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Effects of COMT inhibitors on striatal dopamine metabolism: a microdialysis study

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In vivo microdialysis was used to examine the effect of two new catechol-*O*-methyltransferase (COMT) inhibitors, Ro 40-7592 and OR-611, on extracellular levels of dopamine, dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), and 5-hydroxyindoleacetic acid (5-HIAA) in rat striatum. The interactions of the COMT inhibitors with nomifensine, clorgyline and deprenyl were also studied. Ro 40-7592 (3–30 mg/kg, i.p.) decreased dose-dependently the efflux of HVA, increased that of DOPAC, and tended to increase that of dopamine. Higher doses of OR-611 (30–100 mg/kg, i.p.) also decreased the dialysate level of HVA, increased that of DOPAC, and tended to increase that of dopamine. Ro 40-7592 was about ten-fold as potent as OR-611. Neither of the COMT inhibitors changed dialysate levels of 5-HIAA. An OR-611 dose of 10 mg/kg i.p. had no significant effect, in contrast to Ro 40-7592, on any of the parameters studied; this dose was thus used to differentiate between the effects of central and peripheral COMT inhibition. Both nomifensine (15 mg/kg, i.p.) and clorgyline (4 mg/kg, i.p.) alone elevated extracellular dopamine levels, and lowered those of DOPAC and HVA, though there were quantitative and temporal differences between the drugs. L-Deprenyl (1 mg/kg, i.p.) alone had no significant effect on any of the compounds measured. Ro 40-7592 (10 mg/kg, i.p.) potentiated the effect of nomifensine on dopamine efflux, and it tended to increase clorgyline-induced dopamine efflux. DOPAC levels in dialysates were significantly increased by combinations of Ro 40-7592 and nomifensine or clorgyline, whereas HVA levels remained about as low as they were after Ro 40-7592 alone. Ro 40-7592 had no significant interactions with L-deprenyl. OR-611 (10 mg/kg, i.p.) did not modify the effects on dopamine metabolism of nomifensine, clorgyline or L-deprenyl. These data show that Ro 40-7592 is a potent centrally active COMT inhibitor, whereas OR-611 is principally a peripherally active inhibitor. Use of drugs which inhibit brain COMT can considerable modify dopamine metabolism. COMT inhibitors may be of clinical significance in treating Parkinson's disease.

INTRODUCTION

The most important step for terminating the actions of dopamine (and other catecholamines) in brain synapses involves its uptake into nerve terminals (uptake 1) and perhaps non-neural cells (uptake 2), and its subsequent metabolism by monoamine oxidase (MAO) and catechol-*O*-methyltransferase (COMT) [26,44]. Considerable information is now available about the uptake mechanisms and MAO, largely because potent and specific pharmacological agents have been available for modulating the macromolecules which subserve these functions. It has, for instance, been demonstrated that MAO exists as two isoenzymes, MAO-A and MAO-B, whose molecular structures are now known [4,48]. The former is located within both dopaminergic neurons and non-neural cells, whereas the latter is thought to be located largely in non-neural (glial) cells [11,15,27,42,45].

Although the main function of COMT, *O*-methylation of catechols, was described in the late 1950s [1,2], the significance of this enzyme has been less studied, in part because of the lack of suitable in vivo inhibitors. The recent development of selective and potent COMT inhibitors, however, has restimulated interest in this enzyme and in the potential use of COMT inhibitors as adjunct therapy in Parkinsons's disease [5,7,31,32,33]. There appear to be 2 forms of COMT, soluble and membrane bound, both of which are located extraneuronally and not within dopaminergic terminals [22,23,39]. In rat stria-

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tum the soluble COMT is probably a glial enzyme, whereas membrane bound COMT may be localized in postsynaptic neurons [22,39]. Very recently, the primary structures of these enzyme forms have also been described [6,29,40].

The present series of experiments examined the effects of 2 new COMT inhibitors, Ro 40-7592 and OR-611, on dopamine metabolism in rat striatum, using the technique of in vivo microdialysis. Dialysate levels of dopamine, dihydroxyphenylacetic acid (DOPAC; a MAO-dependent metabolite), homovanillic acid (HVA; a MAO-and COMT-dependent metabolite), and 5-hydroxyindoleacetic acid (5-HIAA; a MAO-dependent metabolite of 5-hydroxytryptamine) were measured. Both of the COMT inhibitors are nitrocatechols, and are selective and potent agents in vitro with K_i values in low nanomolar range [28,32,36,53,54]. After systemic administration, Ro 40-7592–but not OR-611–penetrates the blood–brain barrier easily [30,34,36,53]. We studied the interactions of the COMT inhibitors with nomifensine (a DA uptake inhibitor [18]), clorgyline (a MAO-A inhibitor [21]) and L-deprenyl (a MAO-B inhibitor [25]).

MATERIALS AND METHODS

Animals

Male Sprague–Dawley rats (Charles River Breeding Lab., Wilmington, MA, USA) weighing 270–370 g were exposed to light between 08.00 and 20.00 h, and were given free access to food (Prolab Animal Diet 3000, Agway Inc., Syracuse, NY, USA) and water.

Surgical and microdialysis procedures

For surgery and dialysate collections, animals were anaesthetized with α-chloralose/urethane (50/500 mg/kg i.p. in isotonic saline). Further anaesthesia was provided as required to maintain a stable behavioral level. A heating pad was used to maintain body temperature at 37°C. Each animal was placed in a Kopf stereotaxic frame, its skull exposed, and a burr hole was drilled above the right striatum (A + 0.4, L–2.7 [38]). A concentric dialysis probe (either CMA/10 probe, 500 μm o.d., 4 mm exposed dialysis membrane, Carnegie Medicin, Solna, Sweden; or custom-constructed probe, 210 μm o.d., 4 mm exposed membrane, essentially as described by Parry et al. [37]) was then implanted; the tip extending 6.5 mm below the dura. The probe was perfused with Krebs–Ringer solution (Na^+ 147 mM, K^+ 3.5 mM, Ca^{2+} 1.0 mM, Mg^{2+} 1.2 mM, Cl^- 129 mM, PO_4^{3-} 1 mM, HCO_3^- 25 mM, gassed with O_2/CO_2 (95/5%) to pH 7.35) at a flow rate of 1.0 μl/min, using a CMA microperfusion pump (Carnegie Medicin, Solna, Sweden).

Prior to implanation, recovery of substances by these probes was studied in vitro. Each probe was placed in a beaker containing 100 nM solutions of the relevant amines or metabolites at room temperature. At a perfusion rate of 1.0 μl/min, the in vitro recoveries of the compounds varied as follows for CMA probes: DA 18–24%, DOPAC 21–29%, HVA 24–30%, and 5-HIAA 24–28%; for custom-constructed probes: DA 11–16%, DOPAC 15–21%, HVA 15–22%, and 5-HIAA 13–20%. The recovery data were used only to assess between-probe variability and not to estimate extracellular concentrations in the dialysates.

Following probe implantation, dialysate samples were discarded over the first 120 min in order to allow recovery from the acute effects of the implantation procedure. Dialysate samples were then collected continuously for 20 min periods into tubes containing 5 μl of 0.5 M perchloric acid to minimize decomposition. Following 1 h baseline period, drugs of interest were injected and further dialysate samples collected for 260–280 min. The position of the probe was verified by visual inspection of the brain slices at the end of the experiment.

HPLC analysis

Dialysate levels of dopamine, DOPAC, HVA and 5-HIAA were measured by high-pressure liquid chromatography (HPLC) and electrochemical detection (EC). The compounds were separated on a 3 μm HR-80 column (ESA Inc., Bedford, MA, USA) using a mobile phase consisting of 70 mM sodium phosphate, 1.1 mM heptane sulphonic acid, 0.22 mM EDTA, 4% v/v methanol, and 0.1% v/v tetrahydrofuran. The pH was adjusted to 2.90 with phosphoric acid. The mobile phase was pumped at a rate of 1.3 ml/min using an isocratic Altex or Hitachi pump with dual SSI suppressors in series. An ESA 5020 guard cell with the electrode set at +0.55 V preceded the injector (Rheodyne model 7125 with 20 μl loop; Rheodyne Inc., Cotati, CA, USA). The compounds were detected using an ESA 5100A detector with a screening electrode at +0.01 V and a working electrode set to an oxidation potential of +0.36 V. Chromatograms were simultaneously recorded on a two-channel RYT recorder (BAS, West Lafayette, IN, USA) and a HP 3392A integrator (Hewlett-Packard Co., Avondale, PA, USA). The injection of solutions of Ro 40-7592 or OR-611 did not generate any peaks in this HPLC system.

Drugs and chemicals

OR-611 (entacapone pINN; E-2-cyano-*N*,*N*-diethyl-3-(3,4-dihydroxy-5-nitrophenyl)propenamide) and Ro 40-7592 (4′-methyl-3,4-dihydroxy-5-nitro-benzophenone) were synthesized by Orion Pharmaceutica (Espoo, Finland). The sources of the other drugs were as follows: clorgyline-HCl (RBI, Natick, MA, USA); nomifensine maleate (Hoechst–Roussell Pharmaceuticals Inc., Somerville, NJ, USA); selegiline HCl (L-deprenyl; Chinoin Chemical and Pharmaceutical Works, Budapest, Hungary). The drugs were either dissolved in physiological saline or, if insoluble in saline, suspended in 5% gum arabic. They were injected intraperitoneally (i.p.). The doses refer to free acid or base.

The standards for HPLC analysis were purchased from Sigma Chemical Company (St. Louis, MO, USA). Other chemicals were of analytical grade and obtained from Aldrich Chemical Company (Milwaukee, WI, USA) or Mallinckrodt (Paris, KY, USA).

Statistics

The results are expressed as means ± S.E.M. of the percentage change from baseline levels. The area under the curve (AUC) values [35] for each animal were calculated using Simpson's rule. The statistical significances of differences, in AUC or basal values between various treatment groups, were determined by one-way analysis of variance, followed by Tukey's multiple comparison test. The effects of vehicle, clorgyline and L-deprenyl alone over time were analyzed using a one-way repeated analysis of variance (with Huynh–Feldt adjustment). The level of significance was set at $P < 0.05$.

RESULTS

Effects of COMT inhibitors on striatal dopamine, DOPAC, HVA and 5-HIAA efflux

Basal dialysate levels of dopamine, DOPAC, HVA and 5-HIAA were comparable among the control and treatment groups. The average (±S.E.M.) basal dialysate levels of animals tested ($n = 44$) were as follows: dopamine 72 ± 4 fmol/20 min; DOPAC 38.7 ± 1.8 pmol/20 min; HVA 30.9 ± 1.5 pmol/20 min; 5-HIAA 5.6 ± 0.2 pmol/20 min. After i.p. injection of

the vehicle, the extracellular levels of dopamine, DOPAC and 5-HIAA remained rather stable for the entire collecting period, whereas a slight but significant decrease with time was observed in the HVA efflux ($P < 0.01$; Figs. 1C and 2C).

Administration of 10–30 mg/kg of Ro 40-7592 non-significantly ($P > 0.05$) increased striatal extracellular dopamine levels (Fig. 1A). A non-significant increase in dopamine efflux was also seen after the administration of the highest dose (100 mg/kg) of OR-611 tested (Fig. 2A).

Both Ro 40-7592 and OR-611 dose-dependently increased striatal DOPAC efflux ($P < 0.001$; Figs. 1B and 2B). The maximum increase was $170 \pm 4\%$ of baseline after Ro 40-7592 at 120 min and $160 \pm 9\%$ of baseline after OR-611 at 180 min. Ro 40-7592 was clearly more potent than OR-611; 10 mg/kg (~ 36.6 μmol/kg) of Ro 40-7592 was approximately equipotent with 100 mg/kg (~ 328 μmol/kg) of OR-611.

Ro 40-7592 decreased effectively and dose-dependently extracellular HVA levels ($P < 0.001$; Fig. 1C). After 30 mg/kg of Ro 40-7592, the maximum HVA decrease was to $3.4 \pm 0.5\%$ of basal levels at 180 min, and no clear recovery was seen during the period of collections. OR-611 also caused a dose-dependent reduction in HVA efflux ($P < 0.001$; Fig. 2C). The maximum decrease was to $9.0 \pm 1.2\%$ of baseline, and some recovery was seen during the 280 min collection period. Again, Ro 40-7592 was about 10 times more potent than OR-611.

Neither Ro 40-7592 nor OR-611 had any effect on striatal 5-HIAA efflux (data not shown).

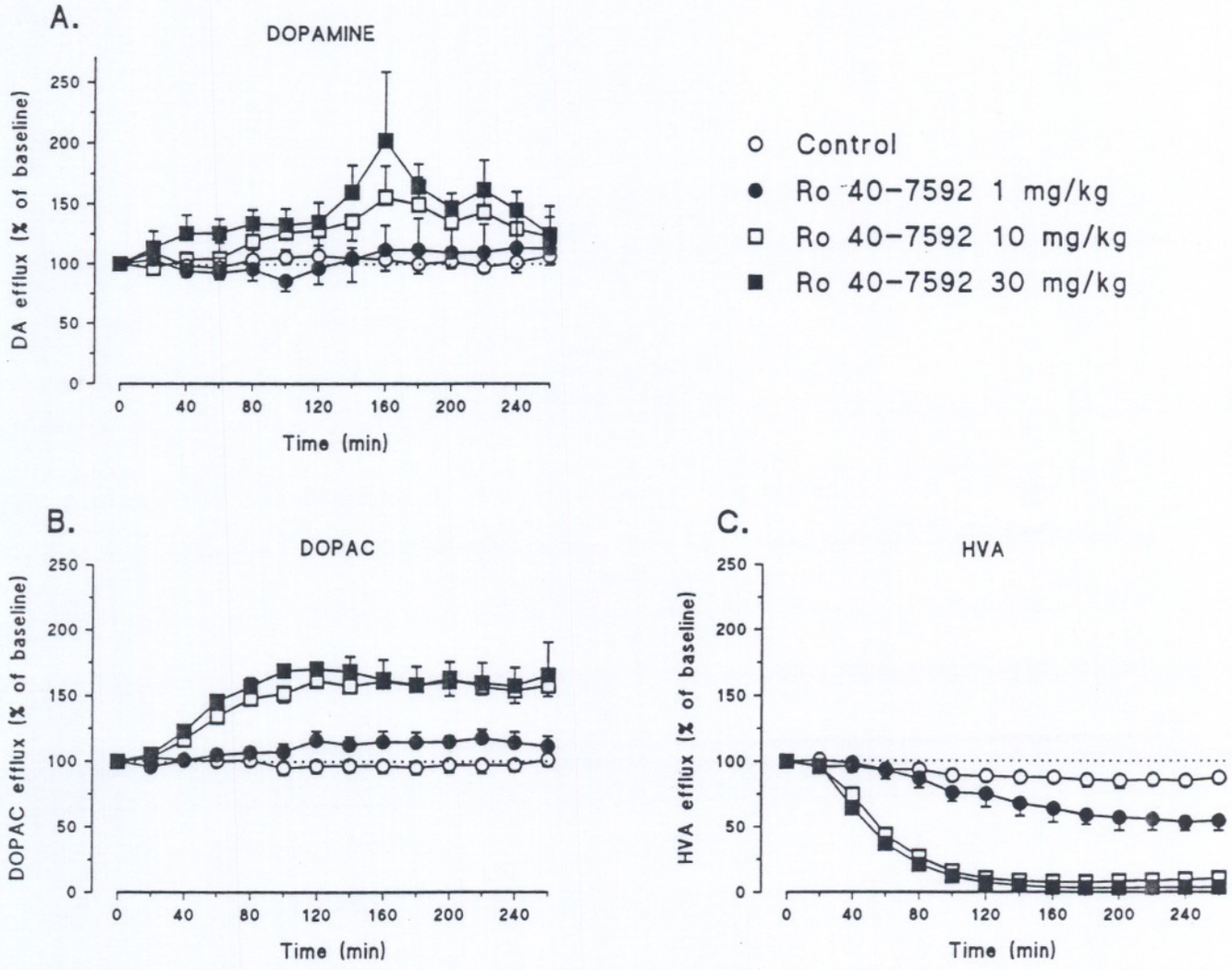

Fig. 1. Dose-response effects of Ro 40-7592 (0, 1, 10 and 30 mg/kg) on striatal extracellular levels of dopamine (A), DOPAC (B) and HVA (C). Injections were given i.p. at time = 0 min. Dialysis samples were collected for 20 min periods and assayed by HPLC-EC. Data are given as mean ($\pm$S.E.M.) percent variation of basal (preinjection) values, $n = 6$ for each group except for 1 mg/kg group where $n = 4$. See text for statistics.

Effects of nomifensine and COMT inhibitors on striatal dopamine, DOPAC, HVA and 5-HIAA efflux

Average baseline dialysate levels for the compounds studied were of the same magnitude as noted above, and no significant differences were found among the various treatment groups. Nomifensine (15 mg/kg, i.p.) given alone significantly increased striatal dopamine efflux; its maximum effect was to raise dopamine levels to $649 \pm 108\%$ of baseline 40 min after its injection ($P < 0.05$, compared to the control group which is omitted from Fig. 3 for clarity). Ro 40-7592 (10 mg/kg) significantly ($P < 0.01$, compared to nomifensine alone) potentiated the effect of nomifensine on striatal dopamine efflux, whereas OR-611 (10 mg/kg) was ineffective (Fig. 3A).

Nomifensine decreased striatal DOPAC efflux to $52.0 \pm 3.7\%$ of baseline after 280 min ($P < 0.001$, compared to the control group). After rats received the combination of nomifensine and Ro 40-7592, dialysate DOPAC levels first increased slightly, and then decreased to baseline values (Fig. 3B). This combination effect was significantly different from those of Ro 40-7592 ($P < 0.001$) or of nomifensine ($P < 0.001$) alone. OR-611 did not significantly modify nomifensine-induced decrease in dialysate DOPAC levels (Fig. 3B).

Nomifensine alone did not change dialysate HVA level until 140 min after its administration, after which time a significant decrease was observed (i.e. compared with values in the control group; $P < 0.001$). After rats

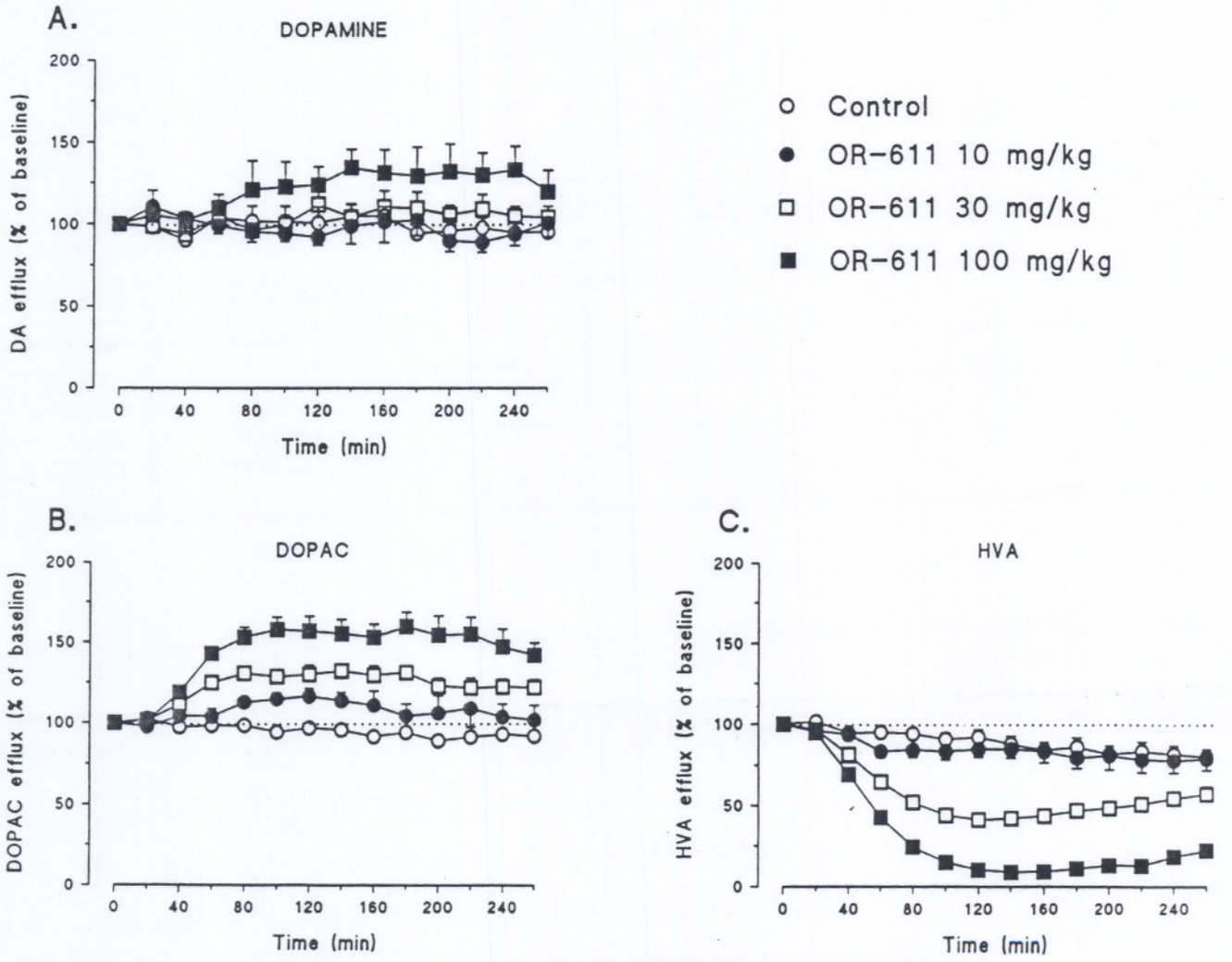

Fig. 2. Dose-response effects of OR-611 (0, 10, 30 and 100 mg/kg) on striatal extracellular level of dopamine (A), DOPAC (B) and HVA (C). Injections were given i.p. at time = 0 min. Dialysis samples were collected for 20 min periods and assayed by HPLC-EC. Data are given as mean ($\pm$ S.E.M.) percent variation of basal (preinjection) values, $n = 6$ for each group except for 100 mg/kg group where $n = 4$. See text for statistics.

received the combination of nomifensine and Ro 40-7592, dialysate HVA levels remained as low as they were after Ro 40-7592 alone (Fig. 3C). There were no significant interactions between nomifensine and OR-611 regarding HVA efflux (Fig. 3C).

Nomifensine and COMT inhibitors given together did not significantly modify dialysate 5-HIAA levels (data not shown).

Effects of clorgyline and COMT inhibitors on striatal dopamine, DOPAC, HVA and 5-HIAA efflux

Basal dialysate levels of dopamine, DOPAC, HVA and 5-HIAA (Table I) were moderately lower in rats receiving clorgyline plus COMT inhibitors, as well as in those described in all following experimental groups, probably due to lower in vitro recovery of the compounds when our custom-constructed probes were used. Pretreatment of rats with clorgyline (4 mg/kg) significantly increased baseline dopamine levels (Table I). Clorgyline also significantly decreased baseline levels of DOPAC, HVA and 5-HIAA (Table I). During the period of collections (280 min), the striatal dopamine efflux of clorgyline-pretreated rats did not change significantly, whereas the efflux of DOPAC, HVA and 5-HIAA all further decreased ($P < 0.001$).

The administration of Ro 40-7592 (10 mg/kg) to clorgyline-pretreated rats moderately increased dialysate dopamine levels. This change, however, was not significantly greater than that produced by Ro 40-7592 alone (as percent scale), although it was significantly greater than that produced by clorgyline alone (Table I). After rats received the combination of clorgyline

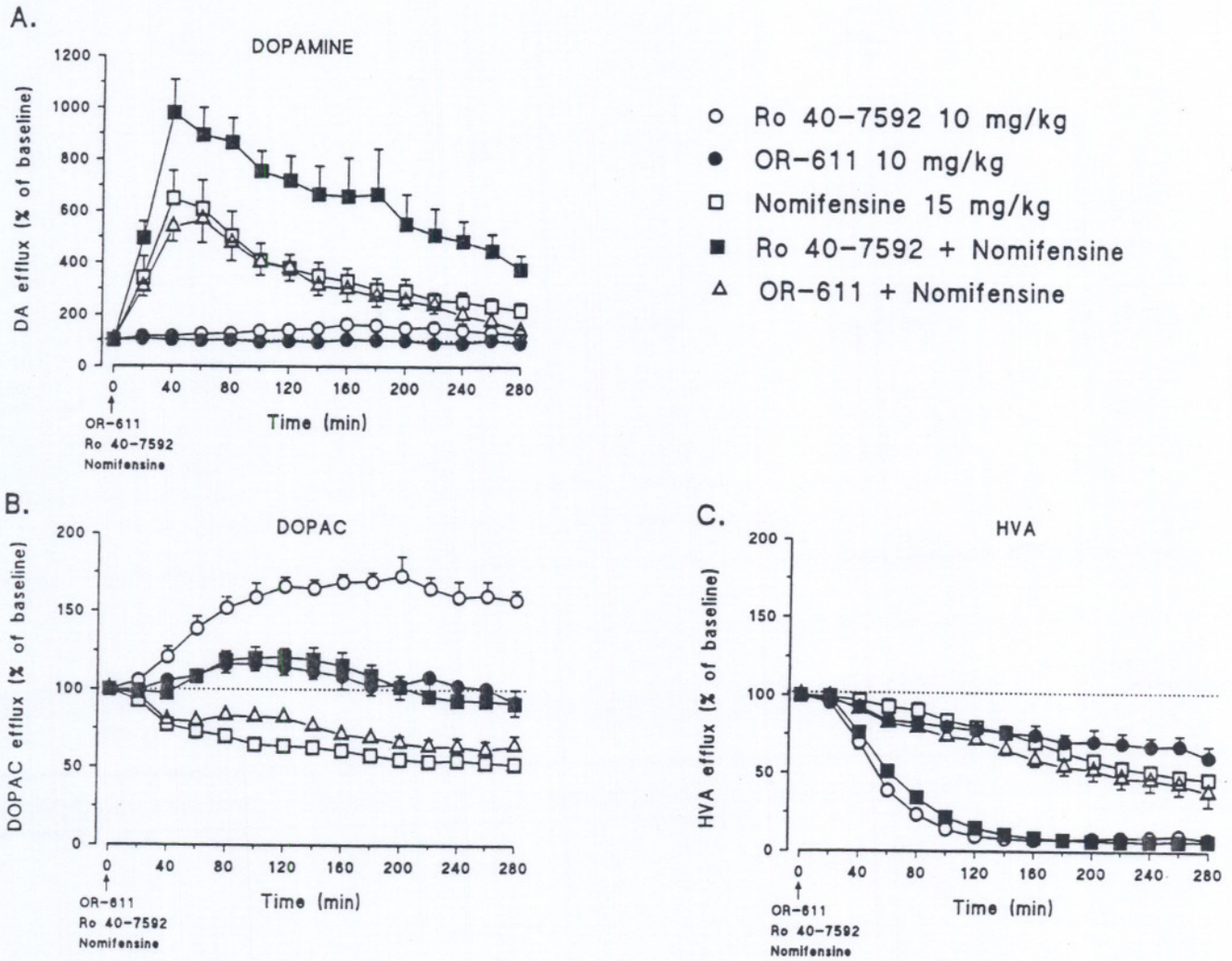

Fig. 3. Effects of Ro 40-7592 or OR-611 on nomifensine-induced alterations in dopamine (A), DOPAC (B) and HVA (C) dialysate levels in rat striatum. Ro 40-7592 (10 mg/kg), OR-611 (10 mg/kg) or vehicle were administered together with nomifensine (15 mg/kg) i.p. at time = 0 min. Dialysis samples were collected for 20 min periods and assayed by HPLC-EC. Data are given as mean (±S.E.M.) percent variation of basal (preinjection) values, $n = 6$–8 for each group. See text for statistics.

TABLE I

Effects of Ro 40-7592 or OR-611 on clorgyline-induced alterations in dopamine, DOPAC, HVA, and 5-HIAA dialysate levels in rat striatum

Each value represents means ± S.E.M. of 6 rats. Clorgyline (4 mg/kg, i.p.) was given 180 min before Ro 40-7592 (10 mg/kg, i.p.) or OR-611 (10 mg/kg, i.p.). Dialysis samples were collected for 20 min periods over 280 min and assayed by HPLC-EC. Basal values represent the last values before administration of COMT inhibitor. Statistical analyses of the data were performed using one-way analysis of variance followed by Tukey's multiple comparison test.

Compound	*Treatment*	*Basal (pmol / 20 min)*	*AUC (% basal × 280 min)*
DA	Ro 40-7592	0.038 ± 0.005	32000 ± 1100
	OR-611	0.049 ± 0.008	27000 ± 500
	Clorgyline	0.144 ± 0.006	26600 ± 900
	Clorgyline + Ro 40-7592	0.115 ± 0.016 ***	35800 ± 2300 †††
	Clorgyline + OR-611	0.136 ± 0.008 ###	27200 ± 800
DOPAC	Ro 40-7592	30.3 ± 5.0	41600 ± 1300
	OR-611	29.1 ± 5.4	28800 ± 1400
	Clorgyline	4.1 ± 0.6	23100 ± 1300
	Clorgyline + Ro 40-7592	6.0 ± 1.3 ***	35100 ± 1500 †††, **
	Clorgyline + OR-611	6.1 ± 1.2 ###	25900 ± 700
HVA	Ro 40-7592	25.3 ± 2.2	8600 ± 600
	OR-611	22.0 ± 3.8	21400 ± 1000
	Clorgyline	2.6 ± 0.4	21700 ± 800
	Clorgyline + Ro 40-7592	2.9 ± 0.3 ***	7300 ± 400 †††
	Clorgyline + OR-611	3.8 ± 0.7 ###	18500 ± 700 †
5-HIAA	Ro 40-7592	4.72 ± 0.61	28500 ± 1600
	OR-611	5.61 ± 0.67	28100 ± 700
	Clorgyline	2.61 ± 0.35	23500 ± 1200
	Clorgyline + Ro 40-7592	2.92 ± 0.50	23600 ± 900 *
	Clorgyline + OR-611	3.37 ± 0.45 #	24400 ± 700 #

* $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ compared to Ro 40-7592.
\# $P < 0.05$ and ### $P < 0.001$ compared to OR-611.
† $P < 0.05$ and ††† $P < 0.001$ compared to clorgyline.

and Ro 40-7592, dialysate DOPAC levels were intermediate between those in animals given clorgyline and those given Ro 40-7592 alone (Table I). The combination of clorgyline and Ro 40-7592 did not further decrease HVA levels ($P > 0.05$, compared to Ro 40-7592; $P < 0.001$, compared to clorgyline). Similarly, dialysate levels of dopamine and DOPAC after treatment with the combination of clorgyline and OR-611 (10 mg/kg) did not significantly differ from those produced by either drug alone (Table I). HVA levels were slightly lower in rats given the combination (clorgyline plus OR-611) than in those receiving clorgyline alone; these levels, however, did not differ significantly from those produced by OR-611 alone (Table I). Neither Ro 40-7592 nor OR-611 significantly modified the striatal 5-HIAA efflux in clorgyline-pretreated rats when compared to clorgyline alone (Table I).

Effects of L-deprenyl and COMT inhibitors on striatal dopamine, DOPAC, HVA and 5-HIAA efflux

Pretreatment with L-deprenyl (1 mg/kg) did not significantly modify the baseline levels of dopamine, DOPAC, HVA and 5-HIAA, compared with those in vehicle-pretreated rats (Table II).

There were no significant interactions between L-deprenyl and COMT-inhibitors (10 mg/kg) on the striatal dialysate levels of either dopamine, DOPAC, HVA or 5-HIAA (Table II); the changes seen after the combinations were very similar to those seen after administration of each of the COMT inhibitors alone.

DISCUSSION

These data show that the new COMT inhibitors can significantly modify striatal dopamine metabolism in vivo, apparently without affecting 5-hydroxy-tryptamine metabolism; they thus support the previous conclusions, based on in vitro studies, about the selectivity of those agents [36,54]. Previous studies have shown that oral administration of Ro 40-7592 to rats dose-dependently inhibits brain COMT activity, decreases striatal (tissue) levels of HVA, increases those of DOPAC, but does not change striatal levels of dopamine [30,52]. OR-611 (10 mg/kg orally) slightly and transiently inhibited striatal COMT activity without significantly changing striatal levels of HVA (after levodopa) [28,34,36]. Based on these studies, it has been proposed that Ro 40-7592 is a potent centrally (and peripherally) active COMT

TABLE II

Effects of Ro 40-7592 or OR-611 on L-deprenyl-induced alterations in dopamine, DOPAC, HVA, and 5-HIAA dialysate levels in rat striatum

Each value represents means ± S.E.M. of 5 rats. L-deprenyl (1 mg/kg, i.p.) was given 180 min before Ro 40-7592 (10 mg/kg, i.p.) or OR-611 (10 mg/kg, i.p.). Dialysis samples were collected for 20 min periods over 280 min and assayed by HPLC-EC. Basal values represent the last values before administration of COMT inhibitor. Statistical analyses of the data were performed using one-way analysis of variance followed by Tukey's multiple comparison test.

Compound	*Treatment*	*Basal (pmol / 20 min)*	*AUC (% basal × 280 min)*
DA	Ro 40-7592	0.040 ± 0.006	30400 ± 1600
	OR-611	0.044 ± 0.008	28000 ± 700
	L-deprenyl	0.035 ± 0.003	30600 ± 2500
	L-deprenyl + Ro 40-7592	0.042 ± 0.003	26400 ± 900
	L-deprenyl + OR-611	0.048 ± 0.010	24600 ± 1400
DOPAC	Ro 40-7592	32.9 ± 5.3	41100 ± 1500
	OR-611	34.1 ± 4.3	28000 ± 1300
	L-deprenyl	35.9 ± 0.5	28200 ± 900
	L-deprenyl + Ro 40-7592	39.7 ± 8.9	42900 ± 3100 †††
	L-deprenyl + OR-611	37.5 ± 4.1	29600 ± 1700
HVA	Ro 40-7592	26.3 ± 2.3	8500 ± 700
	OR-611	27.0 ± 3.5	21700 ± 600
	L-deprenyl	28.2 ± 1.7	24400 ± 1100
	L-deprenyl + Ro 40-7592	30.7 ± 4.3	8300 ± 500 †††
	L-deprenyl + OR-611	29.3 ± 2.9	21900 ± 800
5-HIAA	Ro 40-7592	6.33 ± 0.47	28000 ± 1300
	OR-611	5.95 ± 0.70	28200 ± 800
	L-deprenyl	5.94 ± 0.42	29500 ± 900
	L-deprenyl + Ro 40-7592	6.62 ± 1.11	28900 ± 1300
	L-deprenyl + OR-611	6.56 ± 0.88	29400 ± 1500

††† $P < 0.001$ compared to L-deprenyl.

inhibitor, whereas OR-611's effect is principally peripheral. The present in vivo results regarding Ro 40-7592 are compatible with this hypothesis. Our results also suggest that, at doses of 10 mg/kg or less, OR-611 lacks a significant central effect; at higher doses, however, it can penetrate the blood–brain barrier sufficiently to modify the striatal dopamine metabolism. The central potency of OR-611 seems to be about one-tenth that of Ro 40-7592 (i.e. based on their effects on striatal DOPAC of HVA efflux), whereas their potencies are much closer in producing peripheral actions [34] (Kaakkola and Wurtman, unpublished results). For studies on drug interactions, we thus used a dose of 10 mg/kg for both Ro 40-7592 and OR-611, since this dose could differentiate between peripheral and central COMT inhibition. The inactivity of OR-611 in our interaction studies (Fig. 3; Tables I and II) suggests that the effects of Ro 40-7592 in combination with other drugs result from central, and not peripheral COMT inhibition.

It has previously been proposed that, when dopaminergic neurons are functioning normally, about 70–80% of HVA in striatum originates from DOPAC and about 20–30% from 3-methoxytyramine [47,49]. Our present in vivo findings with COMT inhibitors agree with this conclusion. When the formation of HVA was almost completely inhibited by a centrally and peripherally active COMT inhibitor (Ro 40-7592), a compensatory increase in extracellular DOPAC level was observed (Fig. 1). This increase was smaller, however, than the decrease in HVA level. There was also a tendency for dopamine efflux to increase after COMT inhibition (Fig. 1), suggesting that a portion of extracellular dopamine is indeed metabolized primarily by COMT (via 3-methoxytyramine route). Unfortunately, we could not obtain reliable measurements of extracellular 3-methoxytyramine levels using our present HPLC system to determine the percent of released dopamine metabolized by *O*-methylation.

We found that both nomifensine and clorgyline, if given alone, elevated extracellular dopamine levels as described previously [8,9,10,19,20,24]. These findings support the view that it is the re-uptake mechanism and the subsequent metabolism by MAO-A which mainly eliminate the dopamine released from presynaptic terminals by normal nerve activity. When re-uptake was inhibited, however, a COMT inhibitor (Ro 40-7592) caused a further elevation in the extracellular dopamine level (Fig. 3A). A similar, though less robust relationship was observed when both MAO-A and COMT was inhibited (Table I). Presumably, the re-uptake of dopamine and its storage within presynaptic terminals are both still operative when MAO-A is inhibited, so that administering both a MAO-A and a COMT inhibitor has less effect on dopamine metabolism than administering a dopamine uptake blocker and a COMT inhibitor. When dopamine re-uptake and/or MAO-A activity are inhibited, however, there is a shift to extraneuronal metabolism, so that the importance of COMT is accentuated. In unpublished observations, we have found in addition that the new COMT inhibitors also potentiate the effect of levodopa on striatal dopamine levels and release (Kaakkola and Wurtman, unpublished), and on behavioral actions [13,30,36]. In this case, peripheral COMT has also some significance. These findings suggest that combinations of COMT inhibitors with MAO-A inhibitors or dopamine uptake blockers may have a clinical utility; they may also raise the possibility of an increased risk of toxic interactions,

however, as observed in rats given COMT inhibitors and nomifensine or amphetamine [43]. It would be also important to study the effect of COMT inhibitors under other conditions of increased dopaminergic nerve activity (e.g. after administration of neuroleptics, amphetamine or of L-tyrosine, all of which can increase extracellular levels of dopamine [12,50,51]).

The MAO-B inhibitor L-deprenyl, at a selective dose of 1 mg/kg, did not change baseline levels of dopamine or its metabolites. This finding is consistent with the results reported Kato et al. [24] and Butcher et al. [8]. Even when combined with a COMT inhibitor, L-deprenyl failed to affect striatal dopaminergic metabolism. Our in vivo results with both clorgyline and L-deprenyl support the view that dopamine in rat striatum is mainly deaminated by MAO-A (as demonstrated by other methods [3,11,16,46]). MAO-B could have some role in dopamine metabolism when re-uptake or MAO-A is inhibited or when there is an enhanced release of dopamine [8,14,17,41].

In conclusion, our microdialysis studies show that Ro 40-7592 is a potent, centrally active COMT inhibitor, whereas OR-611 is primarily a peripherally acting COMT inhibitor. Although re-uptake and MAO-A seem to be the main routes for dopamine metabolism in rat striatum under conditions of normal nerve activity, the significance of COMT may be more pronounced under conditions of increased dopamine release and/or inhibition of other metabolic pathways. Our results warrant for further experimental and clinical research with these new COMT inhibitors.

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