclopride and [<sup>125</sup>I]iodolisuride injection, a rapid rise in striatal radioactivity concentration followed by a gradual decrease was observed. Striatum-to-cerebellum concentration ratios reached their maximum values at 60 min (S/C =  $8.0 \pm 0.5$ ) and 120 min (S/C =  $4.5 \pm 0.4$ ) for [<sup>3</sup>H]raclopride and [<sup>125</sup>I]iodolisuride, respectively.

The half-life of the clearance of the radioligands in cerebellum was calculated by fitting a two exponential function to the experimental data. As shown in Table 1, the rate of tissue clearance



**FIG. 1.** Time course of the effect of nomifensine and  $\alpha$ -methyl-p-tyrosine (AMPT) on total dopamine (A), dihydroxyphenylacetic (DOPAC) (B), and homovanilic acid (HVA) (C) concentration. Values are the mean  $\pm$  SD from one rat striata ( $n = 6$  at 15, 30, 60, 120, and 240 min after tracer injection;  $n = 2$  at 45 and 90 min after tracer injection).

estimated in cerebellum was in the order: [<sup>3</sup>H]raclopride > [<sup>125</sup>I]NCQ298 > [<sup>125</sup>I]iodolisuride.

The effect of nomifensine and AMPT treatment on striatal-to-cerebellum concentration ratios of [<sup>125</sup>I]NCQ 298, [<sup>125</sup>I]iodolisuride, and [<sup>3</sup>H]raclopride calculated 240, 120, and 60 min after tracer injection, respectively, is shown in Figure 3. The S/C of [<sup>3</sup>H]raclopride was significantly reduced ( $-34\%$ ,  $p = 0.0002$ ) by nomifensine pretreatment. In the same conditions a slight, decrease of [<sup>125</sup>I]iodolisuride S/C ( $-13\%$   $p = 0.051$ ) was observed. Finally no effect on the *in vivo* S/C of the high affinity [<sup>125</sup>I]NCQ298 was observed. Treatment with AMPT did not changed [<sup>125</sup>I]NCQ298 S/C, but slightly increased those of [<sup>3</sup>H]raclopride ( $+11\%$ ,  $p = 0.052$ ) and [<sup>125</sup>I]iodolisuride ( $+10\%$   $p = 0.16$ ).

The plots of the striatal and cerebellar DV of [<sup>125</sup>I]NCQ298, [<sup>125</sup>I]iodolisuride, and [<sup>3</sup>H]raclopride in control rats are shown in Figure 4. In both striatum and cerebellum  $\int_0^T C_t(t)dt/C_t(T)$  become a linear function of  $\int_0^T C_p(t)dt/C_t(T)$  approximately 15 min after [<sup>125</sup>I]iodolisuride or [<sup>3</sup>H]raclopride injection, and 60 min after [<sup>125</sup>I]NCQ298 injection. The values of the slope of the straight line, that is, the DV, and the  $k3/k4$  ratios calculated from the striatal to

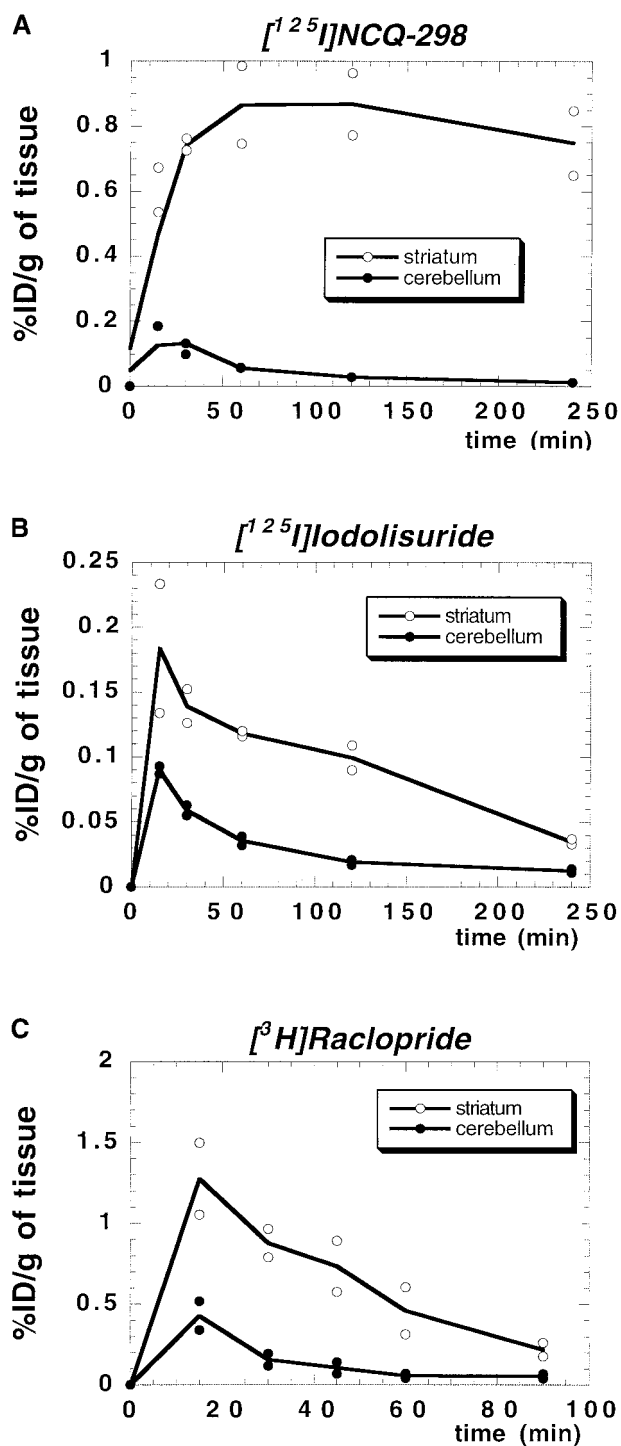


FIG. 2. Time course of [ $^{125}\text{I}$ ]NCQ-298 (A), [ $^{125}\text{I}$ ]lisuride (B), and [ $^3\text{H}$ ]raclopride (C) in striatum and cerebellum of control rats ( $n = 2$  at each time point).

cerebellar DV ratios of control and drug-treated animals according to Equation (3), are shown in Table 2. Cerebellar DV of [ $^3\text{H}$ ]raclopride was slightly increased by both AMPT and nomifensine pretreatment. On the contrary, no changes in cerebellar distribution volume were observed for the two radioiodinated tracers. In agreement with results of S/C measurements, nomifensine pretreatment reduced the [ $^3\text{H}$ ]raclopride and [ $^{125}\text{I}$ ]iodolisuride binding

TABLE 1. [ $^{125}\text{I}$ ]NCQ298, [ $^{125}\text{I}$ ]iodolisuride, and [ $^3\text{H}$ ]Raclopride Clearance Rate from Cerebellum

Tracer	$\beta$ ( $\text{min}^{-1}$ )
[ $^{125}\text{I}$ ]NCQ298	$0.013 \pm 0.008$
[ $^{125}\text{I}$ ]Lisuride	$0.003 \pm 0.002$
[ $^3\text{H}$ ]Raclopride	$0.020 \pm 0.015$

The rate of tissue wash-out of the radiotracers was calculated fitting a bi-exponential function to the cerebellar data. Values are the mean estimate  $\pm$  standard errors of the estimates ( $n = 2$  at each time point).

indexes by  $-33\%$  and  $-8\%$ , respectively, without affecting the binding indexes of the high affinity tracer [ $^{125}\text{I}$ ]NCQ298. A slight increase in [ $^3\text{H}$ ]raclopride and [ $^{125}\text{I}$ ]iodolisuride  $k_3/k_4$  values was observed in animals pretreated with AMPT ( $+11\%$  and  $+7\%$ , respectively).

## DISCUSSION

In both *in vitro* and *in vivo* receptor studies, endogenous dopamine can compete with the radioligand for the binding sites, and produces errors in the estimates of binding parameters, that is,  $B_{\text{max}}$  and  $K_d$ , whenever such competition is overlooked. Thus, for an accurate measurement of receptor density and affinity, using radioligand uptake and bio-mathematical models, the effects of synaptic dopamine on radiotracer binding must be taken into account. In *in vivo* studies, this effect depends on the relative affinity of the radioligand and of the neurotransmitter for the receptor and the synaptic concentration of endogenous neurotransmitter, and the relative rate of delivery of the radiotracer between tissue and plasma (33). In this study we have evaluated the effect of changes in extracellular dopamine concentration on the *in vivo* binding of  $D_2$  dopamine receptor radioligands that differ for their *in vitro* affinity, chemical class, and tissue clearance.

The concentration of dopamine in the intersynaptic cleft, where most dopamine receptors are distributed, was changed using pharmacological challenges. In particular, dopamine concentration was reduced by the false dopamine precursor AMPT and enhanced by the dopamine reuptake blocker nomifensine. After AMPT pretreatment, we found a sharp decrease of tissue dopamine concentration over approximately 1 h until a stabilization of approximately the 40% of the initial value was achieved. A parallel decrease of dopamine metabolites was also observed. The decrease in total dopamine concentration induced by AMPT promotes a parallel and proportional reduction in the concentration of extracellular dopamine (12, 19, 47). Conversely, no changes either in the total concentration of dopamine neither of its metabolites was found in rats treated with nomifensine. The lack of increase in dopamine concentration after nomifensine pretreatment is in agreement with the increase in the extracellular fraction of dopamine and not in its global concentration induced by the blockade of dopamine reuptake sites. In fact, like other dopamine reuptake blockers, nomifensine, increases dopamine concentration in the synaptic cleft (extracellular fraction) with no effect on the intracellular pools (presynaptic dopamine), where dopamine concentration is more than 1,000 times higher than in the synaptic cleft (31, 39) and confirm the hypothesis that the effect observed on tracers binding depends on the concentration of dopamine in the synaptic cleft. Several groups measured the effect of nomifensine on extracellular dopamine by *in vivo* voltametry or intracerebral dialysis. These studies indicate that

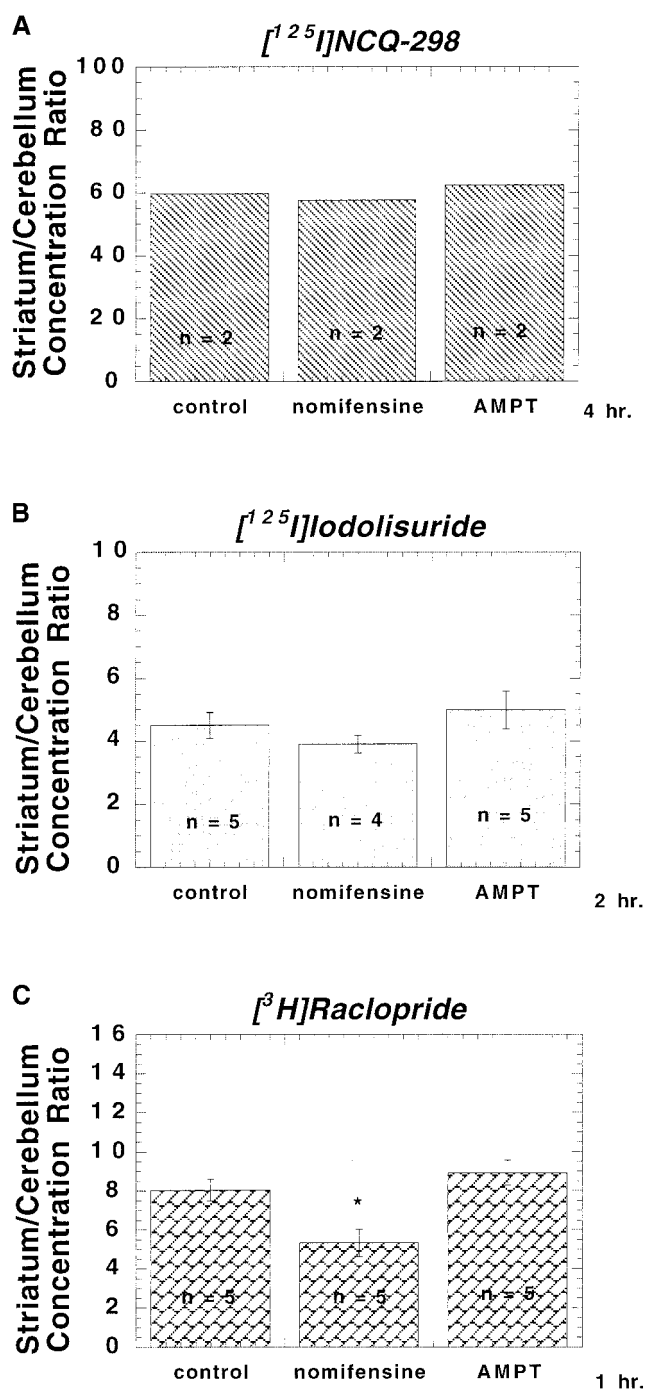


FIG. 3. Striatal to cerebellar radioactivity concentration ratios from control, nomifensine (15 mg/kg IP) and  $\alpha$ -methyl-p-tyrosine (AMPT, 250 mg/kg IP) pretreated rats. Animals were killed 2 h after [<sup>125</sup>I]NCQ-298 (A) or [<sup>125</sup>I]iodolisuride (B) injection and 1 h after [<sup>3</sup>H]raclopride injection (C). Both drugs were administered 1 h before tracer injection. Left and right striata were pooled for each determination. Bars indicated mean with SD and n = total number of animals per group.

nomifensine, when administered at doses similar to those used in this study, induces an increase of extracellular dopamine that peaks between 20 and 40 min after drug administration. The effect becomes stable at approximately 60–90 min after administration of

nomifensine and determines dopamine levels that are only three- to sixfold higher than control values (5, 12, 47).

The *in vivo* binding of [<sup>125</sup>I]NCQ298 was not affected by either AMPT or nomifensine pretreatment. These findings are in agreement with the results of a previous study on the effects of low doses of amphetamine (1 mg/kg) on the *in vivo* binding of [<sup>125</sup>I]epidepride, which like [<sup>125</sup>I]NCQ298 is a high affinity benzamide (23). On the contrary, most of the studies on high affinity spiperone derivatives indicate that their *in vivo* binding is enhanced by drugs such as amphetamine, bupropion, and methylphenidate, which increase intrasynaptic concentration of dopamine, or by the unilateral stimulation of the substantia nigra, and reduced by treatment with reserpine or AMPT that decrease dopamine levels in the synaptic cleft (2, 6, 27, 28). The differences between high affinity benzamides and spiperone derivatives might be related to their differences with respect to D<sub>2</sub> dopamine receptors. In fact, spiperone derivatives and benzamides not only have different affinities for D<sub>2</sub>, D<sub>3</sub>, and D<sub>4</sub> dopamine receptor subtypes, but also bind to overlapping yet distinct portions of the dopamine D<sub>2</sub> receptors (18, 44). The interaction with a specific domain of D<sub>2</sub> dopamine receptor might explain the peculiar behavior of spiperone derivatives. Spiperone derivatives have an *in vivo* binding that is modified in a direction opposite to that expected on the basis of dopamine-radioligand competition as indicated by most of the studies, that is, increased binding in the presence of increased dopamine concentration and reduced binding when dopamine concentration is decreased. The reduction in spiperone derivatives binding after *d*-amphetamine pretreatment reported by some authors (7, 33, 51) may be consequent to the high doses of amphetamine used to modify the extracellular concentration of dopamine (51), or to the specificity of the assumptions and experimental condition in the other reports (7, 33).

Pretreatment with nomifensine or AMPT induced a slight but not significant effect in the *in vivo* binding of [<sup>125</sup>I]iodolisuride, indicating that this tracer is only barely sensitive to extracellular dopamine. In a previous study with [<sup>123</sup>I]IBZM, a tracer with an *in vitro* affinity similar to that of [<sup>125</sup>I]iodolisuride (0.46 nM and 0.27 nM, respectively [25, 32]), a significant increase in the striatal clearance of the tracer after the administration of amphetamine was reported (26). The lower sensitivity of iodolisuride to extracellular dopamine can be related to its slow tissue-to-plasma washout constant, as indicated by its slow cerebellar clearance.

In agreement with previous studies in rats, monkeys, and human subjects, our findings indicate that the *in vivo* binding of [<sup>3</sup>H]raclopride in the rat striatum is significantly inhibited by pharmacological challenges that increase extracellular dopamine concentration. In contrast with most previous experiments based on pharmacological treatments that increased dopamine concentration up to 20 times, producing neurochemical and behavioral effects more pronounced than those observed in physiological condition, in this study we used a pharmacological protocol that induced a moderate increase in extrasynaptic dopamine concentration (5, 12, 47, 49). In animals treated with AMPT, in which the reduction in total dopamine concentration is approximately 40% of control values at the time of tracer injection, we did not find the 30–40% increase in [<sup>3</sup>H]raclopride binding previously described in rats pretreated with reserpine, but we observed only an 11% increase in radioligand binding. This discrepancy is easily explained by the different pharmacological challenge used to reduce dopamine concentration. Reserpine alone or associated with AMPT depletes the dopamine storage by up to 90% of the initial values, and determines an impairment of the dopamine system comparable to that found in

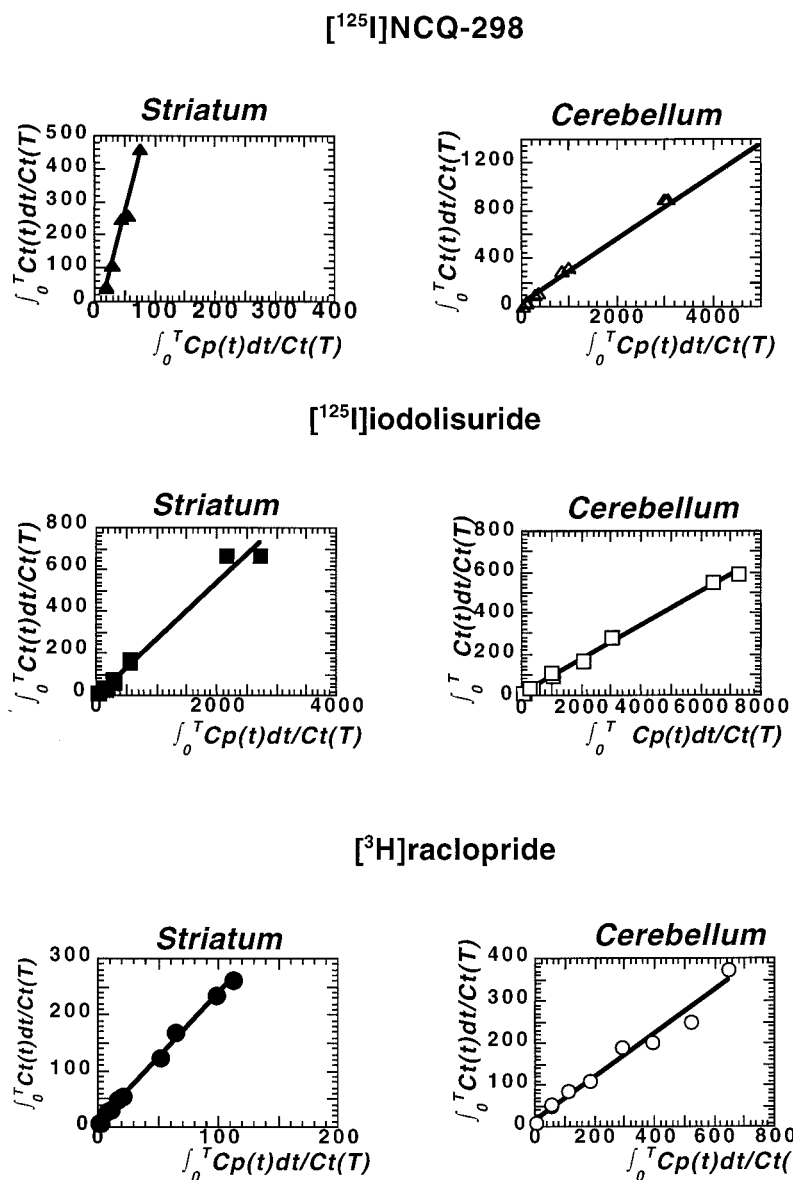


FIG. 4. Graphical analysis of the time course of [<sup>125</sup>I]NCQ-298, [<sup>125</sup>I]iodolisuride, and [<sup>3</sup>H]raclopride in striatum (left side) and cerebellum (right side).  $C_p(t)$  is the plasma radioactivity concentrations at time  $t$ , and  $C_t(T)$  is the radioactivity concentration in the region of interest at time  $T$  ( $n = 2$  at each time point).

patients with Parkinson's disease. Pharmacological treatment with vigabatrin or citalopram, that is, with drugs that reduce extracellular dopamine concentration in striatum, increase [<sup>11</sup>C]raclopride binding up to 20–40% of control values (8, 11). However, the reduction in extracellular dopamine concentration induced by both drugs, measured by microdialysis in the case of citalopram (8) or estimated by mathematical modeling in the case of vigabatrin (11), was higher than that induced by AMPT pretreatment. Interestingly, [<sup>11</sup>C]raclopride binding was not affected in animals in which the tracer was administered 60 min after citalopram, when extracellular dopamine was only slightly decreased (approximately 20% of control values), but only in animals pretreated at 180 min before tracer injection, when dopamine levels were significantly reduced (approximately 50–60% of controls). Results on AMPT pretreatment suggests that the sensitivity of [<sup>3</sup>H]raclopride to changes in endogenous dopamine may not be sufficient to clearly detect a condition of moderate reductions in dopamine release, synthesis, or reuptake. Thus, the use of [<sup>11</sup>C]raclopride for the *in vivo* measurement of reduction in synaptic dopamine concentration requires further investigation.

As already observed after reserpine pretreatment (43), both nomifensine and AMPT increase the cerebellar  $DV$  of [<sup>3</sup>H]raclopride, probably by increasing tracer delivery to brain tissue; however, the analysis used to evaluate the effect of pharmacological challenges on striatal binding are insensitive to changes in tracer delivery.

## CONCLUSION

Results of the study indicate that the rank order of tracer sensitivity to change in extracellular dopamine concentration was raclopride > iodolisuride > NCQ298, which is in good agreement with the *in vitro*  $K_d$  and the tissue-to-plasma efflux constants of the tracers examined. [<sup>125</sup>I]NCQ298, a radiotracer with a picomolar *in vitro* affinity and a slow tissue clearance, is barely sensitive to the endogenous dopamine concentration; thus, it is a good radiotracer for the *in vivo* measurement of total  $D_2$  dopamine receptor density with emission tomography and appropriate biomathematical models. On the other hand, [<sup>123</sup>I]iodolisuride, a tracer with a subnanomolar affinity and a slow tissue to plasma clearance, is only

**TABLE 2. Distribution Volumes (DV) and Binding Potential (k3/k4) Obtained from the Slope of the Plot of the Graphical Analysis from Striatum and Cerebellum of Drug-Treated and Control Rats**

Tracer	Control	Nomifensine	AMPT
<sup>125</sup> I]NCQ298			
DV (Striatum) <sup>a</sup>	7.0 ± 0.5, r = 0.98	6.1 ± 0.3, r = 0.99	7.3 ± 0.6, r = 0.97
DV (Cerebellum) <sup>a</sup>	0.27 ± 0.01, r = 0.99	0.26 ± 0.01, r = 0.98	0.27 ± 0.01, r = 0.99
K3/K4 <sup>b</sup>	23.05	22.50	25.90
<sup>125</sup> I]Iodolisuride			
DV (Striatum) <sup>a</sup>	0.27 ± 0.01, r = 0.98	0.25 ± 0.18, r = 0.96	0.27 ± 0.02, r = 0.96
DV (Cerebellum) <sup>a</sup>	0.080 ± 0.001, r = 0.98	0.080 ± 0.002, r = 0.99	0.080 ± 0.002, r = 0.99
K3/K4 <sup>b</sup>	2.30	2.12	2.44
<sup>3</sup> H]Raclopride			
DV (Striatum) <sup>a</sup>	2.31 ± 0.04, r = 0.99	3.24 ± 0.07, r = 0.99	3.3 ± 0.4, r = 0.9
DV (Cerebellum) <sup>a</sup>	0.510 ± 0.002, r = 0.99	1.01 ± 0.04, r = 0.97	0.67 ± 0.03, r = 0.98
K3/K4 <sup>b</sup>	3.50	2.20	3.94

<sup>a</sup> The distribution volume (DV) expressed in mL/g was determined as the slope of the line fitted to the plot of  $\int_0^T Ct(t)dt/Ct(T)$  vs.  $\int_0^T Cp(t)dt/Ct(T)$  for experimental times  $T$  higher than  $t'$ , where  $t'$  is the time at which equilibrium is achieved. In regions containing receptors, i.e., the striatum, the slope is equal to  $K1/k2 \times [1 + NS + (k3/k4)]$ ; in regions devoid of receptors the slope is equal to  $K1/k2 \times (1 + NS)$ .

<sup>b</sup>  $k3/k4$  were calculated assuming that the radioactivity in the cerebellum corresponds to the free tracer concentration and that the nonspecific binding is negligible. Values are the estimate ± standard errors of the estimates ( $n = 2$  animals each time point).

minimally affected by changes in extracellular dopamine concentration. However, after pharmacological treatment or in pathological conditions in which changes in extracellular dopamine concentration may be postulated, results of receptors availability should be carefully interpreted. Finally, the good responsiveness of [<sup>11</sup>C]raclopride to changes in extracellular dopamine make this tracer, so far, a unique candidate for the *in vivo* PET measurement of functional responsiveness of dopaminergic system to pharmacological challenges and the modulation of striatal dopamine release by GABAergic, cholinergic, or serotonergic systems (8, 10, 11, 13, 48). However, the low sensitivity of [<sup>11</sup>C]raclopride to reduction of intrasynaptic dopamine and its moderate sensitivity to increase in dopamine concentration must be carefully considered in the process of developing its use under pharmacological and behavioral paradigms.

The development of radiotracers with nanomolar affinity for D<sub>2</sub> receptors and responsiveness to changes in endogenous dopamine concentration higher than that of [<sup>3</sup>H]raclopride would be useful to measure the effect of the stimulation or inhibition of the dopaminergic system induced by pharmacological challenges both in physiological and pathological conditions.

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