ELSEVIER

European Journal of Pharmacology 273 (1995) 215–221

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Effects of tolcapone, a novel catechol-*O*-methyltransferase inhibitor, on striatal metabolism of L-DOPA and dopamine in rats

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Received 1 August 1994; revised 21 October 1994; accepted 1 November 1994

Abstract

In vivo brain microdialysis was used to assess the effects of tolcapone, a novel central and peripheral inhibitor of catechol-*O*-methyltransferase on striatal 3,4-dihydroxyphenyl-L-alanine (L-DOPA) and dopamine metabolism. The oral administration of 30 mg/kg of tolcapone failed to change dopamine output but elicited a marked and long-lasting decrease of the extracellular levels of homovanillic acid (HVA) and 3-methoxytyramine with a concomitant increase of 3,4-dihydroxyphenylacetic acid (DOPAC). The administration of L-DOPA (20 and 60 mg/kg p.o.) + benserazide (15 mg/kg p.o.) resulted in a dose-dependent increase of dialysate levels of L-DOPA and 3-*O*-methyl-DOPA. Tolcapone (30 mg/kg p.o.), given as adjunct to both doses of L-DOPA, markedly enhanced the elevation of extracellular L-DOPA, while it completely prevented the formation of 3-*O*-methyl-DOPA. In another experiment, the administration of L-DOPA + benserazide (30 + 15 mg/kg p.o.) resulted in increased extracellular levels of dopamine, DOPAC, HVA and 3-methoxytyramine. The co-administration of tolcapone (30 mg/kg p.o.) further increased dopamine and DOPAC levels, whereas HVA and 3-methoxytyramine effluxes were reduced. These findings support the notion that tolcapone has the ability to enhance striatal dopamine neurotransmission by increasing L-DOPA bioavailability through peripheral and central inhibition of L-DOPA O-methylation, as well as by blocking the central conversion of dopamine into 3-methoxytyramine.

Keywords: Dopamine; Microdialysis; Catechol-*O*-methyltransferase inhibition; L-DOPA (3,4-dihydroxyphenyl-L-alanine); Tolcapone

1. Introduction

Catechol-*O*-methyltransferase (EC 2.1.1.6.), an enzyme present in both peripheral tissues and in glial cells of the brain, catalyzes the transfer of a methyl group from *S*-adenosyl-L-methionine to endogenous and exogenous substrates containing a catechol moiety (Axelrod, 1966; Guldberg and Marsden, 1975). This enzyme plays an important role in the metabolism of 3,4-dihydroxyphenyl-L-alanine (L-DOPA), catecholamine neurotransmitters (e.g. dopamine, noradrenaline and adrenaline) and their metabolites, as well as on the O-methylation of catechol steroids and xenobiotic drugs, e.g. apomorphine. The catechol-*O*-methyltransferase enzyme, whose crystallographic structure has been recently elucidated (Vidgren et al., 1994), was reported to exist in two isoforms, one of which is soluble and the other membrane bound (Rivett et al., 1983; Bertocci et al., 1991; Roth, 1992).

The discovery of potent and selective catechol-*O*-methyltransferase inhibitors has led to a possible novel strategy in the treatment of Parkinson's disease (Männistö and Kaakkola, 1989; Da Prada et al., 1991). In fact, when L-DOPA is administered together with a peripheral aromatic amino acid decarboxylase (EC 4.1.1.28) inhibitor, such as benserazide in Madopar or carbidopa in Sinemet, a large part of the amino acid is converted in the periphery by catechol-*O*-methyltransferase to 3-*O*-methyl-DOPA (Messiha et al., 1972; Nutt and Fellman, 1984). This L-DOPA metabolite has a half-life (approximately 15 h) much longer than that of L-DOPA (approximately 1 h) and, for this reason, 3-*O*-methyl-DOPA accumulates in the plasma (Kuruma et al., 1971; Cedarbaum, 1987). High plasma levels of 3-*O*-methyl-DOPA could compete with the transport

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Elsevier Science B.V.
SSDI 0014-2999(94)00682-2

of L-DOPA into the brain and might play a role in the pathogenesis of the side effects occurring under long-term L-DOPA therapy (Reches et al., 1982; Da Prada et al., 1984).

Among the new catechol-*O*-methyltransferase inhibitors, tolcapone (Ro 40-7592) is currently under clinical development as adjunct to Madopar and Sinemet for the therapy of Parkinson's disease patients. Tolcapone markedly blocks the peripheral conversion of L-DOPA into 3-*O*-methyl-DOPA, as revealed by reduced plasma and brain 3-*O*-methyl-DOPA levels and increased plasma and brain L-DOPA bioavailability. (Zürcher et al., 1990, 1993). In addition, tolcapone is able to cross the blood-brain barrier and therefore to inhibit central catechol-*O*-methyltransferase, as demonstrated by the elevation of 3,4-dihydroxyphenylacetic acid (DOPAC) and by the decreased brain levels of homovanillic acid (HVA) and 3-methoxytyramine (Zürcher et al., 1991; Männistö et al., 1992). The central catechol-*O*-methyltransferase inhibitory action of tolcapone should play a relevant role by blocking the central O-methylation of L-DOPA thus further improving its bioavailability, and in addition by inhibiting the conversion of dopamine into 3-methoxytyramine.

In the present study, the effects of tolcapone co-administered with L-DOPA + benserazide, on the striatal L-DOPA and dopamine 3-*O*-methylation, has been investigated using the technique of in vivo microdialysis in freely moving rats.

2. Materials and methods

2.1. Animals

Male albino rats (Fü-albino, 270–300 g) were maintained under an artificial light/dark cycle of 12 h (light on at 6 a.m.) in a temperature and humidity controlled environment. Food and water were available ad libitum.

2.2. Surgery

Rats were anesthetized with sodium pentobarbital (40 mg/kg, Vetanarcol, Veterinaria AG, Zürich, Switzerland). The technique used to prepare and implant the dialysis tubes was essentially that described by Imperato and Di Chiara (1984). Briefly, dialysis fibers (Filtral 12 AN69 HF, Hospal-Industrie, Meyzieu, France), were covered with epoxy glue except for 4 mm corresponding to each caudate, to confine the dialysis to the area of interest. Dialysis fibers were transversally inserted through the dorsal caudate nucleus (coordinates A +1, V −5 from bregma) (Paxinos and Watson, 1986) and stainless steel tubes were glued to both ends of the fiber and then secured to the skull using dental cement (Sevriton, De Trey Dentsply, Konstanz, Germany). After surgery, rats were allowed to recovery for 24 h in their home cages with free access to food and water.

2.3. Brain dialysis

Experiments were started between 8:00 and 9:00 a.m. One stainless steel tube was connected to a microinfusion pump (CMA 100, Schmidlin, Neuheim-Sarbach, Switzerland) by PE-10 tubing (800 × 0.28 mm) and modified Ringer's solution (147 mM NaCl, 3 mM KCl, 1.3 mM $CaCl_2$ and 1 mM $MgCl_2$) was perfused through the dialysis probe at a flow rate of 2.5 μl/min for dopamine and metabolites and of 2 μl/min for L-DOPA and 3-*O*-methyl-DOPA. The outflow of the dialysis tube was connected to an electrically actuated sample injector (Valco, model EC10W, Schmidlin, Neuheim-Sarbach, Switzerland) by a fused silica tube (800 × 0.1 mm, MicroQuartz, München, Germany). The sample injector was controlled by a digital valve sequence programmer (Valco, model DVSP) set to remain in the load position for 20 min and then to move to the inject position for 20 s. The injection volume was 50 μl for dopamine and metabolites and 20 μl for L-DOPA and 3-*O*-methyl-DOPA. Drugs were administered when the levels of dopamine had become stable or 2 h after the initiation of the perfusion for L-DOPA and 3-*O*-methyl-DOPA assay. Experiments lasted for 6 h after drug administration, and at the end rats were killed by decapitation, brains were removed and the site of the dialysis tube was verified by light microscopy.

2.4. Analytical procedure

Dialysate concentrations of dopamine, DOPAC, HVA and 3-methoxytyramine were measured by high performance liquid chromatography (HPLC) with electrochemical detection. The compounds were separated by a reverse phase column (Nucleosil 5 C18, 150 × 4.6 mm, Macherey-Nagel, Düren, Germany). The mobile phase, consisting of 0.04 M sodium acetate adjusted to pH 4.1 with acetic acid, 0.1 mM EDTA, 0.6 mM octanesulfonic acid, and 15% (v/v) methanol, was delivered at a flow rate of 1.25 ml/min by means of a HPLC pump (Kontron, model 420). Detection of the compounds was achieved by means of a coulometric detector (ESA, model 5100A) equipped with an analytical cell ESA, model 5011; the potentials of the two electrodes were maintained at +450 and −200 mV.

The HPLC system used for L-DOPA and 3-*O*-methyl-DOPA assay consisted of a reverse phase column (Spherisorb ODS-2, 3 μm, 125 × 4 mm, Stagroma, Wallisellen, Switzerland) and a coulometric detector,

with the two electrodes set at +450 and −250 mV. The mobile phase, consisting of 0.05 M sodium dihydrogen phosphate adjusted to pH 2.2 with 85% orthophosphoric acid, 0.1 mM EDTA, 0.52 mM octyl sodium sulfate, and 7% (v/v) acetonitrile, was delivered at a flow rate of 0.8 ml/min.

Chromatograms were simultaneously recorded on an integrator (Hitachi, model D-2500) and on a two channel recorder (Kipp and Zonen, model BD 41).

2.5. Drugs and chemicals

Tolcapone (Ro 40-7592, 3,4-dihydroxy-4'-methyl-5-nitrobenzophenone), L-DOPA and benserazide were synthetized at F. Hoffmann-La Roche (Basel, Switzerland). All drugs, suspended in saline containing 1% Tween 80 using a glass homogenizer, were administered orally in a final volume of 2 ml/kg.

The standards for HPLC were purchased from Sigma (St. Louis, MO). Octyl sodium sulfate was obtained from Eastman Kodak (Rochester, USA) and acetonitrile from Rathburn (Walkerburn, Scotland). Other reagents were of analytical grade and purchased from Merck (Darmstadt, Germany) and Fluka (Buchs, Switzerland).

2.6. Statistical analysis

For each rat the three samples collected before drug administration were averaged and represented baseline values. The subsequent values were expressed as percentage of the baseline (= 100%). In the case of L-DOPA and 3-*O*-methyl-DOPA only absolute values (pmol/20 μl) were used, since the baseline concentrations were under the detection limit of our assay technique. The area under the curve (AUC) was calculated for each animal. Data were then analyzed by one-way analysis of variance (ANOVA) for repeated measures. Following a significant overall effect, pair-wise comparisons were made with the Newman-Keuls test.

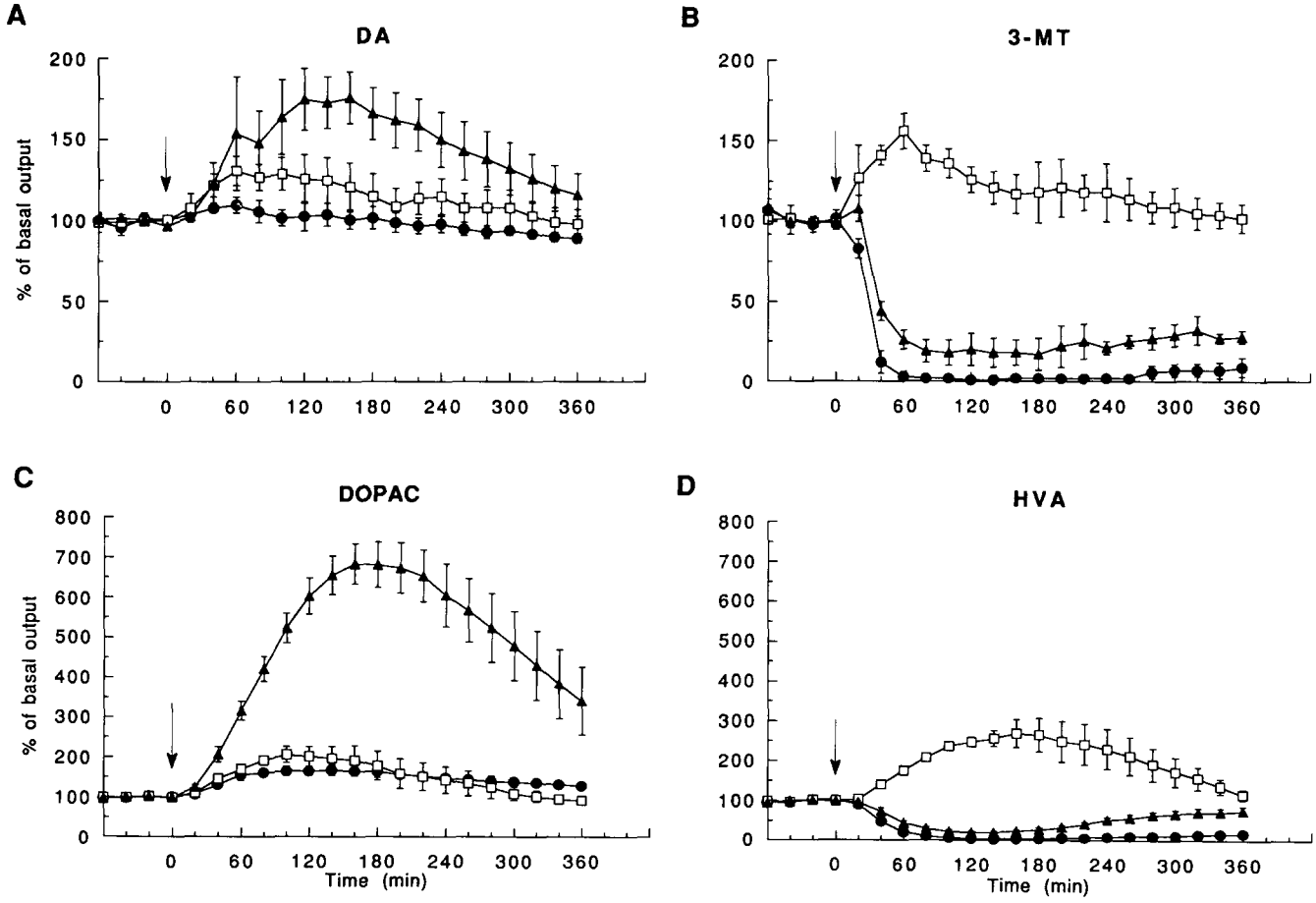

Fig. 1. Effects of tolcapone (30 mg/kg, ●) and L-DOPA + benserazide (30 + 15 mg/kg), alone (□) or in combination with tolcapone (30 mg/kg, ▲), on striatal dialysate levels of dopamine (A), 3-methoxytyramine (B), DOPAC (C), and HVA (D). All drugs were administered orally at time 0 (arrow). Data are means ± S.E.M. of five rats, expressed as percent of basal output (average of the three samples before drug administration). See text for statistics.

3. Results

3.1. Effects of tolcapone on striatal extracellular levels of dopamine, DOPAC, HVA, and 3-methoxytyramine

Oral administration of tolcapone (30 mg/kg) produced no significant effect on dialysate levels of dopamine (Fig. 1a), but increased outflows of DOPAC ($P < 0.001$) and decreased HVA ($P < 0.001$) as well as 3-methoxytyramine ($P < 0.001$) (Fig. 1b,c,d). The reductions of HVA and 3-methoxytyramine were particularly pronounced, since HVA decreased by 95% and 3-methoxytyramine became hardly detectable, 2 h and 1 h post dosing, respectively, and both metabolites remained markedly decreased throughout the experiment.

3.2. Effect of tolcapone on striatal extracellular changes of dopamine and metabolites induced by L-DOPA + benserazide

Striatal extracellular levels of dopamine, DOPAC, HVA and 3-methoxytyramine were increased ($P <$ 0.001 versus baseline) after L-DOPA + benserazide administration (30 + 15 mg/kg) (Fig. 1). The maximum increase of dopamine (31%) was obtained 60 min after dosing with a subsequent decrease towards basal levels (Fig. 1a). 3-Methoxytyramine extracellular levels showed time course changes similar to dopamine (Fig. 1b). The increase of HVA extracellular levels was more pronounced and delayed compared to that of DOPAC (Fig. 1c,d).

The addition of tolcapone (30 mg/kg) to L-DOPA + benserazide resulted in a further increase ($P < 0.05$)

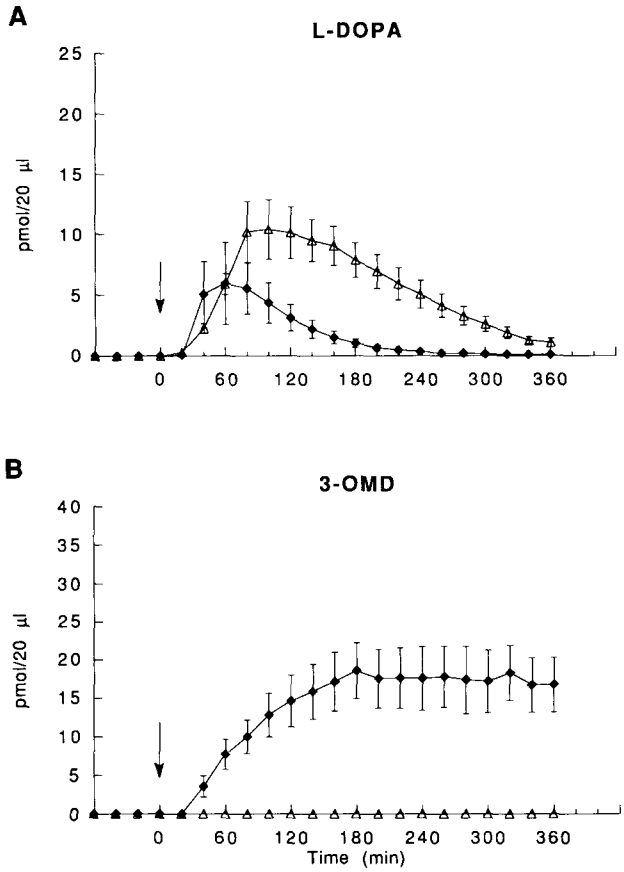

Fig. 2. Effects of L-DOPA + benserazide (20 + 15 mg/kg), alone (♦) or in combination with tolcapone (30 mg/kg, △) on striatal dialysate levels of L-DOPA (A) and 3-*O*-methyl-DOPA (B). All drugs were administered orally at time 0 (arrow). Data are means ± S.E.M. of six rats. The basal concentrations of the two compounds were not detectable, thus the values are expressed as absolute values. See text for statistics.

Table 1
Effects of tolcapone, alone or in combination with L-DOPA + benserazide, on dopamine, DOPAC, HVA and 3-methoxytyramine dialysate levels in rat striatum

	Treatment	Basal levels (pmol/20 min)	AUC (% basal × 360 min)
Dopamine	Tolcapone	0.789 ± 0.184	36 888 ± 1 559
	L-DOPA	0.615 ± 0.078	42 346 ± 3 119
	L-DOPA + tolcapone	0.669 ± 0.096	54 549 ± 4 053 [a]
DOPAC	Tolcapone	58.70 ± 6.72	53 885 ± 2 379
	L-DOPA	57.65 ± 12.10	55 103 ± 6 551
	L-DOPA + tolcapone	58.21 ± 7.27	175 589 ± 17 085 [b]
HVA	Tolcapone	33.47 ± 2.46	6 385 ± 1 701
	L-DOPA	35.15 ± 9.12	73 228 ± 8 357
	L-DOPA + tolcapone	40.25 ± 3.55	19 368 ± 2 052 [b]
3-Methoxytyramine	Tolcapone	0.257 ± 0.096	4 971 ± 879
	L-DOPA	0.208 ± 0.042	44 581 ± 2 717
	L-DOPA + tolcapone	0.190 ± 0.044	12 165 ± 2 403 [c]

Each value represents the mean ± S.E.M. of five rats. Basal values are the means of the three preadministration samples. Tolcapone (30 mg/kg) and L-DOPA (30 mg/kg) were administered orally at the same time. L-DOPA was given together with benserazide (15 mg/kg) ([a] $P < 0.05$, [b] $P < 0.01$, [c] $P < 0.001$ versus L-DOPA, one-way ANOVA followed by Newman-Keuls multiple comparison test).

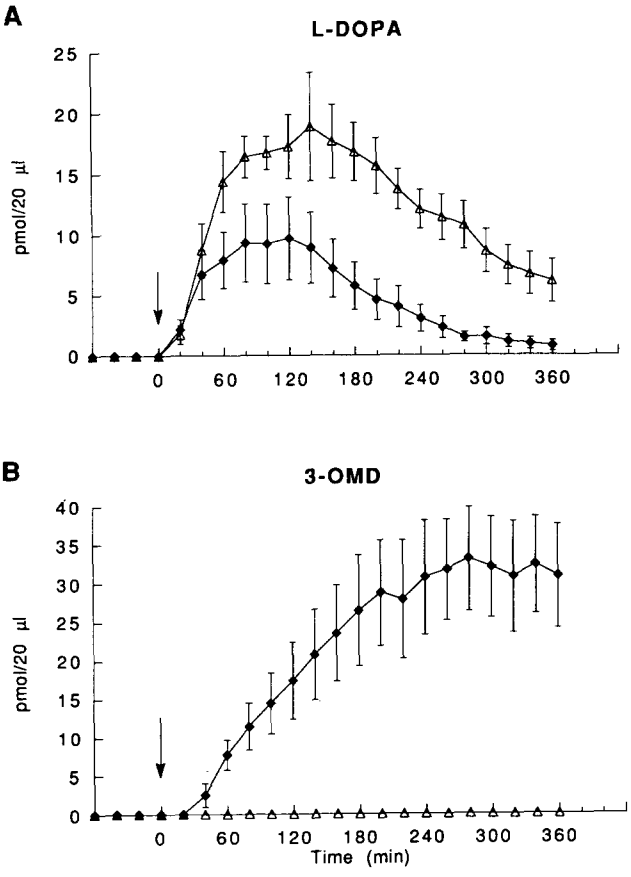

Fig. 3. Effects of L-DOPA + benserazide (60 + 15 mg/kg), alone (♦) or in combination with tolcapone (30 mg/kg, △) on striatal dialysate levels of L-DOPA (A) and 3-*O*-methyl-DOPA (B). All drugs were administered orally at time 0 (arrow). Data are means ± S.E.M. of six rats. The basal concentrations of the two compounds were not detectable, thus the values are expressed as absolute values. See text for statistics.

of dopamine levels over that obtained without the catechol-*O*-methyltransferase inhibitor (Fig. 1a, Table 1). A maximum increase of 75% was observed and dopamine levels were still elevated 6 h post-dosing. The increased output of DOPAC was also enhanced by tolcapone ($P < 0.01$ compared to L-DOPA) (Fig. 1c, Table 1), whereas the effluxes of HVA and of 3-methoxytyramine were strongly and long-lastingly decreased ($P < 0.01$ and $P < 0.001$, respectively, compared to the effect of L-DOPA; Fig. 1b,d, Table 1).

3.3. Effect of tolcapone on striatal extracellular changes of L-DOPA and 3-O-methyl-DOPA levels induced by L-DOPA + benserazide

Two different doses of L-DOPA (20 and 60 mg/kg), together with a fixed dose of benserazide (15 mg/kg) were tested. Basal concentrations of L-DOPA and 3-*O*-methyl-DOPA were not detectable. L-DOPA at the dose of 20 mg/kg increased the outflow of L-DOPA ($P < 0.001$), which returned at a level lower than the detection limit within 4 h, and induced a progressive raise of 3-*O*-methyl-DOPA levels ($P < 0.001$), which were stable between 3 and 6 h post dosing (Fig. 2). When tolcapone (30 mg/kg) was co-administered with this dose of L-DOPA, nearly a 5-fold potentiation of the L-DOPA increase was observed ($P < 0.05$) (Table 2), with a complete recovery only at 6 h (Fig. 2a). 3-*O*-Methyl-DOPA formation was fully abolished by the addition of tolcapone (Fig. 2b). The administration of L-DOPA at the dose of 60 mg/kg produced approximately a 4-fold increase of L-DOPA AUC when compared to that obtained with the dose of 20 mg/kg ($P < 0.05$, Table 2). Also at this relatively high dose of L-DOPA, the addition of tolcapone further enhanced L-DOPA bioavailability (approximately 2.5 times, $P < 0.05$ compared to L-DOPA 60 mg/kg alone, Table 2) and its levels were still elevated 6 h after dosing (Fig. 3a). The formation of 3-*O*-methyl-DOPA, which was markedly increased by 60 mg/kg L-DOPA ($P < 0.001$ versus baseline), was again completely blocked when the amino acid was co-administered together with tolcapone (Fig. 3b).

4. Discussion

In the present study, we investigated the effects of the oral administration to rats of the novel catechol-

Table 2
Effects of two different doses of L-DOPA + benserazide, without or with tolcapone, on L-DOPA and 3-O-methyl-DOPA dialysate levels in rat striatum

	Treatment	AUC (pmol × 360 min)
L-DOPA	L-DOPA (20 mg/kg)	406 ± 92
	L-DOPA (20 mg/kg) + tolcapone	1967 ± 402 [a]
	L-DOPA (60 mg/kg)	1727 ± 561 [a]
	L-DOPA (60 mg/kg) + tolcapone	4368 ± 632 [b,c]
3-*O*-Methyl-DOPA [d]	L-DOPA (20 mg/kg)	4986 ± 903
	L-DOPA (60 mg/kg)	7742 ± 1299 [a]

Each value represents the mean ± S.E.M. of six rats. Basal concentrations of the compounds were not detectable. L-DOPA (20 and 60 mg/kg) together with a fixed dose of benserazide (15 mg/kg) was administered orally without or with tolcapone (30 mg/kg) ([a] $P < 0.05$ versus L-DOPA 20 mg/kg, [b] $P < 0.05$ versus L-DOPA 60 mg/kg, [c] $P < 0.05$ versus L-DOPA 20 mg/kg + tolcapone, one-way ANOVA followed by Newman-Keuls multiple comparison test; [d]3-O-methyl-DOPA levels were not detectable when tolcapone was added to both doses of L-DOPA).

O-methyltransferase inhibitor tolcapone, together with L-DOPA + benserazide, on L-DOPA and dopamine striatal metabolism.

In spite of the marked changes in dopamine metabolism, i.e. strong reduction of the 3-*O*-methylated compounds HVA and 3-methoxytyramine and increase of DOPAC, no changes in dopamine levels were observed when tolcapone was administered alone. This finding, consonant with previous microdialysis studies (Acquas et al., 1992; Kaakkola and Wurtman, 1992) might imply that, in the absence of L-DOPA, only a small proportion of extraneuronal dopamine is primarily *O*-methylated to 3-methoxytyramine. However, since it has been previously demonstrated (Kaakkola and Wurtman, 1992) that tolcapone potentiates the nomifensine-induced increase of dopamine, it can be concluded that an elevation of extracellular dopamine may occur after central catechol-*O*-methyltransferase blockade, and that in part this dopamine is rapidly taken up and deaminated intraneuronally by monoamine oxidase (flavin containing, EC 1.4.3.4).

The addition of tolcapone to L-DOPA + benserazide notably enhanced the striatal L-DOPA bioavailability. This effect was greater for the low dose of L-DOPA (20 mg/kg) compared to the high dose (60 mg/kg) of the compound. It is worth remarking that tolcapone not only increased the L-DOPA peak over that achieved with L-DOPA alone, but also modified the time course, delaying the return to baseline. Our data are in line with those of Brannan et al. (1992) obtained in anesthetized rats with a higher dose of L-DOPA. The increase in L-DOPA bioavailability measured after tolcapone is due to the inhibition of both peripheral and central *O*-methylation of the amino acid, and this L-DOPA-sparing effect is reflected by the fact that tolcapone completely prevented the increase of striatal 3-*O*-methyl-DOPA outflow after both doses of L-DOPA. Although the major part of the *O*-methylation of L-DOPA occurs in the periphery, the central catechol-*O*-methyltransferase blockade might play a role in raising brain L-DOPA bioavailability. This notion is supported by the observation that entacapone, a peripheral catechol-*O*-methyltransferase inhibitor (Nissinen et al. 1992), when co-administered with L-DOPA only slightly increased L-DOPA dialysate levels compared to the effect of L-DOPA alone, and could only in part reduce 3-*O*-methyl-DOPA formation (Törnwall et al., 1994).

The increased dopamine levels observed after L-DOPA + benserazide were markedly potentiated by the simultaneous administration of tolcapone. The extent of the response we observed was akin to that achieved by Acquas et al. (1992) using a similar experimental paradigm, but slightly less pronounced than that measured in other studies (Brannan et al., 1992; Kaakkola and Wurtman, 1993), in which, however, higher doses of L-DOPA were injected intraperitoneally. The higher dopamine outflow, observed when tolcapone was co-administered with L-DOPA, seems to be mainly due to increased brain L-DOPA bioavailability. However, the increased dopamine efflux could also be the consequence of a reduced conversion of dopamine into 3-methoxytyramine, via blockade of brain catechol-*O*-methyltransferase. As already observed by other investigators (Wood et al., 1987; Brown et al., 1991), the 3-methoxytyramine dialysate levels measured in this study were nearly one third of those of dopamine and considerably lower than those of HVA and DOPAC. Rats treated with L-DOPA + benserazide showed an increase in 3-methoxytyramine extracellular levels which displayed the same time profile of dopamine, thus further confirming that dialysate 3-methoxytyramine levels are a good index of dopamine release in a wide range of pharmacological manipulations (Brown et al., 1991). However, when rats were co-administered with tolcapone, a clear dissociation between dopamine and 3-methoxytyramine extracellular levels was observed, with a dopamine increase and a marked 3-methoxytyramine reduction. Therefore, it is likely that following L-DOPA administration, and in conditions of central catechol-*O*-methyltransferase inhibition by tolcapone, a reduced conversion of dopamine into 3-methoxytyramine may also contribute to increase the extracellular levels of the neurotransmitter.

In conclusion, tolcapone has proven to be very effective in increasing the striatal extracellular levels of L-DOPA and dopamine in the rat, when given in combination with L-DOPA + benserazide. The increased L-DOPA bioavailability produced by tolcapone, associated with a reduced catabolism of dopamine in the synaptic cleft, will be of great importance for improving the treatment of Parkinsonian patients with motor fluctuations, possibly leading to a reduction of L-DOPA doses and a prolongation of interdose intervals. Moreover, data from our laboratory have proven that tolcapone, by means of its central catechol-*O*-methyltransferase-inhibitory activity, is able to increase rat brain *S*-adenosyl-L-methionine levels and to counteract the L-DOPA-induced *S*-adenosyl-L-methionine decrease (Da Prada et al., 1994), an effect which could be important in the treatment of Parkinson's disease-associated depression.

Acknowledgements

We would like to thank Mr. Flavian D'Agostini and Mrs. Martine Buhler for technical assistance, Dr. J.G. Richards for critical reading of the manuscript, Mrs. M. Wdonwicki for secretarial work.

References

Acquas, E., E. Carboni, R.A.H. De Ree, M. Da Prada and G. Di Chiara, 1992, Extracellular concentrations of dopamine and metabolites in the rat caudate after oral administration of a novel catechol-*O*-methyltransferase inhibitor Ro 40-7592, J. Neurochem. 59, 326.

Axelrod, J., 1966, Methylation reactions in the formation and metabolism of catecholamines and other biogenic amines, Pharmacol. Rev. 18, 95.

Bertocci, B., V. Miggiano, M. Da Prada, Z. Dembic, H.W. Lahm and P. Malherbe, 1991, Human catechol-*O*-methyltransferase: cloning and expression of the membrane-associated form, Proc. Natl. Acad. Sci. USA 88, 1416.

Brannan, T., J. Martínez-Tica and M.D. Yahr, 1992, Catechol-*O*-methyltransferase inhibition increases striatal L-dopa and dopamine: an in vivo study in rats, Neurology 42, 683.

Brown, E.E., G. Damsma, P. Cumming and H.C. Fibinger, 1991, Interstitial 3-methoxytyramine reflects striatal dopamine release: an in vivo microdialysis study, J. Neurochem. 57, 701.

Cedarbaum, J.M., 1987, Clinical pharmacokinetics of anti-parkinsonian drugs, Clin. Pharmacokinet. 13, 141.

Da Prada, M., H.H. Keller, L. Pieri, R. Kettler and W.E. Haefely, 1984, The pharmacology of Parkinson's disease: basic aspects and recent advances, Experientia 40, 1165.

Da Prada, M., G. Zürcher, R. Kettler and A. Colzi, 1991, New therapeutic strategies in Parkinson's disease: inhibition of MAO-B by Ro 19-6327 and of COMT by Ro 40-7592, Adv. Behav. Biol. 39, 723.

Da Prada, M., J. Borgulya,, A. Napolitano and G. Zürcher, 1994, Improved therapy of Parkinson's disease by tolcapone, a central and peripheral COMT inhibitor with an *S*-adenosyl-L-methionine sparing effect, Clin. Neuropharmacol. 17 (Suppl. 3), S26.

Guldberg, H.C. and C.A. Marsden, 1975, Catechol-*O*-methyl transferase: pharmacological aspects and physiological role, Pharmacol. Rev. 27, 135.

Imperato, A. and G. Di Chiara, 1984, Trans-striatal dialysis coupled to reverse phase high performance liquid chromatography with electrochemical detection: a new method for the in vivo release of endogenous dopamine and metabolites, J. Neurosci. 4, 966.

Kaakkola, S. and R.J. Wurtman, 1992, Effects of catechol-*O*-methyltransferase inhibitors on striatal dopamine metabolism, Brain Res. 587, 241.

Kaakkola, S. and R.J. Wurtman, 1993, Effects of catechol-*O*-methyltransferase inhibitors and L-3,4-dihydroxyphenylalanine with or without carbidopa on extracellular dopamine in rat striatum, J. Neurochem. 60, 137.

Kuruma, I., G. Bartholini, R. Tissot and A. Pletscher, 1971, The metabolism of L-3-*O*-methyldopa, a precursor of dopa in man, Clin. Pharmacol. Ther. 12, 678.

Männistö, P.T. and S. Kaakkola, 1989, New selective COMT inhibitors: useful adjuncts for Parkinson's disease?, Trends Pharmacol. Sci. 10, 54.

Männistö, P.T., P. Tuomainen and R.K. Tuominen, 1992, Different in vivo properties of three new inhibitors of catechol-*O*-methyltransferase in the rat, Br. J. Pharmacol. 105, 569.

Messiha, F.S., T.H. Hsu and J.R. Bianchine, 1972, Peripheral aromatic L-amino acid decarboxylase inhibitors in parkinsonism. I. Effect on *O*-methylated metabolites of L-dopa-2-^{14}C, J. Clin. Invest. 51, 452.

Nissinen, E., I.B. Linden, E. Schultz and P. Pohto, 1992, Biochemical and pharmacological properties of a peripherally acting catechol-*O*-methyltransferase inhibitor, entacapone, Naunyn-Schmied. Arch. Pharmacol. 346, 262.

Nutt, J.G. and J.H. Fellman, 1984, Pharmacokinetics of levodopa, Clin. Neuropharmacol. 7, 35.

Paxinos, G. and C. Watson, 1986, The Rat Brain in Stereotaxic Coordinates, 2nd ed. (Academic Press, London).

Reches, A., L.R. Mielke and S. Fahn, 1982, 3-*O*-Methyldopa inhibits rotations induced by levodopa in rats after unilateral destruction of the nigrostriatal pathway, Neurology 32, 887.

Rivett, A., A. Francis and J.A. Roth, 1983, Distinct cellular localization of membrane-bound and soluble forms of catechol-*O*-methyltransferase in brain, J. Neurochem. 40, 215.

Roth, J.A., 1992, Membrane-bound catechol-*O*-methyltransferase: a re-evaluation of its role in the *O*-methylation of catecholamine neurotransmitters, Physiol. Biochem. Pharmacol. 120, 1.

Törnwall, M., S. Kaakkola, P. Tuomainen, A. Kask and P.T. Männistö, 1994, Comparison of two new inhibitors of catechol-*O*-methylation: a microdialysis study in rats, Br. J. Pharmacol. 112, 13.

Vidgren, J., L.A. Svensson and A. Lijas, 1994, Crystal structure of catechol-*O*-methyltransferase, Nature 368, 354.

Wood, P.L., H.S. Kim and M.R. Marien, 1987, Intracerebral dialysis: direct evidence for the utility of 3-MT measurements as an index of dopamine release, Life Sci. 41, 1.

Zürcher, G., H.H. Keller, R. Kettler, J. Borgulya, E.P. Bonetti, R. Eigenmann and M. Da Prada, 1990, Ro 40-7592, a novel, very potent, and orally active inhibitor of catechol-*O*-methyltransferase: a pharmacological study in rats, Adv. Neurol. 53, 497.

Zürcher, G., J. Dingemanse and M. Da Prada, 1991, Ro 40-7592, a potent inhibitor of extracerebral and brain catechol-*O*-methyltransferase: preclinical and clinical findings, in: New Developments in Therapy of Parkinson's Disease, eds. A. Agnoli and G. Campanella (John Libbey CIC, Roma) p. 37.

Zürcher, G., J. Dingemanse and M. Da Prada, 1993, Potent COMT inhibition by Ro 40-7592 in the periphery and in the brain, Adv. Neurol. 60, 641.