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Central action of benserazide after COMT inhibition demonstrated in vivo by PET*

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Summary. Positron emission tomography (PET) following intravenous administration of β-[^{11}C]-L-DOPA provides a method of assessing regional cerebral uptake and utilization of levodopa. Cerebral levodopa kinetics in the rhesus monkey were investigated after the inhibition of catechol-O-methyltransferase (COMT) with RO 40-7592, and after coadministration of the peripheral aromatic L-amino acid decarboxylase (AADC) inhibitors benserazide and carbidopa. Pretreatment with RO 40-7592 (10 mg/kg), benserazide (10 mg/kg) or carbidopa (3.5 mg/kg) did not change striatal k_3, which mainly reflects the ability for the brain tissue to convert [^{11}C]-L-DOPA to [^{11}C]-dopamine, although the brain's uptake of radioactivity increased substantially after pretreatment with the AADC inhibitors. When benserazide was coadministered with RO 40-7592 (10 mg/kg) a dose-dependent decrease in striatal k_3 was measured with an apparent ED_{50} of 3 mg/kg. No such effect was indicated after pretreatment with the combination of RO 40-7592 (10 mg/kg) and carbidopa (3.5 mg/kg). The possible negative interactions of coadministration with COMT inhibitors and predominantly peripherally acting AADC inhibitors must be considered when used in the therapy of Parkinson's disease.

Keywords: Tomography, monkey, L-DOPA, aromatic L-amino acid decarboxylase, catechol-O-methyltransferase, Parkinson's disease.

Introduction

Catechol-O-methyltransferase (COMT) O-methylates a wide variety of catechols (Guldberg and Marsden, 1975), the highest activities being found in the liver and kidney (Jefferey and Roth, 1985). In vivo, methylation of catecholamines and related substances almost exclusively results in the formation of m-

* A contribution from the Uppsala University PET centre

0-methylated metabolities by the transfer of the methyl group from S-adenosyl-L-methionine to the hydroxyl groups of the catechols (Axelrod, 1966).

The use of COMT inhibitors is assumed to have potential therapeutic benefits in clinical disorders such as depression and Parkinson's disease (PD). They may also be used to improve the signal-to-noise ratio for [^{18}F]fluoro-L-DOPA PET scans (Hartvig et al., 1991 a). Several COMT inhibitors have recently been synthesized and pharmacologically evaluated (Nissinen et al., 1988; Borgulya et al., 1989). RO 40-7592 (3,4-dihydroxy-4-methyl-5-nitrobenzophenone) has been shown to be a potent peripherally and centrally acting reversible inhibitor of COMT (Zürcher et al., 1990).

As an adjunct to conventional levodopa therapy in PD, the administration of a COMT inhibitor is expected to increase levodopa bioavailability and prolong its half-life in plasma by reducing the peripheral conversion of L-DOPA to 3-O-methyldopa (3-OMD) (Nutt, 1987). However, to reduce the peripheral conversion to dopamine, L-DOPA is administered combined with an inhibitor of extracerebrally-acting aromatic L-amino acid decarboxylase (AADC). At present two AADC inhibitors are used for this purpose: benserazide ([N-(DL-seryl)-N-(2,3,4-trihydroxybenzyl)hydrazine], available as 28.5 mg/100 mg L-DOPA, Madopar®) and carbidopa (α-methyldopahydrazine], available as 10 and 25 mg/100 mg L-DOPA, Sinemet®). Both of these, as well as one metabolite of benserazide (2,3,4-trihydroxybenzylhydrazine), have been shown to act as substrates of COMT (Hagan et al., 1980). Thus, COMT inhibition is also in itself expected to increase the bioavailability of these AADC inhibitors.

Recently, ^{11}C-labelled levodopa has been used in studies on presynaptic dopaminergic function in vivo with PET (Tedroff et al., 1991; Hartvig et al., 1991 b). In the present investigation we used PET and β-^{11}C-labelled L-DOPA to investigate possible interactions between the peripheral AADC inhibitors and RO 40-7592 on the intracerebral uptake and utilization of levodopa.

Materials and methods

Animals

Ten female rhesus monkeys of unknown age weighing 6–10 kg from the Primate Laboratory of Reproductive Research, University of Uppsala were used in the study. The animals were anaesthetized with ketamine (Parke-Davis; 10 mg/kg/h i.m).

The study was approved by the Ethics Committee for Animal Research of the University of Uppsala.

Drugs

The following drugs were used: L-3,4-dihydroxyphenylalanine, levodopa (Hoffman-La Roche Basel, Switzerland) dissolved in 5.5% glucose and pH adjusted to 3–4. Benserazide, (Hoffman-La Roche, Basel, Switzerland) dissolved in polyethyleneglycol 1 ml, propyleneglycol 1 ml, and sterile water 1 ml, and carbidopa (Merck, Sharp and Dohme, USA) dissolved in sterile water. Benserazide and carbidopa were injected i.v. 15 minutes prior to the PET scan. RO 40-7592 (Hoffman-La Roche, Basel, Switzerland) was dissolved in polyethyle-

neglycol 1 ml, propyleneglycol 1 ml, and sterile water 1 ml, and 10 mg/kg was injected i.v. 30 minutes before the investigation.

Injection schedules

All ten monkeys were subject to baseline investigations. Six animals were pretreated with RO 40-7592 10 mg/kg, benserazide 10 mg/kg, or carbidopa 3.5 mg/kg, two animals receiving each drug, respectively. The 3.5 mg/kg of carbidopa administered was supposed to be equipotent, with respect to extracerebral AADC inhibition, to 10 mg/kg benserazide. Four monkeys were pretreated with a combination of RO 40-7592 10 mg/kg and increasing doses of benserazide (1, 3, 5, 10 mg/kg), one dose for each monkey. Additionally, one of the monkeys was investigated a third time after pretreatment with RO 40-7592 10 mg/kg and 3.5 mg/kg carbidopa.

Radiochemistry

The radionuclide ^{11}C was produced by the Tandem accelerator at The Svedberg Laboratory, Uppsala University. [^{11}C]-L-DOPA labelled in the β-position was synthesized as described elsewhere (specific activity 2–17 Ci/mmol) (Bjurling et al., 1990). After analysis for identity and radiochemical and chemical purity, a buffer solution of [^{11}C]-L-DOPA was filtered through a 0.22 μm filter before intravenous administration to the monkeys. To saturate compartments located extracerebrally, 3 mg of levodopa solution was added to the solution containing the radioactive substance before injection (Hartvig et al., 1991 b). The radioactivity injected varied from 0.9 to 4.2 mCi.

PET

PET was performed with the monkeys lying with the head fixed in a two ring PET system (Scanditronix PC 384-3B). The monkeys were positioned in the scanner with the aid of a laser than situated at a predetermined distance from the axial planes. The brain scanner allows simultaneous acquisition of data from three consecutive 14 mm slices with an in-plane resolution of 7.6 mm full width at half-maximum. The radioactive substance was administered i.v. given via a catheter in the hind leg of the monkey. Immediately after administration, a series of scans were started to obtain data on regional distribution. Each investigation continued for approximately 50 minutes. PET images were reconstructed for each sequential measurement. The striatum (40–50 pixels, 2.5–3.1 cm^2) and tissue in the back of the brain in the section containing the striatum (250 pixels) were chosen as regions-of-interest (ROI) from PET scan images and compared with an atlas of the brain of the rhesus monkey made from cryosectioning. The ROI chosen from the back of the brain was used as reference tissue. The radioactivities measured in these regions at different time intervals were corrected for physical decay to the time of administration. The tissue radioactivities measured were normalized for injected radioactivity to the body weight of the monkeys "uptake" and expressed as:

$$\frac{\text{tissue radioactivity/cm}^3}{\text{injected radioactivity/g body weight}}$$

By approximating 1 gram $\approx$ 1 cm^3 uptake 1, will correspond to homogeneous dilution of the radioactivity in the whole body of the monkey.

Calculations

Data were quantified according to a reduced three-compartment model using reference tissue, as presented elsewhere (Tedroff et al., 1991). The graphical solution of the model

Table 1. Effects on striatal k_3 and reference tissue uptake of ^{11}C after pretreatment with benserazide, carbidopa or RO 40-7592

Investigation	k_3 (min^{-1})	uptake
Baseline ± SD	0.0105 ± 0.0011	0.59 ± 0.14
Benserazide 10 mg/kg i.v.	0.0103	0.94
	0.0099 N.S	1.06 $p < 0.005$
Carbidopa 3.5 mg/kg i.v.	0.0113	0.97
	0.0105 N.S	1.01 $p < 0.005$
RO 40-7592 10 mg/kg i.v.	0.0121	0.42
	0.0094 N.S	0.47 N.S

yields a unidirectional first-order rate constant, k_3, which directly relates to the ability of the brain tissue to decarboxylate the tracer by the action of AADC. Since AADC has a low affinity for L-DOPA, the dose of L-DOPA adminstered does not influence the calculation of k_3 (Tedroff et al., 1990).

Linear regression analysis was determined by the best least-square fit. The results from each investigation with drugs were compared to the ten baseline investigations by means of Student's t-test.

Results

For the ten baseline scans, striatal k_3 was calculated to 0.0105 min^{-1} ± 0.0011 (S.D.) (range 0.009–0.012 min^{-1}). Average uptake in the reference tissue 5–45 minutes after injection of the radioactivity was 0.59 ± 0.14 (S.D.) (range 0.40–0.79) homogeneous dilution of radioactivity.

Pretreatment with RO 40-7592 produced no change in striatal k_3 or the uptake in the reference tissue. Following pretreatment with benserazide (10 mg/kg) or carbidopa (3.5 mg/kg), uptake in the reference tissue increased significantly but striatal k_3 remained unchange (Table 1).

Pretreatment with RO 40-7592 and benserazide did not further enhance the uptake of radioactivity in the brain. When the animals were pretreated with the two drugs, striatal k_3 decreased dose-dependently and when 10 mg/kg benserazide was coadministered with the COMT inhibitor there was almost no decarboxylation of [^{11}C]-L-DOPA in the striatum (Fig. 1, Table 2).

Coadministration of carbidopa (3.5 mg/kg) and RO 40-7592 in one monkey did not significantly decrease striatal k_3 (Table 2).

Discussion

RO 40-7592 has been shown to abolish elevations of 3-OMD, after administration of levodopa in both plasma and brain and thus markedly elevate levodopa bioavailability (Zürcher et al., 1990). In mice, oral R0 40-7592 30 mg/kg causes a complete suppression of 3-OMD formation for several hours after

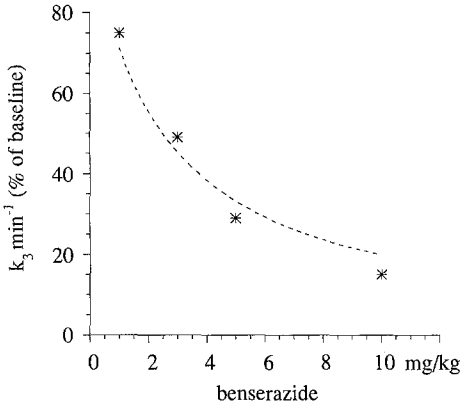

Fig. 1. Calculated striatal k_3 (y-axis) in four monkeys after pretreatment with the COMT inhibitor RO 40-7592 (10 mg/kg) and increasing doses of benserazide (x-axis). Each point represents one monkey (see also Table 2). Values are expressed as percentages of corresponding baseline investigations made without the addition of drug

administration of L-DOPA. Following the administration of oral RO 40-7592 10 mg/kg in monkeys (Macaca Sylvana) plasma levodopa AUC increases more than 6-fold with a simultaneous inhibition of 3-OMD formation (Muggli et al., 1989).

In this investigation we found no significant change in the distribution of radioactivity in the brain following pretreatment with RO 40-7592. 3-OMD can readily penetrate the blood brain barrier using the same transport system as L-DOPA (Wade and Katzman, 1971). Since PET only measures total radioactivity, suppression of [^{11}C]-3-OMD formation will increase the relative amount of [^{11}C]-L-DOPA without affecting the total amount of radioactivity entering the brain.

As expected, pretreatment with benserazide or carbidopa markedly increases

Table 2. Effects on striatal k_3 and reference tissue uptake of ^{11}C after pretreatment with RO 40-7592 (10 mg/kg) and the AADC inhibitors benserazide or carbidopa

Dose	k_3 (min^{-1})	Uptake	Baseline k_3 (min^{-1})	Baseline uptake
Benserazide				
1 mg/kg	0.0072	0.64	0.0099	0.45
3 mg/kg	0.0050	1.16	0.0108	0.66
5 mg/kg	0.0028	0.59	0.0102	0.45
10 mg/kg	0.0014	0.85	0.0091	0.64
Carbidopa				
3.5 mg/kg	0.0103	0.75	0.0118	0.43

brain uptake of radioactivity. Extracerebral AADC inhibition will decrease the amount of radioactivity being converted to [^{11}C]-dopamine, which does not easily penetrate the blood-brain barrier (Oldendorf, 1971). Although uptake increases, kinetic analysis revealed that high doses of benserazide (10 mg/kg) or carbidopa (3.5 mg/kg) do not alter the striatal k_3, which is compatible with a predominantly extracerebral action using the present doses.

Striatal k_3 decreased dose-dependently following the combined administration of benserazide and RO 40-7592. When RO 40-7592 and benserazide (10 mg/kg) were coadministered almost no striatal accumulation of radioactivity was measured. The PET image and the intracerebral kinetics were similar to those seen after pretreatment with NSD 1015, an AADC inhibitor which enters the brain (Tedroff et al., 1991). Benserazide and carbidopa have been shown to the potent competitive inhibitors of COMT in previous investigations (Hagan et al., 1980). Both benserazide and the metabolite 2,3,4-trihydroxybenzylhydrazine are better substrates for COMT than carbidopa or levodopa itself. It has previously been shown that benserazide, after high doses given to experimental animals, enters the brain and inhibits the conversion of L-DOPA to dopamine (Plechter, 1973).

Based on the foregoing, we believe that COMT inhibition will increase plasma levels of benserazide and its active metabolite leading to entry of substantial amounts of AADC inhibiting drug into the brain. This effect was not found after coadministration of 10 mg/kg RO 40-7592 and 3.5 mg/kg carbidopa, an equipotent dose of carbidopa to the 10 mg/kg of benserazide which combined with RO 40-7592 almost completely suppressed striatal k_3. Carbidopa has not been shown to enter the brain even in high doses (Plechter, 1973) and the affinity as well as V_{max} of COMT for carbidopa are lower compared to benserazide (Hagan et al., 1980). However, both benserazide and carbidopa may presumably without preceding COMT inhibition inhibit cerebral AADC, if administered in much higher doses than used in the present investigation.

The results of the present investigation may have clinical implications. The therapeutic benefits of levodopa therapy in Parkinson's disease are generally agreed to be due to the action of dopamine formed from the administered L-DOPA. The necessity of keeping adequate plasma levels of the amino acid to maintain clinical effect is well established (Lees, 1989). As the ability for the remaining dopaminergic neurons to convert levodopa to dopamine is crucial for the therapeutic benefit, substantial inhibition of brain AADC activity will most likely reduce the clinical response of the drug. Although the doses of benserazide used to markedly inhibit striatal AADC after COMT inhibition were considerably higher than those used in clinical praxis, the possible cerebral AADC inhibiting effects after long-term coadministration of COMT inhibitors and benserazide must be considered.

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