Inhibition of catechol-*O*-methyl transferase by L-dopa and decarboxylase inhibitors

High doses of L-dihydroxyphenylalanine (L-dopa) have been found to exert beneficial effects in patients with Parkinson's disease (Barbeau & McDowell, 1970). A leading hypothesis concerning its mechanism of action is that it may serve as a precursor for catecholamines, which are deficient in the basal ganglia of such patients (Horney-kiewicz, 1966). Most of a dose of L-dopa is rapidly degraded by mechanisms including decarboxylation and *O*-methylation, and frequent large doses are needed in patients (Wurtman, Chou & Rose, 1970). In attempts to reduce the dosage and expense of L-dopa therapy, to enhance its effectiveness, and to reduce side-effects, inhibitors of the peripheral decarboxylation of L-dopa have been used experimentally and clinically with some success (Porter, 1971). L-Dopa as a substrate of catechol-*O*-methyl transferase (EC 2.1.1.6) can apparently severely tax normal methylation reactions since it can deplete tissue concentrations of the methyl donor *S*-adenosyl-methionine and interfere with the methylation of substances *in vivo* (Chalmers, Baldessarini & Wurtman, 1971). Prevention of these biochemical side effects by blocking the methylation of L-dopa has yet not been undertaken in patients, largely because known inhibitors of COMT are too weak, short acting or toxic. Since L-dopa is a substrate for COMT and since most of the available inhibitors of L-aromatic amino-acid decarboxylase (EC 4.1.1.26) now in use in parkinsonian patients are hydrazine derivatives of catechols or polyphenols (Pletscher & Bartholini, 1971; Porter, 1971), several such compounds were tested *in vitro* for activity as inhibitors of COMT.

The assay method was that of Creveling & Daly (1971) with minor modifications

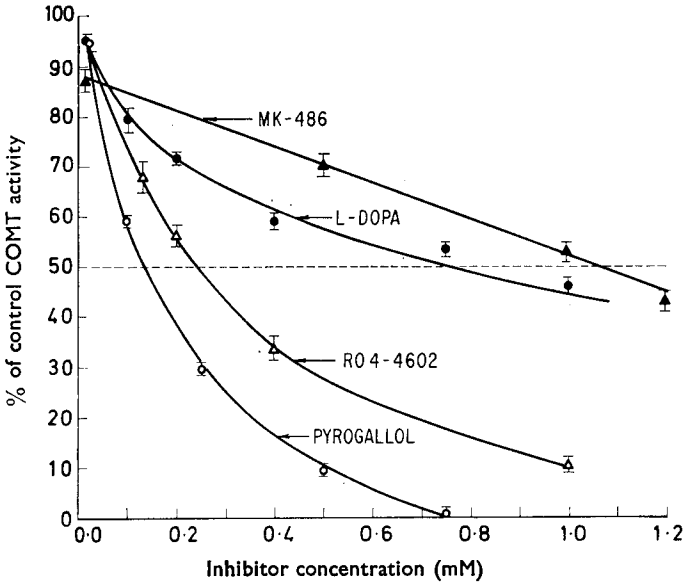

FIG. 1. Inhibition of COMT activity *in vitro*. Drugs were incubated at 10^{-6} to 10^{-3}M with preparations of rat liver in the presence of [^{3}H]-L-noradrenaline (0·25 μCi, 1 mM) and compared with controls containing no inhibitor. Data were obtained as velocity of methylation = nCi of [^{3}H]normetanephrine recovered after 30 min, less a "boiled-enzyme blank" and are expressed as mean % of control $\pm$ s.e. (N = 3 to 10). All differences from control at concentrations $\geq 10^{-5}$M are statistically significant ($P<0{\cdot}05$, or less). Drugs were MK-486 (▲), L-dopa (●), RO4-4602 (△) and pyrogallol (○).

Thus, compounds were tested for their ability to prevent the formation of [^{3}H]-normetanephrine from the catechol substrate, [1(7)-^{3}H]noradrenaline (New England Nuclear, 10 Ci/m mol) in the presence of crude extracts of tissue homogenized in isotonic KCl and centrifuged at 48 000 $\times$ *g* for 20 min. The [^{3}H]normetanephrine produced in 30 min was extracted into toluene: isoamyl alcohol (3: 2, vol) and counted by scintillation spectrometry. [^{3}H]Normetanephrine accounted for over 90% of the recovered ^{3}H, and migrated during paper chromatography with authentic normetanephrine as a single peak of radioactivity.

When preparations of rat liver containing COMT activity were tested with several structural analogues of the catechols at concentrations ranging from 10^{-6} to 10^{-3}M and with a single substrate concentration (1mM) above K_m, the following results were obtained (Fig. 1). The compound N^1-(DL-seryl)-N^2-(2,3,4-trihydroxybenzyl) hydrazine (RO4-4602) was a very active inhibitor of COMT, and very nearly as potent as the well known inhibitor, pyrogallol (Sigma), of which it is a structural analogue. The catechol compounds, L-dopa and L-α-methyl-dopa-α-hydrazine (MK-486), were much weaker inhibitors. The apparent ID50 values were close to 10^{-4}M and about 10^{-3}M for the polyphenols and the catechol compounds, respectively, while the K_m for [^{3}H]noradrenaline was found to be about 2 $\times$ 10^{-4}M. Both of the polyphenols and the catechols accepted [^{14}C]methyl groups from labelled *S*-adenosyl-methionine and thus are substrates of COMT. The kinetics of their inhibitory effects appear to involve competitive effects of the catechols and mixed competitive and non-competitive effects for the polyphenols. Very similar results were obtained with preparations of COMT from rat brain. In contrast, L-α-methyldopa and L-3-*O*-methyldopa were very weak inhibitors (15% inhibition at 10^{-3}M) and DL-α-methyl tyrosine (Sigma) and ascorbic acid (Fisher) were even less effective.

The metabolic inhibitors MK-486 and RO4-4602 are much more active against decarboxylase (ID50 in the order of 10^{-9} to 10^{-8}M; Porter, 1971). However, their effects on COMT might have some significance *in vivo* since doses of 1000 mg or more have been given to patients without apparent ill effects, and this possibility is being investigated. Evidence for the activity of L-dopa as an apparent inhibitor of *O*-methylation *in vivo* in rats (Chalmers & others, 1971), as well as in patients (Frère & Barbeau, 1971) has already been presented.

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Effect of reserpine on the transport of 5-hydroxytryptamine to the rat brain

Bulat & Supek (1967; 1968a) have surveyed previous work on the passage of 5-hydroxytryptamine (5-HT) across the blood-brain barrier, the subject of which has been a matter of dispute for some time, and have demonstrated the penetration of 5-HT through the blood-barrier 10 min after an intravenous injection. The concentration of 5-HT in the rat brain was directly dependent on dose.

The uptake of 5-HT by platelets against a concentration gradient has been reported (Stacey, 1961). Reserpine can affect platelet 5-HT in two ways: either by depleting the 5-HT or by inhibiting its uptake (Lahti & Platz, 1969; Alivisatos, Ungar & others, 1970). We have found a very low dose of reserpine (5 μg/kg) to have no depleting effect on endogenous 5-HT; however, the same dose of reserpine prevented a significant uptake of exogenous 5-HT by the platelets. Under these conditions we examined the transport of 5-HT to the brain of reserpinized rats.

Male Sprague-Dawley rats (190–220 g) were injected into the tail vein with 10 mg/kg of 5-HT creatine sulphate in saline. When reserpine was used, the animals received 5 μg/kg in 30% propylene glycol intraperitoneally 18 h before the 5-HT. Control animals were injected with saline or the propylene glycol. The animals were killed 10 min after the 5-HT injection by cutting the jugular vein, and blood samples were collected in siliconized containers using oxalate as an anticoagulant. 5-HT in platelet-rich plasma and platelets were extracted according to Crawford & Rudd (1962), except the re-extraction was with heptane and 0·01N HCl after which the aqueous phase was acidified to 3N with HCl (conc.). Extraction of brain 5-HT was according to Wiegland & Perry (1961). The fluorescence of 5-HT was measured on an Aminco-Bowman spectrofluorometer at 540 nm (activation 295 nm) using a Kodak screen (Wise, 1967).

In the perfusion studies, the animals were anaesthetized with ether after which the right carotid artery was tied off distally and a polyethylene tube inserted above the ligation; 10 ml of isotonic saline was then perfused into the artery. The same procedure was performed on the left carotid artery and the animals were then decapitated to remove the brain which appeared to be free of blood.

Reserpine neither depleted nor inhibited the uptake of endogenous 5-HT in blood platelets (Table 1). Furthermore, it exerted no effect on the concentration of endogenous 5-HT in the rat brain. After administration of 10 mg/kg of 5-HT, the absolute platelet content of 5-HT was significantly lower in reserpinized animals than in animals receiving no reserpine, indicating a blockade of 5-HT uptake in the platelets by the reserpine. This blockade, however, was not complete since the value obtained in reserpinized animals injected with exogenous 5-HT was much higher than that of the controls receiving reserpine only.

In animals injected only with 5-HT, the brain 5-HT content was significantly increased above the control values (Table 1). These results substantiated the findings of Bulat & Supek (1967; 1968a,b) that 5-HT does cross the blood-brain barrier.