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BIA 3-202, a novel catechol-*O*-methyltransferase inhibitor, enhances the availability of L-DOPA to the brain and reduces its *O*-methylation

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Abstract

1-[3,4-Dihydroxy-5-nitrophenyl]-2-phenyl-ethanone (BIA 3-202) is a new long-acting catechol-*O*-methyltransferase (COMT) inhibitor with limited access to the brain. The present study evaluated the interference of BIA 3-202 upon levels of L-3,4-dihydroxyphenylalanine (L-DOPA) and metabolites in plasma (3-*O*-methyl-L-DOPA) and brain [3-*O*-methyl-L-DOPA, dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA)] in rats orally treated with L-DOPA (20 mg/kg) plus benserazide (30 mg/kg). At different time points (1, 3 and 6 h) after the administration of BIA 3-202 (0, 3, 10 and 30 mg/kg) or L-DOPA plus benserazide, rats were sacrificed and the right striatum was quickly dissected out and stored for the assay of L-DOPA, 3-*O*-methyl-L-DOPA, dopamine and amine metabolites. Levels of L-DOPA, 3-*O*-methyl-L-DOPA, dopamine, DOPAC and HVA in the striatum in L-DOPA plus benserazide-treated rats were higher than in vehicle-treated rats. However, this increase in striatal L-DOPA, dopamine, DOPAC and HVA was, in a dose- and time-dependent manner, even higher ($P < 0.05$) in rats given BIA 3-202 (3, 10 and 30 mg/kg). This effect was accompanied by a marked decrease in 3-*O*-methyl-L-DOPA levels in the striatum of L-DOPA plus benserazide-treated rats. Increases in levels of L-DOPA and decreases in 3-*O*-methyl-L-DOPA levels in plasma also accompanied the administration of BIA 3-202. BIA 3-202 did not significantly affect levels of DOPAC and HVA in the striatum in vehicle-treated rats. It is concluded that administration of BIA 3-202 enhances the availability of L-DOPA to the brain by reducing its *O*-methylation in the periphery, which may prove beneficial in parkinsonian patients treated with L-DOPA plus an aromatic amino acid decarboxylase inhibitor.

Keywords: L-3,4-dihydroxyphenylalanine (L-DOPA); 3-*O*-methyl-3,4-dihydroxyphenylalanine (3-*O*-methyl-L-DOPA); Brain; BIA 3-202

1. Introduction

In recent years, several nitrocatechol derivatives have been developed and found to be highly selective and potent inhibitors of catechol-*O*-methyltransferase (COMT), both in vitro and in vivo conditions (Männistö and Kaakkola, 1989; Männistö et al., 1988; Zürcher et al., 1990, 1993). Some of these compounds have already been used as adjuncts to L-3,4-dihydroxyphenylalanine (L-DOPA) therapy in Parkinson's disease (Davis et al., 1995; Dingemanse et al., 1995; Kaakkola et al., 1994; Roberts et al., 1993; Waters et al., 1997). The rationale for the use of COMT inhibitors is based on their capacity to slow the elimination of L-DOPA from the plasma, through inhibition of its *O*-methylation to 3-*O*-methyl-L-DOPA, which also competes with L-DOPA in gaining access to the brain (Bonifati and Meco, 1999). In contrast to entacapone, tolcapone is known to cross the blood–brain barrier and to inhibit brain COMT (Da Prada et al., 1994; Männistö, 1994). This has been suggested to be an advantage over compounds which do not cross the blood–brain barrier, since it may improve the availability of released dopamine in the biophase (Männistö and Kaakkola, 1990; Napolitano et al., 1995). Due to unacceptable liver toxicity of tolcapone, only entacapone is currently used for the treatment of patients afflicted with Parkinson's disease. One potential problem with entacapone concerns its relatively short half-life (0.3 h) (Keranen et al., 1994). To circumvent this problem, entacapone is recommended to be administered together with L-DOPA, up to 10 times a day.

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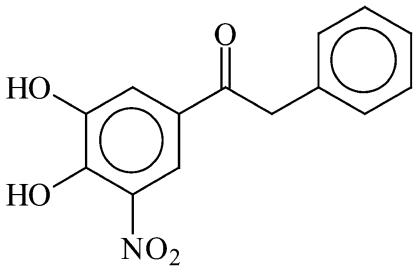

Fig. 1. Chemical structure of 1-[3,4-dihydroxy-5-nitrophenyl]-2-phenyl-ethanone (BIA 3-202).

1-[3,4-Dihydroxy-5-nitrophenyl]-2-phenyl-ethanone (BIA 3-202; Fig. 1) is a novel selective COMT inhibitor endowed with long duration of action, and this effect is unique in a series of the higher homologues (Benes et al., 2000; Vieira-Coelho et al., 2000). The present study evaluated the interference of BIA 3-202 upon levels of L-DOPA and metabolites in plasma (3-*O*-methyl-L-DOPA) and brain (3-*O*-methyl-L-DOPA, dopamine and amine metabolites) in rats orally treated with L-DOPA plus benserazide. It is reported that administration of BIA 3-202 enhances the availability of L-DOPA to the brain by reducing its *O*-methylation, which may prove beneficial in parkinsonian patients treated with L-DOPA plus an aromatic amino acid decarboxylase inhibitor.

2. Materials and methods

2.1. Animals

Male Wistar rats obtained from Harlan (UK) and weighing 180–280 g, were used. Rats were kept eight per cage, under controlled environmental conditions (12 h light/dark cycle, room temperature 22 ± 2°C and humidity 50 ± 5%). Food and tap water were allowed ad libitum and the experiments were all carried out during daylight hours.

2.2. Experimental procedure

Male Wistar rats (200–250 g; Harlan), fasted overnight, were administered orally with BIA 3-202 (0, 3, 10 and 30 mg/kg), suspended in 0.5% carboxymethylcellulose (4 ml/kg). Thirty minutes later, one arm was administered orally with L-DOPA (20 mg/kg) plus benserazide (30 mg/kg) and the second arm with vehicle (0.5% carboxymethylcellulose, 4 ml/kg). One, three and six hours after BIA 3-202 or vehicle, rats were anaesthetised with sodium pentobarbitone (60 mg/kg, i.p.) and blood samples taken by heart puncture. The right striatum was quickly dissected out and stored in perchloric acid, 0.2 M, for subsequent assay of L-DOPA, 3-*O*-methyl-L-DOPA, dopamine, DOPAC and HVA. Blood samples were centrifuged for 15 min at 3000 × *g* (4°C) and the plasma samples were stored at −80°C till the assay of L-DOPA and 3-*O*-methyl-L-DOPA. Blood samples were centrifuged for 15 min at 3000 × *g* (4°C) and the plasma samples were stored at

Table 1
Rat striatal levels (in nmol/g) of L-DOPA, 3-*OM*-L-DOPA, dopamine (DA), DOPAC and HVA at 1, 3 and 6 h after the administration of vehicle or L-DOPA (20 mg/kg) plus benserazide (30 mg/kg)
Values are means ± S.E.M. of four independent determinations; n.d.—not detectable.

Time (h)	Vehicle	L-DOPA + benserazide
L-DOPA		
1	n.d.	2.9 ± 0.6
3	n.d.	1.5 ± 0.2[a]
6	n.d.	0.4 ± 0.4[a]
3-OMD		
1	n.d.	3.9 ± 0.6
3	n.d.	15.4 ± 0.9[a]
6	n.d.	12.8 ± 0.7[a]
DA		
1	7.0 ± 0.9	18.6 ± 1.7[b]
3	8.6 ± 0.7	15.7 ± 0.8[b]
6	9.5 ± 0.9	12.3 ± 0.4[a,b]
DOPAC		
1	1.4 ± 0.2	4.2 ± 0.5[b]
3	2.2 ± 0.2	5.8 ± 0.4[b]
6	2.3 ± 0.4	2.8 ± 0.1
HVA		
1	2.1 ± 0.3	4.7 ± 0.3[b]
3	2.4 ± 0.3	10.8 ± 0.4[a,b]
6	2.5 ± 0.4	4.5 ± 0.3[b]

[a] $P > 0.05$; significantly different from corresponding values at 1-h treatment.
[b] $P > 0.05$; significantly different from corresponding values in vehicle-treated rats.

Table 2
Rat striatal levels (in nmol/g) of dopamine (DA), DOPAC and HVA at 1, 3 and 6 h after the administration of vehicle or BIA 3-202
Values are means ± S.E.M. of four independent determinations.

BIA 3-202 (mg/kg)	DA	DOPAC	HVA
1 h			
0	7.0 ± 0.9	1.4 ± 0.2	2.1 ± 0.3
3	8.3 ± 0.9	2.1 ± 0.3	2.4 ± 0.2
10	7.8 ± 1.2	1.9 ± 0.3	1.9 ± 0.3
30	9.1 ± 2.0	2.7 ± 0.7	2.5 ± 0.2
3 h			
0	8.6 ± 0.7	2.1 ± 0.2	2.4 ± 0.3
3	9.9 ± 0.5	2.3 ± 0.2	2.8 ± 0.1
10	11.5 ± 0.4[a]	2.7 ± 0.1	2.8 ± 0.2
30	13.4 ± 1.2[a]	3.9 ± 0.4[a]	2.9 ± 0.2
6 h			
0	9.5 ± 0.9	2.3 ± 0.4	2.5 ± 0.4
3	8.1 ± 0.8	2.1 ± 0.4	2.2 ± 0.1
10	11.2 ± 0.7[a]	2.7 ± 0.2	3.3 ± 0.2
30	14.1 ± 0.5[a]	3.3 ± 0.4	3.5 ± 0.1

[a] $P > 0.05$; significantly different from corresponding control values.

−80°C till the assay of L-DOPA and 3-*O*-methyl-L-DOPA. All animals interventions were performed in accordance with the European Directive number 86/609, and the rules of the "Guide for the Care and Use of Laboratory Animals," 7th edn., 1996, Institute for Laboratory Animal Research (ILAR), Washington, DC.

2.3. Assay of catechol derivatives

Assay of L-DOPA, 3-*O*-methyl-L-DOPA, dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) was carried out by means of HPLC with electrochemical detection (Soares-da-Silva et al., 2000). In brief, aliquots of 50 μl were injected into the chromatograph. The chromatographic system consisted of a pump (Gilson 307) and a stainless steel 5-μm ODS2 column (Biophase; Bioanalytical Systems, West Lafayette, IN) of 25-cm length and 4.6-mm diameter; samples were injected by means of an automatic sample injector (Gilson 231) connected to a Gilson dilutor (Gilson 401). The mobile phase was a degassed solution of 0.1 mM citric acid; 0.5 mM sodium octylsulphate; 0.1 M sodium acetate; 0.17 mM Na_2EDTA; 1 mM dibutylamine and 10% v/v methanol, adjusted to pH 3.5 with 2 M PCA and pumped at a rate of 1.0 ml/min. The detection was carried out electrochemically with a glassy carbon electrode, an Ag/AgCl reference electrode and an amperometric detector (Gilson 142); the detector cell was operated at 0.75 V.

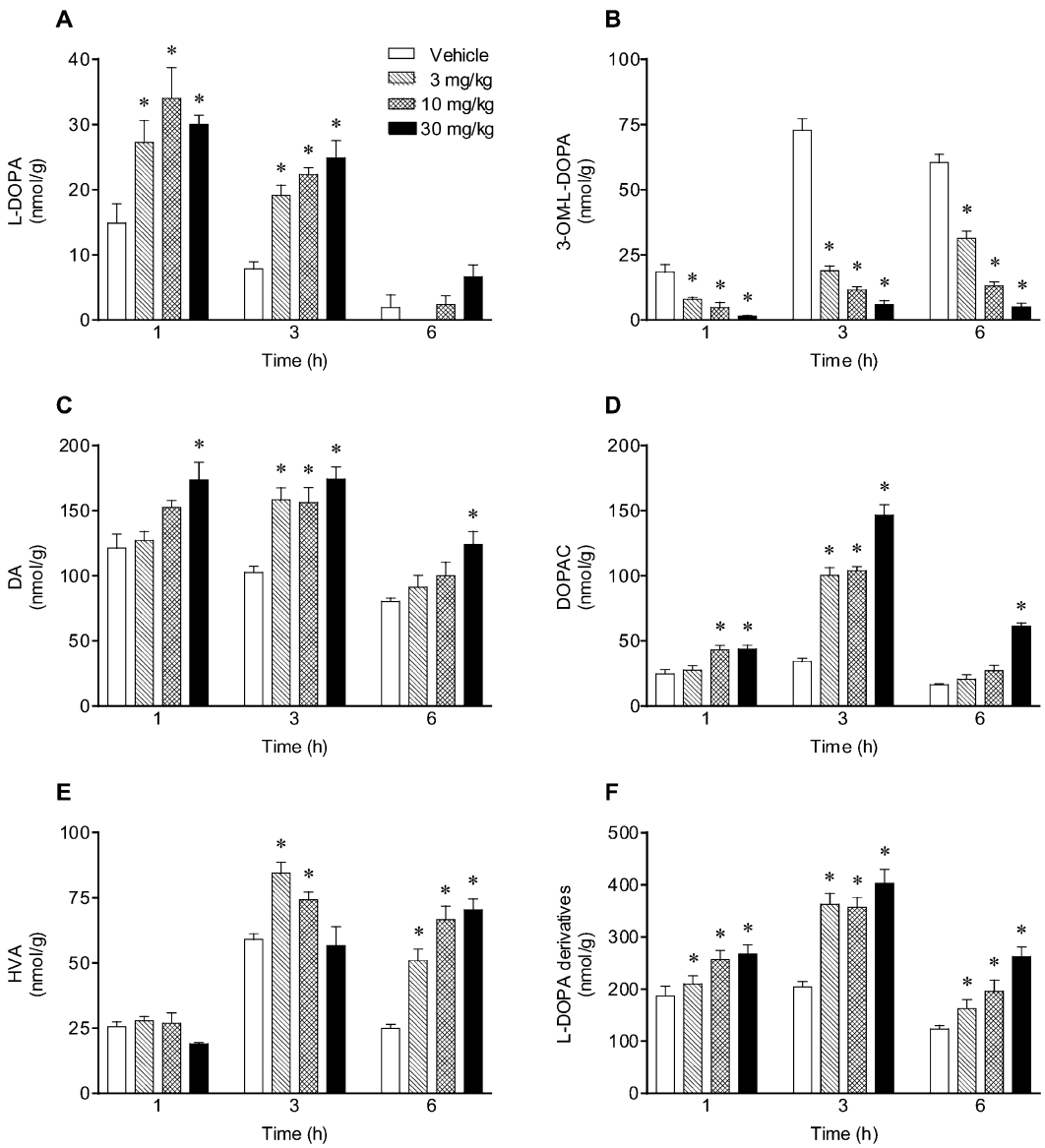

Fig. 2. Striatal levels of L-DOPA, 3-*O*-methyl-L-DOPA, dopamine, DOPAC, HVA and total amounts of L-DOPA derivatives in brain (dopamine, DOPAC and HVA), in vehicle- or BIA 3-202-treated rats. Rats were given L-DOPA (20 mg/kg) plus benserazide (30 mg/kg) 30 min after the administration of vehicle or BIA 3-202, and killed 1, 3 and 6 h after the administration of the COMT inhibitor. Columns represent means of five to six experiments per group; vertical lines indicate S.E.M. Significantly different from corresponding values in vehicle-treated rats ($^{*}P < 0.05$).

The current produced was monitored using the Gilson Unipoint HPLC software. The lower limit of detection of L-DOPA, dopamine, DOPAC and HVA ranged from 350 to 1000 fmol.

2.4. Drugs

BIA 3-202 was synthesised in the Department of Chemistry (BIAL). L-DOPA and benserazide were obtained from Sigma (St. Louis, MO).

2.5. Statistics

Results are mean ± S.E.M. Statistical comparisons were made using two-way analysis of variance and Newman–Keuls tests for post hoc comparisons. All tests were performed in Statistica software version 5.1 StatSoft (Tulsa, OK).

3. Results

Levels of L-DOPA and 3-*O*-methyl-L-DOPA were not detectable in the striatum of vehicle-treated rats (Table 1). In rats given L-DOPA plus benserazide, striatal levels of L-DOPA declined with time, whereas levels of 3-*O*-methyl-L-DOPA peaked 3 h after L-DOPA administration. In these rats, levels of dopamine, DOPAC and HVA were significantly ($P < 0.05$) higher than in vehicle-treated animals. Levels of dopamine in the striatum of L-DOPA plus benserazide-treated rats peaked 1 h after L-DOPA administration, whereas DOPAC and HVA peaked at 3 h.

In the next series of experiments, rats were given the vehicle or increasing doses of BIA 3-202 (3, 10 and 30 mg/kg) and the brains removed at selected time points (1, 3 and 6 h). As shown in Table 2, the effect of BIA 3-202 was a significant rise in striatal dopamine, particularly evident at 3 and 6 h after BIA 3-202 administration with the highest doses (10 and 30 mg/kg). Levels of DOPAC and HVA were not affected by BIA 3-202, with the exception of a significant rise in DOPAC levels at 3 h with the highest dose.

In rats treated with L-DOPA plus benserazide, the effect of BIA 3-202 was a dose-dependent increase in striatal L-DOPA and dopamine, accompanied by a marked attenuation in striatal 3-*O*-methyl-L-DOPA (Fig. 2). The effect of BIA 3-202 on L-DOPA, dopamine and 3-*O*-methyl-L-DOPA peaked at 3 h, this being particularly evident for dopamine and 3-*O*-methyl-L-DOPA. At 6 h after the administration of BIA 3-202, a marked attenuation in striatal 3-*O*-methyl-L-DOPA was still evident, but this was no longer the case for L-DOPA and dopamine, except at the highest dose of BIA 3-202. In general, changes in striatal levels of DOPAC and HVA followed the pattern described for dopamine. The total amount of L-DOPA derivatives in brain (dopamine, DOPAC and HVA), a rough measure of L-DOPA availability to the brain, indicates a dose- and time-dependent effect of BIA 3-202 in increasing the availability of L-DOPA to the brain (Fig. 2F).

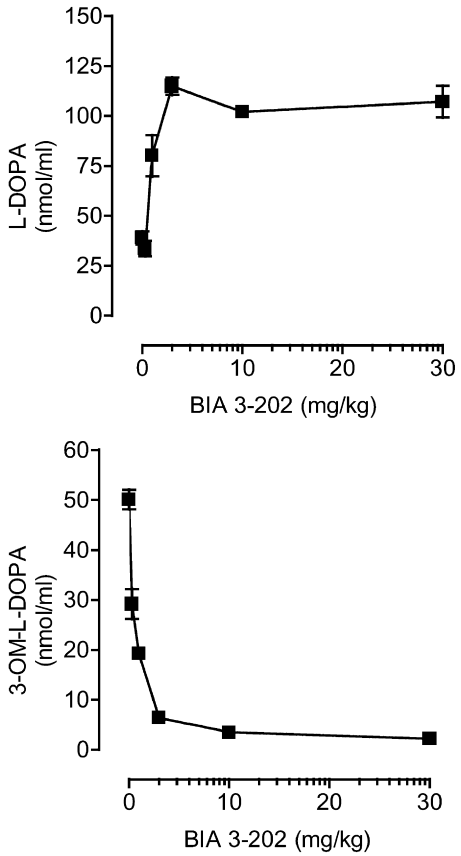

Fig. 3. Effect of BIA 3-202 (0.3, 1, 3, 10 and 30 mg) on plasma levels of L-DOPA and 3-*O*-methyl-L-DOPA in rats treated with L-DOPA (20 mg/kg) plus benserazide (30 mg/kg), 3 h after the administration of the COMT inhibitor. Symbols represent means of five to six experiments per group; vertical lines indicate S.E.M. Significantly different from corresponding values in vehicle-treated rats ($^{*}P < 0.05$).

Fig. 3 shows the effects of increasing doses of BIA 3-202 (0.3, 1, 3 10 and 10 mg/kg) on levels of L-DOPA and 3-*O*-methyl-L-DOPA in plasma of rats treated with L-DOPA plus benserazide, at 3 h after the administration of BIA 3-202. This time-point was chosen because it represented the T_{max} for L-DOPA. As can be observed, BIA 3-202 produce a dose-dependent increase in plasma L-DOPA accompanied by a marked decrease in circulating 3-*O*-methyl-L-DOPA.

4. Discussion

The results presented here clearly show that BIA 3-202 enhances in a time- and dose-dependent manner the availability of L-DOPA to the brain and reduces its *O*-methylation to 3-*O*-methyl-L-DOPA, which may prove beneficial in parkinsonian patients treated with L-DOPA plus an aromatic amino acid decarboxylase inhibitor.

The profile of changes in striatal tissue levels of L-DOPA and dopamine in rats treated with L-DOPA plus benserazide, a progressive decline from 1 to 6 h, is in agreement with the short half-life of L-DOPA (Tolosa et al., 1998). On the other hand, the inverted U shape profile of striatal tissue levels of DOPAC and HVA is compatible with the metabolic pathway of striatal dopamine, deamination of dopamine followed by *O*-methylation of DOPAC to HVA (Kopin, 1985). The sustained maintenance of striatal levels of 3-*O*-methyl-L-DOPA after administration of L-DOPA plus benserazide is consistent with the suggestion that most of it had its origin in peripheral tissues (Männistö et al., 1992).

BIA 3-202 is a novel long-acting COMT inhibitor with limited access to the brain (Benes et al., 2000; Vieira-Coelho et al., 2000). Studies performed to investigate the duration of its inhibitory effect upon liver COMT showed that BIA 3-202 was a particularly long-acting compound. Notably, inhibition of liver COMT by BIA 3-202 (30 mg/kg, p.o.) at 9 h was approximately 70% (Benes et al., 2000; Vieira-Coelho et al., 2000). At t_{max} (1 h after the administration), BIA 3-202 was much more potent in inhibiting liver COMT than brain COMT with ED_{50}s (in mg/kg) of 0.7 ± 1.1 and 5.3 ± 1.1, respectively. The inhibitory effect of BIA 3-202 upon brain COMT was a short-living one, with a complete recover in enzyme activity at 3 h post administration (Benes et al., 2000; Vieira-Coelho et al., 2000). When given alone, BIA 3-202 (from 3 to 30 mg/kg) was found to fail to alter levels of HVA in the brain. This agrees with the finding that BIA 3-202 does not produce marked inhibition of brain COMT, at least up to a level which affects the metabolism of endogenous dopamine in steady-state conditions. The increases in brain dopamine observed with the 10 and 30 mg/kg BIA 3-202 were particularly evident at 3 and 6 h after administration. Because these changes were not accompanied by decreases in HVA, it is unlikely that their origin is related to a decrease in the *O*-methylation of dopamine.

The effect of BIA 3-202 in rats treated with L-DOPA plus benserazide was a dose-dependent increase in striatal L-DOPA and dopamine, accompanied by a marked attenuation in striatal 3-*O*-methyl-L-DOPA. The changes in brain L-DOPA, dopamine and 3-*O*-methyl-L-DOPA, were particularly evident at 3 h after the administration of L-DOPA plus benserazide. Concomitantly, it was observed that BIA 3-202 produced a dose-dependent increase in levels of L-DOPA in plasma, accompanied by marked reductions in levels of 3-*O*-methyl-L-DOPA in plasma. Taken together, these results clearly indicate that inhibition of COMT by BIA 3-202 reduces the *O*-methylation of L-DOPA to 3-*O*-methyl-L-DOPA in the periphery, enhances the availability of L-DOPA to the brain and its subsequent conversion to dopamine. The finding that changes in DOPAC and HVA levels in brain parallel those observed for dopamine suggest that BIA 3-202 failed to significantly alter the metabolism of the amine in the brain, namely *O*-methylation processes. In fact, the changes in DOPAC and HVA levels in brain are compatible with metabolic processes described for the amine (Kopin, 1985). In this respect, it is interesting to mention that effective inhibition of brain COMT is usually accompanied by decreases in HVA and increases in DOPAC levels (Acquas et al., 1992; Kaakkola and Wurtman, 1992), which was not the case with BIA 3-202. This is in line with the findings described for entacapone, a short-acting COMT inhibitor with limited access to the brain, which was also found to markedly decrease 3-*O*-methyl-L-DOPA levels in brain, but failed to alter HVA levels (Männistö et al., 1992). Using an experimental protocol similar to that described here, with the exceptions that the dose of L-DOPA was much higher (50 mg/kg) and carbidopa was used instead of benserazide, Männistö et al. (1992) showed that increases in striatal dopamine by entacapone and tolcapone at 3 h post-treatment were of the same magnitude as those describe here for BIA 3-202. In that study, the decrease in striatal 3-*O*-methyl-L-DOPA by 30 mg/kg tolcapone (93.7% reduction) was greater than that with 30 mg/kg entacapone (80.2% reduction). This was in part attributed to the fact that entacapone is mainly a peripheral COMT inhibitor, whereas tolcapone inhibits both peripheral and central COMT. BIA 3-202, under similar experimental conditions (3 h post-treatment with 30 mg/kg), proved to be a potent inhibitor of COMT, while decreasing striatal 3-*O*-methyl-L-DOPA by 95.2%.

In conclusion, the novel COMT inhibitor BIA 3-202 enhances the availability of L-DOPA in the brain by reducing its *O*-methylation, which may prove beneficial in parkinsonian patients treated with L-DOPA plus an aromatic amino acid decarboxylase inhibitor.

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