Cholinergic Neuronal Modulation Alters Dopamine D_2 Receptor Availability *In Vivo* by Regulating Receptor Affinity Induced by Facilitated Synaptic Dopamine Turnover: Positron Emission Tomography Studies with Microdialysis in the Conscious Monkey Brain

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To evaluate the cholinergic and dopaminergic neuronal interaction in the striatum, the effects of scopolamine, a muscarinic cholinergic antagonist, on the striatal dopaminergic system were evaluated multi-parametrically in the conscious monkey brain using high-resolution positron emission tomography in combination with microdialysis. L-3,4-Dihydroxyphenylalanine (L-[β-^{11}C]DOPA) and 2β-carbomethoxy-3β-(4-fluorophenyl)-tropane ([β-^{11}C]CFT) were used to measure dopamine synthesis rate and dopamine transporter (DAT) availability, respectively. For assessment of dopamine D_2 receptor binding *in vivo*, [^{11}C]raclopride was applied because this labeled compound, which has relatively low affinity to dopamine D_2 receptors, was hypothesized to be sensitive to the striatal synaptic dopamine concentration. Systemic administration of scopolamine at doses of 10 and 100 μg/kg dose-dependently increased both dopamine synthesis and DAT availability as measured by L-[β-^{11}C]DOPA and [β-^{11}C]CFT, respectively. Scopolamine decreased the binding of [^{11}C]raclopride in a dose-dependent manner. Scopolamine induced no significant changes in dopamine concentration in the striatal extracellular fluid (ECF) as determined by microdialysis. However, scopolamine dose-dependently facilitated the striatal ECF dopamine induced by the DAT inhibitor GBR12909 at a dose of 0.5 mg/kg. Scatchard plot analysis *in vivo* of [^{11}C]raclopride revealed that scopolamine reduced the apparent affinity of dopamine D_2 receptors. These results suggested that the inhibition of muscarinic cholinergic neuronal activity modulates dopamine turnover in the striatum by simultaneous enhancement of the dynamics of dopamine synthesis and DAT availability, resulting in no significant changes in apparent "static" ECF dopamine level but showing a decrease in [^{11}C]raclopride binding *in vivo* attributable to the reduction of affinity of dopamine D_2 receptors.

Key words: L-[β-^{11}C]DOPA; [^{11}C]raclopride; [β-^{11}C]CFT; positron emission tomography; microdialysis; monkey brain

The neurotransmitter systems do not work in isolation, and they are anatomically and functionally integrated as a network directly (Hattori et al., 1976) or indirectly (Bunney and Aghajanian, 1976) through multisynaptic connections. Although neuropsychiatric and neurodegenerative diseases have been attributed to deficits within a single neurotransmitter system, disease progression might be related to the deficit of the initially affected system to modulate or be modulated by other neurotransmitters. The extrapyramidal motor system, for example, relies on a balance between dopamine and acetylcholine, and disruption in the balance results in motor abnormalities. Positron emission tomography (PET) can evaluate the functional responses of neurotransmitters to pharmacological manipulation, as well as the interactions between neuronal systems. PET has been used to assess the effects of endogenous dopamine (Innis et al., 1992; Dewey et al., 1993a; Carson et al., 1997), NMDA/glutamate (Smith et al., 1997), acetylcholine (Dewey et al., 1993b), serotonin (Dewey et al., 1995), and GABA (Dewey et al., 1992) on striatal [^{11}C]raclopride binding (for review, see Laruelle, 2000). These reports suggested that the changes in striatal synaptic dopamine could be measured noninvasively by PET using [^{11}C]raclopride, which has more moderate affinity for D_2 receptors than [^{11}C]*N*-methyl spiperone (NMSP) (Seeman et al., 1989; Young et al., 1991). The measurement is based on the principle that neurotransmitters might compete with a radiolabeled ligand on the receptors if the affinity of the ligand to the receptor is moderate. In fact, basic studies using rodents demonstrated that increases or decreases in dopamine concentration decreased or increased the *in vivo* binding of [^{3}H]/[^{11}C]raclopride, respectively. (Seeman et al., 1989; Inoue et al., 1991; Young et al., 1991; Ginovart et al., 1997). However, we demonstrated recently that the alternation of [^{11}C]raclopride binding *in vivo* as measured by PET was not regulated simply by the apparent "static" dopamine level in the synapse, i.e., it represents the dynamic balance of release and reuptake rates of dopamine (Tsukada et al., 1999a, 2000a). Thus, in the conscious monkey brains in combination with animal PET and microdialysis, we showed that indirect dopamine modulators such as benztropine (a muscarinic cholinergic antagonist) and ketanserine (a 5-HT_2 antagonist) reduced [^{11}C]raclopride binding in the striatum with much smaller degree of increase in synaptic dopamine than those induced by methamphetamine and GBR12909 (Tsukada et al., 1999a). In addition, ketamine, a noncompetitive NMDA receptor antagonist, reduced [^{11}C]raclopride binding in the striatum without any significant change in the synaptic dopamine concentration (Tsukada et al., 2000a).

The aim of the present study was to explore the regulatory mechanisms between cholinergic and dopaminergic neuronal systems multi-parametrically using PET in combination with L-3,4-dihydroxyphenylalanine (L[β-^{11}C]DOPA), [^{11}C]raclopride, and 2β-carbomethoxy-3β-(4-fluorophenyl)tropane ([β-^{11}C]CFT) in the conscious monkey brain. Scopolamine, a muscarinic cholinergic antagonist, was used as a modulator of the dopaminergic neuronal system instead of benztropine, which has a slight dopamine transporter (DAT) inhibitory effect in addition to its muscarinic cholinergic receptor inhibitory action (Coyle and Snyder, 1969). *In vivo* Scatchard plot analysis was applied to evaluate the

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effects of scopolamine on binding parameters of [11C]raclopride to dopamine D_2 receptors. Microdialysis studies were conducted to assess the effects of scopolamine on dopamine concentrations in the striatal extracellular fluid (ECF).

MATERIALS AND METHODS

Animals and drugs. Young-adult male rhesus monkeys (*Macaca mulatta*; $n = 4$) weighing from 5.5 to 6.5 kg were used for the PET measurements. Monkeys were maintained and handled in accordance with recommendations of the United States National Institutes of Health and also the guidelines of the Central Research Laboratory, Hamamatsu Photonics. They were trained to sit on a chair twice a week over a period of >3 months. Magnetic resonance images (MRI) of all monkeys were obtained with a Toshiba MRT-50A/II (0.5 T) under pentobarbital anesthesia. The stereotactic coordinates of PET and MRI were adjusted based on the orbitomeatal (OM) line with a specially designed head holder (Takechi et al., 1994). At least 1 month before the PET study, an acrylic plate, with which monkey was fixed to a monkey chair, was attached to the head under pentobarbital anesthesia as described previously (Onoe et al., 1994).

Scopolamine hydrobromide was obtained from Kyorin Pharmaceutical Co. Ltd. (Tokyo, Japan). Precursors for labeling of [11C]raclopride and [β-11C]CFT were purchased from Research Biochemicals (Natick, MA). The enzymes for L[β-11C]DOPA synthesis, alanine racemase (EC 5.1.1.1.), D-amino acid oxidase (EC 1.4.3.3.), and β-tyrosinase (EC 4.1.99.2), were purchased from Ikeda Food Research Co. Ltd. (Hiroshima, Japan).

Synthesis of [11C]-labeled compounds. Carbon-11 (11C) was produced by $^{14}N(p,\alpha)^{11}C$ nuclear reaction using a cyclotron (HM-18; Sumitomo Heavy Industry, Tokyo, Japan) at Hamamatsu Photonics PET center and obtained as $[^{11}C]CO_2$. [β-11C]CFT was labeled with 11C by *N*-methylation of its nor-compound with [11C]methyl iodide prepared from $[^{11}C]CO_2$. [11C]Raclopride was synthesized by *O*-methylation of its precursor with [11C]methyl iodide. The radiochemical and chemical purities used here were greater than 98 and 99%, respectively, and the specific radioactivity ranged from 107 to 141 GBq/μmol for [β-11C]CFT and from 54.2 to 77.8 GBq/μmol for [11C]raclopride, respectively.

L-[β-11C]DOPA was synthesized using a combination of organic synthesis and multi-enzymatic procedures (Bjurling et al., 1990) using an automated synthesizer (Harada et al., 2000). The radiochemical and chemical purities of L-[β-11C]DOPA were better than 98 and 99%, respectively.

After analysis for identification, the solution was passed through a 0.22 μm pore size filter before intravenous administration to the monkey.

PET scan. Data were collected on a high-resolution PET scanner (SHR-7700; Hamamatsu Photonics, Hamamatsu, Japan) with a transaxial resolution of 2.6 mm full-width at half-maximum and a center-to-center distance of 3.6 mm (Watanabe et al., 1997). The PET camera allowed 31 slices for imaging to be recorded simultaneously.

After an overnight fast, animals were fixed to the monkey chair with stereotactic coordinates aligned parallel to the OM line. A cannula was implanted into the posterior tibial vein of the monkey for administration of [11C]-labeled ligands, and another cannula was put into the femoral artery of the other leg to obtain arterial blood samples for scans with [11C]raclopride and [β-11C]CFT.

During PET scans, heart rate, respiration rate, blood pressure, and body temperature were continuously monitored using a life monitoring system (Nihon Kohden, Tokyo, Japan). The levels of carbon dioxide ($Paco_2$), blood oxygen (Pao_2), and pH of arterial blood were measured with a Stat Profile blood gas analyzer (Nova Biochemical, Waltham, MA).

All four monkeys were subjected to PET scans with L-[β-11C]DOPA, [11C]raclopride, and [β-11C]CFT. Three PET scans with either [11C]-labeled compound were serially performed in the same animal in 1 d. At 30 min after administration of saline, a [11C]-labeled compound was injected through the posterior tibial vein cannula. For second and third scans, at 30 min after administration of scopolamine (10 or 100 μg/kg), the same [11C]-labeled compound was injected every 3 hr. Because of the very short half-life of 11C (20.4 min), the radioisotope used in these studies, a time lag of at least 3 hr between scans provided sufficient time for decay of the radioactivity in the monkeys (~1/400 of injected dose), so that the level of radioactivity associated with the previous injection of [11C]-labeled compound would not interfere with the next scan.

PET scans with [11C]raclopride and L-[β-11C]DOPA were performed for 64 min with six time frames at 10 sec intervals, six time frames at 30 sec, 12 time frames at 1 min, followed by 16 time frames at 3 min. For [β-11C]CFT study, PET scans were performed with an additional nine time frames at 3 min.

Kinetic analysis of in vivo *binding.* Regions of interest (ROI), i.e., the striatum and cerebellum, were identified according to MR images of each monkey brain, and the time–activity curves of L-[β-11C]DOPA, [11C]raclopride, and [β-11C]CFT in ROIs were obtained as described previously (Tsukada et al., 1999a,b, 2000a,b).

To measure the input function of [11C]raclopride and [β-11C]CFT to the brain, arterial blood samples were obtained every 8 sec from 10 to 66 sec, followed by 96, 156, 246, and 336 sec, and then 20, 30, 45, and 60 min after tracer injection. For [β-11C]CFT, additional samples were taken at 75 and 90 min. Blood samples were centrifuged to separate plasma and weighed, and their radioactivity was measured. For metabolite analysis, methanol was added to some plasma samples (sample/methanol, 1:1) obtained at 42 and 66 sec and 5.6, 10, 30, 45, 60, 75, and 90 min after tracer injection, followed by centrifugation. The obtained supernatants were developed with thin-layer chromatography (TLC) plates (AL SIL G/UV; Whatman, Kent, UK) with a mobile phase of ethylene dichloride/diethyl ether/ethanol/triethylamine, 20:20:1:1. The ratio of unmetabolized fraction was determined using a phosphoimaging plate (BAS-1500 MAC; Fuji Film Co. Ltd., Tokyo, Japan). The r_f values of [11C]raclopride and [β-11C]CFT were 0.41 and 0.52, respectively. The input functions of unmetabolized [11C]raclopride and [β-11C]CFT were calculated using the data obtained by correction of the ratio of the unmetabolized fraction to total radioactivity.

For quantification of *in vivo* binding of [11C]raclopride and [β-11C]CFT, a kinetic three-compartment analysis method was applied as described previously (Huang et al., 1986). The time–activity curves of plasma and of each region were fitted to a three-compartment model with the least-square fitting method using the constrained K_1/k_2 ratio to the distribution volume in the cerebellum. The values of binding potential (BP) of [11C]raclopride and [β-11C]CFT were calculated by determining the ratio of the estimated k_3 value (association rate) to the estimated k_4 value (dissociation rate) (Tsukada et al., 1999a,b, 2000a,b).

For quantification of L-[β-11C]DOPA utilization rate constant in the striatum of the monkey brain, a graphical analysis method was applied to calculate dopamine synthesis rate (k_3) as described previously (Tedroff et al., 1991; Tsukada et al., 1996b, 2000a,b).

Scatchard plot analysis. Saturation experiments were performed to examine the effects of scopolamine on *in vivo* binding parameters (B_{max} and K_d) of [11C]raclopride (Farde et al., 1989; Tsukada et al., 1996a). Thirty minutes after administration of scopolamine (10 and 100 μg/kg), [11C]raclopride was injected into monkeys under carrier-free conditions or together with various amounts (from 3 to 300 μg/kg) of carrier raclopride. The total radioligand concentration of [11C]raclopride in the cerebellum was used as an estimate of the free radioligand concentration (F) in the striatum. Specific binding (B) was defined as radioactivity in the striatum reduced by F. In the case of [11C]raclopride, the curve for B was fitted to a set of three exponential functions to determine the time point at which B reached a peak (Farde et al., 1989). The values for B and F at these time points were used in Scatchard analysis in which the ratio of B/F was plotted against B (Scatchard, 1949). The apparent *in vivo* B_{max} and K_d values were analyzed using LIGAND software (Munson and Rodbard, 1980).

Microdialysis analysis. Microdialysis was performed in the conscious state in the same monkeys used for PET studies as described previously (Tsukada et al., 1999a,b, 2000a,b). A guide cannula was implanted (anterior, 21 mm; lateral, 3.0 mm) according to the individual MR images with reference to the stereotactic brain atlas of Snider and Lee (1961), during the procedure for attachment of the acrylic plate. A microdialysis probe with a membrane region 250 μm in diameter and 3 mm in length (Eicom A-I-25–03; Eicom, Tokyo, Japan) was inserted into the striatal region (17.0 mm below the dura matter) of the monkey brain via the guide cannula. The probe was initially perfused with Krebs'–Henseleit solution (in mM: 118.5 NaCl, 4.7 KCl, 1.2 KH_2PO_4, 1.2 $MgSO_4$, 1.25 $CaCl_2$, 25.0 $NaHCO_3$, and 5.6 glucose, pH 7.4) at a rate of 10 μl/min to remove dopamine overflow from the damaged tissue. The perfusion rate was decreased to 5 μl/min 2 hr after insertion of the probe, 75 μl samples were collected every 15 min, and the content of dopamine was measured by HPLC with electrochemical detection. To verify the exact positioning of the probe, 5 μl of China ink was injected via the guide cannula at the end of the experiments. Animals were anesthetized with sodium pentobarbital and decapitated. The brains were quickly removed, coronal sections were cut on a cryostat, and the location of the probe implantation site was determined visually.

The averaged data obtained from 0 to 120 min before administration of saline or scopolamine were used as " baseline" data. Saline or scopolamine (10 and 100 μg/kg) was administered 120 min after the start of sampling. GBR12909 at a dose of 0.5 mg/kg was administered 30 min after saline or scopolamine (10 and 100 μg/kg). The striatal ECF dopamine level was expressed as percentage of baseline.

Statistical analysis. Results are expressed as means ± SD. Comparison between conditions was performed using the paired, two-tailed Student's t test, and a probability level of $p < 0.05$ was considered statistically significant.

RESULTS

Under the control conditions with saline administration, the summated PET images from 37 to 64 min after injection and the time–activity curves indicated high uptake of L-[β-11C]DOPA (Fig. 1*A*), [11C]raclopride (Fig. 2*A*), and [β-11C]CFT (Fig. 3*A*) in the striatum, and low uptake in the cerebellum of the conscious monkey brain. The striatal radioactivity associated with L-[β-11C]DOPA reached a peak 5 min after injection and remained at this elevated level to the end of the scan (Fig. 1*A*). The maximum accumulation of radioactivity in the striatum occurred ~10 min after injection of [11C]raclopride and decreased gradually thereafter (Fig. 2*A*). The time–activity curve of [β-11C]CFT in the striatum increased with time during the experimental period (Fig. 3*A*). In the cerebellum, the time–activity curves of these labeled compounds showed peak

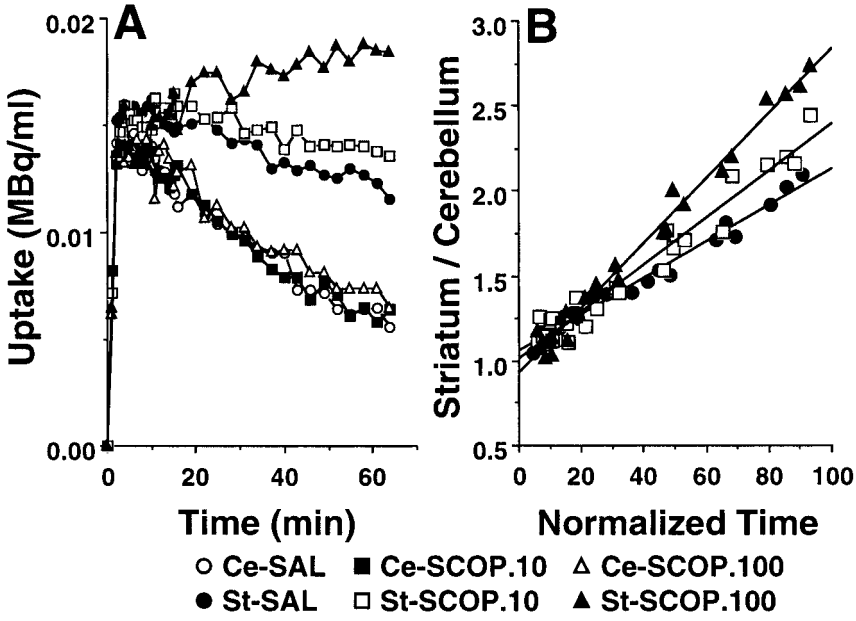

Figure 1. Effects of scopolamine on the time–activity curves of L-[β-^{11}C]DOPA in the brain (*A*) and the k_3 value (*B*). Saline or scopolamine (10 or 100 μg/kg) was administered 30 min before L-[β-^{11}C]DOPA injection. PET scan was started immediately after tracer injection, and image data were collected for 64 min. *A*, ROIs were identified according to MRI of the same animal. The radioactivity in each striatum (*St*) and cerebellum (*Ce*) were plotted against time after tracer injection. *B*, The time course of changes in radioactivity in the striatum as a ratio to that in the cerebellum was expressed as a function of the normalized integral of each cerebellar radioactivity. The slope of the calculated regression line represents k_3 value.

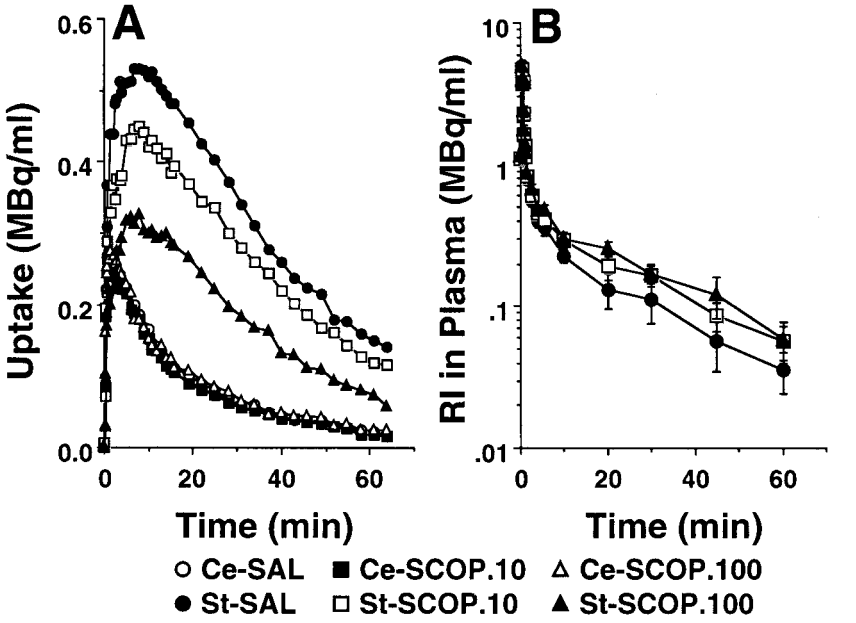

Figure 2. Effects of scopolamine on the time–activity curves of [^{11}C]raclopride in the brain (*A*) and in the arterial plasma (*B*). Saline or scopolamine (10 or 100 μg/kg) was administered 30 min before [^{11}C]raclopride injection. PET scan was started immediately after tracer injection, and image data were collected for 64 min. *A*, ROIs were identified according to MRI of the same animal. The radioactivity in each striatum (*St*) and cerebellum (*Ce*) were plotted against time after tracer injection. *B*, Time-activity curve of unmetabolized [^{11}C]raclopride. Unmetabolized [^{11}C]raclopride was calculated by correction of relative to total radioactivity with the ratio of the unmetabolized fraction at each time point.

values within 5 min after injection, followed by gradual decreases with time (Figs. 1*A*, 2*A*, 3*A*).

In plasma, the curves of total radioactivity associated with [^{11}C]raclopride (Fig. 2*B*) and [β-^{11}C]CFT (Fig. 3*B*) showed peaks ~30 sec after slow bolus intravenous injection and declined rapidly thereafter. Metabolite analysis by TLC and a phosphoimaging system indicated that [^{11}C]raclopride and [β-^{11}C]CFT were gradually metabolized to very polar metabolites, which remained at the origin, and the ratios of radioactivity in unmetabolized labeled compounds to the in total (unmetabolized plus metabolized) were 0.72 and 0.22 at 60 min after injection, respectively (data not shown). The input functions of unmetabolized [^{11}C]-labeled compounds were calculated using the data obtained by correction of total radioactivity relative to the metabolic ratio (data not shown). In general, the input functions of [^{11}C]raclopride and [β-^{11}C]CFT into the brain were not significantly affected by administration of scopolamine (Figs. 2*B*, 3*B*).

Systemic intravenous administration of scopolamine at doses of 10 and 100 μg/kg caused dose-dependent increases in the uptake of L-[β-^{11}C]DOPA in the striatum with no significant changes in the radioactivity curves in the cerebellum (Fig. 1*A*). These alterations in the striatal kinetics of L-[β-^{11}C]DOPA caused dose-dependent enhancement of the kinetic values of dopamine synthesis rate (k_3), as calculated using the time–activity curve of the cerebellum as the input function (Figs. 1*B*, 4*A*).

The administration of scopolamine (10 and 100 μg/kg) resulted in a dose-dependent reduction of [^{11}C]raclopride uptake in the striatum, with no significant changes in those in the cerebellum or arterial plasma (Fig. 2*A*,*B*). These alterations in the striatal kinetics of [^{11}C]raclopride caused a dose-dependent decrease in the binding potential (BP = k_3/k_4) as calculated using each plasma time–activity curve as an input function (Fig. 4*B*).

The administration of scopolamine (10 and 100 μg/kg) caused a dose-dependent increase in the uptake of [β-^{11}C]CFT in the striatum with no significant changes in the radioactivity curves of arterial plasma (Fig. 3*A*,*B*), indicating the dose-dependent increase in the BP of [β-^{11}C]CFT as shown in Figure 4*C*.

As shown in Figure 5, the effects of scopolamine (10 and 100 μg/kg) on dopamine level in the striatal ECF were evaluated by microdialysis in the monkey brain. Microdialysis was performed

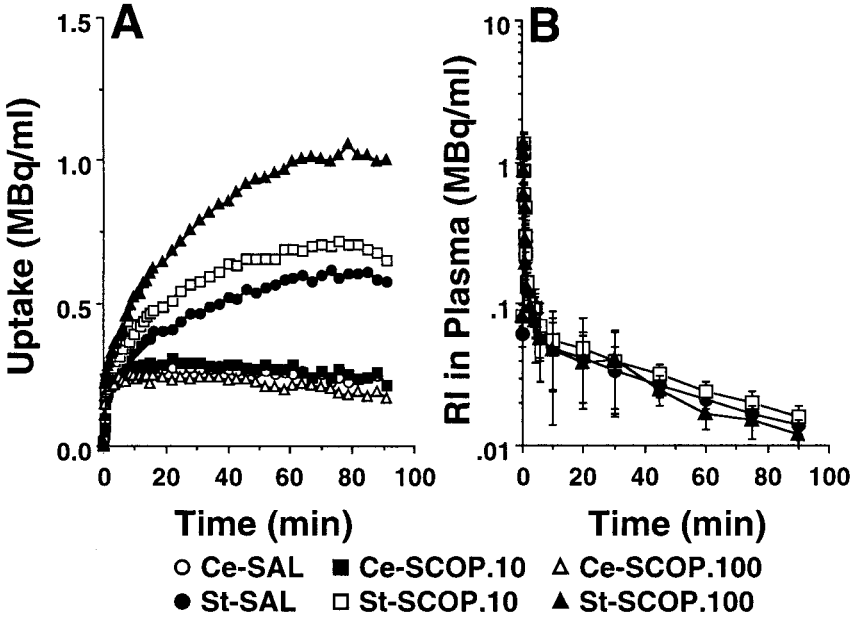

Figure 3. Effects of scopolamine on the time–activity curves of [β-[11]C]CFT in the brain (*A*) and in the arterial plasma (*B*). Saline or scopolamine (10 or 100 μg/kg) was administered 30 min before [β-[11]C]CFT injection. PET scan was started immediately after tracer injection, and image data were collected for 91 min. *A*, ROIs were identified according to MRI of the same animal. The radioactivity in each striatum (*St*) and cerebellum (*Ce*) were plotted against time after tracer injection. *B*, Time–activity curve of unmetabolized [β-[11]C]CFT. Unmetabolized [β-[11]C]CFT was calculated by correction of relative to total radioactivity with the ratio of the unmetabolized fraction at each time point.

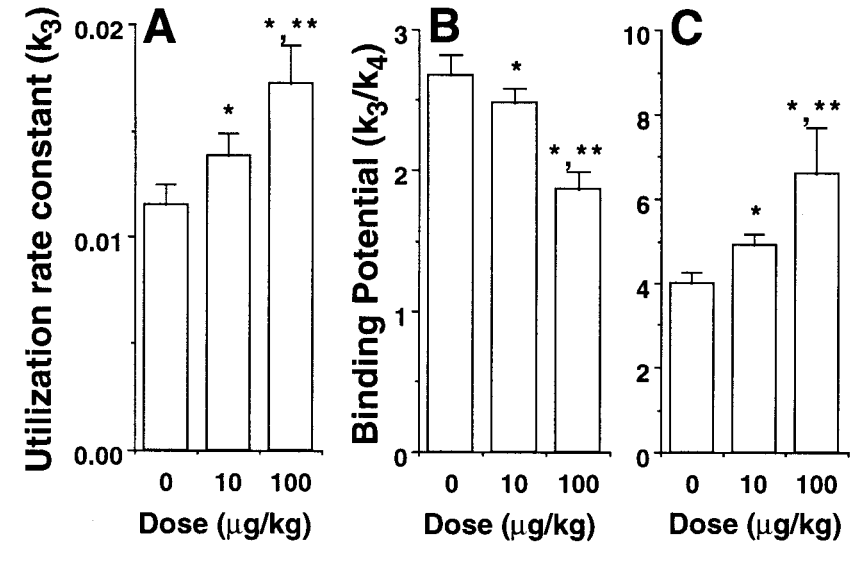

Figure 4. Effects of scopolamine on dopamine synthesis rate, D_2 receptor binding, and transporter availability, as measured with L-[β-[11]C]DOPA (*A*), [[11]C]raclopride (*B*), and [β-[11]C]CFT (*C*), respectively. *A*, The time course of changes in radioactivity in the striatum (*St*) as a ratio of that in the cerebellum (*Ce*) was expressed as a function of the normalized integral of each cerebellar radioactivity. The values of k_3 are represented by the slope of the calculated regression line shown in Figure 1*B*. *B*, *C*, The time–activity curves of unmetabolized [[11]C]raclopride and [β-[11]C]CFT in arterial plasma, shown in Figures 2*B* and 3*B*, were used as input functions into the brain, and the three-compartment model was fitted to the time–activity curve of specific binding in the striatum. The binding potential was calculated as the ratio of the association rate (k_3) to the dissociation rate (k_4). Data are expressed as means ± SD for four animals per treatment condition. $^{*}p < 0.05$ versus respective saline control (dose of 0). $^{**}p < 0.05$ versus respective scopolamine at a dose of 10 μg/kg.

simultaneously with PET scans of [[11]C]raclopride. The baseline level of dopamine was 6.5 ± 1.9 fmol/μl ($n = 4$) in the striatum of conscious monkeys. Administration of scopolamine at any dose used here resulted in no significant changes in the striatal ECF dopamine level (Fig. 5*A*). When GBR12909, a specific DAT inhibitor, was administered at a dose of 0.5 mg/kg in saline-treated animals, it significantly increased dopamine level in the striatal ECF of the monkey brain (Fig. 5*B*). Interestingly, scopolamine preadministered 30 min before the administration of GBR12909 at the same dose as used in the saline condition further facilitated the GBR12909-induced striatal ECF dopamine enhancement in a dose-dependent manner (Fig. 5*B*).

To clarify the mechanism(s) by which scopolamine modulates dopamine D_2 availability *in vivo*, [[11]C]raclopride was injected into monkeys together with various amounts (from 3 to 300 μg/kg) of unlabeled carrier ligand raclopride. [[11]C]Raclopride with carrier ligand was injected 30 min after the administration of scopolamine (10 and 100 μg/kg). The addition of increasing amounts of unlabeled carrier ligand dose-dependently reduced the amounts of bound radiolabeled ligand (Fig. 6*A*). In these studies, a significant decrease in radioactivity of bound [[11]C]raclopride in the striatum over the time span of the PET study was found, and the results were similar in both saline- and scopolamine-treated groups. In contrast, in the cerebellum, no changes were observed in the amount of radioactivity of [[11]C]raclopride over the range of amount of unlabeled carrier added in both cases (Fig. 6*A*). The free radioligand concentration (*F*) in the striatum was assumed to be comparable with the radioligand concentration in the cerebellum. The specific binding (*B*) in the striatum was calculated by subtracting the radioligand concentration measured in the cerebellum from total binding in the striatum. For Scatchard analysis of the binding of [[11]C]raclopride *in vivo*, equilibrium values for *B* and *F* were obtained when the *B* value was maximum. The maximum accumulation occurred between 12 and 15 min after injection of [[11]C]raclopride. The Scatchard plot revealed a linear curve for [[11]C]raclopride in saline-treated, as well as scopolamine-treated, monkeys (Fig. 6*B*). The administration of scopolamine resulted in dose-dependent reduction of the slopes of the curves determined with [[11]C]raclopride, suggesting the alteration of the affinity ($1/K_d$) of D_2 receptors (Fig. 6*B*). In contrast, scopolamine did not affect the intercept with the *x*-axis in [[11]C]raclopride, which provided the maximum number of binding sites (B_{max}) of D_2 receptors (Fig. 6*B*).

DISCUSSION

This is the first study to demonstrate the effects of muscarinic cholinergic modulation on the striatal dopamine neuronal activity

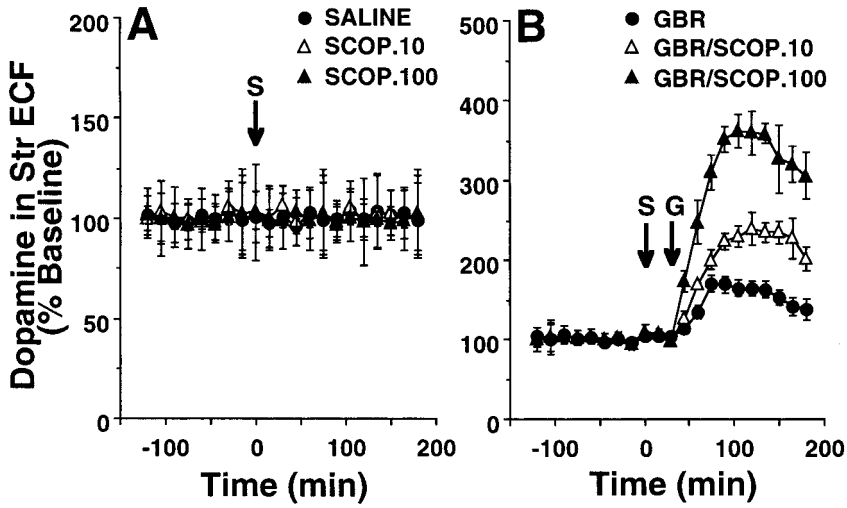

Figure 5. Effects of scopolamine (*A*) and/or GBR12909 (*B*) on dopamine concentration in the striatal ECF of the monkey brain. A microdialysis probe was inserted into the striatal region via the guide cannula. The probe was perfused with Krebs'–Henseleit solution (pH 7.4) at a rate of 5 μl/min. Samples were collected every 15 min, and the content of dopamine was measured by HPLC with electrochemical detection. The averaged data obtained from 0 to 120 min without any infusion were used as baseline data. Saline or scopolamine at doses of 10 or 100 μg/kg was administered 120 min after the start of sampling (*arrow* with *S*). GBR12909 at a dose of 0.5 mg/kg was administered 30 min after saline or scopolamine administration (*arrow* with *G*). The striatal ECF dopamine level was expressed as percentage of baseline.

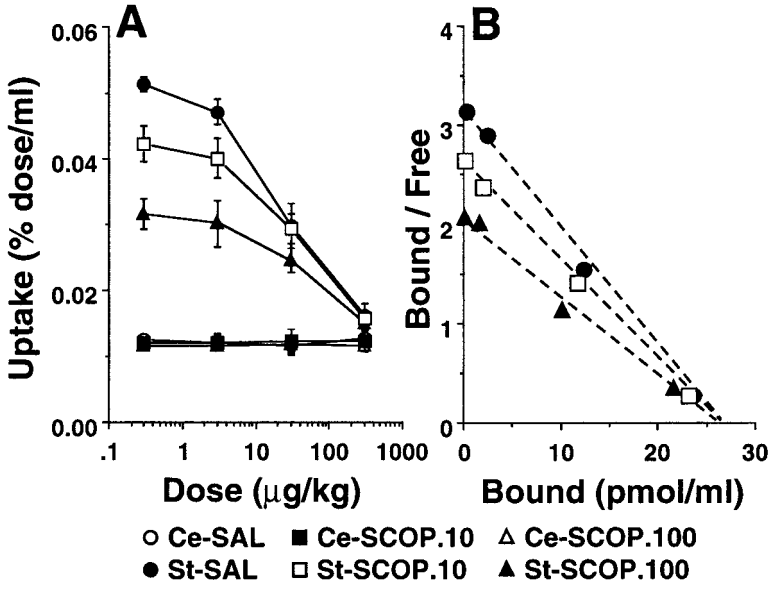

Figure 6. Saturation studies (*A*) and Scatchard plot analysis (*B*) of *in vivo* binding of [11C]raclopride in the monkey brain. Monkeys were administered saline or scopolamine (10 or 100 μg/kg, i.v.) 30 min before injection of [11C]raclopride under carrier-free conditions or with various doses of carrier ligand ranging from 3 to 300 μg/kg. *A*, The radioactivities in striatum and cerebellum at 12–15 min after tracer injection were expressed as percentage of dose per milliliter. Data are expressed as means ± SD for four animals per treatment group. Radioactivity in the striatum (*open symbols*) or cerebellum (*filled symbols*). *B*, The total radioligand concentration in the cerebellum was used as the free radioligand concentration (*Free*) in the striatum. Specific binding (*Bound*) was defined as radioactivity in the striatum reduced with *Free*. The average values ($n = 4$ for each points) for *Bound* and *Free* were used in a Scatchard analysis in which the ratios *Bound*/*Free* were plotted against *Bound*.

by simultaneous multi-parametric assessment of dopamine synthesis, D_2 receptor binding and DAT availability as measured by PET in the same nonhuman primates in the conscious state.

We reported previously that benztropine, a muscarinic cholinergic antagonist with slight DAT inhibitory activity, induced the reduction of [11C]raclopride binding in the conscious monkey brain as measured by PET (Tsukada et al., 1999a). These observations were consistent with the previous report of inhibition of [11C]raclopride binding by cholinergic blockade induced by scopolamine in anesthetized baboons (Dewey et al., 1993b). Although these authors speculated that the reduced [11C]raclopride binding was attributable to the increase in synaptic dopamine level via neuronal interactions, it did not demonstrate a clear relationship between the doses administered and the reduction in magnitude of [11C]raclopride binding (Dewey et al., 1993b). The lack of microdialysis data as measured in the same animals used in the PET study impaired interpretation of the previous data. The change in synaptic dopamine level does not always represent a reasonable explanation for the alterations of dopamine D_2 receptor availability *in vivo* as measured by PET with [11C]raclopride. As we have demonstrated previously, although administration of benztropine-induced elevation of dopamine concentration in the striatal ECF as measured by microdialysis, the magnitude of the elevation of dopamine level by benztropine was much lower and of shorter duration than those evoked by direct dopamine enhancers, such as GBR12909 and methamphetamine (Tsukada et al., 1999a). However, benztropine induced significant reduction of [11C]raclopride in the striatum of conscious monkeys to a similar extent as that induced by the direct dopamine enhances (Tsukada et al., 1999a). Furthermore, benztropine also decreased [18F]NMSP (Dewey et al., 1990), which was expected to be more stable against alterations of dopamine concentration than [11C]raclopride because of its 10 times higher affinity to dopamine D_2 receptors. As shown in the present study, scopolamine did not alter the apparent static dopamine concentration in the striatal ECF; however, scopolamine reduced the *in vivo* binding of [11C]raclopride. Amphetamine reduced the *in vivo* binding of both [3H]/[11C]raclopride (Dewey et al., 1991, 1993a; Young et al., 1991) and [3H]/[18F]NMSP (Dewey et al., 1991; Logan et al., 1991; Young et al., 1991). Isoflurane has been reported to increase synaptic dopamine levels (Opacka-Juffry et al., 1991); however, it decreased the *in vivo* binding of [11C]raclopride to a much lesser extent than that of [11C]NMSP (Kobayashi et al., 1995). Subanesthetic doses of ketamine, a noncompetitive antagonist of NMDA receptors, decreased the striatal binding of [11C]raclopride in human subjects, suggesting that ketamine increased the striatal dopamine concentration by ketamine (Smith et al., 1997). However, the results of previous microdialysis studies demonstrated that NMDA antagonists [ketamine and (+)-5-methyl-10,11-dihydro-5H-dibenzo(a,d)cyclohepten-5,10-imine hydrogen maleate (MK-801)] increased (Moghaddam et al., 1990; French 1994; Verma and Moghaddam, 1996), decreased (Kashihara et al., 1990), or did not change (Bacopoulos et al., 1979;

Koshikawa et al., 1988; Onoe et al., 1994; Tsukada et al., 2000a) dopamine concentration in the striatum. It is of interest that MK-801 significantly increased stereotypic behavior but did not affect the striatal dopamine concentration (Weihmuller et al., 1991). Our previous studies indicated that ketamine paradoxically altered dopamine D_2 receptor availability as measured by [11C]raclopride (increased binding) and [11C]NMSP (decreased binding) without any apparent change in dopamine concentration in the striatal ECF (Onoe et al., 1994; Tsukada et al., 2000a). Modulation using reserpine, which depletes endogenous dopamine, induced the increased binding of [3H]/[11C]raclopride (Inoue et al., 1991; Ginovart et al., 1997) but the decreased binding of [3H]NMSP (Inoue et al., 1991) (for review, see Laruelle, 2000). Interestingly, [11C]raclopride binding *in vivo* was also markedly reduced in the human striatum during video game playing with hand movement, and the degree of reduction showed a reverse correlation with the performance of the game, showing at most 50% reduction of binding (Koepp et al., 1998). In this study, the reduction of [11C]raclopride was simply attributed to the increased dopamine in the striatal synaptic cleft. However, in the animal experiment with conscious monkeys, 50% reduction of [11C]raclopride required the administration of methamphetamine at 1 mg/kg or more, which corresponds to an almost 100-fold higher dose than that used by drug abusers daily (Tsukada et al., 1999a). Together, the alteration of affinity or availability change of receptor sites induced by the neuronal interactions should also be taken into account for the alteration of dopamine D_2 receptor availability *in vivo* as measured by PET with [11C]raclopride, as well as [11C]NMSP. In fact, the present results obtained by Scatchard plot analysis *in vivo* demonstrated that cholinergic modulation elicited alteration of the apparent affinity ($1/K_d$) of dopamine D_2 receptors, without any alteration of the apparent maximum numbers of binding sites (B_{max}) of dopamine D_2 receptors, resulting in the reduced binding potential $BP = k_3/k_4 = B_{max}/K_d$ of [11C]raclopride *in vivo* in the monkey brain as measured by PET.

It was hypothesized previously that the altered affinity of dopamine D_2 receptors measured by [11C]raclopride accounted for the apparent static concentration of endogenous dopamine in the synaptic cleft (Farde et al., 1995). Although a reduction in affinity of [11C]raclopride was consistent with a competitive model between endogenous dopamine and [11C]raclopride binding to receptors, the present results did not support the previous hypothesis, because similar analytical procedures revealed that scopolamine administration resulted in decreased affinity of dopamine D_2 receptors as measured by [11C]raclopride without any changes in apparent static dopamine concentration in the striatal ECF as measured by microdialysis. The present results obtained from the assessments of dopamine synthesis and DAT availability provide the important insight into these mechanisms. Systemic administration of scopolamine produced the simultaneous and dose-dependent enhancement of dopamine synthesis and DAT availability as measured by L-[β-11C]DOPA and [β-11C]CFT, respectively. Previous studies demonstrated that the utilization rate constant (k_3) of L-[β-11C]DOPA was increased by enhancement of the activity of tyrosine hydroxylase (EC 1.14.16.2) (Tsukada et al., 1996b), which is the rate-limiting enzyme in the synthesis of catecholamines (Nagatsu et al., 1964), accompanied with increased dopamine release into the synaptic cleft (Tsukada et al., 1994b). As observed in the present study, administration of scopolamine increased the utilization rate constant (k_3) of L-[β-11C]DOPA in a dose-dependent manner, suggesting that dopamine synthesis was enhanced in addition to dopamine release into the synaptic cleft of the striatum. However, microdialysis assay indicated no significant increase in dopamine concentration in the striatal ECF in the present study. One possible explanation for this discrepancy is that the change in dopamine concentration in ECF measured by microdialysis does not reflect the "true" dopamine release into the synaptic cleft as suggested previously using voltammetry (Kuhr et al., 1984; Kuhr and Wightman, 1986; May, 1988; Grace, 1993). This is unlikely however, because our previous study demonstrated that the same microdialysis assay could detect the enhancement of dopamine synthesis in the neurons (Tsukada et al., 1994a,b), the facilitation of dopamine release by methamphetamine (Tsukada et al., 1999a), and also the increase in the synaptic dopamine level induced by the inhibition of DAT by cocaine and GBR12909 (Tsukada et al., 1999a,b). Then the resulting increase in dopamine concentration in the striatal ECF was confirmed by measurements of "cold" endogenous dopamine (Tsukada et al., 1994a, 1999a,b) and [11C]-labeled "hot" dopamine converted from L-[β-11C]DOPA (Tsukada et al., 1994b). The precise mechanisms of enhanced DAT availability by scopolamine remain unclear yet. Because of the slow kinetics of [β-11C]CFT in the brain, its delivery is often affected by the change in regional cerebral blood flow (rCBF); that is, the increased rCBF might result in the enhanced uptake of [β-11C]CFT as observed in the present study. However, changes in rCBF might not account for the enhanced DAT availability by scopolamine, because our previous result demonstrated that the administration of scopolamine at the doses of 10 and 100 μg/kg decreased, not increased, rCBF in the conscious monkey brain (Tsukada et al., 1997). Some compensatory mechanisms with negative feedback system might be involved in this enhanced DAT availability for the regulation of increased dopamine release. Our recent results also revealed that ketamine infusion dose-dependently decreased [11C]raclopride binding with no significant changes in dopamine concentration in the striatal ECF as observed in the case of scopolamine, and also that ketamine increased both dopamine synthesis and DAT availability as measured by L-[β-11C]DOPA and [β-11C]CFT, respectively (Tsukada et al., 2000a). Microdialysis using the DAT inhibitor GBR12909 demonstrated that preadministration of scopolamine further facilitated the increase in striatal ECF dopamine level induced by DAT inhibition. The present results further indicated the facilitation of dopamine turnover by scopolamine and also strongly supported the usefulness of the combined use of L-[β-11C]DOPA and [β-11C]CFT for the assessment of dopamine turnover. Together, these results suggested that the modulation of muscarinic cholinergic, as well as glutamatergic, neuronal activities altered dopamine turnover in the striatum by simultaneous enhancement of the dynamics of dopamine synthesis and DAT availability to the same extent, resulting in no apparent marked changes in ECF dopamine concentration as measured by microdialysis.

In conclusion, the present results revealed that scopolamine reduced dopamine D_2 receptor binding *in vivo* by increasing dopamine turnover rate, not by elevating apparent static dopamine concentration in the synaptic cleft, resulting in the altered receptor affinity or availability. That is, the regulatory mechanism of dopamine neuronal transmission might be explained by the "rate" theory defined as the dynamics of dopamine binding to receptors and synaptic turnover of dopamine, not by the conventional "occupancy" theory. These results further support our hypothesis that alteration of the binding of radiolabeled ligands *in vivo* as measured by PET might not simply be modulated by the apparent static synaptic concentration of dopamine (Tsukada et al., 1999a, 2000a). These observations will be important for research and diagnosis of neuropsychiatric and neurodegenerative diseases using the functional imaging modalities of PET and single photon emission tomography with labeled compounds.

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