

## **Effect of chronic L-DOPA administration on catecholamine metabolism in the rat**

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### **Summary**

1. L-DOPA was administered to rats by twice daily intraperitoneal injection for a period of eleven days. The daily dose was gradually increased from 250 mg/kg to 1 g/kg. The animals were killed 12 h after the last injection and tissue catechol-*O*-methyl transferase, DOPA-decarboxylase and monoamine oxidase activity determined.
2. Catechol-*O*-methyl transferase activity was reduced in the liver (46%) and red blood cells (38%) but was approximately doubled in the heart.
3. Liver DOPA-decarboxylase activity was reduced by approximately 25%.
4. L-DOPA administration did not significantly affect the activity of any of the enzymes studied in brain tissue.

### **Introduction**

Tolerance develops to the antiparkinsonian effect of L-DOPA in some patients (Lenman, personal communication) and to some of the side effects in the majority of patients (Cotzias, Papavasiliou & Gellene, 1969; Brogden, Speight & Avery, 1971). One possible explanation for this tolerance is an alteration in activity of one or more of the enzymes involved in metabolism of either L-DOPA or the dopamine formed by its decarboxylation. Most of a dose of L-DOPA administered to laboratory animals is rapidly metabolized by decarboxylation or 3-*O*-methylation (Wurtman & Romero, 1972). Decreased activity of the former and/or increased activity of the latter pathway could reduce the pharmacological effects of L-DOPA. Wurtman & Romero (1972) observed that prior administration of L-DOPA decreased *O*-methylation of intracisternally administered noradrenaline and increased the percentage of deaminated metabolites. These authors favour the view that depletion of tissue levels of *S*-adenosylmethionine may have been responsible for this effect. However, tissue catechol-*O*-methyltransferase (COMT) activity was not determined in their experiments. A reduction of COMT activity in the red blood cells of parkinsonian patients receiving chronic L-DOPA therapy has however been reported (Weiss, Cohn & Chase, 1971).

Dopamine is also inactivated by *O*-methylation as well as being oxidized by monoamine oxidase (MAO). An increased rate of metabolism of dopamine in the brain could also result in tolerance to the effects of L-DOPA. To our knowledge, the effect of long term L-DOPA administration on cerebral monoamine oxidase activity has not been reported. In this study we have determined DOPA-decarboxylase, COMT and MAO activities of tissue preparations from rats treated chronically with L-DOPA.

## Methods

### *Administration of L-DOPA*

Female Wistar rats, weighing 150–160 g at the beginning of the experiments, were used throughout. L-DOPA was administered twice daily by intraperitoneal injection, according to the following dose schedule: days 1–3, 125 mg/kg; days 4–7, 250 mg/kg; days 8–11, 500 mg/kg.

### *Preparation of tissues*

Twelve hours after the last injection rats were killed by cervical dislocation. The thorax was opened and approximately 4 ml blood was collected by cardiac puncture, with a siliconed pipette and added to 0.1 ml (2,500 units) of heparin in a centrifuge tube. After centrifugation at 3,000 *g* for 5 min at 4° C, the plasma was removed by aspiration. The red cells were washed with 0.9% w/v NaCl solution (saline) and again centrifuged. Finally the cells were lysed with ice-cold distilled water (1:5) and stored at –20° C until assay.

The brain, heart and approximately 2 g of liver were removed as rapidly as possible and were each homogenized in 10 ml of ice cold 0.5 M phosphate buffer pH 8.0. Brain homogenates were stored at –20° C until required for assay. Liver and heart homogenates were centrifuged at 100,000 *g* for 60 min at 0–4° C and the supernatants stored at –20° C.

### *Determination of catechol-O-methyl transferase (COMT) activity*

This was determined on all four tissues which had been prepared as described above. The method used was essentially that described by Creveling & Daly (1971), with 3,4-dihydroxybenzoic acid as substrate. To a 15 ml glass stoppered centrifuge tube, kept in ice, was added 0.01 ml of 0.5 M magnesium chloride, 0.05 ml of a solution containing 1  $\mu$ Ci/ml of *S*-adenosyl-L-methionine-methyl-<sup>14</sup>C (specific activity of 0.51 mCi/mmol—Radiochemical Centre, Amersham) plus 0.5  $\mu$ M of *S*-adenosyl-L-methionine and 300  $\mu$ l of tissue preparation plus 0.5 M phosphate buffer pH 8.0. The reaction was started by the addition of 0.1 ml of 0.01 M 3,4-dihydroxybenzoic acid. After incubation at 37° C for between 15 and 40 min depending upon the tissue being studied, 0.1 ml of 1 N HCl was added and the *O*-methylated product extracted into 10 ml of toluene. Following centrifugation, 5 ml of the toluene phase was transferred to counting vials containing 10 ml of phosphor and the radioactivity determined.

### *Determination of DOPA-decarboxylase activity*

DOPA-decarboxylase activity was determined on the liver and brain preparations only, since in preliminary experiments, the activity of heart was found to be too low to be accurately determined by this method. The method used was a modification of that described by Lovenberg, Weissbach & Udenfriend (1961). All reagents were dissolved in 0.1 M sodium phosphate buffer, pH 7.0. A typical reaction mixture contained L-DOPA (200  $\mu$ g/ml), pyridoxal-5'-phosphate (5  $\mu$ g/ml), iproniazid phosphate (100  $\mu$ g/ml) and buffer and tissue sufficient to make a final volume of 3 ml. The reaction mixtures, in 15 ml glass stoppered centrifuge tubes were pre-incubated at 37° C before addition of the substrate. After incubation the reaction was stopped by immersion of each tube in boiling water for 30 s and the

precipitated protein was separated by centrifugation. Aliquots (0.5 ml) of each supernatant were diluted to 4.5 ml with water and passed down columns (3 cm  $\times$  0.5 cm) of Amberlite CG50 resin (100–200 mesh) buffered at pH 7.0. The columns were washed with 5 ml water and the dopamine, eluted with 5 ml of 0.5 N HCl, was determined spectrophotofluorimetrically (Lovenberg *et al.*, 1961).

#### *Determination of monoamine oxidase activity*

MAO activity was assayed on brain homogenates only. The method used was that described by Krajl (1965), with the modification that the reaction was stopped with 0.6 M perchloric acid instead of 10% trichloroacetic acid (Century & Rupp, 1968).

#### **Results**

The results are shown in Figs. 1 and 2. Chronic L-DOPA administration reduced the COMT activity of liver and red blood cells by 46 and 38% respectively (Fig. 1). In contrast, the COMT activity in cardiac tissue was approximately doubled (Fig. 1).

Dopa-decarboxylase activity was reduced by approximately 25% in liver but was not altered in brain (Fig. 2).

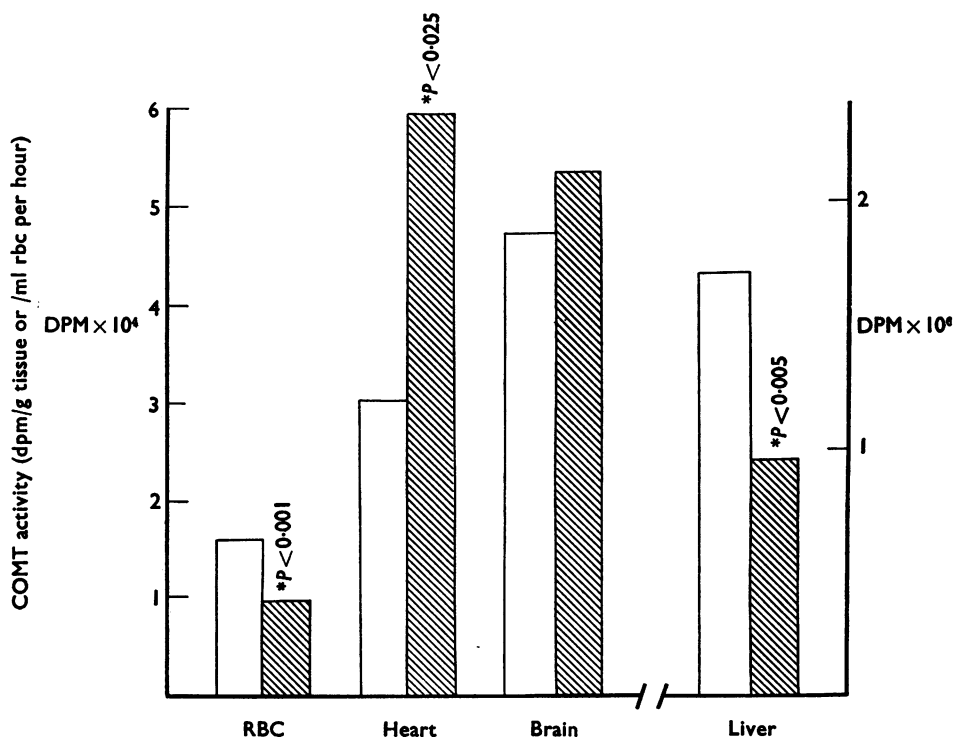


FIG. 1. Effect of chronic L-DOPA administration on tissue catechol-O-methyl transferase (COMT) activity. Open columns control, hatched columns L-DOPA-treated animals.

The amount of 4-hydroxyquinoline formed from kynuramine (per g brain/h) was 126  $\mu\text{g}$  in control and 137  $\mu\text{g}$  in animals treated with L-DOPA indicating no change in MAO activity in the brain.

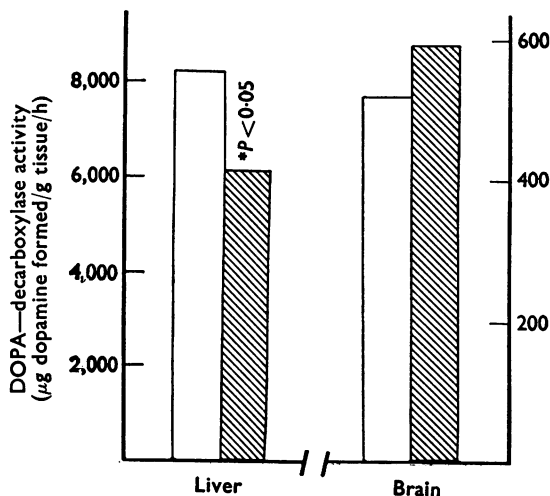


FIG. 2. Effect of chronic L-DOPA administration on liver and brain DOPA-decarboxylase activity. Open columns control, hatched columns L-DOPA-treated animals.

## Discussion

Our results show that chronic administration of L-DOPA reduces the COMT activity of liver and red blood cells. Since the incubation medium contained excess *S*-adenosylmethionine the possibility that the reduced activity could have been due to an L-DOPA-induced depletion of this cofactor can be excluded. It is also unlikely that the inhibition could have been due to L-DOPA itself as the animals were killed 12 h after the last dose of the drug and according to Wurtman & Romero (1972) less than 20% of injected L-DOPA can be detected in mice 1 h after its intraperitoneal administration. In contrast, the COMT activity in cardiac tissue was approximately doubled. It is difficult to assess what effect these changes might have on the pharmacological effects of L-DOPA since the reduced activity in liver and red blood cells would tend to increase the half-life of L-DOPA while the increased activity found in heart would tend to antagonize the effects on that particular organ since any of this amino-acid taken up by cardiac tissue would be more rapidly *O*-methylated and any catecholamine formed by its decarboxylation would also be more rapidly inactivated. No change in activity was found in brain.

DOPA-decarboxylase activity was found to be reduced in liver but was not altered in brain (Fig. 2). This observation is in keeping with the findings of Dairman & Udenfriend (1971) who reported a lowering of aromatic L-amino acid decarboxylase in the kidney, but no change in the brain or adrenals of rats given L-DOPA for 7 days. A reduced rate of decarboxylation may explain tolerance to some effects of L-DOPA but not to the antiparkinsonian effect as the activity in brain was found to be unchanged.

That tolerance to the effects of L-DOPA might be due to an increased activity of cerebral MAO was shown to be unlikely by the finding that the MAO activity of brain was unchanged. It is interesting to note in this respect, however, that the serum level of MAO activity has been reported to be increased in patients given L-DOPA for a period of 2–3 months (Tryding, Tufvesson & Nilsson, 1971). Also, Tarver & Spector (1970) found elevated MAO activity in the vasculature of rabbits treated for several days with L-DOPA. However, it seems unlikely that a change in activity of this particular enzyme in the periphery could affect the central effects of L-DOPA.

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