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INHIBITION OF CATECHOL-*O*-METHYL TRANSFERASE BY CATECHOLS AND POLYPHENOLS*

ROSS J. BALDESSARINI† and ELLEN GREINER

Psychiatric Research Laboratories, Department of Psychiatry, Massachusetts General Hospital and Harvard Medical School, Boston, Mass. U.S.A.

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Abstract—The effects of the polyphenolic decarboxylase inhibitor, N^1-(*d,l*-seryl)-N^2-(2,3,4-Trihydroxybenzyl)-hydrazine (RO4-4602), were compared with the effects of dopa and pyrogallol as inhibitors of catechol-*O*-methyl transferase (COMT). The apparent K_m for [^{3}H]norepinephrine as the substrate was 0·36 mM. The catechol amino acid, L-dihydroxyphenylalanine (L-dopa), a substrate of COMT, was a weak inhibitor of COMT (K_i = 0·42 mM) and appeared to be competitive with norepinephrine; D-dopa was slightly less potent. In contrast, the polyphenol pyrogallol exhibited partially noncompetitive inhibition of COMT with respect to the substrate (K_i = 30 μM). RO4-4602 exhibited similar noncompetitive inhibitory kinetics (K_i = 60 μM). In contrast to dopa and pyrogallol, RO4-4602 appears to be a relatively poor substrate for COMT. *In vivo*, RO4-4602 led to markedly increased recovery from brain of labeled catechol products of peripherally administered [^{3}H]L-dopa, probably partly due to inhibition of decarboxylation as well as methylation. Furthermore, RO4-4602 led to increased recovery of catechol products of [^{3}H]norepinephrine in the heart for as long as 3–5 hr. RO4-4602 was found to be a weak inhibitor of monoamine oxidase *in vitro* (IC_{50} about 1 mM) and inactive *in vivo* (up to 100 mg/kg, i.p.). Thus, RO4-4602, an analog of pyrogallol, has similar COMT-inhibitory characteristics to the latter compound *in vitro* and appears to inhibit COMT *in vivo*. This property may be partly responsible for its beneficial effects in patients treated with L-dopa.

L-DIHYDROXYPHENYLALANINE (L-dopa) can exert beneficial neurological effects in Parkinson's disease, but can also induce dyskinesias and psychotic side effects.[1] A leading hypothesis concerning the mechanism of action of this catechol-amino acid is related to its physiological role as a precursor of the catecholamines, which are deficient in the basal ganglia of Parkinsonian patients.[2] L-Dopa normally does not accumulate in animal tissues,[3] and frequent large doses of L-dopa are required to produce notable increases of dopamine levels in the brain[4] or to ameliorate the signs of Parkinsonism.[5] These observations probably reflect the rapid metabolism of L-dopa by several processes, including decarboxylation and *O*-methylation.[6,7] In attempts to enhance the clinical efficacy of L-dopa, and to reduce side effects and expense, inhibitors of L-aromatic amino acid decarboxylase (EC 4.1.1.26) activity outside the brain have been used with some success.[1,6,8]

As a substrate of catechol-*O*-methyl transferase (COMT, EC 2.1.1.6), L-dopa can apparently severely tax normal methylation processes. In doses encountered clinically, it can deplete tissues of the important methyl donor, *S*-adenosyl-methionine (SAMe)

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in the rat and interfere with the methylation of biologically important substances,[9] and can decrease blood concentrations of SAMe in patients.[10] Furthermore, large doses of L-methionine, which presumably increase the availability of SAMe,[11] are reported to interfere with the beneficial effects of L-dopa and increase its toxicity in Parkinsonian patients.[12] It follows that blocking the methylation of L-dopa might enhance the availability of this precursor for conversion to catecholamines in the nervous system, decrease the formation of methylated metabolites, and also prevent certain of the side effects of L-dopa. The use of this approach in patients has been impeded, since presently known inhibitors of COMT are too weak, short-acting or toxic. We have demonstrated previously that such an approach can enhance the accumulation of labeled catecholamines in the rat brain following parenteral injection of [^{3}H]L-dopa with pyrogallol.[13] Furthermore, we have investigated several catecholic and polyphenolic compounds as possible inhibitors of COMT *in vitro*, including substances currently used in patients as inhibitors of L-aromatic amino acid decarboxylase,[14] and we now present further investigations of these compounds.

MATERIALS AND METHODS

Animals. Young adult (190–210 g) male albino rats (Charles River Company, Wilmington, Mass.) were maintained *ad lib.* on Charles River Laboratory chow and water.

Chemicals. The following radiochemicals and drugs were used: L-[G-^{3}H]dihydroxyphenylalanine (dopa; 10 Ci/m-mole) and [7-^{3}H]*d,l*-norepinephrine (12 Ci/m-mole; New England Nuclear Corp., Boston, Mass.) were chromatographically pure (>95 per cent) prior to use; D-dopa and pyrogallol (Sigma Chemical Company, St. Louis, Mo.); L-dopa and N^1-(*d,l*-seryl)-N^2-(2,3,4-trihydroxybenzyl)-hydrazine (RO4-4602) were donated by Hoffman–La Roche Pharmaceutical Company, Nutley, N.J.; *S*-adenosyl-methionine iodide (SAMe) and authentic reference compounds used for chromatography (CalBiochem Company, Los Angeles, Calif.); pargyline–HCl was donated by Abbott Laboratories, No. Chicago, Ill.

Assay of COMT. The assay method was that of Ross and Haljasmaa[15] and Creveling and Daly[16] with only minor modifications.[14] Thus, compounds were tested for their ability to prevent the formation of [^{3}H]normetanephrine from the catechol substrate, [7-^{3}H]*d,l*-norepinephrine (1 mM), in the presence of SAMe (1 mM), an inhibitor of monoamine oxidase (pargyline, 0·2 mM), Mg^{2+} (2 mM), cysteine (0·04 mM) and 100-μl portions (equivalent to approx. 20 mg liver and 50 mg brain) of extracts of tissue homogenized in 2 (brain) or 4 (liver) vol. of isotonic KCl and centrifuged at 48,000 *g* for 20 min. The reaction was conducted at 37° for 30 min at pH 7·8 (phosphate buffer) in a reaction volume of 500 μl. After adding 2·5 and 10·0 μmoles of unlabeled normetanephrine and norepinephrine, respectively, the [^{3}H]normetanephrine produced was extracted into toluene–isoamyl alcohol (3:2, v/v) from the aqueous reaction mixture with NaCl-saturated borate buffer (pH 10) added, and was counted by scintillation spectrometry.[16] The labeled material produced by liver or brain homogenates and extracted into the organic phase was found to migrate in a single peak (>90 per cent) of radioactivity, corresponding in R_f to authentic normetanephrine, during ascending chromatography on paper or on thin-layer cellulose plates in *n*-butanol–

acetic acid–water (25:4:10, v/v) and on the thin-layer plates in *n*-butanol–ethanol–water (4:1:1, v/v). The rate of production of [^{3}H]normetanephrine was found to be approximately linear with time of incubation for at least 45 min, and with up to twice the amount of extract of liver or brain used in the assay.

*Effects of RO*4-4602 in vivo. Rats were injected intraperitoneally with the drug dissolved in 1 ml of isotonic saline, or with saline alone. Either [^{3}H]-labeled L-dopa or *d,l*-norepinephrine was injected intraperitoneally or intracisternally,[17] and at various times the animals were decapitated and their brains or hearts were removed, chilled on ice, and homogenized in 5 vol. of ice-cold 0·4 N perchloric acid. The tissue extracts were then prepared for alumina column chromatography;[17] after the columns were washed and eluted with strong acid (usually 5 ml of 2 N HCl), the ^{3}H in the eluates was counted by scintillation spectrometry. The elution procedure was found to return over 90 per cent of the radioactivity of [^{3}H]-labeled dopa, dopamine or norepinephrine, or of unlabeled dihydroxymandelic acid as estimated by native fluorescence.[17]

In other animals, levels of SAMe in brain or liver were estimated by a double isotopic enzymatic derivative method previously described.[18]

RESULTS

Experiments in vitro. The production of [^{3}H]normetanephrine in the assay of COMT appeared to follow saturable Michaelis–Menten kinetics with increasing concentrations of the substrate [^{3}H]*d,l*-norepinephrine, and the apparent Michaelis constant (K_m)was 0·36 mM with respect to this substrate. When L-dopa, a substrate of COMT, was present, it appeared to inhibit the reaction in competition with norepinephrine when the data were analyzed according to Lineweaver and Burk,[19] and the inhibitory constant (K_i) for L-dopa was 0·42 mM (Table 1). When the

TABLE 1. KINETIC ANALYSIS OF INHIBITION OF COMT*

Inhibitor	Method of analysis	$K_i \pm$ S.E.M. (mM)	Kinetics
L-Dopa	Lineweaver – Burk	0·42 ± 0·01	Competitive with substrate
Pyrogallol	Lineweaver – Burk	0·026 ± 0·001	Noncompetitive
Pyrogallol	Dixon	0·027 ± 0·001	Noncompetitive

* Extracts of rat liver equivalent to 20 mg of wet tissue were assayed for COMT activity as described in Materials and Methods in the presence of various concentrations of substrate, [^{3}H]*d,l*-norepinephrine from 0·2 to 1·0 mM, and at several concentrations of each inhibitor. Data were obtained for three to six determinations of velocity of methylation (micromoles of [^{3}H]normetanephrine formed per hour per assay) and were analyzed according to Lineweaver and Burk[19] or Dixon[20] and plotted by linear regression analysis (cf. Figs. 1 and 2). The apparent mean K_m for NE was 0·35 ± 0·01 mM.

D- and L-isomers of dopa were compared, D-dopa was found to be slightly less potent than the natural isomer as an inhibitor of COMT. For example, COMT activity was 59·5 ± 0·2 and 73·8 ± 0·08 per cent of control ($P < 0·01$; $N = 3$) with 1 mM L- and D-dopa respectively. (These and subsequent data are mean ± S.E.M.)

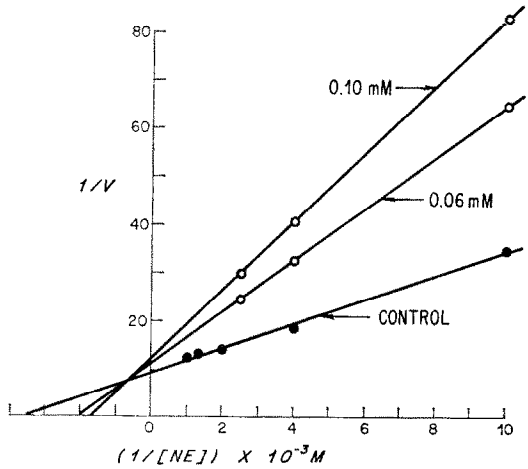

FIG. 1. Inhibition of COMT by RO4-4602 *in vitro*. Extracts of rat liver were assayed for COMT activity in the presence of RO4-4602 and analyzed as described for Table 1, The data were plotted by linear regression analysis and analyzed according to Lineweaver and Burk.[1] Reaction velocity (V) is in micromoles per hour per assay. Substrate, [^{3}H]norepinephrine (NE) ,was present at 0·1–1·0 mM. Points are mean values for three determinations. Width of circles = ± S.E.M. Apparent mean K_i = 0·80 mM.

When the inhibitory effects of pyrogallol were analyzed by the same method, inhibition (apparent K_i = 26 μM) appeared to be partly noncompetitive with respect to the substrate norepinephrine (Table 1). When other data were analyzed by the method of Dixon,[20] a similar noncompetitive inhibition was found (Table 1, apparent K_i = 27 μM). When RO4-4602 was studied by the same methods, again evidence of noncompetitive inhibition of COMT was found and the apparent K_i was 80 μM by one method of analysis (Fig. 1) and 45 μM by another (Fig. 2). Pyrogallol and RO4-4602 had inhibitory effects on COMT activity in brain extracts similar to those observed with liver. Thus, at 0·1 and 1·0 mM, RO4-4602 inhibited COMT in the brain extracts to 62·7 ± 2·7 and 4·9 ± 2·6 per cent of controls respectively ($P < 0{\cdot}01$; N = 3). The inhibitory kinetics for both pyrogallol (Table 1) and RO4-4602 (Figs. 1 and 2) were noncompetitive with respect to the substrate, norepinephrine, whether the data were analyzed according to Lineweaver and Burk[19] or Dixon,[20] and the apparent V_{max} was decreased, although in no case did the plots of $1/V$ vs. $1/[S]$ or of $1/V$ vs. $[I]$ converge on the abscissa.

In order to estimate the ability of the various inhibitors of COMT to accept methyl groups, they were incubated with [methyl-^{14}C]SAMe, and no other added substrate for COMT, and compared with control incubations lacking any added substrate. The radioactivity of methylated products so formed and extracted into toluene–isoamyl alcohol was 9–18 times above blank in the case of L-dopa or pyrogallol and only twice the blank with RO4-4602 (Table 2). When ethylacetate was used to extract the [methyl-^{14}C]-labeled products, fewer counts were recovered, but again more ^{14}C was recovered with L-dopa or pyrogallol as substrate than with RO4-4602. Although the efficiency of extraction of the methylated products of RO4-4602 into the organic phase was much lower than for L-dopa or pyrogallol, the estimated production of

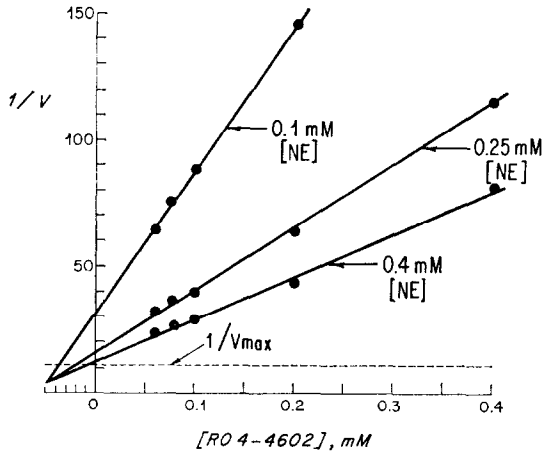

FIG. 2. Inhibition of COMT by RO4-4602 *in vitro*. Extracts of rat liver were assayed for COMT activity in the presence of RO4-4602 as described for Table 1. The data were plotted by linear regression analysis and analyzed according to Dixon.[20] Substrate, [^{3}H]norepinephrine (NE), was present at 0·10, 0·25 or 0·40 mM and RO4-4602 was present from 0·05 to 0·40 mM. Reaction velocity is in micromoles per hour per assay; V_{max} was estimated in a separate experiment. Points are mean values of three determinations. Width of circles = ± S.E.M. Apparent mean K_i = 0·045 mM.

methylated products with RO4-4602 was only 6 and 12 per cent of that with L-dopa or pyrogallol respectively (Table 2).

Experiments in vivo. When 25 μCi [^{3}H]dopa was given intraperitoneally 30 min after doses of RO4-4602 as small as 10 mg/kg (5·2-fold), significant ($P < 0{\cdot}001$ at $N = 5$ or 6) and dose-related (8·4-fold at 100 mg/kg) increases of labeled catechol products were recovered from the brain 30 min later. When [^{3}H]norepinephrine was

TABLE 2. RECOVERY OF [METHYL-^{14}C]-LABELED PRODUCTS OF COMT INHIBITORS*

	^{14}C	
Substrate	Uncorrected (dis/min × 10^{-3})	Corrected for blank and recovery (dis/min × 10^{-3})
L-dopa	465·7 ± 5·5	476·3
Pyrogallol	234·3 ± 2·4	241·0
RO4-4602	40·9 ± 1·1	29·1
None (blank)	26·1 ± 0·2	0

* The [methyl-^{14}C]-labeled products of several inhibitors of COMT were recovered after incubation of the compounds (0·5 mM) with 1 μCi [methyl-^{14}C]SAMe in the presence of rat liver extracts for 45 min, addition of salt-saturated borate buffer (pH 10), and extraction into toluene–isoamylol (3:2, v/v) and counting. Assay conditions are as described for COMT assay in Materials and Methods, except that [^{3}H[norepinephrine was omitted. Data are mean thousands of dis./min ± S.E.M. of [^{14}C]methylated products per assay for triplicate determinations. Extraction efficiencies were estimated by re-equilibrating portions of the organic phase with appropriate volumes of freshly prepared aqueous phase, lacking added radioactivity, and counting the distribution of the presumed labeled methylated products. The extraction efficiencies for the products of L-dopa, pyrogallol and RO4-4602 were 92·3, 86·4 and 50·9 per cent, respectively.

TABLE 3. RECOVERY OF [^{3}H]CATECHOLS IN HEART AFTER RO4-4602*

Dose of RO4-4602 (mg/kg)	^{3}H-catechols (dis/min $\times 10^{-3}$/g $\pm$ S.E.M.)
0	30·5 $\pm$ 2·5
10	41·1 $\pm$ 3·7†
25	50·7 $\pm$ 5·0‡
50	58·5 $\pm$ 3·1‡
100	98·7 $\pm$ 10·8§
250	108·6 $\pm$ 7·6§
500	163·6 $\pm$ 12·4§

* Labeled catechol products were selective recovered from rat hearts as described in Materials and Methods for animals given by intraperitoneal injection: RO4-4602, 60 min, and 10 μCi [^{3}H]norepinephrine, 30 min prior to sacrifice. Data are mean dis./min ($\times 10^{-3}$) of [^{3}H]catechols per gram of heart $\pm$ S.E.M. for five to seven rats. Statistical significance by *t*-test is indicated as follows: † $P < 0.05$; ‡ $P < 0.01$; § $P < 0.001$.

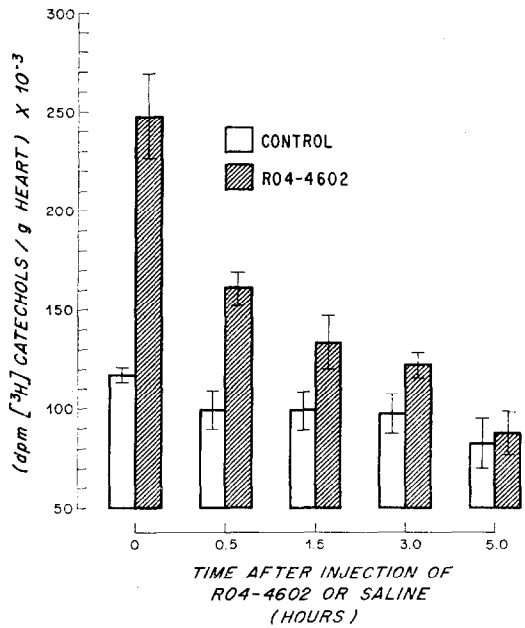

FIG. 3. Effect of RO4-4602 on [^{3}H]catechols in rat heart liver *in vivo*. Groups of five rats were given a 100 mg/kg dose of RO4-4602 or saline alone intraperitoneally. At various times from 5 min (zero time) to 5 hr later, 10 μCi [^{3}H]norepinephrine was given intraperitoneally; 30 min later, the animals were killed and [^{3}H]catechols were selectively recovered from their hearts as described in Materials and Methods. Data are mean dis./min ($\times 10^{-3}$) per gram $\pm$ S.E.M.

given by intracisternal injection after 50 or even 250 mg/kg of RO4-4602 intraperitoneally, the quantity of [^{3}H]catechols remaining in brain 75 min after the intracisternal injection was not significantly increased. However, when [^{3}H]norepinephrine was given intraperitoneally, the quantity of labeled catechols recovered from heart was greatly increased after doses of RO4-4602 as low as 10–25 mg/kg (Table 3). The latter experiment was repeated with a dose of 100 mg/kg of RO4-4602, and the time between injection of this drug and the intraperitoneal administration of [^{3}H]norepinephrine was varied. In this case, the quantity of [^{3}H]catechols recovered from the heart 30 min after injection of the tracer was greatly increased (up to 2·5 times control) during the first 30–60 min after injection of RO4-4602, and small increases were sustained for about 3–5 hr (Fig. 3).

The administration of RO4-4602 in doses ranging from 25 to 292 mg (1 m-mole) per kg intraperitoneally acutely or twice over a 24-hr period led to insignificant changes in SAMe levels in brain or liver 60 or 90 min after the final administration of the drug. In contrast, pyrogallol, as noted previously,[11] produced marked decreases of the methyl donor in brain and significant decreases in liver.

DISCUSSION

The results of the present experiments confirm that COMT activity in rat liver or brain extracts can be assayed by the use of labeled norepinephrine as substrate, followed by selective recovery of labeled normetanephrine. This reaction appeared to follow saturable kinetics, with an apparent K_m value (0·36 mM) in good agreement with results previously reported for norepinephrine as substrate and rat liver extracts as the source of enzyme.[21,22]

L-Dopa (Table 1) appeared to act as a weak competitive inhibitor of COMT, as expected, since it is a substrate for the enzyme; D-dopa was even slightly less potent. Since L-dopa does apparently interfere with the methylation of other catechols *in vivo* in large doses comparable to those given to Parkinsonian patients,[9] this effect may occur by competition with other substrates and is reflected in a transient decrease of tissue methyl donor levels.[9] It is also reported that chronic treatment of Parkinsonian patients with L-dopa can lead to sustained decreases of COMT activity in erythrocytes by an uncertain mechanism which does not appear to depend on the presence of a readily dialyzable small molecule.[23] Since D-dopa is not readily converted to pharmacologically active catecholamine products by L-aromatic amino acid decarboxylase,[24] it might possibly have useful COMT-inhibitory properties *in vivo*, with less of the side effects produced by L-dopa. Unfortunately, its potency as a COMT inhibitor *in vivo* appears to be severely limited. Furthermore, it is known that in treating Parkinsonian patients, D,L-dopa is about half as effective as the pure L-isomer,[1,5] which suggests that D-dopa does not appreciably potentiate the actions of L-dopa in such patients, as might be expected if it prevented the *O*-methylation of L-dopa, the therapeutically more active isomer.

Pyrogallol appeared to exhibit partially noncompetitive inhibitory effects on the methylation of norepinephrine. This behavior is not readily explained, but both competitive and noncompetitive inhibitory effects of pyrogallol on COMT have been reported previously, and the degree of noncompetitive effect is said to increase with increasing purification of the enzyme and with increased pre-incubation.[21] Similar

inhibition was noted with the pyrogallol analog, RO4-4602 (a 2,3,4-polyphenol), and noncompetitive or "mixed" kinetics have been reported also for other types of trebly ring-substituted aromatic inhibitors of COMT.[22] *In vitro*, pyrogallol (K_i approx. 30 μM) and RO4-4602 (K_i approx. 60 μM) appear to have similar potency in inhibiting COMT (Table 1, Figs. 1 and 2). One possible explanation for the pattern of "partial" noncompetitive inhibition observed with RO4-4602 as well as pyrogallol is that two types of inhibition might occur (as suggested previously for pyrogallol and other trebly substituted aromatic compounds[22]): competitive inhibition for polyphenolic substrates of COMT, and noncompetitive inhibition by a methylated product. For example, the 2-*O*-methylated product of pyrogallol and several other aromatic compounds with a methoxy group interposed between two adjacent hydroxyl groups are clearly classical noncompetitive inhibitors of COMT *in vitro* and they are active *in vivo*.[22] On the other hand, it is not clear whether appreciable quantities of an *O*-methylated product of RO4-4602 would accumulate *in vitro* or *in vivo*. Evidence against this is that relatively little methyl-labeled product of [^{3}H]SAMe and RO4-4602 (in contrast to L-dopa or pyrogallol) was recovered after incubation with liver extract *in vitro* (Table 2), and RO4-4602 *in vivo* does not appear to lower tissue levels of SAMe significantly.

In vivo, RO4-4602 was very effective in potentiating the recovery from brain of labeled catechol products derived from [^{3}H]L-dopa, as described previously.[6,8] Presumably, a significant proportion of this increase was due to the lack of formation of amines from L-dopa in peripheral tissues by the well known action of this drug as an inhibitor of dopa decarboxylation.[6,8] However, since a greater recovery from heart of labeled catechols derived from injected [^{3}H]norepinephrine was also observed (Table 3, Fig. 3), it is probable that RO4-4602 is also an effective inhibitor of COMT activity *in vivo*, and possible that this action contributed to the observed increase in brain of catechol products of [^{3}H]dopa. The method of recovery of labeled catechol products (alumina column chromatography) in all of the present experiments *in vivo* does not require that the products be intact amines, so that the effects *in vivo* are not explainable by an action of the hydrazine derivative RO4-4602 as an inhibitor of monoamine oxidase (MAO). Furthermore, it has been suggested previously that in contrast to many hydrazine compounds, RO4-4602 is a poor inhibitor of MAO *in vivo*;[8] we have confirmed this observation and found that the drug is active against MAO *in vitro* only at very high concentrations (IC_{50} in the order of mM). It is possible that an action against COMT may contribute to the reported ability of RO4-4602 to potentiate the conversion of L-dopa to catecholamine products,[25] to potentiate the clinical efficacy of L-dopa and to reduce its side effects in Parkinsonian patients.[1,6,26] The clinically effective daily doses of RO4-4602 in man are in the order of 5 mg/kg or less orally,[26] while high parenteral doses (10–25 mg/kg or more) were required to produce clear COMT-inhibitory effects *in vivo* in the rat (Table 3, Fig. 3). On the other hand, doses in the order of 100 mg/kg are also required in laboratory animals to produce maximal inhibition of decarboxylase activity.[6,8] Furthermore, since doses of RO4-4602 in the order of 1000 mg or more have been tolerated in patients without apparent ill effect,[25] the possibility that this drug or a 3-*O*-methylated or other derivative of it may be a useful COMT inhibitor in man should be considered.

The use of inhibitors of COMT with L-dopa in Parkinsonian patients might help to reduce the required dose of L-dopa and to reduce any side effects produced by

methylated products of the metabolism of L-dopa. We have shown previously that pretreatment of rats with pyrogallol prior to intraperitoneal injection of [^{3}H]L-dopa led to greater accumulations of unmetabolized L-dopa and its catecholamine products, but decreases of *O*-methylated metabolites in the brain.[13] Whether RO4-4602 as presently used in man[26] may have similar effects through COMT inhibition is not yet clear. Most of the available inhibitors of COMT (including pyrogallol, desmethylpapaverine, tropolones, catecholacetamides, gallic acid esters and substituted benzoates) are of limited usefulness due to lack of potency and duration of action or to toxicity. Furthermore, some inhibitors are also substrates of COMT, and in the large doses required for COMT inhibition *in vivo*, they can utilize methyl groups and deplete tissue levels of the methy donor, SAMe,[11] potentially adding further to any untoward metabolic effects resulting from the depletion of SAMe by L-dopa.

It has also been suggested that methylated products of L-dopa might be responsible for certain of the neurological side effects of L-dopa therapy in Parkinsonism, such as the dyskinesias or involuntary movements which often occur after use of L-dopa.[1,27] Since many methylated amines are known to be psychotomimetic,[28] it is also conceivable that certain of the psychiatric side effects of L-dopa therapy could result from actions of methylated metabolites. It was reported that when Parkinsonian patients were given large doses of L-methionine with L-dopa, their neurological and psychiatric status worsened;[12] it is also known that increased availability of L-methionine can enhance the synthesis of the methyl donor, SAMe.[11,28] Furthermore, it has been reported recently that the COMT inhibitor, *n*-butylgallate, in high doses (750 mg/day) may potentiate therapeutic effects of L-dopa in Parkinsonism and decreases neurological (dyskinetic) and perhaps psychiatric side effects ("nervousness") and can possibly even alleviate dyskinetic symptoms of Huntington's chorea and spasmodic torticollis.[29]

In conclusion, the polyphenolic decarboxylase inhibitor RO4-4602 appears to act also as an inhibitor of catechol-*O*-methylation. Its effects are similar to those of pyrogallol *in vitro* and it appears to be active *in vivo*. In contrast, L-dopa appears to be a competitive substrate of COMT, but a relatively weak inhibitor, and D-dopa is even weaker. It is possible that some of the effectiveness of RO4-4602 in Parkinsonian patients receiving L-dopa therapy may be due to its effects on methylation as well as decarboxylation.

REFERENCES

1. A. Barbeau and F. A. McDowell, *L-Dopa and Parkinsonism*, F. A. Davis Company, Philadelphia (1970).
2. O. Horneykiewicz, *Pharmac. Rev.* **18,** 925 (1966).
3. A. H. Anton and D. F. Sayre, *J. Pharmac. exp. Ther.* **145,** 326 (1964).
4. H. Weil-Malherbe, H. S. Posner and G. R. Bowles, *J. Pharmac. exp. Ther.* **132,** 278 (1961).
5. G. C. Cotzias, P. S. Papavasiliou and R. Gellene, *New Engl. J. Med.* **280,** 337 (1969).
6. F. Bartholini and A. Pletscher, *J. Pharmac. exp. Ther.* **161,** 14 (1968).
7. R. J. Wurtman, C. Chou and C. Rose, *J. Pharmac. exp. Ther.* **174,** 351 (1970).
8. C. C. Porter, *Fedn Proc.* **30,** 871 (1971).
9. J. P. Chalmers, R. J. Baldessarini and R. J. Wurtman, *Proc. natn. Acad. Sci. U.S.A.* **68,** 662 (1971).
10. S. Matthysse, J. F. Lipinski and V. Shih, *Clinica chim. Acta* **35,** 253 (1971).
11. R. J. Baldessarini, *Biochem. Pharmac.* **15,** 741 (1966).
12. L. A. Pearce and L. D. Waterbury, *Neurology* **21,** 410 (1971).
13. R. J. Baldessarini and K. V. Chace, *Eur. J. Pharmac.* **17,** 163 (1972).

14. R. J. BALDESSARINI, *J. Pharm. Pharmac.* **24,** 78 (1972).
15. S. B. Ross and Ö. HALJASMAA, *Acta pharmac. tox.* **21,** 205 (1964).
16. C. R. CREVELING and J. W. DALY, in *Methods of Biochemical Analysis* (Suppl., *Analysis of Biogenic Amines and Related Enzymes*) (Ed. D. GLICK), p. 153. Wiley, New York (1971).
17. R. J. BALDESSARINI, J. F. LIPINSKI and K. V. CHACE, *Biochem. Pharmac.* **21,** 77 (1972).
18. R. J. BALDESSARINI and I. J. KOPIN, *J. Neurochem.* **13,** 769 (1966).
19. H. H. LINEWEAVER and D. BURK, *J. Am. chem. Soc.* **56,** 658 (1934).
20. M. DIXON, *Biochem. J.* **55,** 170 (1953).
21. J. R. CROUT, *Biochem. Pharmac.* **6,** 47 (1961).
22. B. NIKODEJEVIC, S. SENOH, J. W. DALY and C. R. CREVELING, *J. Pharmac. exp. Ther.* **174,** 83 (1970).
23. J. L. WEISS, C. K. COHN and T. N. CHASE, in *Proc. First Annual Meeting Society for Neuroscience*, abstr. 18·4. Washington, D.C. (1971).
24. W. LOVENBERG, H. WEISSBACH and S. UDENFRIEND, *J. biol. Chem.* **237,** 89 (1962).
25. R. TISSOT, G. BARTHOLINI and A. PLETSCHER, *Archs Neurol., Chicago* **20,** 187 (1969).
26. A. BARBEAU, L. GILLO-JOFFROY and H. MARS, *Clin. Pharmac. Ther.* **12,** 353 (1971).
27. A. D. ERICSSON and B. G. WERTMAN, *Neurology* **21,** 1129 (1971).
28. H. E. HIMWICH, S. S. KETY and J. R. SMYTHIES, *Amines and Schizophrenia*, Pergamon Press, Oxford (1967).
29. A. D. ERICSSON, *J. neurol. Sci.* **14,** 193 (1971).