Kinetic Interactions of Dopamine and Dobutamine with Human Catechol-O-methyltransferase and Monoamine Oxidase in Vitro

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ABSTRACT

Dopamine and dobutamine are often infused together into acutely ill patients requiring temporary support of cardiac and renal function, but whether these catecholamines affect the metabolic clearance of each other is not established. We determined the kinetics of dopamine and dobutamine as substrates and inhibitors of each other, i.e., apparent \( V_{\text{max}} \), \( K_m \), and \( K_i \), with crude preparations of human blood mononuclear cell catechol-O-methyltransferase (COMT) and platelet monoamine oxidase (MAO) at pH 7.4 and 37°C. Values of \( V_{\text{max}} \) for dopamine and dobutamine as substrates for COMT were 0.45 and 0.59 nmol of 3-O-methyl product formed per milligram of protein per minute, whereas those for \( K_m \) were 0.44 and 0.05 mM, respectively. Dopamine and dobutamine were competitive inhibitors of each other in this reaction. The \( K_i \) for dopamine as an inhibitor of dobutamine methylation was 1.5 mM, whereas that for dobutamine as an inhibitor of dopamine methylation was 0.015 mM. Dopamine but not dobutamine was a substrate for MAO. The \( V_{\text{max}} \) for dihydroxyphenylacetaldehyde formation from dopamine was 0.29 nmol/mg protein/min and the \( K_m \) for dopamine was 0.38 mM. Dobutamine was a noncompetitive inhibitor of dopamine oxidation in this reaction (\( K_i = 1.19 \) mM). The high apparent \( K_m \) and \( K_i \) values derived for dopamine and dobutamine when tested with these two human enzymes in vitro suggest that these catecholamines do not interfere with the metabolism of each other when both are infused together at therapeutic concentrations.

Dopamine and dobutamine are catecholamines commonly infused together to treat critically ill patients with shock and/or heart failure. Dopamine is a biogenic amine given at low doses to maintain or enhance renal and splanchnic perfusion, whereas dobutamine is a chemically related synthetic inotrope employed to enhance cardiac output without increasing systemic vascular resistance (Latifi et al., 2000). Whether dopamine and dobutamine affect the systemic clearance of each other when both compounds are administered together is unclear from reports of three pharmacokinetic studies (Banner et al., 1989, 1991; Schwartz et al., 1991).

Catechol-O-methyltransferase (COMT) and monoamine oxidase (MAO) are the two enzymes primarily responsible for the initial metabolic disposition of infused catecholamines in the blood of mammals (Kopin, 1985). Moreover, formation of 3-O-methyldobutamine catalyzed by COMT appears to be the main route for dobutamine bio- disposition in the dog (Murphy et al., 1976), and we have recently shown that 3-O-methyldobutamine is a major product of infused dobutamine metabolism in humans (Yan et al., 2002). To our knowledge there is no published information as to whether dobutamine serves as a substrate for MAO in humans.

To evaluate the roles of COMT and MAO in the metabolic clearance of coinfused dopamine and dobutamine and in possible metabolic interactions between these two inotropes in humans, we now report kinetic studies of dopamine and dobutamine used both as substrates and as inhibitors of each other in assays of human blood mononuclear cell COMT and platelet MAO activities. Characterizing COMT and MAO activities from these two cell types in vitro may reflect certain functional properties of these two major enzymes of catecholamine metabolism in vivo. Our results suggest that the apparent \( K_i \) concentrations for dopamine and dobutamine are so high for both COMT and MAO, relative to their therapeutic concentrations in human plasma, that these agents will not affect the metabolic clearance of each other by these enzymes when both drugs are coinfused clinically. Moreover, our observation that dobutamine serves as a substrate for human blood mononuclear cell COMT but not for platelet MAO is consistent with O-methylation constituting a major metabolic pathway for dobutamine disposition in humans.

ABBREVIATIONS: COMT, catechol-O-methyltransferase; MAO, monoamine oxidase; DOPAL, 3,4-dihydroxyphenylacetaldehyde; 3-MT, 3-methoxytyramine; HPLC, high-performance liquid chromatography; EC, electrochemical detection; 3-O-MD, 3-O-methyldobutamine; SAM, S-adenosylmethionine; S-COMT, soluble catechol-O-methyltransferase; M-COMT, membrane-bound catechol-O-methyltransferase.
**Materials and Methods**

**Blood Samples.** Blood to assess mononuclear cell COMT activity with both dopamine and dobutamine (Fig. 1) was obtained in compliance with an investigational protocol approved by the Institutional Review Board at Rainbow Babies and Children’s Hospital for ongoing studies of dopamine and dobutamine in acutely ill infants and children. As part of a larger sample used for clinical laboratory tests, 3 ml of blood from each patient was introduced into a tube containing EDTA, and mononuclear cells were isolated and stored at −70°C as previously described (Allen et al., 1992). Patients without life-threatening diagnoses consisted of six males and six females, aged 1 month to 13 years, with hematocrits ranging from 31 to 44. The lowest hematocrit in the six infants less than 1 year old was 33, a value within the normal range for this patient population.

Four normal adults, two of them investigators, volunteered to donate blood used to isolate and store mononuclear cells and platelets at −70°C for kinetic studies of COMT and MAO (Table 1; Figs. 2–4).

**Chemicals and Reagents.** S-Adenosylmethionine (SAM), adenosine deaminase (EC 3.5.4.4, bovine spleen, 78 U/mg of protein), 3-methoxytyramine (3-MT), 3-methoxy-4-hydroxybenzylamine, dopamine, 3,4-dihydroxybenzylamine, epinephrine, Na2EDTA, L-ascorbic acid, Tris base, Tris-HCl, Amberlite CG-50 (H+ form, wet mesh 100–200), 2,4-dinitrophenylhydrazine, monoamine oxidase (MAO) (EC 1.4.3.6, bovine plasma, 40–100 U/mg protein) and bovine serum albumin were obtained from Sigma (St. Louis, MO). Sodium octylsulfate was purchased from Eastman Kodak (Rochester, NY), Ficoll Hypaque from Amersham Pharmacia Biotech (Piscataway, NJ), partially purified catecholamine-O-methyltransferase (COMT) (EC 2.1.1.6, porcine liver, 2200 U/mg protein) from Calbiochem (La Jolla, CA), and dobutamine from Sigma/RBI (Natick, MA). HPLC-grade HPLC-EC system. HPLC-EC was carried out with an LC-400 Bioanalytical Systems (West Lafayette, IN) liquid chromatograph equipped with a carbon/carbon electrode and interfaced with a Vari-an Instruments (Sunnyvale, CA) model 2510 pump. The potential of the working electrode was maintained at +700 mV versus an Ag+/AgCl reference electrode. Separations were achieved in a reverse-phase system with a Bioanalytical Systems phase II ODS stainless steel prepacked column used as the stationary phase (100 mm × 3.2 mm i.d., particle size 3 μm). The mobile phase consisted of 880 ml of 0.069 M acetic acid/2 mM Na2EDTA adjusted to pH 4.5 with 5 M NaOH prior to addition of 120 ml of acetonitrile; this solution was passed through nitrocellulose filters and degassed with N2 prior to use. With the flow rate set at 0.8 ml/min at 25°C, 3-O-methyldobutamine eluted at 21 min. Fractions containing this compound were pooled, evaporated to dryness, and stored at −70°C.

**Residues from many chromatographic separations were taken up in 0.5 ml of mobile phase and rechromatographed for further purification. Fractions containing the single large peak eluting at 21 min were combined, evaporated to dryness, dissolved in 0.5 ml of water, extracted into 5 ml of methylene chloride, back extracted with 1 ml of 0.1 M HCl, and vortex-mixed. After centrifugation, the upper aqueous layer was removed, evaporated to dryness, taken up in 0.5 ml of water, added to 0.5 ml of 1.33 M sodium borate buffer, pH 11, containing 1% (w/v) Na2EDTA, extracted into 4 ml of chloroform, and evaporated to dryness. This material, stored at −70°C, was shown to be >99% pure 3-O-methyldobutamine by mass spectrometry, and its extinction coefficient, E in water, was 5.73 OD (Yan et al., 2002).

**Assays of Human Blood Mononuclear Cell COMT Activity.** Isolation of mononuclear cells from 3 ml of human blood, their storage at −70°C, and sonication of the thawed cells at 4°C were accomplished as previously reported (Allen et al., 1992). COMT activity also was assayed as described by these investigators except that 0.55-ml reaction mixtures were run for 45 instead of 10 min and the O-methylated products were extracted with 5 ml of methylene chloride instead of ethyl acetate (Yan et al., 2002). Briefly, sonicated cell pellet protein (0.34–0.44 mg) was preincubated with 218 μM SAM, 10.9 mM MgCl2, and 3 units of adenosine deaminase (30 μg) in 0.5 ml of reaction mixture at pH 7.4 in a 37°C shaking water bath. Reactions were initiated by addition of 0.05 ml of dopamine or dobutamine used either alone or in various combinations at concentrations shown in Figs. 2 and 3. Reactions were stopped by addition of 0.5 ml of 1.33 M borate buffer, pH 11, containing 1% (w/v) Na2EDTA and immediately extracted with 5 ml of methylene chloride.

To analyze for the dopamine reaction product, 50 μl of 2 μg/ml 3-methoxy-4-hydroxybenzylamine internal standard was added before the borate buffer and methylene chloride extraction. After evaporation of the lower organic phase, the 3-methoxytyramine product was dissolved in 0.2 ml of 0.1 M perchloric acid and quantitated by HPLC-EC (Allen et al., 1992).

For the dobutamine reaction product, i.e., 3-O-methyldobutamine, exactly 4 ml of the methylene chloride-extracted reaction sample was dried under vacuum without prior addition of an internal standard.

![Fig. 1. Relationship between dopamine and dobutamine as substrates for mononuclear cell COMT.](image-url)
To correct for product loss during processing, another mixture of identical composition was processed in parallel except that the 3-O-methyl-dobutamine external standard was substituted for dobutamine in the original incubation mixture. Residues were reconstituted in 0.2 ml of mobile phase and 0.1 ml was used for HPLC-EC analysis under chromatographic conditions described above for isolation of urinary 3-O-methyl-dobutamine.

Assays of Human Blood Platelet MAO Activity. Preparation of human platelets, determinations of MAO activity, and calculations of formed DOPAL product were done as reported by Ogata et al. (1992). Thus, for platelet isolation, 1 ml of isosotic phosphate buffer (0.145 M NaCl, 0.01 M Na₂HPO₄, 3.14 mM Na₂EDTA, pH 7.4) was added to 2 ml of whole blood in a polypropylene tube containing 7.5 mg of Na₂EDTA. The sample was mixed gently and centrifuged at 700 g for 3 min at 20°C. The supernatant containing platelet-rich plasma was transferred with a plastic pipette to another polypropylene tube. The red cell pellet was washed three more times with isotonic phosphate buffer, and the four supernatant solutions were combined and centrifuged at 700 g for 20 min at 4°C.

For determination of MAO activity, the resulting pellet was suspended in 0.35 ml of 0.1 M sodium phosphate buffer containing 0.1 mM Na₂EDTA and 0.10 mM ascorbic acid, all adjusted to pH 7.4, and the mixture was sonicated at 70 W for 15 s. Exactly 20 μl of dopamine, dobutamine, or dopamine/dobutamine mixture together at various concentrations (shown in Fig. 4) was added to 100 μl of platelet sonicate mixture and 390 μl of 0.1 M sodium phosphate buffer/0.1 mM Na₂EDTA/0.10 mM ascorbic acid, pH 7.4 solution in a glass tube, and reactions were run for 20 min at 37°C. Measured MAO activity was identical for crude enzyme preparations, whether they were composed standard deviations plotted in Figs. 2 to 4 by use of Deltagraph software. Data were analyzed by both Lineweaver-Burk and Dixon plots (Segel, 1975). Linear regression was used to estimate apparent kinetic constants and determine the types of inhibition observed. Values for Kᵢ were derived from Lineweaver-Burk plots, whereas those for Kᵢ were obtained from Dixon plots; estimates of Vₘₐₓ were made from both types of plots (Figs. 2–4).

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Substrate</th>
<th>Apparent Kᵢ (mM)</th>
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Results

Both Dopamine and Dobutamine Are Substrates for Human Blood Mononuclear Cell COMT. Except for the catecholamine substrate, the assay used to monitor conversion of dobutamine to 3-O-methyl-dobutamine was identical to that used to test 3-methyltyramine formation from dopamine (Allen et al., 1992; Yan et al., 2002). Formation of 3-O-methyl-dobutamine from dobutamine exhibited the same optima as did generation of 3-methyltyramine from dopamine with respect to pH and concentrations of SAM and MgCl₂. Production of 3-O-methyl-dobutamine at pH 7.4 and 37°C increased linearly with time up to 60 min, with sonicate protein concentrations ranging between 1 and 6 mg/ml. Moreover, sonicates from 12 pediatric patients in the intensive care unit displayed a positive correlation between the rates of 3-O-methyl-dobutamine generation from dobutamine and 3-methyltyramine formation from dopamine (Fig. 1; r = 0.72, n = 12, p < 0.001). Although dobutamine exhibited about 3-fold the activity of dopamine in this assay, COMT activity of these crude preparations showed marked variation between patients and could be assessed with either catecholamine substrate.

Dopamine and Dobutamine Act as Substrates and Competitive Inhibitors of Each Other with Respect to Human Blood Mononuclear Cell COMT. Kinetic data for dopamine and dobutamine tested as substrates and inhibitors of COMT at pH 7.4 and 37°C are shown in Figs. 2 and 3, and the kinetic constants derived from these data are summarized in Table 1. Both dopamine and dobutamine were substrates for COMT. Values of Vₘₐₓ for dopamine and dobutamine were 0.45 and 0.59 nmol of 3-O-methyl product formed per milligram of protein per minute, whereas those for Kᵢ were 0.44 and 0.05 mM, respectively. Dopamine and dobutamine acted as competitive inhibitors of each other. Thus, the Kᵢ for dobutamine as an inhibitor of dopamine methylation was 0.015 mM, whereas the Kᵢ for dopamine as an inhibitor of dobutamine methylation was 1.5 mM.

Dobutamine Is Not a Substrate for Human Platelet MAO. Dopamine is a known substrate for human platelet MAO (Kopin, 1985), but we found no evidence for an electrochemically detectable product when dobutamine was tested as a substrate. Thus, dobutamine, purified to about 99.9% homogeneity by HPLC-EC, was tested at 2 mM (5 times the Kᵢ concentration for dopamine in this MAO assay system), and the reaction was run for 3 h at pH 7.4 and 37°C instead of the usual 20 min. The HPLC elution time used to detect extracted products also was increased from 10 to 40 min. No dobutamine-dependent electrochemically detectable peaks were observed under these conditions except for a small peak

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attributed to DOPAL that accounted for the 0.01% contamination found in the purified dobutamine substrate.

**Dobutamine Is a Noncompetitive Inhibitor of Dopamine Oxidation by Human Platelet MAO.** Kinetic data for MAO with dopamine as the substrate and dobutamine as the inhibitor are shown in Fig. 4; the derived kinetic constants are depicted in Table 1. Dopamine exhibited a $V_{\text{max}}$ of 0.29 nmol of dihydroxyphenylacetaldehyde formed per milligram of protein per minute and a $K_m$ of 0.38 mM in this reaction. Dobutamine acted as a noncompetitive inhibitor of dopamine oxidation with a $K_i$ of 1.19 mM.

**Discussion**

Results of this in vitro kinetic study of crude preparations of human blood mononuclear cell COMT and platelet MAO,
two major enzymes of catecholamine catabolism, suggest two conclusions. First, they provide no evidence that dopamine and dobutamine affect each other by COMT in vivo. Second, at high concentrations, dobutamine is not a substrate or an inhibitor of this enzyme (Sweeney and Nellans, 1995). Apparent $K_m$ values for dopamine and dobutamine $O$-methylation by COMT (0.44 mM and 0.05 mM) differed by nearly 10-fold, whereas those for $V_{max}$ were similar. Thus, the catalytic efficiency ($V_{max}/K_m$) for dobutamine was about 10-fold that for dopamine, suggesting that the former is a better substrate for mononuclear cell COMT. The $K_m$ found for dopamine is similar to values of 0.51 mM and 0.79 mM reported previously (Creveling et al., 1972; Allen et al., 1992).

Dobutamine was a more potent inhibitor of dopamine methylation by mononuclear cell COMT than the converse (Table 1). The expected degree of competitive inhibition can be estimated from the equation,

$$i = \frac{[I]}{[I] + K_i \left(1 + \frac{[S]}{K_m}\right)}$$

where $i$ is the fraction inhibited, $[I]$ the inhibitor concentration, $[S]$ the substrate concentration, $K_i$ the inhibition constant, and $K_m$ the Michaelis constant for substrate (Segel, 1975). To determine the degree of inhibition in vivo for a typical infusion of 2 $\mu$g/kg/min for dopamine and 10 $\mu$g/kg/min for dobutamine, we used plasma concentrations of 0.135 $\mu$M for dopamine and 0.33 $\mu$M for dobutamine, mean values from previous studies (Kates and Leier, 1978; Jarnberg et al., 1981; Ruffolo, 1987; Banner et al., 1989, 1991; Schwartz et al., 1991) as well as from our own data. If values of $K_m$ and $K_i$ found in the presence of high cofactor and cosubstrate concentrations (Table 1) are the same in vivo, the above relationship predicts that COMT activity with dobutamine would be inhibited 0.01% by dopamine and COMT activity with dopamine inhibited 2% by dobutamine. This analysis suggests that dopamine and dobutamine do not interfere with the metabolism of one another by COMT in vivo.

A similar approach can be used to estimate whether dobutamine acting as a noncompetitive inhibitor of dopamine oxidation by platelet MAO in vitro might interfere with dopamine oxidation by MAO in vivo. For noncompetitive inhibition, the equation for degree of inhibition is $i = [I]/(K_i + [I])$. Here, the concentration of dopamine, $[I]$, found clinically is 0.33 $\mu$M and $K_i$ for dopamine shown in Table 1 is 1.19 mM. The calculated degree of platelet MAO inhibition by dobutamine is a clinically insignificant 0.028%. This estimate assumes that dobutamine has a similar $K_i$ value for MAO in the major organs of dopamine metabolism (Weinshilboum, 1978; Sladek-Chelgren and Weinshilboum, 1981; Boudikova et al., 1990). Values of $K_m$ and $V_{max}$ for dopamine oxidation by platelet MAO (Table 1) were similar to those reported previously, i.e., $K_m \approx 0.1$ to 0.22 mM (Donnelly and Murphy, 1977; Ogata et al., 1992) and $V_{max} \approx 0.06$ to 0.51 nmol/min/mg protein (Glover et al., 1977).

Conclusions drawn from this in vitro kinetic study are valid only to the extent that the substrate specificities and the apparent values of $K_m$ and $K_i$ derived for dopamine and dobutamine with crude preparations of human blood mono-
nuclear COMT and platelet MAO mimic these properties of the same enzymes in their major metabolic tissues in vivo. To avoid contamination by transfused erythrocytes in acutely ill pediatric patients, we assayed COMT activity in blood mononuclear cells because they are more likely to reflect COMT activity in individuals from whom blood samples are obtained (Fig. 1). Former studies including our own have shown that the relative activities of COMT in human blood erythrocytes and monocytes correlated not only with each other, but also with the activity of this enzyme in organs having higher specific activities, such as lungs, kidney, and liver (Weinshilboum, 1978; Sladek-Chelgren and Weinshilboum, 1981; Boudikova et al., 1990; Allen et al., 1992, 1997). Mannisto and Kaakkola (1999) have discussed the relevant properties of COMT and COMT inhibitors in a recent review. In humans, just a single COMT gene activated by two distinct promoters encodes two polypeptides, soluble COMT (S-COMT) and membrane-bound COMT (M-COMT). That these enzymes differ by only 50 amino acids, 20 of which comprise a membrane-anchoring domain, argues strongly that their substrate specificities should be identical. This contention is supported further by studies of the three-dimensional crystal structure and catalytic reaction mechanism of rat S-COMT which closely resembles the human enzyme. S-COMT and M-COMT are variably expressed in different tissues; especially high levels occur in liver, kidney, and intestine. S-COMT dominates in most tissues except the brain, and the human recombinant enzyme has a relatively high K_m and K_i values derived for dopamine and dobutamine (Lautala et al., 2001). This value approximates the apparent K_m of 0.44 mM found for human mononuclear cell COMT (Table 1), whereas the K_m reported for the less expressed M-COMT was lower (0.015 mM). A recent investigation comparing relative K_m values for methylation of various substrates by a recombinant human S-COMT indicated that dopamine had a K_m of 0.188 mM as compared with 0.024 mM found for dobutamine (Lautala et al., 2001). Although these apparent K_m values were about half those we obtained, the ratio of their values favoring dopamine was nearly the same, i.e., 7.9 versus 9.8 (Table 1). Thus, despite differences in enzyme sources, preparations, and assay conditions, it seems likely that the substrate specificity and apparent K_m and K_i values derived for dopamine and dobutamine with crude preparations of human mononuclear cell COMT reflect identical properties of COMT in the major metabolic tissues for disposition of these infused catecholamines.

Although liver and kidney express the highest activities of MAO in tissues outside the central nervous system, the role of this enzyme in the peripheral disposition of pharmacological doses of infused dopamine and dobutamine is unclear. MAO exists in two forms, MAO A and MAO B, which are outer mitochondrial membrane flavin-binding enzymes encoded by separate genes on the X chromosome (see reviews by Kopin, 1985; Shih and Chen, 1999; Abell and Kwan, 2000). MAO B, the only form expressed by human blood platelets, reflects the distribution of MAO B activity in peripheral human tissues such as liver, kidney, and monocytes. Therefore, dopamine oxidation by MAO B in the major peripheral tissues of dopamine oxidation by humans is likely to be accomplished with an apparent K_m value similar to that shown for platelets in Table 1. Moreover, dobutamine would not be a substrate for MAO B in any tissue. MAO A, the only form present in term placenta, is found in human liver but not in blood platelets or mononuclear cells. Although studies with selective inhibitors indicate that endogenous dopamine is oxidized by human MAO A (Kopin, 1985), there is yet no comparative kinetic data about the oxidation of pharmacological levels of dopamine and dobutamine by MAO A in human liver, even though both forms of MAO can be separated from that organ (Denney et al., 1982). Indeed, the primary role of MAO A may be to protect the fetus from transfer of biogenic or bioactive amines across the placenta, whereas MAO B protects against certain xenobiotics reaching the bloodstream from dietary or exogenous sources (Abell and Kwan, 2000).

So how do our findings relate to published pharmacokinetic studies? Our data, calculated to clinically achieved concentrations, are most consistent with those of Banner et al. (1991), which show no alterations in the pharmacokinetics of dobutamine in the presence of dopamine. There is, however, little agreement with the first report of Banner et al. (1989) or that of Schwartz et al. (1991), which indicate significant pharmacokinetic changes when dopamine and dobutamine are administered together. Displacement from plasma protein binding sites, invoked to explain these changes, is unlikely to cause major interactions between dopamine and dobutamine. Although these catecholamines may compete for binding to plasma proteins leading to transient increases in free displaced drug in plasma and free drug clearance, the concentration of free drug in plasma and systemic clearance at steady state should remain unchanged, even though the concentration of total drug in plasma may decrease.

One factor contributing to the differences between pharmacokinetic studies is the large interindividual variation in pharmacokinetic data, especially in pediatric populations, that makes such studies difficult to interpret. Interindividual variation may result from differences in the prescribed and infused dose, exposure to catecholamine therapy, patient age, and organ perfusion among critically ill children. One study from our institution showed that the actual dose of dopamine infused was as much as 25% lower than the dose ordered and that an age-related decrease in dopamine clearance occurred in patients between 2 months and 2 years of age (Allen et al., 1997). The latter observation confirms a previous report (Notterman et al., 1990) and diminished dopamine clearance also has been associated with alterations in renal and/or hepatic function (Zaritsky et al., 1988; Notterman et al., 1990).

In summary, our kinetic studies of dopamine and dobutamine metabolism by crude preparations of human blood monocyte COMT and platelet MAO in vitro suggest that these two drugs do not affect the metabolism of each other by COMT and MAO when both catecholamines are present together at therapeutic concentrations in vivo. Dobutamine was a better substrate than dopamine for mononuclear cell COMT, and both drugs at high concentrations competitively inhibited each other in this reaction. dopamine but not dobutamine was a substrate for platelet MAO, and high concentrations of dobutamine actually inhibited dopamine oxidation by this enzyme in a noncompetitive fashion.
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