ELSEVIER

European Journal of Pharmacology 334 (1997) 31–38

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Effects of monoamine oxidase and catechol-*O*-methyltransferase inhibition on dopamine turnover: A PET study with 6-[^{18}F]L-DOPA

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Received 17 February 1997; revised 7 July 1997; accepted 11 July 1997

Abstract

The consequences of monoamine oxidase and catechol-*O*-methyltransferase inhibition on the effective turnover of dopamine were investigated using 6-[^{18}F]L-3-4-dihydroxyphenylalanine (6-[^{18}F]L-DOPA) and positron emission tomography. The effective dopamine turnover was expressed as the ratio between the rate of reversibility of 6-[^{18}F]L-DOPA trapping (k_{loss}) and the rate of uptake of 6-[^{18}F]L-DOPA (K_i) in the striatum of normal cynomolgus monkeys. The monkeys received 6-[^{18}F]L-DOPA scans, untreated or after pretreatment with either the peripheral catechol-*O*-methyltransferase inhibitor nitecapone; the peripheral and central catechol-*O*-methyltransferase inhibitor tolcapone; the monoamine oxidase inhibitors deprenyl or pargyline; a combination of tolcapone and the monoamine oxidase inhibitors. Tolcapone alone or combined with the monoamine oxidase inhibitors produced a significant decrease in the dopamine turnover (55 to 65%). Neither nitecapone nor monoamine oxidase inhibition alone produced significant changes. These results may have implications for the use of central catechol-*O*-methyltransferase inhibitors added to routine levodopa therapy in parkinsonian patients.

Keywords: PET (positron emission tomography); 6-[^{18}F]L-DOPA (6-[^{18}F]L-3-4-dihydroxyphenylalanine); Catechol-*O*-methyltransferase inhibition; Monoamine oxidase inhibition; Dopamine turnover

1. Introduction

Clinically, inhibition of monoamine metabolism could be expected to have therapeutic effects on Parkinson's disease. By reducing the metabolism of dopamine, enzyme inhibition should preserve already depleted striatal dopamine. Within the last decade, several specific inhibitors of the two main enzymes of monoamine metabolism, monoamine oxidase and catechol-*O*-methyltransferase have been developed that are suitable for human use (Backstrom et al., 1990; Da Prada et al., 1990). Typically, monoamine oxidase inhibitors are used as antidepressants, although recently they have been given as adjuvants to therapy in Parkinson's disease and Alzheimer's disease because of their putative neuroprotective actions in neurodegenerative illnesses (Yu, 1994). The catechol-*O*-methyltransferase inhibitors tolcapone and entacapone, are now in clinical trials as adjuvants to levodopa therapy in Parkinson's disease (Maj et al., 1990; Da Prada et al., 1994). The usefulness of catechol-*O*-methyltransferase inhibitors in Parkinson's disease derives from the inhibition of peripheral *O*-methylation of levodopa. Inhibition of catechol-*O*-methyltransferase increases the bioavailability of administered levodopa in the blood but little in vivo data, if any, exist on the effects of these agents on the central metabolism of dopamine.

In this pilot study, we took advantage of the unique capability of positron emission tomography (PET) and 6-[^{18}F]L-3-4-dihydroxyphenylalanine (6-[^{18}F]L-DOPA), a tracer specific for presynaptic dopaminergic function, to evaluate, in vivo, the effects of inhibition of dopamine metabolism in the nigro–striatal system. The attachment of ^{18}F to L-DOPA does not substantially alter its biological properties (Garnett et al., 1980; Chiueh et al., 1983; Cum-

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ming et al., 1993). In the striatum, 6-[^{18}F]L-DOPA is decarboxylated through the action of the aromatic amino acid decarboxylase into 6-[^{18}F]-dopamine and the accumulation of 6-[^{18}F]-dopamine in the vesicles of the dopaminergic terminals is responsible for most of the striatal activity after 6-[^{18}F]L-DOPA administration, with small contributions by 6-[^{18}F]-dopamine metabolites (Firnau et al., 1987; Melega et al., 1990b). Thus, 6-[^{18}F]L-DOPA provides an index of the function of the nigro–striatal dopamine system. Using a group of young, normal cynomolgus monkeys, we examined the effects of catechol-*O*-methyltransferase and monoamine oxidase inhibition alone and in combination on the peripheral and central handling of 6-[^{18}F]L-DOPA. Catechol-*O*-methyltransferase inhibition was achieved with nitecapone (OR462, Orion Pharmaceutica), a peripheral catechol-*O*-methyltransferase inhibitor and tolcapone (RO 40-7592, Hoffman LaRoche), a catechol-*O*-methyltransferase inhibitor that can also cross the blood brain barrier. Monoamine oxidase inhibition was achieved with either deprenyl, a specific monoamine oxidase-B inhibitor, or pargyline, a non-specific monoamine oxidase inhibitor. Since there was no significant difference in their effects on the 6-[^{18}F]L-DOPA measures, data from the two monoamine oxidase inhibitors were combined. In addition, the effects of combined inhibition of catechol-*O*-methyltransferase with tolcapone and the monoamine oxidase inhibitors were evaluated. The peripheral effects of these drugs were reflected in the changes in 6-[^{18}F]L-DOPA plasma metabolism. The central effects were evaluated through their actions on the uptake rate constant of 6-[^{18}F]L-DOPA into the striatum (K_i) (Martin et al., 1989) and on the rate of reversibility of 6-[^{18}F]L-DOPA trapping (i.e. the rate of loss of radioactivity from the striatum) (k_{loss}) (Holden et al., 1997). During extended PET studies, the loss of striatal radioactivity is likely due to the diffusion of the metabolites of 6-[^{18}F]-dopamine out of the striatum. Inhibition of the central metabolism of dopamine and 6-[^{18}F]-dopamine, either by monoamine oxidase or catechol-*O*-methyltransferase inhibition, will decrease the loss rate constant, through preservation of synaptic dopamine or its fluorinated analogue. An index of effective dopamine turnover can be derived from the ratio of the rate of loss of striatal activity to the rate of uptake of striatal activity, k_{loss}/K_i. This index of effective dopamine turnover can be expected to be reduced after enzyme inhibition of monoamine oxidase and catechol-*O*-methyltransferase.

Some of the data reported have been previously published to validate the extended graphical analysis of 6-[^{18}F]L-DOPA (Holden et al., 1997).

2. Materials and methods

Eight juvenile cynomolgus monkeys (macaca fascicularis) were studied. Five animals were scanned without pharmacological intervention (A: Untreated); seven after pretreatment with nitecapone (B: Nitecapone); seven after pretreatment with tolcapone (C: Tolcapone); six after pretreatment with a monoamine oxidase inhibitor (D: monoamine oxidase inhibition); and five with a combination of tolcapone and monoamine oxidase inhibitor (E: Tolcapone + monoamine oxidase inhibition). The monoamine oxidase inhibitors used were either deprenyl (3.3 ± 0.4 mg/kg), which is selective for monoamine oxidase-B, or pargyline (35.8 ± 6.7 mg/kg), which acts on monoamine oxidase-A and monoamine oxidase-B. Half the animals received deprenyl and half received pargyline in each of groups D and E. However, since there was no significant difference between the results obtained with these two inhibitors in any of the measurements considered, we pooled the data obtained with the two monoamine oxidase inhibitors into a single group for both studies D and E. Tolcapone (28.9 ± 5 mg/kg, gift of Hoffman LaRoche) and nitecapone (20.8 ± 3 mg/kg, gift of Orion Pharmaceutica) were dissolved in 1–1.5 ml of DMSO before administration. All the test substances were given intraperitoneally 60–90 min prior to 6-[^{18}F]L-DOPA. The minimum interval between scans for one animal was 8 weeks, but this interval was increased to 3 months after monoamine oxidase inhibition. All animal procedures were approved by the Commitee on Animal Care of the University of British Columbia.

Prior to the scan, the animal fasted overnight and was preanesthetized with ketamine and pentobarbital for insertion of the intravenous and intraarterial lines. After intubation, the animal was maintained under isofluorane anesthesia for the duration of the PET study. The peripheral decarboxylase inhibitor carbidopa (2.5–3.5 mg/kg intraperitoneally, gift of Merck, Sharp and Dohme) was administered 45–75 min prior to every study. PET scans were obtained with the UBC/TRIUMF PETT VI scanner (Evans et al., 1983). This tomograph has an in plane resolution of about 9 mm full width at half maximum and an averaged axial resolution of 11.3 mm allowing the simultaneous acquisition of 7 planes, 14 mm apart center to center. A transmission scan was acquired for attenuation correction. Twenty four 10-min time frames were acquired, starting at the intravenous injection of a 5 mCi bolus of 6-[^{18}F]L-DOPA (Adam et al., 1986). At the end of the dynamic sequences, 4 interleaved sets of planes were acquired to provide a 3 mm axial sampling of the striatal region (Sossi et al., 1993).

Arterial samples were obtained throughout the study to determine the total plasma radioactivity. The fractions of 6-[^{18}F]L-DOPA and its metabolites, mainly 3-*O*-methyl-6-[^{18}F]L-DOPA, 6-[^{18}F]-homovanillic acid, 6-[^{18}F]-dihydroxy-phenylacetic acid and sulfated conjugates were determined by high performance liquid chromatography as previously described (Melega et al., 1990a; Chan et al., 1992) at 10, 30, 60, 120 and 180 min post 6-[^{18}F]L-DOPA injection. The plasma metabolite data were expressed as a

fraction of total plasma activity and plasma time activity courses computed separately. Plasma activity courses were expressed in μCi/ml and normalized by the amount of mCi injected per kg of body weight. Each drug condition was compared to the untreated condition using repeated measures analysis of variance.

Elliptical regions of interest were placed on the left and right striatum and on the left and right occipital cortices. Time activity curves were obtained for each region. The striatal region of interest values were adjusted by a correction factor for variation in axial positioning determined by a method similar to the previously reported method in human subjects (Sossi et al., 1993). Briefly, the axial distribution of the striatal regions of interest measured in the data sets acquired following the dynamic study was fitted to a Gaussian function with the full width at half maximum fixed at a previously determined value of 16 mm. The correction factor was calculated as the ratio between the peak of the fitted curve and the striatal value corresponding to the axial position where the dynamic sequence was acquired. Using this correction factor, the striatal time activity curves were maximized to the values that would have been obtained if the striatum had been centered exactly in an axial plane. The mean correction factor was 1.06 (range 1.0–1.22). The left and right occipital values were averaged.

The uptake rate constant K_i was calculated for the left and right striatum using the conventional graphical analysis of the data acquired between 30 and 120 min using the arterial plasma 6-[^{18}F]L-DOPA as the input function (Martin et al., 1989). The reversibility of 6-[^{18}F]L-DOPA trapping, expressed as a first-order rate constant, k_{loss}, was calculated using an extended graphical analysis of the time-courses acquired between 30 and 200 min post 6-[^{18}F]L-DOPA injection (Patlak and Blasberg, 1985; Holden et al., 1997). The fitting method and its theoretical basis have been described previously (Holden et al., 1997). The influx rate constant K_c was calculated using the conventional graphical analysis of the data between 30 and 120 min post injection, using the averaged occipital radioactivity time course as the input function (Brooks et al., 1990). Finally, both the striatal and cortical time activity curves were averaged between 90 and 120 min and the striatum/occipital cortex ratio estimated. An index of effective dopamine turnover was obtained by calculating the ratio of the rate of loss of striatal radioactivity k_{loss} to the uptake rate of 6-[^{18}F]L-DOPA into the striatum, K_i.

There were no significant differences between the left and right striatal measurements. Thus, left and right were averaged and are reported as a single value per animal for each of the measurements. The uptake rate constants K_i and K_c, the loss rate constant k_{loss}, the striatum/cortex

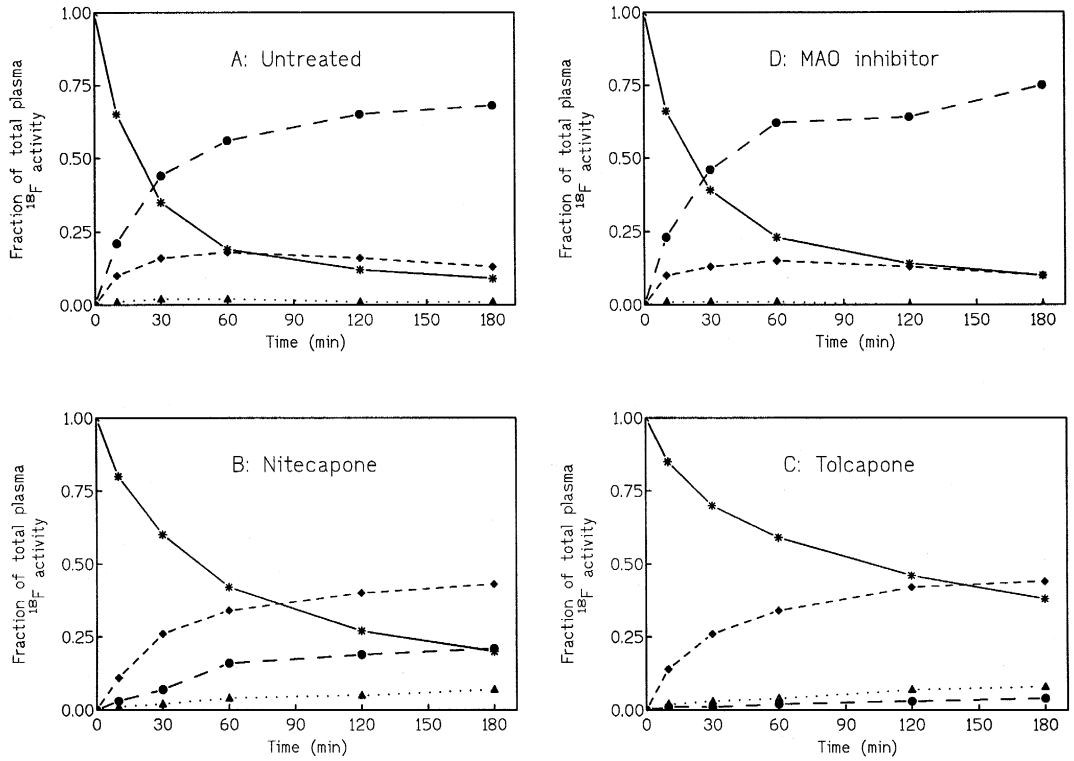

Fig. 1. Fractions of the total plasma ^{18}F activity made up by 6-[^{18}F]L-DOPA (∗), 3-*O*-methyl-6-[^{18}F]L-DOPA (○), sulfated conjugates () and 6-[^{18}F]L-dihydroxy-phenylacetic acid () as a function of time in representative animals in each of the conditions A (untreated), B (nitecapone pretreatment), C (tolcapone pretreatment) and D (monoamine oxidase inhibition). Condition E (tolcapone + monoamine oxidase inhibition) plasma metabolic profile is similar to that of condition C. Note the increase in plasma 6-[^{18}F]L-DOPA, 6-[^{18}F]L-dihydroxy-phenylacetic acid and sulfated conjugates and decrease in 3-*O*-methyl-6-[^{18}F]L-DOPA fractions with nitecapone and tolcapone pretreatments.

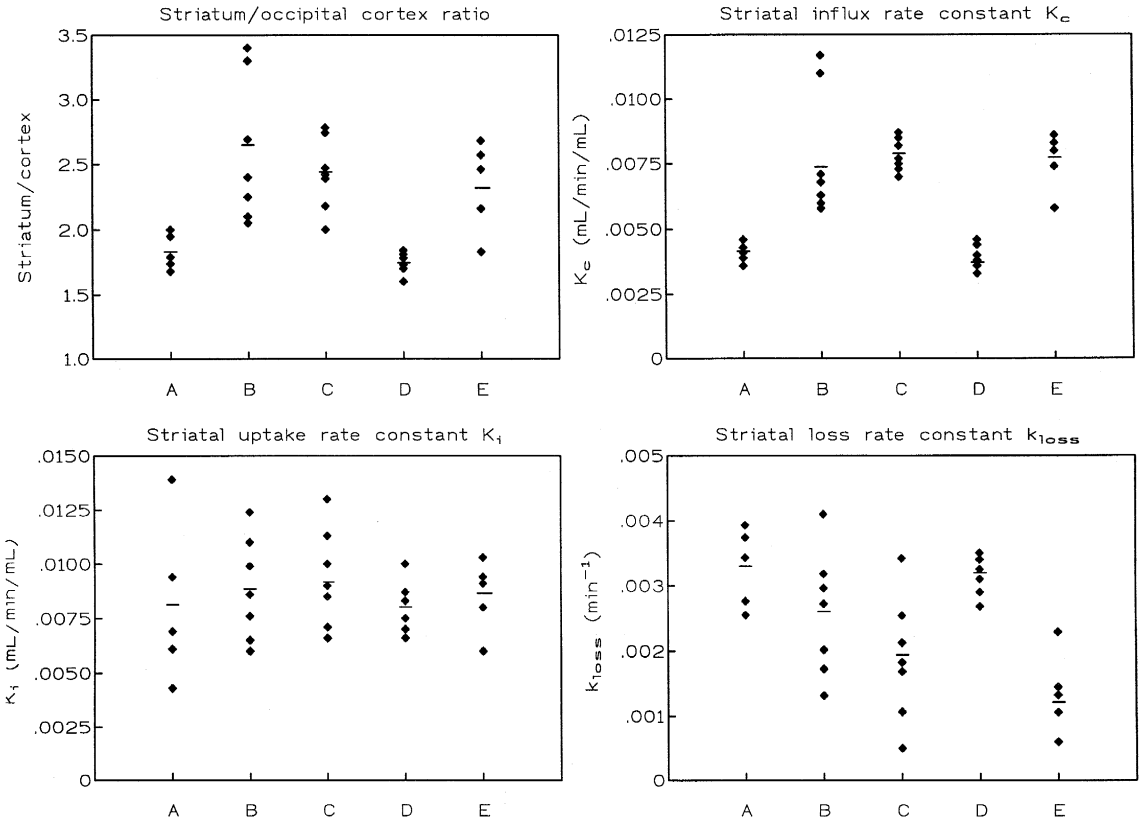

Fig. 2. Scatter plots of the effects of catechol-*O*-methyltransferase or monoamine oxidase inhibitors pretreatment on the striatum/cortex ratio (top left), influx rate constant K_c (top right), uptake rate constant K_i (bottom left) and rate of reversibility k_{loss} (bottom right). Data are represented as the averaged left–right striatal value for each animal. The horizontal line represent the mean. A: untreated; B: nitecapone pretreatment; C: tolcapone pretreatment, D: monoamine oxidase inhibitor pretreatment and E: tolcapone + monoamine oxidase inhibitor pretreatment. Please see text for significance.

ratios and the dopamine turnover index k_{loss}/K_i for each of the drug treatments were compared to the untreated condition using an analysis of variance (ANOVA) followed by Student's unpaired *t*-test between groups. No correction was made for multiple comparisons.

3. Results

The peripheral plasma data are summarized in Fig. 1. No significant differences were found in the plasma 6-[^{18}F]L-DOPA and metabolite time courses of the animals treated with the monoamine oxidase inhibitors alone compared to the untreated group. There was a significant increase ($P < 0.0001$) in the fraction of plasma 6-[^{18}F]L-DOPA and a significant decrease in the fraction of plasma 3-*O*-methyl-6-[^{18}F]L-DOPA accompanied by an increase in the fraction of 6-[^{18}F]L-dihydroxy-phenylacetic acid and sulfated conjugates in all the animals that received a catechol-*O*-methyltransferase inhibitor (nitecapone or tolcapone). Fig. 1 shows the plasma metabolic profiles of representative monkeys in conditions A (untreated), B (nitecapone), C (tolcapone) and D (monoamine oxidase inhibition). Condition E (Tolcapone + monoamine oxidase inhibition) is not shown since its plasma metabolic profile is similar to condition C. For simplicity, only the fractions of 6-[^{18}F]L-DOPA, 3-*O*-methyl-6-[^{18}F]L-DOPA, sulfated conjugates and 6-[^{18}F]L-dihydroxy-phenylacetic acid are displayed. The others (including 6-fluoro-homovanillic acid) represented less than 5% of the total radioactivity.

The brain data are summarized in Figs. 2 and 3 and Table 1. Pretreatment with monoamine oxidase inhibitors

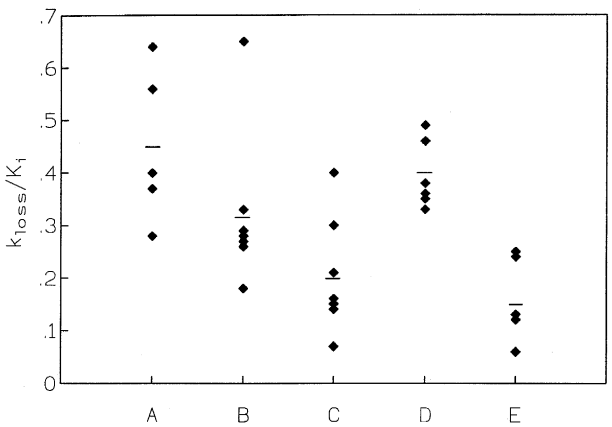

Fig. 3. Scatter plots of the effects of catechol-*O*-methyltransferase and monoamine oxidase inhibitors pretreatment on the index of dopamine turnover k_{loss}/K_i. Same legend as for Fig. 2.

Table 1
6-[^{18}F]L-DOPA data for each of the treatment condition

	Striatum/cortex	K_c (ml/min/ml)	K_i (ml/min/ml)	k_{loss} (min^{-1})	k_{loss}/K_i
Untreated $N = 5$	1.86 ± 0.19	0.0042 ± 0.0003	0.0081 ± 0.0037	0.0033 ± 0.0006	0.45 ± 0.15
Nitecapone $N = 7$	2.61 ± 0.58 [a]	0.0072 ± 0.0010 [c]	0.0089 ± 0.0024	0.0027 ± 0.0009	0.32 ± 0.15
Tolcapone $N = 7$	2.42 ± 0.28 [b]	0.0079 ± 0.0006 [c]	0.0093 ± 0.0023	0.0019 ± 0.0010 [a]	0.20 ± 0.11 [e]
Deprenyl/pargyline $N = 6$	1.74 ± 0.08	0.0038 ± 0.0004	0.0079 ± 0.0013	0.0031 ± 0.0003	0.40 ± 0.06
Tolc. + deprenyl/pargyline $N = 5$	2.34 ± 0.34 [a]	0.0076 ± 0.0011 [c]	0.0086 ± 0.0017	0.0013 ± 0.0006 [d]	0.16 ± 0.08 [f]

Data are mean ± standard deviation.
[a] $P = 0.02$. [d] $P = 0.0008$.
[b] $P = 0.0032$. [e] $P = 0.0074$.
[c] $P < 0.0001$. [f] $P = 0.005$.

did not affect the uptake rate of 6-[^{18}F]L-DOPA into the striatum, or the rate of reversibility of 6-[^{18}F]L-DOPA trapping or the effective dopamine turnover. None of the drug pretreatments significantly changed the uptake rate constant K_i of 6-[^{18}F]L-DOPA calculated with the 6-[^{18}F]L-DOPA plasma input function. However, there was a significant increase in the striatum/cortex ratio (respectively 40%, $P = 0.02$; 30%, $P = 0.0032$ and 26%, $P = 0.025$) and influx rate constant K_c (respectively 79%, $P < 0.0001$; 96%, $P < 0.0001$ and 90%, $P < 0.0001$) in each of the conditions B, C and E when the animals received a catechol-*O*-methyltransferase inhibitor pretreatment. There was no significant difference from control in the loss rate constant k_{loss} or the index of dopamine turnover k_{loss}/K_i after pretreatment with the peripheral catechol-*O*-methyltransferase inhibitor nitecapone. There was a significant decrease in k_{loss} and the index of effective dopamine turnover k_{loss}/K_i in the group receiving tolcapone alone (respectively: 43%, $P = 0.02$ and 55%, $P = 0.0074$) and the group receiving the combination tolcapone + monoamine oxidase inhibitor (respectively: 59%; $P = 0.0008$ and 65%; $P = 0.005$) compared to the untreated group.

4. Discussion

Both catechol-*O*-methyltransferase and monoamine oxidase inhibitors have been proposed and tried as adjuvants to levodopa therapy in Parkinson's disease. Although clinical trials of recently developed catechol-*O*-methyltransferase inhibitors unanimously suggest that peripheral catechol-*O*-methyltransferase inhibition potentiates levodopa therapy (Mannisto and Kaakkola, 1990; Da Prada et al., 1994), results concerning monoamine oxidase inhibition are more ambiguous with both reported benefits as well as no change (Birkmayer and Riederer, 1984; Elizan et al., 1989; Kofman, 1991; Myllyla et al., 1991). In spite of the wide use of these inhibitors, little data exist on their effects on central dopamine function in primates. The aim of this study was to investigate, in vivo, the effects of an acute dose of catechol-*O*-methyltransferase and/or monoamine oxidase inhibitor on the metabolism of 6-[^{18}F]L-DOPA, a tracer specific for the dopamine presynaptic neurons and a widely used marker of dopamine function.

The peripheral plasma data are in good agreement with previous PET studies on the effects of catechol-*O*-methyltransferase inhibitors, i.e. an increase in the plasma availability of 6-[^{18}F]L-DOPA with concomitant decrease in 3-*O*-methyl-6-[^{18}F]L-DOPA and increases in the fractions of both 6-[^{18}F]L-dihydroxy-phenylacetic acid and sulfated conjugates (Linden et al., 1988; Maj et al., 1990; Kaakkola et al., 1990; Laihinen et al., 1992; Doudet et al., 1997). At the doses employed, the plasma levels of 6-[^{18}F]L-DOPA were lower and the plasma levels of 3-*O*-methyl-6-[^{18}F]L-DOPA higher after nitecapone compared to tolcapone (Fig. 1). Studies by others using nitecapone at various dosages have also failed to reduce the plasma concentrations of 3-*O*-methyl-6-[^{18}F]L-DOPA to less than 15–20% of their untreated values (Linden et al., 1988; Kaakkola et al., 1990). Tolcapone inhibits *O*-methylation of 6-[^{18}F]L-DOPA both peripherally and, in sufficient dosage, centrally. In contrast, nitecapone does not cross the blood brain barrier and 6-[^{18}F]L-DOPA can still be *O*-methylated in the brain. The higher plasma concentrations of 3-*O*-methyl-6-[^{18}F]L-DOPA after nitecapone pretreatment as compared to tolcapone may be due to the diffusion of the central *O*-methylation products of 6-[^{18}F]L-DOPA out of the brain (Doudet et al., 1991).

As anticipated, monoamine oxidase inhibition alone had no effect on the peripheral or central metabolism (i.e. decarboxylation and storage processes) of 6-[^{18}F]L-DOPA, as L-DOPA is not a substrate for monoamine oxidase. In addition, monoamine oxidase B, the primary monoamine catabolic enzyme in primates, is located extracellularly to the dopaminergic neurons and thus, would not interfere with the decarboxylation and storage processes of dopamine or 6-[^{18}F]-dopamine in the dopaminergic terminals. However, neither 6-[^{18}F]L-DOPA rate of reversibility nor the effective turnover were affected by monoamine oxidase inhibition. It is possible that the doses of inhibitors used were not sufficient to produce enough enzyme inhibition. Indeed, keeping in mind the animal's welfare, we did not attempt to block completely the monoamine oxidase enzy-

matic processes. Alternatively, it is generally accepted that, under physiological conditions, only small quantities of active enzyme are necessary to handle endogenous dopamine metabolism in normal conditions and that the enzyme has spare capacity for its substrate. Nomoto and Fukuda (1993) demonstrated that selective inhibition of monoamine oxidase-B activity did not provoke changes in behavior or microdialysis concentrations of dopamine metabolites in either normal or MPTP-treated marmosets. Changes could only be elicited when exogenous pharmacological doses of L-DOPA/benserazide (10/5 mg/kg) was administered in combination. The dose of 6-[^{18}F]L-DOPA administered as the tracer (1–3 mg) during the PET studies was likely to be too small to produce a measurable effect.

On the other hand, catechol-*O*-methyltransferase inhibition produced measurable effects on dopamine metabolism even in the absence of co-administered pharmacological amounts of levodopa (Napolitano et al., 1995). Increases in the striatum/cortex ratio and the influx rate constant K_c (calculated with the cortical time course as the input function) after catechol-*O*-methyltransferase treatment have been reported by others (Sawle et al., 1994; Ruottinen et al., 1995; Ishikawa et al., 1996). Apart from reflecting the increased bioavailability of 6-[^{18}F]L-DOPA, the changes in both measures are partly a consequence of the decrease in circulating 3-*O*-methyl-6-[^{18}F]L-DOPA that represents most of the non-specific background activity. Significant increases in these two measures were found in each condition that included catechol-*O*-methyltransferase pretreatment. We did not find a significant change in K_i in our normal cynomolgus monkeys; this concurs with data from others (Sawle et al., 1994; Ruottinen et al., 1995; Ishikawa et al., 1996). Since K_i reflects the rate of uptake and decarboxylation of 6-[^{18}F]L-DOPA and the storage of 6-[^{18}F]-dopamine in the dopaminergic terminals, our data suggest that, at the doses used, catechol-*O*-methyltransferase inhibition does not affect these processes.

The measurable loss of striatal radioactivity during the extended positron emission tomographic studies likely reflects the catabolism of 6-[^{18}F]-dopamine by the action of monoamine oxidase and catechol-*O*-methyltransferase and the diffusion of 6-[^{18}F]-dopamine metabolites out of the striatum (Barrio et al., 1990). Inhibition of central dopamine metabolism would be expected to reduce this loss. We recently described a simple fitting method, based on an extension of the classical graphical analysis of Patlak and Blasberg (1985) to calculate this rate of loss, k_{loss} (Holden et al., 1997). Thus, k_{loss} alone could be considered an index of DA turnover. However, we reasoned that an index of turnover should take into consideration, not only the loss of striatal radioactivity, k_{loss}, but also the initial accumulation of 6-[^{18}F]L-DOPA, as measured by K_i. Thus, we used the ratio k_{loss}/K_i as the index of the effective turnover.

The index of dopamine turnover estimated in these PET studies differs from the conventional concept of neurotransmitter turnover. 6-[^{18}F]L-DOPA cannot trace the dopamine turnover as such. Under normal circumstances, dopamine is synthesized from the decarboxylation of L-DOPA made in the nerve terminals from the hydroxylation of tyrosine. Exogenous L-DOPA, whether as a therapeutic dose or as a labelled tracer, enters this pathway in the middle and thus is not sensitive to the regulation of the hydroxylation step, the rate limiting step in the synthesis of dopamine. Rather, 6-[^{18}F]L-DOPA tests the relative strengths of the uptake (K_i) and elimination processes (k_{loss}) present in the striatum. Though these processes may not act on endogenous L-DOPA at the same rates, our index of effective turnover can still provide information about the variations of dopamine storage capacity between subjects or pharmacological interventions. A change in the K_i/k_{loss} index would then reflect an active physiological process.

In the case of this particular pharmacological intervention, since K_i remained unchanged, the change in the index of dopamine turnover showed the same trends as k_{loss}. As predicted, the peripheral inhibitor nitecapone failed to significantly reduce k_{loss} or k_{loss}/K_i. However, catechol-*O*-methyltransferase inhibition with the peripheral AND central inhibitor tolcapone significantly reduce k_{loss} (43%) and k_{loss}/K_i (55%), suggesting that tolcapone was centrally effective in inhibiting not only 6-[^{18}F]L-DOPA metabolism but also 6-[^{18}F]-dopamine metabolism, thus increasing the half life of the trapped radioactivity, presumably 6-[^{18}F]-dopamine.

Interestingly, combined inhibition of catechol-*O*-methyltransferase with tolcapone and monoamine oxidase with either deprenyl or pargyline further reduced k_{loss} (59%) and the effective turnover index (65%). One may speculate, from rodent studies, that in the presence of an increased pool of synaptic 6-[^{18}F]-dopamine due to the inhibition of catechol-*O*-methyltransferase, the quantities of uninhibited monoamine oxidase are insufficient to handle the increase concentrations of substrate; this further increases the half life of trapped radioactivity in the striatum.

In conclusion, selective catechol-*O*-methyltransferase inhibitors and especially catechol-*O*-methyltransferase inhibitors with a central component, such as tolcapone, may provide clinical benefits to parkinsonian patients above and beyond those provided by peripheral inhibitors only. Based on our data in this small group of animals, monoamine oxidase inhibition may potentiate the effects of catechol-*O*-methyltransferase inhibition. However, these data need to be replicated in a larger population and especially in animals with lesions of the nigro–striatal pathway, using selective monoamine oxidase-A and monoamine oxidase-B inhibitors. The choice of the most appropriate monoamine oxidase inhibitor for maximum clinical benefits could be investigated from the battery of new selective and reversible inhibitors of monoamine oxidase-A and monoamine oxidase-B, associated with low side effects, that have recently become available.

Acknowledgements

This work was supported by the Medical Research Council of Canada. We thank Merck, Sharp and Dohme, Canada for their gift of the carbidopa, Orion Pharmaceutica, Finland for their gift of nitecapone and Hoffman LaRoche, Basel, Switzerland for their gift of tolcapone. The authors thank the staff of the UBC/TRIUMF PET program for their assistance and contribution to this work, especially Dr. M.J. Adam and Ms. Salma Jivan for tracer synthesis, Ms. Poppy Schofield and Ms. Teresa Dobko for data acquisition and processing. We are indebted to Ms. Kellie Hewitt for her expert technical assistance during the animal PET studies. Special thanks are due to Dr. J. Love and Mr. Mike Boyd and the personnel of the UBC Animal Care Facilities.

References

Adam, M.J., Ruth, T.J., Grierson, J.R., Abeysekera, B., Pate, B.D., 1986. Routine synthesis of L-[^{18}F]6-fluorodopa with F-18 acetyl hypofluorite. J. Nucl. Med. 27, 1462–1466.

Backstrom, R., Honkanen, E., Pippuri, A., Kairisalo, P., Pystynen, J., Heinola, K., Nissinen, E., Linden, I.B., Mannisto, P.T., Kaakkola, S., Pohto, P., 1990. Synthesis of some novel potent and selective catechol *O*-methyltransferase inhibitors. J. Med. Chem. 32, 841–846.

Barrio, J.R., Huang, S.C., Melega, W.P., Yu, D.C., Hoffman, J.M., Schneider, J.S., Satyamurthy, N., Mazziotta, J.C., Phelps, M.E., 1990. 6-[^{18}F]fluoro-L-DOPA probes dopamine turnover rates in central dopaminergic structures. J. Neurosci. Res. 27, 487–493.

Birkmayer, W., Riederer, P., 1984. Deprenyl prolongs the therapeutic efficacy of combined L-Dopa in Parkinson's disease. In: Hassler, R.G., Christ, J.F. (Eds.), Advances in Neurology, vol. 40. Raven Press, New York, pp. 475–481.

Brooks, D., Salmon, E.P., Mathias, C.J., Quinn, N., Leenders, K.L., Bannister, R., Marsden, C.D., Frackowiak, R.S.J., 1990. The relationship between locomotor disability, autonomic dysfunction and the integrity of the striatal dopaminergic system in patients with multiple system atrophy, pure autonomic failure and Parkinson's disease, studied with PET. Brain 113, 1539–1552.

Chan, G.L.Y., Hewitt, K.A., Pate, B.D., Schofield, P., Adam, M.J., Ruth, T.J., 1992. Routine determination of [F18]-L-6-fluorodopa and its metabolites in blood plasma is essential for accurate positron emission tomography studies. Life Sci. 50 (4), 309–318.

Chiueh, C.C., Zubowska-Grojec, Z., Kirk, K.L., Kopin, I.J., 1983. 6-fluorocatecholamines as false adrenergic neurotransmitters. J. Pharmacol. Exp. Ther. 225, 529–533.

Cumming, P., Leger, G., Kuwabara, H., Gjedde, A., 1993. Pharmacokinetics of plasma 6-[18F]fluoro-L-3,4-dihydroxy-phenylalanine ([18F]FDOPA) in humans. J. Cereb. Blood Flow Metab. 13, 668–675.

Da Prada, M., Kettler, R., Keller, H.H., Cesura, A.M., Richards, J.G., Saura Marti, J., Muggli-Maniglio, D., Wyss, P.C., Kyburz, E., Imhof, R., 1990. From moclobemide to Ro 19-6327 and Ro 41-1049: The development of a new class of reversible, selective MAO-A and MAO-B inhibitors. J. Neural Transm. 29 (Suppl.), 279–292.

Da Prada, M., Borgulya, J., Napolitano, A., Zurcher, G., 1994. Improved therapy of Parkinson's disease with tolcapone, a central and peripheral COMT inhibitor with an *S*-adenosyl-L-methionine-sparing effect. Clin. Neuropharmacol. 17, S26–S37.

Doudet, D.J., McLellan, C.A., Carson, R.E., Adams, H.R., Miyake, H., Aigner, T.G., Finn, R.T., Cohen, R.M., 1991. Distribution and kinetics of 3-*O*-methyl-6-[F18]fluoro-L-DOPA in the rhesus monkey brain. J. Cereb. Blood Flow Metab. 11, 726–734.

Doudet, D.J., Chan, G.L.Y., Holden, J.E., Morrison, K.S., Dobko, T., Wyatt, R.J., Ruth, T.J., 1997. Effects of catechol-*O*-methyltransferase inhibition on the rates of uptake and reversibility of 6-fluoro-L-dopa trapping in MPTP-induced parkinsonism in monkeys. Neuropharmacology 36, 363–371.

Elizan, T.S., Yahr, M.D., Moros, D.A., Mendoza, M.R., Pang, S., Bodian, C.A., 1989. Selegiline use to prevent progression of Parkinson's disease. Arch. Neurol. 46, 1275–1279.

Evans, B.R., Harrop, D., Heywood, J., Mackintosh, R.W., Moore, R.W., Pate, B.D., Rogers, J.G., Ruth, T.J., Sayre, C., Sprenger, H., Van Oers, N., Guang, Y.X., 1983. Engineering development on the aUBC-TRIUMF modified PETT VI positron emission tomograph. IEEE Trans. Nucl. Sci. NS-30, 707–710.

Firnau, G., Sood, S., Chirakal, R., Nahmias, C., Garnett, E.S., 1987. Cerebral metabolism of 6-^{18}F-fluoro-L-3,4-dihydroxyphenylalanine in the primate. J. Neurochem. 48, 1077–1082.

Garnett, E.S., Firnau, G., Nahmias, C., Sood, S., Belbeck, L.W., 1980. Blood–brain barrier transport and cerebral utilization of dopa in living monkeys. Am. J. Physiol. 238, R318–327.

Holden, J.E., Doudet, D.J., Endres, C.J., Chan, G.L.Y., Morrison, K.S., Vingerhoets, F.J.G., Snow, B.J., Pate, B.D., Sossi, V., Buckley, K.R., Ruth, T.J., 1997. Extended graphical analysis of 6-fluoro-L-dopa trapping: Effect of inhibition of catechol-*O*-methyltransferase on the rate of reversibility. J. Nucl. Med., in press.

Ishikawa, T., Dhawan, V., Chaly, T., Robeson, W., Belakhlef, A., Mandel, F., Dahl, R., Margouleff, C., Eidelberg, D., 1996. Fluorodopa positron emission tomography with an inhibitor of catechol-*O*-methyltransferase: Effect of the plasma 3-*O*-methyldopa fraction on data analysis. J. Cereb. Blood Flow Metab. 16, 854–863.

Kaakkola, S., Gordin, A., Jarvinen, M., Wikberg, T., Schultz, E., Nissinen, E., Pentikainen, P.J., Rita, H., 1990. Effect of a novel catechol-*O*-methyltransferase inhibitor, nitecapone, on the metabolism of L-dopa in healthy volunteers. Clin. Neuropharmacol. 13, 436–447.

Kofman, O.S., 1991. Deprenyl: Protective vs. symptomatic effect. Can. J. Neurol. Sci 18, 83–86.

Laihinen, A., Rinne, J.O., Rinne, U.K., Haaparanta, M., Ruotsalainen, U., Bergman, J., Solin, O., 1992. [F18]-6-fluorodopa PET scanning in Parkinson's disease after selective COMT inhibition with nitecapone (OR-462). Neurology 42, 199–203.

Linden, I.B., Nissinen, E., Etemadzadeh, E., Kaakkola, S., Mannisto, P.T., Pohto, P., 1988. Favorable effect of catechol-*O*-methyltransferase inhibition by OR-462 in experimental models of Parkinson's disease. J. Pharmacol. Exp. Ther. 247, 289–293.

Maj, J., Rogoz, Z., Skuza, G., Sowinska, H., Superata, J., 1990. Behavioral and neurochemical effects of Ro 40-7592, a new COMT inhibitor with a potential therapeutic activity in Parkinson's disease. J. Neural Transm. Parkinson's Dis. Dementia Sect. 2, 101–112.

Mannisto, P.T., Kaakkola, S., 1990. Rationale for selective COMT inhibitors as adjuncts in the drug treatment of Parkinson's disease. Pharmacol. Toxicol. 66, 317–323.

Martin, W.R.W., Palmer, M.R., Patlak, C.S., Calne, D.B., 1989. Nigrostriatal function in humans studied with positron emission tomography. Ann. Neurol. 26, 535–542.

Melega, W.P., Hoffman, J.M., Luxen, A., Nissenson, C.H.K., Phelps, M.E., Barrio, J.R., 1990a. The effects of carbidopa in the metabolism of 6-[F18]fluoro-L-dopa in rats, monkeys, and humans. Life Sci. 47, 149–157.

Melega, W.P., Luxen, A., Perlmutter, M., Nissenson, C.H.K., Phelps, M.E., Barrio, J.R., 1990b. Comparative in vivo metabolism of 6-[18F]fluoro-L-dopa and [3H]L-dopa in rats. Biochem. Pharmacol. 39, 1853–1860.

Myllyla, V.V., Sotaniemi, K.A., Vuorinen, J.A., 1991. Selegiline as a primary treatment of Parkinson's disease. Acta Neurol Scand. 84 (Suppl.), 70–72.

Napolitano, A., Zurcher, G., Da Prada, M., 1995. Effects of tolcapone, a novel catechol-*O*-methyltransferase inhibitor, on striatal metabolism of L-DOPA and dopamine in rats. Eur. J. Pharmacol. 273, 215–221.

Nomoto, M., Fukuda, T., 1993. A selective MA*O*-B inhibitor Ro19-6327 potentiates the effects of levodopa on parkinsonism induced by MPTP in the common marmoset. Neuropharmacology 32, 473–477.

Patlak, C.S., Blasberg, R.G., 1985. Graphical evaluation of blood-to-brain transfer constants from multiple-time uptake data. Generalizations. J. Cereb. Blood Flow Metab. 5, 584–590.

Ruottinen, H., Rinne, J.O., Ruotsalainen, U., Bergman, J., Oikonen, V., Haaparanta, M., Solin, O., Laihinen, A., Rinne, U.K., 1995. Striatal [18F]fluorodopa utilization after COMT inhibition with entacapone studied with PET in advanced Parkinson's disease. J. Neural Transm. 10, 91–106.

Sawle, G.V., Burn, D.J., Morrish, P.K., Lammertsma, A.A., Snow, B.J., Luthra, S.K., Osman, S., Brooks, D.J., 1994. The effect of entacapone (OR-611) on brain [18-F]-6-L-fluorodopa metabolism: Implications for levodopa therapy of Parkinson's disease. Neurology 44, 1292–1297.

Sossi, V., Buckley, K.R., Snow, B.J., Holden, J.E., Morrison, K.S., Pate, B.D., Ruth, T.J., 1993. Recovery of the human striatal signal in a slice oriented positron emission tomograph. J. Nucl. Med. 34, 481–487.

Yu, P.H., 1994. Pharmacological and clinical implications of MAO-B inhibitors. Gen. Pharmac. 25, 1527–1539.