British Journal of Pharmacology (1996) 117, 516–520

Ontogenic aspects of liver and kidney catechol-*O*-methyltransferase sensitivity to tolcapone

M.A. Vieira-Coelho & [1]P. Soares-da-Silva

Institute of Pharmacology & Therapeutics, Faculty of Medicine, 4200 Porto, Portugal

1 The present work describes the catechol-*O*-methyltransferase (COMT) activities in the liver and kidney of developing and adult rats (aged 3, 6, 9, 18, 30 and 60 days; $n=5$ per group) and evaluates the enzyme sensitivity to inhibition by tolcapone, a reversible COMT inhibitor.

2 COMT activity, evaluated by the ability to methylate adrenaline to metanephrine, was determined in liver and kidney homogenates prepared in 0.5 mM phosphate buffer (pH = 7.8) containing pargyline (0.1 mM), $MgCl_2$ (0.1 mM), EGTA (1 mM) and S-adenosyl-L-methionine (0.1 mM). V_{max} (in nmol mg^{-1} protein h^{-1}) of liver COMT was found to decrease gradually with age, from 5.3 ± 0.5 at the age of 3 days up to 2.9 ± 0.2 at the age of 60 days; for the same age range, K_m values (in μM; geometric means with 95% confidence limits) increased from 3.3 (1.0, 7.5) up to 13.1 (2.1, 24.1). At the age of 3 days, V_{max} values for kidney COMT (2.6 ± 0.1) were lower than those for the liver COMT. However, V_{max} values for kidney COMT were found to increase up to 6.2 ± 0.6 at the age of 18 days and then declined by 44% at the age of 30 and 60 days. In kidney, aging was also accompanied by an increase in K_m values for COMT (from 2.7 [1.1, 4.3] up to 24.0 [11.7, 36.3]).

3 The sensitivity of liver and renal COMT activity to tolcapone was markedly dependent on the age, 3-days old rats being more sensitive to tolcapone than older animals. The IC_{50} values (in nM) for inhibition of liver COMT by tolcapone increased gradually with age, from 41 (26, 65) at the age of 3 days up to 720 (640, 800) at the age of 60 days. As was found in the liver, IC_{50} values (in nM) for inhibition of kidney COMT by tolcapone also increased with age, from 8 (6, 10) at the age of 3 days up to 177 (131, 240) at the age of 60 days. In all experimental groups, the IC_{50} values for inhibition of liver COMT by tolcapone was higher than those for kidney COMT.

4 In conclusion, these results suggest that aging is accompanied by a decrease in liver and kidney COMT affinity for the substrate (evidenced by the increase in K_m values) and a decrease in sensivity towards inhibition by tolcapone (evidenced by the increase in IC_{50} values). Furthermore, kidney COMT is shown to be more sensitive to inhibition by tolcapone than liver COMT, irrespective of the age of the animal.

Keywords: Catechol-*O*-methyltransferase; kidney; liver; tolcapone; ontogeny

Introduction

Catechol-*O*-methyltransferase (COMT) catalyzes the transfer of a methyl group from S-adenosyl-L-methionine to endogenous and exogenous substrates containing a catechol moiety (Axelrod & Tomchick, 1958). The enzyme is widely distributed in both human and animal tissues and plays an important role in the inactivation of catecholamine neurotransmitters, catechol steroids and xenobiotic catechols (Axelrod, 1966; Trendelenburg, 1988). Two major classes of COMT have been defined based on their subcellular location and include a soluble cytosolic form (S-COMT) and a membrane-bound form (MB-COMT). The S-COMT is generally assumed as the predominant form of the enzyme, as indicated by greater V_{max} values in comparison with those observed for MB-COMT (Guldberg & Marsden, 1975; Kopin, 1986). However, several studies have established MB-COMT as a biochemically distinct entity and showed that this form of the enzyme has a higher affinity for the catechol substrates. The high affinity of MB-COMT for catechol substrates has even been suggested as responsible for the *O*-methylation at low and physiologically relevant concentrations of the catecholamine neurotransmitters, whereas the activity of the S-COMT predominates under conditions which lead to saturation of the MB-COMT (for a review see Roth, 1992).

Until recently, there have been no selective, non-toxic inhibitors of COMT and, to some extent, this has hampered research in the field of *O*-methylation mechanisms of brain and peripheral catecholamines. The discovery, in recent years, of new COMT inhibitors has greatly stimulated COMT research and some of these compounds are already in use as adjuncts to L-DOPA therapy in Parkinson's disease (Roberts *et al.*, 1993). Several nitrocatechol derivatives have been developed and found to be highly selective and potent inhibitors of COMT, both *in vitro* and *in vivo* conditions (Männisto & Kaakkola, 1989). All new nitrocatechol COMT inhibitors are thought to bind well to the enzyme, but for some of them (OR-462 and other OR compounds) the evidence obtained in enzyme kinetic studies did not allow determination of the exact type of inhibition (Männistö & Kaakkola, 1989). Tolcapone is claimed to be a potent and orally active COMT inhibitor (Zürcher *et al.*, 1990a) and to inhibit COMT in a competitive fashion at both peripheral tissues and the central nervous system (Zürcher *et al.*, 1990a, b). To our knowledge, however, no enzyme kinetic studies showing a competitive type of inhibition by tolcapone is available in the literature.

The kidney and liver have special roles in relation to catecholamine metabolism since they excrete as well as metabolize circulating or locally formed catecholamines and their metabolites. In addition, these two organs are endowed with the highest COMT activities in the body (Axelrod *et al.*, 1959) and for this reason are expected to play a central role when inhibition of COMT has to be performed. In the past recent years we have been interested in studying the synthesis and

[1] Author for correspondence.

metabolism of dopamine in epithelial cells of renal tubules (for a review see Soares-da-Silva, 1994), an area where the amine is believed to act as a local hormone exerting diuretic and natriuretic effects (for a review see Lee, 1993). In the course of these studies in the rat kidney, it was soon recognized that locally formed dopamine undergoes rapid deamination to 3,4-dihydroxyphenylacetic acid (DOPAC) which is then methylated to homovanillic acid (HVA); methylation of dopamine to 3-methoxytyramine (3-MT) was found to be comparatively less important (Fernandes & Soares-da-Silva, 1993; Vieira-Coelho *et al.*, 1994). This has prompted us to examine in more detail *O*-methylation mechanisms in the kidney and quite unexpectedly it was observed that renal COMT in adult rats was more sensitive to inhibition by tolcapone than liver COMT (Soares-da-Silva & Vieira-Coelho, 1993). Subsequently, in renal and liver tissues of rats with different ages (30, 60 and 90 days old), it was found that despite similar V_{max} and K_m values for liver and kidney COMT activities in all groups, aging is accompanied by a decreased sensitivity of the enzyme to inhibition by tolcapone (Vieira-Coelho & Soares-da-Silva, 1994).

The present work aimed to examine in more detail liver and kidney COMT activity in developing and adult rats (aged 3, 6, 9, 18, 30 and 60 days) and enzyme sensitivity to inhibition by tolcapone and shows that aging is accompanied by a decrease in liver and kidney COMT affinity for the substrate (shown by the increase in K_m values) and a decrease in sensivity towards inhibition by tolcapone (shown by the increase in IC_{50} values).

Methods

Male Wistar rats (Biotério do Instituto Gulbenkian de Ciência, Oeiras, Portugal) aged 3, 6, 9, 18, 30 and 60 days and weighing 12 ± 2, 16 ± 3, 20 ± 2, 32 ± 4, 150 ± 6, 290 ± 10 g, respectively, were used. Adult rats were kept two per cage under controlled environmental conditions (12 h light/dark cycle and room temperature 24°C); up to the age of 20 days, rats were kept with their mother, the environmental conditions being those described above. Food and tap water were allowed *ad libitum*. The rats were killed by decapitation under ether anaesthesia and the kidneys and liver were rapidly removed through an abdominal midline incision and rinsed free from blood with saline (0.9% NaCl). The tissues were homogenized in 0.5 mM phosphate buffer, pH = 7.8, at 4°C with a Duall-Kontes homogenizer.

COMT activity was evaluated by the ability of homogenates to methylate adrenaline to metanephrine. Aliquots of 0.5 ml of the homogenate were preincubated for 20 min with 0.5 ml of phosphate buffer (0.5 mM); thereafter, the reaction mixture was incubated for 30 min with increasing concentrations of adrenaline (5 to 500 μM) in the presence of a saturating concentration (100 μM; Axelrod & Tomchick, 1958) of the methyl donor (S-adenosyl-L-methionine); the incubation medium contained also pargyline (100 μM), $MgCl_2$ (100 μM) and EGTA (1 mM). The preincubation and incubation were carried out at 37°C, in conditions of light protection, with continuous shaking and without oxygenation. In experiments conducted with the aim of studying the inhibitory effect of tolcapone on COMT activity, tissue homogenates were preincubated for 15 min with increasing concentrations of tolcapone (0.5 to 10,000 nM); the incubation was performed in the presence of a concentration of adrenaline three times the corresponding K_m value, as determined in saturation experiments for each age group. At the end of the incubation period the tubes were transferred to ice and the reaction was stopped by the addition of 100 μl of perchloric acid (2 M). The samples were then centrifuged (200 *g*, 4 min, 4°C), and 500 μl aliquots of the supernatant filtered on Millipore microfilters (MF1) were used for the assay of metanephrine. This procedure allows 99% extraction of catecholamines and their methylated metabolites (Soares-da-Silva *et al.*, 1993).

The assay of metanephrine was carried out by means of h.p.l.c. with electrochemical detection. Aliquots of 50 μl of the filtered supernatant were injected into the chromatograph. The mobile phase was a degassed solution of citric acid (0.1 mM), sodium octylsulphate (0.5 mM) and methanol (8% v/v), adjusted to pH 3.5 with perchloric acid (2 M) and pumped at a rate of 1.0 ml min^{-1}. The detection was carried out electrochemically with a glassy carbon electrode, an Ag/AgCl reference electrode and an amperometric detector (Gilson model 141); the detector cell was operated at 0.75 V. The current produced was monitored using the Gilson 712 HPLC software. The lower limits for detection of metanephrine ranged from 350 to 500 fmol and peak height increased linearly with the concentration of metanephrine. The interassay coefficient of variation was less than 7%.

V_{max} and K_m values for COMT activity were calculated from non-linear regression analysis using the GraphPad Prism statistics software package (Motulsky *et al.*, 1994). For the calculation of the IC_{50} s the parameters of the equation for one site inhibition were fitted to the experimental data (Motulsky *et al.*, 1994). Geometric means are given with 95% confidence limits and arithmetic means are given with s.e.mean. Statistical analysis was performed by one-way analysis of variance (ANOVA) using Newman-Keuls multiple comparison test to compare values.

The protein content in the homogenates was determined by the method of Bradford with human serum albumin as standard (Bradford, 1976). The protein content was similar in all samples (approximately 0.10 mg 50 μl^{-1} homogenate).

Drugs

Drugs used were L-adrenaline (Sigma Chemical Company, St. Louis, MO, USA), metanephrine (Sigma), pargyline hydrochloride (Sigma) and S-adenosyl-L-methionine (Sigma). Tolcapone was kindly donated by Professor Mosé Da Prada (Hoffman La Roche, Basle, Switzerland).

Results

Incubation of liver and kidney homogenates in the presence of increasing concentrations of adrenaline resulted in a concentration-dependent formation of metanephrine (Figure 1). The kinetics, V_{max} and K_m values for liver and kidney COMT are given in Table 1. As shown in this table, V_{max} values for COMT in liver homogenates of 3-days old rats were found to be greater than those observed in older animals. Contrariwise, V_{max} values for COMT in kidney homogenates progressively increased from 3-days to 18-days old rats (2.6 ± 0.1 vs 6.2 ± 0.6 nmol mg^{-1} protein h^{-1}, respectively) and then declined by 44% at the age of 30 and 60 days. Thirty- and 60-days old animals were found to present similar V_{max} values for liver and kidney COMT. The results also show that aging is accompanied by a significant and progressive increase in K_m values (in μM) for COMT in both the liver and the kidney (Table 1).

Tolcapone produced a concentration-dependent decrease in COMT activity in all experimental groups (Figure 2). However, the sensitivity of liver and renal COMT activity to tolcapone was markedly dependent on the age: 3-day old rats being more sensitive to tolcapone than older animals, as shown by the rightward shift of inhibition curves to tolcapone as a function of age. This rightward shift of inhibition curves to tolcapone was slightly more marked in the kidney (22-fold decrease in sensitivity) than in the liver (17-fold decrease in sensitivity). In all experimental groups the IC_{50} values for inhibition of liver COMT by tolcapone were greater than those for renal COMT (Table 2). As indicated in this table, IC_{50} values in both the liver and kidney increased with age. In renal tissues, IC_{50} values for tolcapone were 4 fold lower than in liver homogenates irrespective of the age of the animal, but showed a similar trend to increase with age (Table 2).

Discussion

The results presented here show that COMT in renal tissues of developing rats closely follows that in the liver and both organs are already endowed with marked *O*-methylation activity at birth. These results agree with those of Meister *et al.* (1993) who showed that COMT mRNA is diffusely distributed in renal cortex of both foetal and newborn animals. However, the affinity of both liver and kidney COMT for the substrate in 3-day old rats, as shown by K_m values for adrenaline, is higher than that in older animals. Another interesting difference in liver and kidney COMT between developing animals and adult rats concerns their sensitivity to inhibition by tolcapone. In fact, the results presented here clearly show that the form of COMT with a high affinity for the substrate also presents an enhanced sensitivity towards inhibition by tolcapone, as indicated by lower IC_{50} values for tolcapone; this form of COMT is particularly evident in developing rats till the age of 9 days.

In Roth's review article (Roth, 1992), a personal communication by Da Prada is quoted mentioning that tolcapone has been shown in rat liver homogenates as a more potent inhibitor of MB-COMT than of S-COMT. Though the results obtained in the present work do not allow us to suggest that younger rats are endowed with higher amounts of MB-COMT than older animals, this could be used as an explanation for the observation that *O*-methylation developing rats is more sensitive to inhibition by tolcapone. Another indication that would fit this suggestion is that K_m values differ markedly between MB-COMT and S-COMT; according to Apprile & Malamud (1975) and Tong & d'Iorio (1977) the K_m value for

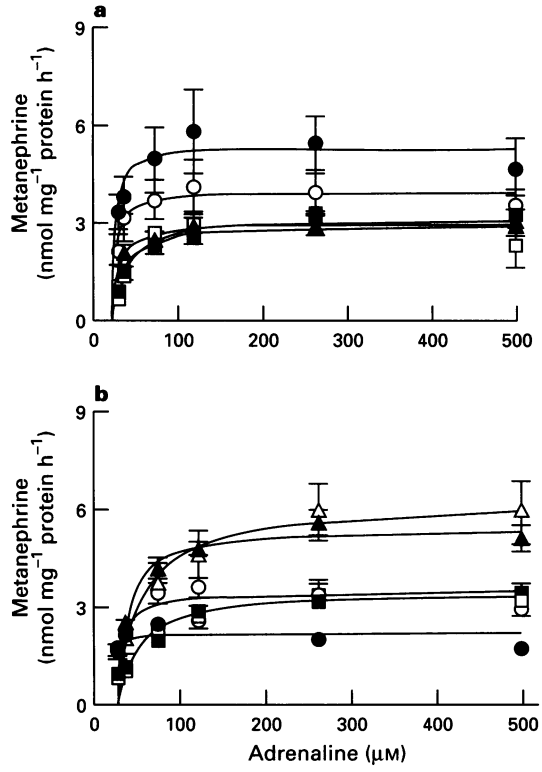

Figure 1 *O*-methylation of increasing concentrations (10 to 500 μM) of adrenaline in homogenates of rat (a) liver and (b) kidney obtained from 3- (●), 6- (○), 9- (▼), 18- (△), 30- (■) and 60- days old rats (□). The results are levels (in nmol mg^{-1} protein h^{-1}) of metanephrine formed from added adrenaline. Each point represents the mean with s.e.mean of four experiments per group.

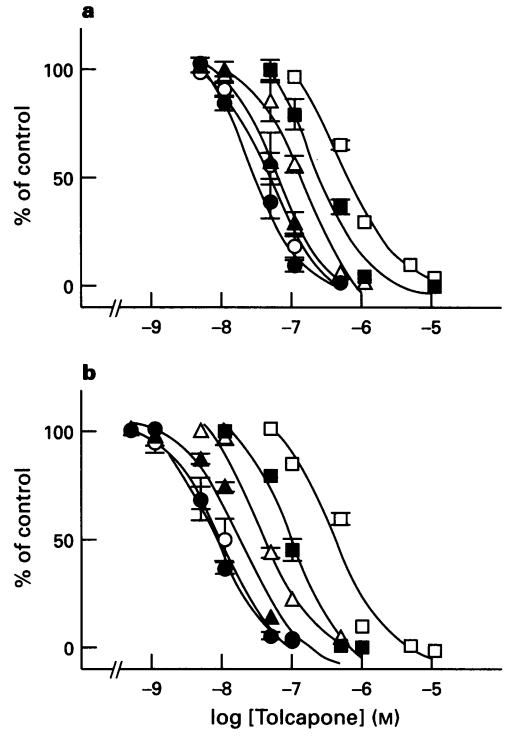

Figure 2 Inhibition curves of COMT activity in (a) liver and (b) kidney homogenates obtained from 3- (●), 6- (○), 9- (▲), 18- (△), 30- (■) and 60-days old rats (□) by tolcapone (0.5 to 10,000 nM). For each age the concentration of adrenaline used was 3 times the corresponding K_m value. Values are means ± s.e.mean of five experiments per group.

Table 1 Kinetics (V_{max} in nmol mg^{-1} protein h^{-1}; K_m in μM) of COMT activity (using adrenaline as substrate) in liver and kidney homogenates of developing and adult rats

Age	*Liver*		*Kidney*	
	V_{max}	K_m	V_{max}	K_m
3-days old	5.3 ± 0.5§	3.3 (1.0, 7.5)	2.6 ± 0.1	2.7 (1.1, 4.2)
6-days old	3.9 ± 0.4	3.5 (1.3, 8.3)	3.4 ± 0.2	5.1 (2.0, 8.2)
9-days old	3.0 ± 0.2*	7.2 (1.0, 13.4)	5.4 ± 0.2*	12.4 (7.9, 16.9)
18-days old	3.1 ± 0.2*	13.2 (2.2, 24.3)	6.2 ± 0.6*	27.0 (4.9, 49.1)
30-days old	3.1 ± 0.1*	16.9 (10.0, 23.9)	3.5 ± 0.1	24.8 (16.0, 33.5)
60-days old	2.9 ± 0.2*	13.1 (2.1, 24.1)	3.5 ± 0.2	24.0 (11.7, 36.3)

Values are arithmetic means ± s.e.mean and geometric means with 95% confidence intervals of 5 experiments per group. Significantly different from V_{max} values in 3 day old rats (*$P<0.05$) and significantly different from values in renal tissues (§$P<0.05$) using the Newman-Keuls test.

Table 2 IC_{50} values (geometric means with 95% confidence limits) of tolcapone for inhibition of COMT activity in liver and kidney homogenates of developing and adult rats

Age	*Liver* IC_{50} (nM)	*Kidney* IC_{50} (nM)
3-days old	41 (26, 65)	8 (6, 10)
6-days old	57 (37, 87)	13 (4, 38)
9-days old	64 (35, 110)	26 (23, 30)
18-days old	160 (93, 280)	45 (41, 50)
30-days old	470 (460, 490)	83 (68, 101)
60-days old	720 (640, 800)	177 (131, 240)

($n = 5$ experiments per group.

MB-COMT is approximately 100 times lower than that for S-COMT. The difference in K_m values between 3 and 60 day old rats is in the kidney by a factor of 8.9 and in the liver by a factor of 3.9. Of course the preparations used in the experiments reported here probably constitute a mixture of MB-COMT and S-COMT, the ratio of which has to be determined in future studies.

Alternatively, it might be suggested that the form of the enzyme preferentially active in the present studies is the S-COMT, the increase in K_m values and the decrease in sensitivity towards tolcapone corresponding to age-dependent changes in the predominant form of the enzyme. In fact, the range of concentrations of adrenaline used (50 to 500 μM) was the same in all experimental groups, saturation occurring usually at 100 μM. These experimental conditions would favour the methylation of adrenaline by a form of COMT with a relative high K_m for the substrate, which has been suggested to correspond to the soluble form (Roth, 1992). This rationale also applies to studies with tolcapone, since the concentration of adrenaline used was always three times the calculated K_m for the corresponding age group. Another argument favouring this view concerns the parallelism of rightward shift of inhibition curves by tolcapone with age in both the liver and kidney. Assuming the form of COMT we are dealing with, in both the liver and kidney, to be mainly S-COMT, then it might be suggested that aging is accompanied by a decrease in the affinity of the enzyme for the substrate and a decrease in enzyme sensitivity to inhibition by tolcapone. This is not only an interesting aspect of the ontogeny of the enzyme, but may also have pharmacological implications when considering the utilization of COMT inhibitors in subjects exhibiting an increased sensitivity to these compounds.

Another aspect we think worth mentioning is that kidney COMT is more sensitive to inhibition by tolcapone than liver COMT, irrespective of the age of the animal. In fact, this different sensitivity was found to occur in all age groups, renal COMT being three times more sensitive to tolcapone than liver COMT. Apart from speculating on some tissue specificity of renal and liver COMT, no other explanation for differences in enzyme sensitivity to tolcapone appears to be obvious. Tolcapone has been suggested to behave as a competitive inhibitor (Zürcher *et al.*, 1990a, b) and, on the basis of the atomic structure of rat liver COMT, the binding of catecholamines to the enzyme was postulated to be very similar to the binding of nitrocatechol inhibitors (Vidgren *et al.*, 1994). Thus, since K_m values for kidney and liver COMT are similar within the same age group it might be assumed that differences in sensitivity to inhibition by tolcapone would be not related to differences in the affinity of the enzyme for the substrate. Again, this aspect could be of some pharmacological relevance if one considers the possibility of obtaining some degree of organ specificity in COMT inhibition.

Altogether, considering the differences in K_m values for COMT and enzyme sensitivity to inhibition by tolcapone during development, the results presented here suggest that the predominant form of COMT in newborn rats is different from that occurring in early adulthood and adult animals. Studies are in progress in order to investigate whether this different form of COMT corresponds to the membrane bound form of COMT, which it has been suggested possesses a higher affinity for the catechol substrates and an increased sensitivity to inhibition by tolcapone.

The helpful discussions and advice of Prof. U. Trendelenburg are gratefully acknowledged.
The present study was supported by grant number PBIC/C/CEN 1139 from the JNICT.

References

APPRILE, J. & MALAMUD, D. (1975). Catechol-O-methyltransferase in mouse liver plasma membranes *Biochem. Biophys. Res. Commun.*, **64,** 1293–1302.

AXELROD, J. (1966). Methylation reactions in the formation and metabolism of catecholamines and other biogenic amines. *Pharmacol Rev.*, **18,** 995–113.

AXELROD, J., ALBERTS, W. & CLEMENTE C. (1959). Distribution of catechol-O-methyltransferase in the nervous system and other tissues. *J. Neurochem.*, **5,** 68–71.

AXELROD, J. & TOMCHICK, R. (1958). Enzymatic O-methylation of epinephrine and other catechols. *J. Biol. Chem.*, **233,** 702–705.

BRADFORD, M.M. (1976). A rapid method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.*, **72,** 248–254.

FERNANDES, M.H. & SOARES-DA-SILVA, P. (1993). Sequential involvement of monoamine oxidase and catechol-O-methyltransferase in the metabolism of newly-formed dopamine in rat renal tissues. In *Cardiovascular and Renal Actions of Dopamine.* ed. Soares-da-Silva P. *Adv. Biosci.*, Vol 88, pp. 21–30. Oxford: Pergamon Press.

GULDBERG, H.C. & MARSDEN, C.A. (1975). Catechol-O-methyltransferase: pharmacological aspects. *Pharmacol. Rev.*, **27,** 135–206.

KOPIN, I.J. (1986). Catecholamine metabolism: basic aspects and clinical significance. *Pharmacol. Rev.*, **37,** 334–364.

LEE, M.R. (1993). Dopamine and the kidney: ten years on. *Clin. Sci.*, **84,** 357–75.

MÄNNISTÖ, P.T. & KAAKKOLA, S. (1989). New selective COMT inhibitors: useful adjuncts for Parkinson's disease? *Trends Pharmacol. Sci.*, **10,** 54–56.

MEISTER, B., BEAN, A.J. & APERIA, A. (1993). Catechol-O-methyltransferase mRNA in the kidney and its appearance during ontogeny. *Kidney Int.*, **44,** 726–733.

MOTULSKY, H.G., SPANNARD, P. & NEUBIG, R. (1994). *GraphPad Prims* (Version 1.0). San Diego, U.S.A.: GraphPad Prism Software Inc.

ROBERTS, J.W., CORA-LOCATELLI, G., BRAVI, D., METMAN, L.V., MOURADIAN, M.M. & CHASE, T.N. (1993). Catechol-O-methyltransferase (COMT) inhibitor Ro 40–7592 prolongs duration of action of levodopa/carbidopa in parkinson patients. *Neurology*, **43,** (4, Suppl. 2): Abst 684S.

ROTH, J.A. (1992). Membrane-bound catechol-O-methyltransferase: A reevaluation of its role in the O-methylation of the catecholamine neurotransmitters. *Rev. Physiol. Biochem. Pharmacol.*, **88,** 1–29.

SOARES-DA-SILVA, P. (1994). Source and handling of renal dopamine: its physiological importance. *News Physiol. Sci.*, **9,** 128–134.

SOARES-DA-SILVA, P., FERNANDES, M.H. & PINTO-DO-Ó, P.C. (1993). Cell inward transport of L-DOPA and 3-O-methyl-L-DOPA in rat renal tubules. *Br. J. Pharmacol.*, **112,** 611–615.

SOARES-DA-SILVA, P. & VIEIRA-COELHO, M.A. (1993). Increased sensitivity of renal catechol-O-methyltransferase to inhibition by tolcapone. *J. Am. Soc. Nephrol.*, **4,** 896.

TONG, J. & D'IORIO, A. (1977). Solubilization and partial purification of particulate catechol-O-methyltransferase from rat liver. *Can. J. Biochem.*, **55,** 1108–1113.

TRENDELENBURG, U. (1988). The extraneuronal uptake and metabolism of catecholamines. In *Catecholamines.* ed. Trendelenburg, U. & Weiner, N. *Handbook of Experimental Pharmacology*, Vol 90/I. pp. 279–320. Berlin: Springer-Verlag.

VIDGREN, J., SVENSSON, L.A. & LILJAS, A. (1994). Crystal structure of catechol-O-methyltransferase. *Nature*, **368,** 354–358.

VIEIRA-COELHO, M.A. & SOARES-DA-SILVA, P. (1994). Sensitivity of liver and kidney catechol-O-methyltransferase to inhibition by tolcapone in developing rats. *Br. J. Pharmacol.*, **112,** 410P.

VIEIRA-COELHO, M.A., FERNANDES, M.H. & SOARES-DA-SILVA, P. (1994). In vivo effects of the monoamine oxidase inhibitors Ro 41-1049 and Ro 19-6327 on the production and fate of renal dopamine. *J. Neural Trans.* {Suppl] **41,** 365–370.

ZÜRCHER, G., COLZI, A. & DA PRADA, M. (1990a). Ro 40-7592: inhibition of COMT in rat brain and extracerebral tissues. *N. Neural Transm.* [Suppl] **32,** 375–380.

ZÜRCHER, G., KELLER, H.H., KETLER, R., BORGULYA, J., BONETTI, E.P., EIGENMANN, R. & DA PRADA, M. (1990b). Ro 40-7592, a novel, very potent and orally active inhibitor of catechol-O-methyltransferase: a pharmacological study in rats. *Adv. Neurol.*, **53,** 497–503.

(Received May 15, 1995
Revised October 2, 1995
Accepted October 4, 1995)