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Altered pattern of dopa metabolism*

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The treatment of Parkinson's disease with dopa was based upon the observations that basal ganglia dopamine content was markedly low in autopsied brain from patients suffering the disease [1,2]. Since it was assumed that dopamine feeding or injections would not restore the amine in the brain because of a blood–brain barrier for catecholamines, the precursor amino acid was employed to provide replacement therapy. It soon became apparent that the bulk of the fed dopa was quickly metabolized, so that only a small dopa blood level to perfuse the brain could be achieved [3,4]. The strategy of employing a decarboxylase inhibitor as an adjunct to this therapy emerged from a recognition that the major metabolic pathway of dopa proceeded via initial decarboxylation [5,6]. Certain decarboxylase inhibitors were found which were demonstrated to inhibit systemic decarboxylase but not central nervous system decarboxylase, since the inhibitor was subject to restricted access to the brain tissue by the blood brain barrier. Additionally, the loss of dopa by vascular decarboxylase activity was also reduced by the inhibitor, increasing brain dopa perfusion [7]. Finally, the inhibition of the systemic decarboxylase activity would reduce the peripheral dopamine production and thus mitigate some of the side effects encountered in dopa therapy.

Recently Sandler *et al.* [8] have reported an alteration in the metabolic pattern of patients who received a decarboxylase inhibitor, MK 486 (α-methyl-α-hydrazino 3,4-dihydroxyphenylpropionate). The primary metabolites of dopa in man are homovanillate and 3,4-dihydroxyphenylacetate. These were sharply decreased after MK 486 treatment and the major metabolic product of dopa metabolism, 3-methoxy-4-hydroxyphenyllactate (VLA), then emerged. They interpreted these findings as an indication of a shift of dopa metabolism from decarboxylation to transamination.

We investigated the metabolism *in vitro* of dopa and report here that MK 486 is an efficient inhibitor of liver cytosol tyrosine aminotransferase (EC 2.6.1.5). Since this enzyme is also capable of accepting dopa [9] and 3-*O*-methyldopa [10] as substrate, we predictably observed an equally strong inhibition of dopa and 3-*O*-methyldopa transamination by MK 486. Nevertheless, Sandler's paradoxical observations could be explained when we observed that, while the liver cytosol aminotransferase activity was sharply decreased at the inhibitor concentrations employed, the mitochondrial transaminase activity was relatively intact. The results in Fig. 1 demonstrate that MK 486 at 5×10^{-6} M inhibits much of the liver cytosol aminotransferase activity, using 3-*O*-methyldopa as a substrate. On the other hand, mitochondrial aminotransferase activity from kidney, liver and other tissue is only weakly inhibited at this concentration. This inhibitor concentration was calculated from dosages commonly employed in human treatment regimens, assuming equal distribution of the MK 486 in all tissues and body fluids.

We have examined the kinetics of these enzyme activities with 3-*O*-methyldopa as substrate. Liver cytosol aminotransferase exhibited a $K_m = 0{\cdot}01$ M, while for the mitochondrial enzyme, a $K_m = 0{\cdot}07$ M (Fig. 2). Examination of the effect of MK 486 on the liver cytosol aminotransferase revealed competitive type inhibition with respect to 3-*O*-methyldopa as substrate.

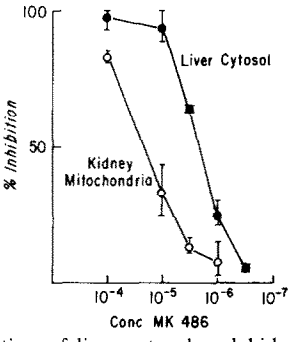

Fig. 1. Inhibition of liver cytosol and kidney mitochondrial amino transferase activity by MK 486. Rat liver and kidneys were homogenized with 4 vol. of 0·15 M KCl containing 0·001 M EDTA (pH 7·2). The cytosol obtained from 15,000 *g* centrifugation was dialyzed against the same KCl–EDTA. Mitochondria were washed twice with this solution and resuspended in 40 per cent of the original volume. Reaction mixtures contained: 0·02 M L-3-*O*-methyldopa, 4·3 mM ketoglutarate, 0·02 mM pyridoxal-5'-phosphate, 200 mM phosphate buffer, pH 7·4, 0·5 ml enzyme source, and MK 486 (plotted in moles/liter) in a volume of 1·75 ml. After 30 min of incubation at 37°, activity was measured by the colorimetric method of Fellman *et al.*[11]. (It was unnecessary to use hydroxylase inhibitor since the product of 3-*O*-methyldopa transamination is not a substrate for *p*-hydroxyphenylpyruvate hydroxylase [10].) Data points represent the average of three or more replicates and the range as shown. Typical uninhibited control values were: 1·56 μmoles 3-methoxy-4-hydroxyphenylpyruvic acid produced by the cytosol from 100 mg liver/30 min and 1·01 μmoles 3-methoxy-4-hydroxyphenylpyruvic acid produced by mitochondria from 250 mg liver/30 min.

The pathway of dopa metabolism is summarized in Fig. 3. The major pathway of dopa in mammals is the decarboxylation pathway leading to two principal urinary products of dopamine, i.e. homovanillate and 3,4-dihydroxyphenylacetate. The decarboxylase inhibitor, MK 486, inhibits not only this pathway but also the liver cytosol tyrosine aminotransferase pathway. Both enzymes operate with loosely bound pyridoxal-5-phosphate [12,13]. In addition, they would both be vulnerable to the inhibitor because of the presumed similarity of their active sites toward aromatic amino acids. The inhibition of liver cytosol aminotransferase is of consequence in connection with the metabolic fate of 3,4-dihydroxyphenylpyruvate, the product of dopa transamination. We recently demonstrated the enzymatic formation of 2,4,5-trihydroxyphenylacetate from the action of *p*-hydroxyphenylpyruvate hydroxylase on 3,4-dihydroxyphenylpyruvate [14]. Furthermore, we identified unequivocally 2,4,5-trihydroxyphenylacetate in the urine of patients being treated with L-dopa. The trihydroxyphenylacetate is easily oxidized and forms a reactive quinone similar to that encountered with 6-hydroxydopamine. Our present findings indicate that this pathway would probably be blocked in patients receiving MK 486. In treated patients, the liver would be perfused by both the inhibitor and dopa after ingestion and transport via portal circulation. Here the transaminase activity would be inhibited.

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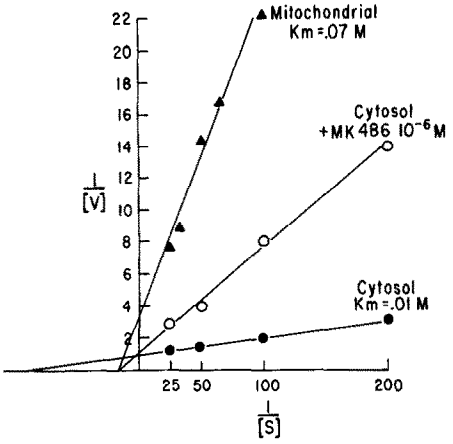

Fig. 2. Kinetic studies of 3-*O*-methyldopa aminotransferase activity. Enzyme source was rat liver. Procedure was as described for Fig. 1, using L-3-*O*-methyldopa concentrations from 0·005 to 0·04 M. Velocity is expressed as absorbance at 380 nm. The figure illustrates the results from a typical experiment; each point represents one data point. At least four such experiments were conducted.

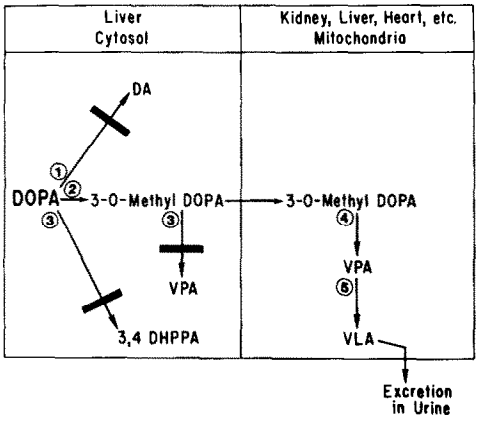

Fig. 3. Alteration of dopa metabolism by therapeutic levels of MK 486. Black bars indicate inhibited pathways. Enzymes are: (1) aromatic amino acid decarboxylase (EC 4.1.1.28); (2) catechol-*O*-methyl transferase (EC 2.1.1.6); (3) tyrosine aminotransferase (EC 2.6.1.5); (4) mitochondrial aminotransferase (EC 2.6.1.1); and (5) aromatic keto-acid reductase (lactic dehydrogenase) (EC 1.1.1.27) [15]. Dopa = 3,4-dihydroxyphenylalanine; DA = 3,4-dihydroxyphenylethylamine; DHPPA = 3,4-dihydroxyphenylpyruvate; 3-*O*-methyldopa = 3-methoxy-4-hydroxyphenylalanine; VPA = 3-methoxy-4-hydroxyphenylpyruvate; and VLA = 3-methoxy-4-hydroxyphenyllacetate.

Thus, MK 486 alters at least two important avenues of metabolism—decarboxylation leading to dopamine and transamination leading ultimately to trihydroxyphenylacetate. With the disruption of both these pathways by the inhibitor, *O*-methylation becomes the principal pathway, giving rise to large amounts of 3-*O*-methyldopa which is a substrate for the relatively uninhibited ubiquitous mitochondrial transaminase. These observations would account for the reported urinary metabolic patterns treated with dopa and the inhibitor MK 486 and also illustrate how misleading it may be to label drugs with such definitive labels as "decarboxylase inhibitors."

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Cyclic AMP metabolism in the cardiac tissue of the spontaneously hypertensive rat

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A strain of spontaneously hypertensive rats (SHR) developed by Okamoto and Aoki [1] is used as a model system for studying the pathogenesis of essential hypertension. Several investigations have been carried out on the properties of the various tissues of SHR animals and their responses to several drugs in comparison to normotensive Wistar rats (NWR). The atria from SHR have been shown to have a greater amount of intrinsic developed tension than NWR atria [2]. The inotropic and chronotropic responses of SHR atria to isoproterenol are reported to be less than those of NWR atria [2]. Since adenosine-3′,5′-monophosphate (cyclic AMP) has been reported to be involved in