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Different *in vivo* properties of three new inhibitors of catechol *O*-methyltransferase in the rat

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1 We compared three new catechol *O*-methyltransferase (COMT) inhibitors (OR-611, Ro 40-7592 and CGP 28014; 10 and 30 mg kg^{-1}, i.p.) in male rats given levodopa (L-DOPA, 50 mg kg^{-1}, i.p.) and carbidopa ((−)-L-α-methyl dopa, 50 mg kg^{-1}, i.p.). In some studies pretreatment with pargyline (80 mg kg^{-1}, i.p.) was used to block the function of monoamine oxidase (MAO).

2 Decreases of hypothalamic and striatal 3-*O*-methyl-dopa (3-OMD) levels were used as measures of the inhibition of peripheral COMT. The inhibition of brain COMT activity was estimated by decreases of hypothalamic and striatal homovanillic acid (HVA) and 3-methoxytyramine (3-MT; after pargyline) levels.

3 The three COMT inhibitors studied had different individual characteristics. OR-611 was primarily a peripherally acting COMT inhibitor, decreasing 3-OMD levels in the striatum (to 31–52%) and in the hypothalamus (to 16–27%) both in the control and pargyline-treated animals at 1 and 3 h. It did not have any effect on brain HVA and 3-MT.

3 Ro 40-7592 was a broad spectrum COMT inhibitor decreasing striatal and hypothalamic 3-OMD (always to <30%), HVA (to <50%) and 3-MT levels (to <23%) significantly both at 1 and 3 h. It was more potent than OR-611.

4 CGP 28014 functioned as a weak COMT inhibitor in the periphery inhibiting 3-OMD formation only at 3 h. In contrast, it was fairly potent in decreasing the brain HVA and 3-MT levels at 1 h (to 37–22% and 42–35% in the striatum, and to 57–33% and 64–35% in the hypothalamus, respectively) but not at 3 h. Since CGP 28014, unlike OR-611 and Ro 40-7592, did not generally increase the brain DOPA, dopamine or DOPAC levels, it was not a typical COMT inhibitor.

Keywords: Catechol *O*-methyltransferase (COMT) inhibitors; brain catecholamines; DOPA metabolism; Parkinson's disease

Introduction

Catechol *O*-methyltransferase (COMT; EC 2.1.1.6) is a ubiquitous enzyme that has both soluble (S-COMT) and membrane-bound (MB-COMT) forms. COMT is one of the key enzymes in the metabolism of L-3,4-dihydroxyphenylalanine (DOPA) and catecholamines. DOPA is converted in the peripheral tissues and brain by dopa decarboxylase (DDC) to dopamine and by COMT to 3-*O*-methyldopa (3-OMD). If DDC is blocked, huge amounts of 3-OMD are formed (Cedarbaum, 1987). Dopamine in the rat (and human) brain is deaminated by monoamine oxidase (MAO) to 3,4-dihydroxy-phenylacetic acid (DOPAC). DOPAC is then *O*-methylated by COMT to the major final metabolite, homovanillic acid (HVA). If MAO is blocked, much 3-MT is formed by COMT from dopamine. Some conjugation reactions of dopamine, DOPAC and HVA also occur in the brain (Kopin, 1985).

The discovery of new COMT inhibitors (Männistö *et al.*, 1988; Borgulya *et al.*, 1989; Waldmeier *et al.*, 1990a) has greatly revitalized COMT research. Nitrocatechol is the key structure in the majority of these compounds. Several of them are very active COMT inhibitors *in vitro* with K_i values in the low nanomolar range. The compounds are highly selective without any action on other methyltransferases, or other catecholamine synthesizing or metabolizing enzymes (Männistö *et al.*, 1988; Borgulya *et al.*, 1989).

CGP 28014 is not a nitrocatechol but a nitropyridine derivative. It has no COMT inhibiting activity *in vitro*, while *in vivo* its action on the metabolism of DOPA and dopamine mimics that of the COMT inhibitors (Waldmeier *et al.*, 1990a,b). The mechanism of action of CGP 28014 is unknown.

We have compared the behaviour of the COMT inhibitors of different origins. There is some knowledge about the pharmacokinetic differences between some of these compounds. Some of them (Ro 41-0960; Ro 40-7592) penetrate the brain (Borgulya *et al.*, 1989), while others (e.g., OR-462; OR-611) enter the brain in very small amounts (Nissinen *et al.*, 1988; Männistö & Kaakkola, 1989; 1990). No similar data are available for CGP 28014. However, it seems to have central activity since it decreased HVA and 3-MT levels in the brain (Waldmeier *et al.*, 1990a,b).

Methods

Animals

Male Wistar rats (Wist/Kuo, 210–235 g; Department of Pharmacology and Toxicology, University of Helsinki) were housed 3–5 per cage at 21 ± 1°C in 12 h light and dark cycles (light on 07 h 00 min). Water and food were available *ad libitum*.

Protocols

A triple treatment of levodopa (50 mg kg^{-1}), carbidopa (50 mg kg^{-1}) and one of the COMT inhibitors (OR-611, Ro 40-7592 or CGP 28014; 0, 10 or 30 mg kg^{-1}) was given 60 or 180 min before the rats were killed. In some experiments, 80 mg kg^{-1} of pargyline hydrochloride was given 60 min before the triple treatment to block MAO and to redirect the dopamine metabolism in the brain. The drugs were injected intraperitoneally (i.p.) in a volume of 0.5 ml 100 g^{-1} animal weight. There were 5–8 animals per treatment group.

After decapitation by guillotine, the brains were removed within 30 s, cooled in liquid nitrogen, and the hypothalamus and striatum were dissected according to Glowinski & Iversen (1966). The dissected tissues were frozen in liquid nitrogen and stored at −80°C until analysed.

Analytical

DOPA, dopamine, noradrenaline (NA), 5-hydroxytryptamine (5-HT) and their metabolites in the hypothalamus and striatum were analysed by high performance liquid chromatography (h.p.l.c.) using electrochemical detection according to

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the method of Wagner et al. (1982) as modified by Männistö et al. (1990). Detection limits were 20 pg per injection for NA, normetanephrine (NMN), dopamine, DOPAC, 5-HT, 5-hydroxyindoleacetic acid (5-HIAA) and 3-MT and 100 pg per injection for 3-OMD and HVA.

Drugs

Carbidopa ((−)-L-α-methyldopa, Orion Pharmaceutica, Espoo, Finland) was suspended in a few drops of Tween 80 and then diluted with saline. Levodopa methylester hydrochloride (L-DOPA) and pargyline hydrochloride were from Sigma Chemical Company, MO, U.S.A. OR-611 (entacapone INN; N,N-diethyl-2-cyano-3-(3,4-dihydroxy-5-nitrophenyl) acrylamide), Ro 40-7592 (3,4-dihydroxy-4'-methyl-5-nitrobenzophenone) and CGP 28014 (N-(2-pyridone-6-yl)-N',N'-di-n-propylformamidine) were synthesized by Ms Aino Pippuri in Orion Pharmaceutica, Espoo, Finland. The purity of the compounds was checked with thin layer chromatography (t.l.c.) and nuclear magnetic resonance (n.m.r.) and was always better than 99%. These drugs were suspended in a few drops of Tween 80 and then diluted with saline. All doses refer to acids or bases.

The following chemicals for h.p.l.c. were purchased from Sigma (St. Louis, MO, U.S.A.): dopamine hydrochloride; DOPAC; HVA; 5-HT creatinine sulphate; NA hydrochloride; DOPA; 3-OMD; 3-MT; NMN; 3,4-dihydroxybenzylamine (internal standard); and 1-octane-sulphonic acid. Other chemicals were of analytical grade and obtained from Merck (Darmstadt, BRD).

Statistics

Arithmetic mean, s.e.mean and s.d. of each group were calculated. Results were analysed usually with one-way (treatment) analysis of variance, followed by a Tukey-Kramer post-hoc test. In case of deviation from the normal variation, a logarithmic transformation ($\log_{10}$) was made before statistics. In rare occasions when the variances between the treatment groups were far from equal, we used Kruskal-Wallis one-way analysis of variance instead. Systat statistical software was used in calculations (Wilkinson, 1990).

Results

Effect of COMT inhibitors on striatal and hypothalamic 3-OMD and HVA levels after levodopa and carbidopa treatment

At 1 h after levodopa (50 mg kg^{-1}) and carbidopa (50 mg kg^{-1}) and each of the three COMT inhibitors, brain 3-OMD levels were dose-dependently decreased by OR-611 and Ro 40-7592 but not by CGP 28014. OR-611 (10 and 30 mg kg^{-1}, respectively) decreased the 3-OMD levels to 52% and 39% in the striatum and to 27% and 10% in the hypothalamus ($P < 0.001$), and Ro 40-7592 (10 and 30 mg kg^{-1}) to 32% and 16% in the striatum and to 21% and 5% in the hypothalamus ($P < 0.001$) (Figure 1). These effects of OR-611 and Ro 40-7592 were sustained for at least 3 h, and even CGP 28014 decreased 3-OMD levels at 3 h (to 60–66%; Tables 1 and 2). Ro 40-7592 was the most effective of the three compounds at both time points.

Striatal and hypothalamic HVA levels, on the other hand, were not affected by OR-611 while Ro 40-7592 (10 and 30 mg kg^{-1}, respectively) decreased them to 44% and 24% in the striatum and to 49% in the hypothalamus. Even CGP 28014 (10 and 30 mg kg^{-1}) decreased HVA to 37% and 22% in the striatum and to 57% and 33% in the hypothalamus at 1 h ($P < 0.001$–0.01) (Figure 1). Ro 40-7592 was still active at 3 h, but CGP 28014 was not (Tables 1 and 2).

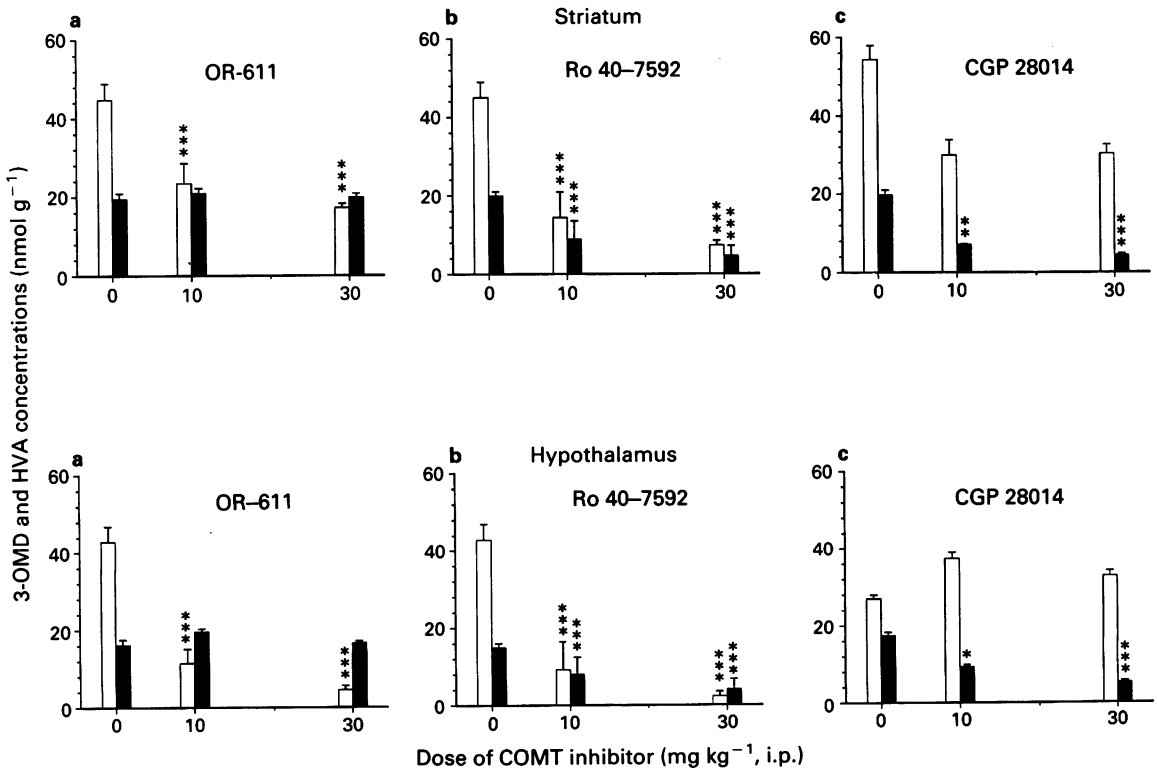

Figure 1 Striatal (upper part) and hypothalamic (lower part) 3-*O*-methyldopa (3-OMD, open columns) and homovanillic acid (HVA, solid columns) concentrations at 1 h after levodopa plus carbidopa (50 mg kg^{-1}, i.p.) and three catechol-*O*-methyltransferase (COMT) inhibitors ((a) OR-611; (b) Ro 40-7592 and (c) CGP 28014; all either 10 or 30 mg kg^{-1}, i.p.). Mean with s.e.mean shown by vertical bars. $n = 19$ in levodopa plus carbidopa group, otherwise $n = 5$–8. Statistics: * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ versus levodopa and carbidopa treatment (effect of COMT inhibitors).

Table 1 Striatal DOPA, 3-O-methyldopa (3-OMD), dopamine and its metabolite concentrations at 3 h after levodopa (50 mg kg^{-1}, i.p.) plus carbidopa (50 mg kg^{-1}, i.p.) and three catechol-*O*-methyltransferase (COMT) inhibitors (10 or 30 mg kg^{-1}, i.p.)

	Striatal concentrations (nmol g^{-1})				
Treatment	*DOPA*	*3-OMD*	*Dopamine*	*DOPAC*	*HVA*
Levodopa (50 mg kg^{-1}) + carbidopa (50 mg kg^{-1})	3.5 ± 0.5	121.7 ± 5.7	104.5 ± 3.3	69.0 ± 4.6	48.4 ± 2.2
Levodopa + carbidopa + OR-611 (10 mg kg^{-1})	7.6 ± 1.5	32.7 ± 5.2***	166.7 ± 9.8***	168.9 ± 4.8***	62.6 ± 4.4**
Levodopa + carbidopa + OR-611 (30 mg kg^{-1})	13.2 ± 3.5	24.1 ± 8.0***	169.1 ± 13.1***	171.9 ± 19.0***	58.7 ± 1.6*
Levodopa + carbidopa + Ro 40-7592 (10 mg kg^{-1})	30.4 ± 5.6***	9.9 ± 0.9***	175.0 ± 13.7***	267.7 ± 7.7***	17.0 ± 1.6***
Levodopa + carbidopa + Ro 40-7592 (30 mg kg^{-1})	22.8 ± 2.0***	7.7 ± 0.5***	176.3 ± 13.1***	276.0 ± 7.7***	11.5 ± 0.5***
Levodopa + carbidopa + CGP 28014 (10 mg kg^{-1})	9.1 ± 2.5	72.9 ± 14.2**	109.0 ± 9.1	104.7 ± 22.0	39.0 ± 6.6
Levodopa + carbidopa + CGP 28014 (30 mg kg^{-1})	10.1 ± 4.1	80.5 ± 4.7***	109.7 ± 12.4	144.6 ± 15.5***	31.3 ± 2.7***

Mean ± s.e.mean. $n = 19$ in levodopa plus carbidopa group, otherwise $n = 5$–8. DOPAC: 3,4-dihydroxy-phenylacetic acid; HVA: homovanillic acid. Statistics: $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ versus levodopa plus carbidopa treatment.

Other amines, DOPA and amine metabolites after levodopa and carbidopa and each of the COMT inhibitors

At 1 h, DOPA and dopamine levels in the striatum and hypothalamus were generally increased (maximally about 2 fold) by OR-611 and Ro 40-7592 (except by 10 mg kg^{-1} in the striatum) but not by CGP 28014 (Tables 3 and 4). All COMT inhibitors elevated generally DOPAC levels (maximally 2 fold). However, the effect of CGP 28014 on striatal DOPAC was not significant at 1 h (Tables 3 and 4).

At 3 h, DOPA levels were significantly elevated only by Ro 40-7592 both in the striatum (7–9 fold) and hypothalamus (3.5–4.5 fold), and by 30 mg kg^{-1} of OR-611 in the latter (3 fold; Tables 1 and 2). Ro 40-7592 and OR-611 also increased dopamine (less than 2 fold) and DOPAC levels (2.5–4 fold) in both brain regions. Even CGP 28014, at 30 mg kg^{-1}, enhanced DOPAC levels (about 2 fold) both in the striatum and hypothalamus (Tables 1 and 2).

NA and NMN as well as 5-HT and 5-HIAA levels were not significantly affected by any treatment in either brain area (not shown).

Table 2 Hypothalamic DOPA, 3-O-methyldopa (3-OMD), dopamine and its metabolite concentrations at 3 h after levodopa plus carbidopa (50 mg kg^{-1}, i.p.) and three different catechol-*O*-methyltransferase (COMT) inhibitors (10 or 30 mg kg^{-1}, i.p.)

	Hypothalamic concentrations (nmol g^{-1})				
Treatment	*DOPA*	*3-OMD*	*Dopamine*	*DOPAC*	*HVA*
Levodopa (50 mg kg^{-1}) + carbidopa (50 mg kg^{-1})	9.6 ± 1.0	127.4 ± 3.8	15.0 ± 0.3	38.1 ± 2.4	25.8 ± 1.1
Levodopa + carbidopa + OR-611 (10 mg kg^{-})	20.8 ± 5.1	30.3 ± 4.3***	25.5 ± 2.6***	89.8 ± 17.8**	29.1 ± 3.8
Levodopa + carbidopa + OR-611 (30 mg kg^{-1})	29.4 ± 4.6***	29.3 ± 10.9***	28.1 ± 2.6***	111.2 ± 13.7***	41.7 ± 2.2***
Levodopa + carbidopa + Ro 40-7592 (10 mg kg^{-1})	43.6 ± 4.1***	8.0 ± 1.4***	30.0 ± 1.3***	163.6 ± 8.3***	12.6 ± 1.1***
Levodopa + carbidopa + Ro 40-7592 (30 mg kg^{-1})	34.0 ± 6.1***	2.4 ± 0.5***	28.3 ± 2.1***	162.4 ± 8.3***	7.1 ± 0.5***
Levodopa + carbidopa + CGP 28014 (10 mg kg^{-1})	13.7 ± 4.1	81.0 ± 14.7***	15.7 ± 3.3	54.1 ± 13.7	21.4 ± 4.9
Levodopa + carbidopa + CGP 28014 (30 mg kg^{-1})	18.3 ± 2.0	81.9 ± 4.3***	18.9 ± 1.3	80.3 ± 7.1	19.2 ± 1.6

Mean ± s.e.mean. $n = 19$ in levodopa plus carbidopa group, otherwise $n = 5$–8. DOPAC: 3,4-dihydroxy-phenylacetic acid; HVA: homovanillic acid. Statistics: $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ versus levodopa plus carbidopa treatment.

Table 3 Striatal DOPA, dopamine and 3,4-dihydroxy-phenylacetic acid (DOPAC) concentrations at 1 h after levodopa (50 mg kg^{-1}, i.p.) and carbidopa (50 mg kg^{-1}, i.p.) and three different catechol-*O*-methyltransferase (COMT) inhibitors (10 or 30 mg kg^{-1}, i.p.)

	Striatal concentrations (nmol g^{-1})		
Treatment	*DOPA*	*Dopamine*	*DOPAC*
Levodopa (50 mg kg^{-1}) + carbidopa (50 mg kg^{-1})	19.8 ± 2.5	109.7 ± 4.6	49.4 ± 3.6
Levodopa + carbidopa + OR-611 (10 mg kg^{-1})	35.5 ± 1.5*	163.3 ± 5.9***	74.4 ± 3.0*
Levodopa + carbidopa + OR-611 (30 mg kg^{-1})	36.5 ± 4.1**	176.3 ± 8.5***	95.2 ± 4.7**
Levodopa + carbidopa + Ro 40-7592 (10 mg kg^{-1})	37.5 ± 5.1	152.1 ± 17.0	82.1 ± 9.5*
Levodopa + carbidopa + Ro 40-7592 (30 mg kg^{-1})	40.6 ± 5.6**	153.4 ± 14.4*	95.2 ± 10.7
Levodopa + carbidopa + CGP 28014 (10 mg kg^{-1})	23.1 ± 4.3	114.9 ± 8.5	61.0 ± 6.2
Levodopa + carbidopa + CGP 28014 (30 mg kg^{-1})	27.5 ± 2.5	128.9 ± 7.2	66.0 ± 4.7

Mean ± s.e.mean. $n = 19$ in levodopa plus carbidopa group, otherwise $n = 5$–8. Statistics: * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ versus levodopa plus carbidopa treatment.

Table 4 Hypothalamic DOPA, dopamine and 3,4-dihydroxy-phenylacetic acid (DOPAC) concentrations at 1 h after levodopa (50 mg kg^{-1}, i.p.) plus carbidopa (50 mg kg^{-1}, i.p.) and three different catechol-*O*-methyltransferase (COMT) inhibitors (10 or 30 mg kg^{-1}, i.p.)

Treatment	*Hypothalamic concentrations* (nmol g^{-1}) *DOPA*	*Dopamine*	*DOPAC*
Levodopa (50 mg kg^{-1}) + carbidopa (50 mg kg^{-1})	23.8 ± 2.5	14.4 ± 0.2	42.5 ± 3.9
Levodopa + carbidopa + OR-611 (10 mg kg^{-1})	42.6 ± 1.5**	26.8 ± 0.7***	81.1 ± 1.5**
Levodopa + carbidopa + OR-611 (30 mg kg^{-1})	50.2 ± 4.6***	27.4 ± 1.3***	99.9 ± 5.2**
Levodopa + carbidopa + Ro 40-7592 (10 mg kg^{-1})	45.3 ± 6.4**	22.2 ± 1.3***	91.0 ± 10.3
Levodopa + carbidopa + Ro 40-7592 (30 mg kg^{-1})	49.1 ± 5.0***	26.3 ± 1.8***	97.0 ± 10.9
Levodopa + carbidopa + CGP 28014 (10 mg kg^{-1})	33.8 ± 1.9	18.9 ± 8.5	61.3 ± 5.9
Levodopa + carbidopa + CGP 28014 (30 mg kg^{-1})	35.0 ± 2.0*	18.3 ± 1.3	75.0 ± 4.8**

Mean ± s.e.mean. $n = 19$ in levodopa plus carbidopa group, otherwise $n = 5$–8. Statistics: * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ versus levodopa plus carbidopa treatment.

Effect of each of the COMT inhibitors on striatal and hypothalamic 3-OMD and 3-MT levels after levodopa and carbidopa in the pargyline pretreated rats

In pargyline pretreated rats, OR-611 (10 and 30 mg kg^{-1}, respectively) suppressed brain 3-OMD levels at 1 h after levodopa and carbidopa treatment to 41% and 31% in the striatum and to 16% and 18% in the hypothalamus ($P < 0.001$). Ro 40-7592 (10 and 30 mg kg^{-1}) was even more effective, decreasing the 3-OMD levels to 14% and 11% in the striatum and to 11% and 8% in the hypothalamus. Again the effect of CGP 28014 was not significant (Figure 2).

CGP 28014 ($P < 0.01$–0.001) and Ro 40-7592 ($P < 0.001$) decreased both striatal (CGP 28014 to 42% and 35% and Ro 40-7592 to 20% and 14%) and hypothalamic (CGP 28014 to 64% and 45% and Ro 40-7592 to 23% and 12%) 3-MT levels at 10 and 30 mg kg^{-1}, respectively. OR-611 had no effect on the brain 3-MT levels (Figure 2).

Few of the rats given pargyline with levodopa and carbidopa survived for 3 h in the pilot studies. Therefore, we did not perform 3 h experiments in the pargyline pretreated rats.

Other amines, DOPA and amine metabolites after levodopa and carbidopa and each of the COMT inhibitors in the pargyline pretreated rats

Even after the pargyline pretreatment, DOPA and dopamine levels in the striatum and hypothalamus were generally further increased by OR-611 (always less than 2 fold) and Ro 40-7592 (usually less than 3 fold) but not by CGP 28014. Both DOPAC and HVA levels were near the detection limit and no effect of any COMT inhibitor was noted (not shown).

NA and 5-HT levels in the hypothalamus were greatly increased by pargyline (2 fold and 5 fold, respectively) and NA levels further significantly elevated by OR-611 (1.2 fold) and Ro 40-7592 (1.5 fold), but never significantly elevated by CGP

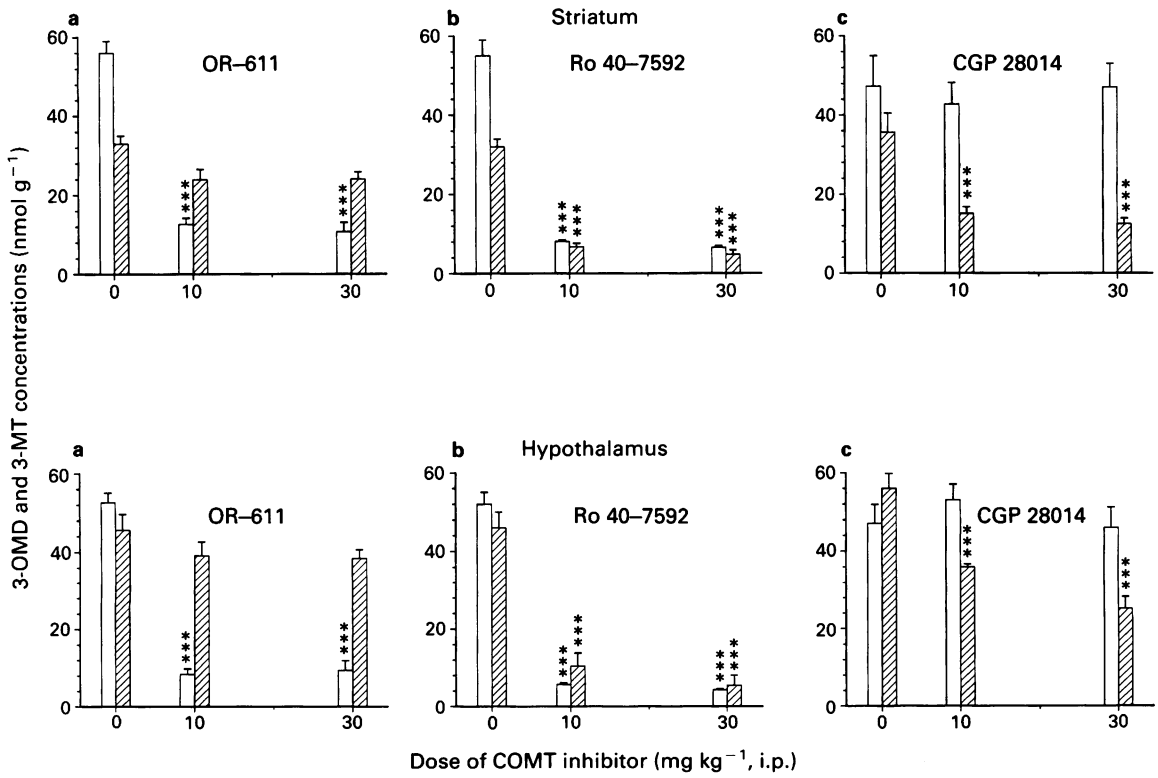

Figure 2 Striatal (upper part) and hypothalamic (lower part) 3-O-methyldopa (3-OMD, open columns) and 3-methoxytyramine (3-MT, hatched columns) concentrations at 1 h after levodopa plus carbidopa (50 mg kg^{-1}, i.p.) in the pargyline-treated (80 mg kg^{-1}, 1 h before levodopa and carbidopa) and three catechol-*O*-methyltransferase (COMT) inhibitors ((a) OR-611; (b) Ro 40-7592 and (c) CGP 28014; all either 10 or 30 mg kg^{-1}, i.p.). Mean with s.e.mean shown by vertical bars. $n = 19$ in levodopa plus carbidopa group, otherwise $n = 5$–8. Statistics: * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ versus levodopa and carbidopa treatment (effect of COMT inhibitors).

28014. Corresponding NMN and 5-HIAA levels were significantly suppressed (not shown).

Pargyline elevated 5-HT levels and decreased 5-HIAA also in the striatum, while changes in NA levels were not significant. Only Ro 40-7592 further enhanced striatal noradrenaline levels (1.7 fold) over those caused by pargyline. Both OR-611 and Ro 40-7592 decreased significantly striatal NMN levels in the pargyline-treated rats (not shown).

Discussion

The major indicators of COMT function in the present study are the concentrations of 3-OMD, HVA and 3-MT in the striatal and hypothalamic tissues. Most of the peripherally administered levodopa does not enter the brain. Therefore most of the 3-OMD is also formed outside the brain. Since 3-OMD readily enters the brain (Kopin, 1985), its amount in the brain tissue is a measure of the peripheral action of COMT. HVA and 3-MT analysed from the brain samples can be considered purely as the COMT-produced brain metabolites of dopamine. They do not easily pass the blood-brain barrier, and therefore the peripherally formed HVA and 3-MT do not significantly affect levels in the brain. Normally, the brain levels of 3-MT, even after levodopa loading, are near or below the electrochemical detection limit. If MAO pathways are blocked (for instance by using pargyline), 3-MT concentrations become easily analysable (Kopin, 1985). Changes in 3-MT can then be used to support the results obtained by measuring HVA levels.

Based on the above definitions, each of the three COMT inhibitors studied behaved differently. OR-611 was principally a peripherally acting COMT inhibitor. It did not decrease but rather increased brain HVA. Even so it increased significantly brain dopamine levels apparently through enhanced DOPA supply. A moderate lowering effect on 3-OMD lasted at least 3 h. Although it produced little, if any, inhibition of brain COMT, it was able to potentiate the action of levodopa in behavioural studies (Etemadzadeh *et al.*, 1989).

Ro 40-7592 was a broad-spectrum COMT inhibitor acting effectively both in the periphery and brain, as described by Zürcher *et al.* (1990). It was also long-acting, still having nearly maximum efficacy at 3 h. Ro 40-7592 closely resembled one of its chemical relatives, Ro 41-0960, which has been thoroughly studied (Borgulya *et al.*, 1989).

CGP 28014 was a poor COMT inhibitor in the periphery but quite an efficient COMT inhibitor in the brain, preventing both HVA and 3-MT formation. It was, however, less effective than Ro 40-7592. This kind of brain selectivity of CGP 28014 has not been emphasized before. In the studies of Waldmeier and coworkers (1990a,b), CGP 28014 inhibited 3-OMD and HVA formation to a similar extent both after oral and intraperitoneal administration. Therefore the route of intake does not explain the difference between our and their results.

At the present time, we cannot resolve the disparity in our results and those of Waldmeier and coworkers (1991a,b). However, the weak and delayed effect of CGP 28014 on peripheral COMT was a constant finding. Most of the original experiments on CGP 28014 were done without levodopa treatment. Even in rare cases where levodopa was given, it was administered without the inhibitor of peripheral dopa decarboxylase (DDC) (Waldmeier *et al.*, 1990a,b). Therefore, most of the DOPA was metabolized to dopamine in the peripheral tissues. Only minor amounts of DOPA were metabolized to 3-OMD or transported to the brain. In the present study, levodopa and carbidopa were always given together. Levodopa coadministered with carbidopa increased DOPA and dopamine levels in the striatal microdialysis fluid 7.8 and 3.3 times, respectively, over those after levodopa alone (Nakashima *et al.*, 1991). Accordingly, in our studies the levels of 3-OMD in the brain were already high at 1 h and were still increasing at 3 h. Even so, the inhibitory effect of CGP 28014 on 3-OMD formation became significant only when at least 3 h had elapsed from the administration of carbidopa. At this time, DOPA levels in the brain were already decreased to one-half or less than those at 1 h.

It is hard to explain how the presence of carbidopa or DOPA would abolish the effect of CGP 28014 on peripheral COMT. The weak COMT inhibiting activity of carbidopa and/or DOPA in the peripheral tissues (Baldessarini, 1972; Kaakkola *et al.*, 1991) would rather have the opposite effect (LeWitt, 1989).

The mechanism of action of CGP 28014 is unknown. It inhibits COMT *in vitro* only in millimolar concentrations. The major metabolite of CGP 28014, 2-amino-6-hydroxypyridine, is not a COMT inhibitor (Waldmeier *et al.*, 1990a,b). In the present study, DOPA, dopamine and DOPAC levels were increased very little by CGP 28014 in the striatum or hypothalamus. In this respect CGP 28014 differed from the real COMT inhibitors, the nitrocatechol derivatives. CGP 28014 may inhibit the transfer of the COMT substrates to the enzyme, i.e., it may inhibit uptake$_2$, as already discussed by Waldmeier *et al.* (1991a). Since peripheral uptake$_2$ is not identical to the brain uptake$_2$ (Trendelenburg, 1989), we suggest that CGP 28014 preferentially inhibits the central type uptake$_2$.

The majority of dopamine in the rat brain is metabolized by neuronal monoamine oxidase-A (MAO-A, Oreland *et al.*, 1983; Kato *et al.*, 1986). Hence, if uptake$_2$ is inhibited, the subsequent shortage of dopamine in the glial cells, where MAO-B prevails (Kopin, 1985), would normally not decrease DOPAC formation. However, when animals are loaded with levodopa, as in the present study, the capacity of MAO-A can be surpassed, and MAO-B becomes important (Männistö & Tuomainen, 1991). Therefore, uptake$_2$ inhibition would decrease the entry of dopamine to glial MAO-B. Indeed, in the present study, less DOPAC was seen after CGP 28014 than after OR-611 or Ro 40-7592.

In conclusion, the three compounds studied behave differently: OR-611 is an effective, but mainly peripheral COMT inhibitor. Ro 40-7592 is even more potent and long acting, and has an additional inhibitory effect on COMT in the brain. CGP 28014 is probably not a COMT inhibitor at all. However, it preferentially inhibits HVA and 3-MT formation in the brain but inhibits only weakly 3-OMD formation in the periphery.

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