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SEROTONIN SYNTHESIS WITH RAT BRAIN SYNAPTOSOMES

EFFECTS OF L-DOPA, L-3-METHOXYTYROSINE AND CATECHOLAMINES*

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Abstract—Experiments were done with a fraction of rat brain containing mitochondria and synaptosomes. L-DOPA‖ (K_i 0·3 mM) and L-3-methoxytyrosine (K_i 0·5 mM) were found to be competitive inhibitors of tryptophan accumulation, while dopamine and *l*-noradrenaline had no effect on the accumulation of tryptophan. Furthermore, L-DOPA (K_i 5·6 μM) was about 100 times more potent than L-3-methoxytyrosine in inhibiting the synthesis of 5HT from tryptophan. The inhibition of 5HT synthesis by L-DOPA appeared to be competitive to tryptophan and was not linear at concentrations of L-DOPA higher than 1 μM. L-DOPA also interfered with the rate of deamination of 5HT synthesized *in vitro*. Dopamine and *l*-noradrenaline also inhibited the synthesis of 5HT.

THE CONCENTRATIONS of 5-hydroxy-3-indoleacetic acid (5HIAA) in cerebrospinal fluid[1] and urine,[2] as well as the serotonin (5HT) content of thrombocytes[3] are reported to be depressed in patients with Parkinson's disease during treatment with L-3,4-dihydroxyphenylalanine (L-DOPA). These findings suggest that L-DOPA can interfere with the metabolism of 5HT in man. It has been reported that L-DOPA, or more likely its product dopamine, can act as a "false transmitter" and displace 5HT bound intraneuronally.[4,5] However, displacement of 5HT alone would not adequately explain the decreased levels of both 5HT and 5HIAA after prolonged treatment with high doses of L-DOPA, and inhibition of 5HT synthesis might also be a likely additional effect. Therefore we examined the effects of L-DOPA, L-3-methoxytyrosine, and catecholamines on the metabolism of 5HT in mitochondrial fractions of rat brain containing nerve ending particles.

MATERIALS AND METHODS

Materials and methods were described in detail in the previous communication.[6] Crude mitochondrial fractions (P_2), isolated by differential centrifugation were assayed for tryptophan-5-monooxygenase activity according to the method of

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‖ Abbreviations used: Try: L-tryptophan, 5HTP: L-5-hydroxytryptophan, 5HT: Serotonin, 5HIAA: 5-Hydroxy-3-indoleacetic acid, L-DOPA: L-dihydroxyphenylalanine.

Ichiyama.[6,7] However, in the present experiments with L-DOPA or L-3-methoxytyrosine complete reaction mixtures without L-[1-^{14}C]-Try were preincubated at 37° for 5 min and the reaction was started by the addition of substrate and stopped after 15 min. Then $^{14}CO_2$ was recovered and counted as described previously.[6,7] Accumulation of L-[^{3}H-(G)]-Try was determined by recovery of nerve endings by filtration on Millipore filters.[6]

RESULTS

As previously described[8] the accumulation of L-[^{3}H-(G)]-Try by a crude mitochondrial fraction occurred rapidly within the first 5 min and then the radioactivity recovered by Millipore filtration remained constant or decreased slightly with longer incubation time (Fig. 1). When L-DOPA (0·5 mM) was introduced simultaneously

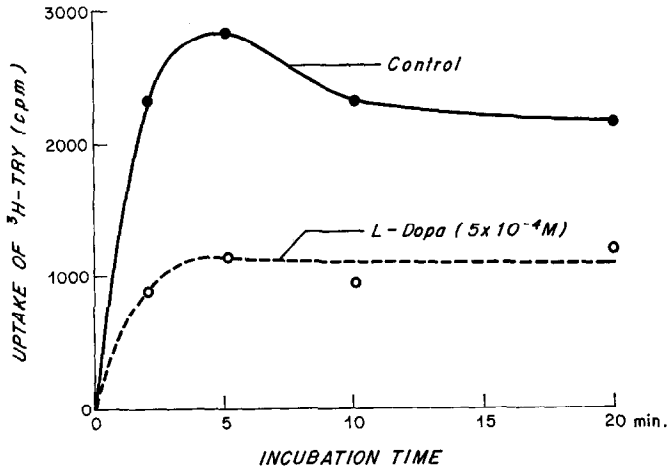

FIG. 1. Effect of L-DOPA on the time course of uptake of L-[^{3}H-(G)]-tryptophan by crude mitochondrial. P_2 fractions (1·5 mg) were incubated with L-[^{3}H-(G)]-Try, (124,000 counts/min) (initial concentration 0·5 mM) and the effect of 0·5 mM L-DOPA on the time course of accumulation was measured. Results are expressed as counts/min retained on Millipore filters. Blank values were obtained by incubation of the samples at 0° (166 ± 15 counts/min) (S.E.M.) (N = 4).

with L-[^{3}H-(G)]-Try the accumulation of Try was decreased and the degree of inhibition remained constant for 20 min (Fig. 1). The kinetics of the effect of L-DOPA and L-3-methoxytyrosine upon the "initial" accumulation of L-[^{3}H-(G)]-Try were determined. The inhibition of L-[^{3}H-(G)]-Try accumulation by L-DOPA was competitive and apparent K_i (± S.E.M.) was 0·30 ± 0·03 mM (N = 7) (Fig. 2a). L-3-methoxytyrosine also inhibited the accumulation of L-[^{3}H-(G)]-Try competitively and its apparent K_i (± S.E.M.) was 0·51 ± 0·09 mM (N = 5) (Fig. 2b). However, neither dopamine nor *l*-noradrenaline in concentrations of 0·1 mM had an effect on the "initial" accumulation of L-[^{3}H-(G)]-Try at 2 μM or 0·5 mM.

When the effects of L-DOPA and L-3-methoxytyrosine on the synthesis of 5HT from Try were examined, tissue fractions (P_2)[6] were preincubated for 5 min with the drugs; L-[1-^{14}C]-Try was then introduced and the $^{14}CO_2$ formed during 15 min of

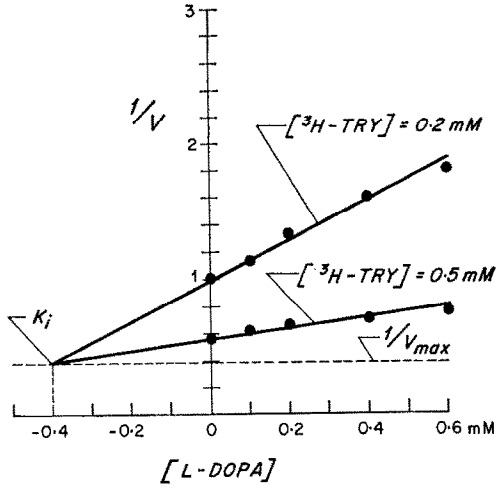

FIG. (2a)

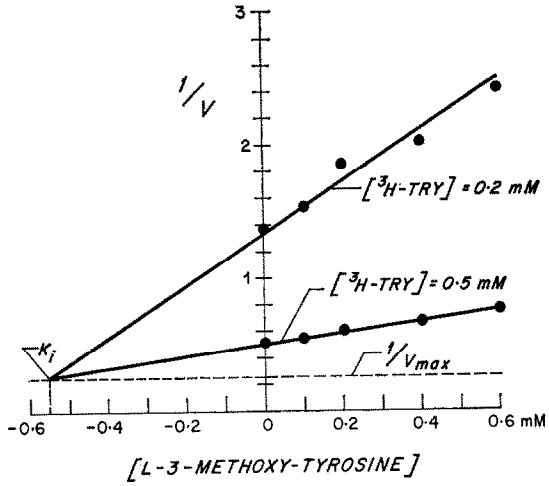

FIG. (2b)

FIG. 2. Kinetic analysis of the inhibition of L-[^{3}H-(G)]-tryptophan uptake by L-DOPA and L-3-methoxytyrosine. In both experiments crude mitochondrial fractions (1·6 mg) were incubated for 5 min with L-[^{3}H-(G)-Try (0·2 or 0·5 mM) with and without drugs.
In both experiments data were calculated as 1/velocity (nmmoles/min) vs. inhibitor concentration. (a) L-DOPA was added (0·1–0·6 mM) and in this experiment K_i was 0·4 mM. (b) L-3-methoxy-tyrosine was added (0·1–0·6 mM) and K_i was 0·55 mM.

incubation was captured and counted.[6] Kinetic analysis of the effect of L-DOPA on the formation of $^{14}CO_2$ from L-[1-^{14}C]-Try with crude mitochondria by the method of Dixon[9] indicated that the inhibition of $^{14}CO_2$ formation was linear only at low concentrations of L-DOPA (< 1 μM) (Fig. 3). Apparent K_i ($\pm$ S.E.M.) for the inhibition of $^{14}CO_2$ formation was 5·8 $\pm$ 0·09 μM ($N = 5$), and this inhibition was

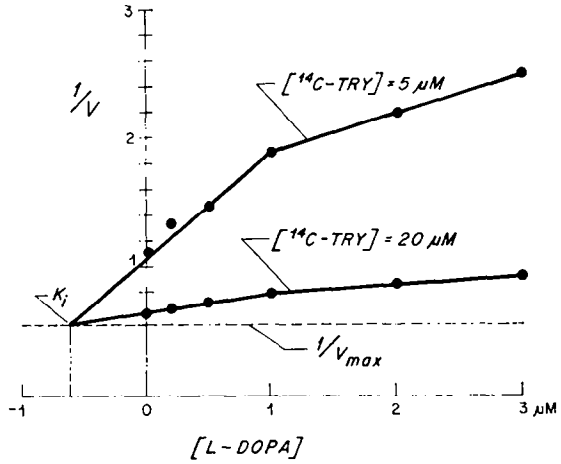

FIG. 3. Effect of L-DOPA on the formation of $^{14}CO_2$ from L-[1-^{14}C]-tryptophan with crude mitochondrial fractions. P_2 fractions (1·6 mg) were incubated with or without L-DOPA for 5 min. L-[1-^{14}C]-Try (5 g 20 μM) was then added and the formation of $^{14}CO_2$ was estimated. Data were calculated as 1/velocity (pmole $^{14}CO_2$/min) vs. L-DOPA concentration (0·3–3 μM).

apparently competitive to Try (Fig. 3). Addition of L-3-methoxytyrosine also led to a decreased formation of $^{14}CO_2$, although the concentrations required were about 100 times higher than those with L-DOPA. The inhibition by L-3-methoxytyrosine was competitive and K_i ($\pm$ S.E.M.) was 0·48 $\pm$ 0·08 mM ($N = 5$) (Fig. 4).

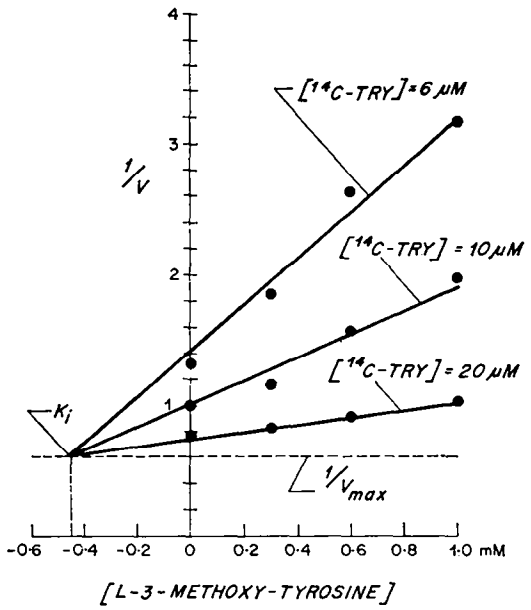

FIG. 4. Effect of L-3-methoxytyrosine on the formation of $^{14}CO_2$ from L-[1-^{14}C]-tryptophan with crude mitochondria. P_2 fractions (1·4 mg) were incubated as in Fig. 3. L-[1-^{14}]-Try concentration was 6·10 and 20 μM and the effect of L-3-methoxytyrosine (0·3–1·0 mM) on the formation of $^{14}CO_2$ was determined. Data were calculated as in Fig. 3.

To examine whether L-DOPA might affect the metabolism of [^{3}H-(G)]-5HT synthesized *in vitro* from L-[^{3}H-(G)]-Try, the tritiated metabolites of the 5-hydroxyindoleamine pathway were separated by column chromatography as previously described.[6] L-DOPA decreased the formation of tritiated 5-hydroxyindole compounds, but [^{3}H-(G)]-5HT was lowered more than [^{3}H-(G)]-5HIAA (Table 1). In some experiments [^{3}H-(G)]-5HT was not detectable and only [^{3}H-(G)]-5HIAA could be found.

TABLE 1. EFFECT OF L-DOPA ON THE FORMATION OF ^{3}H-5HT AND ^{3}H-5HIAA FROM L-[^{3}H-(G)]-TRYPTOPHAN BY CRUDE MITOCHONDRIA

	Product formed in pmoles ± S.E.M., ($N = 3$)		
	[^{3}H]-5HT	[^{3}H]-5HIAA	Total
Control	7·55 ± 1·22	22·27 ± 1·54	29·82
L-DOPA 5 μM	0·51 ± 0·06	7·42 ± 0·14	7·93

L-[H-(G)]-tryptophan, 20 μM (0·5 μc) was incubated with P_2 fractions for 30 min. [^{3}H]-5HT and [^{3}H]-5HIAA were then separated by column chromatography as described in Methods. Results are expressed as pmoles of product formed in 30 min (± S.E.M.). Blank values were obtained by incubations at 0° or by incubation at 37° under *N*. ($N = 3$.)

Crude mitochondrial fractions were incubated with 12·5 μM nialamide and the effect of the exogenous catecholamines on the formation of $^{14}CO_2$ from L-[1-^{14}C]-Try was determined. Both dopamine and l-noradrenaline inhibited the formation of $^{14}CO_2$ but dopamine was somewhat more potent (Fig. 5). Nialamide itself in this concentration had no effect on the rate of formation of $^{14}CO_2$.[6]

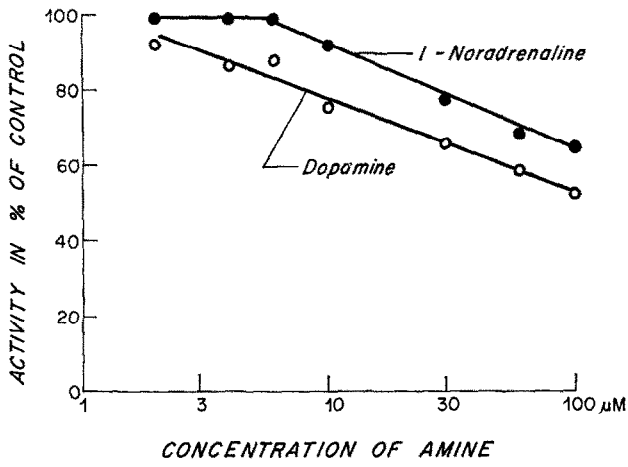

FIG. 5. Effect of exogenous catecholamines on the formation of $^{14}CO_2$ from L-[1-^{14}C]-tryptophan by crude mitochondria. P_2 fractions were incubated for 20 min with or without exogenous catecholamine. L-[1-^{14}C]-Try concentration was 10 μM. All incubations contained 12·5 μM nialamide 1 mM Na-ascorbate and 1 mM diNa-EDTA. Formation of $^{14}CO_2$ in control incubations was 1·33 pmoles/min. Each point represents the average of two determinations.

DISCUSSION

L-DOPA and L-3-methoxytyrosine were competitive inhibitors of Try accumulation, suggesting that these two amino acids and Try are transported through the synaptosomal membrane by a similar mechanism. This suggestion is consistent with previous findings that many amino acids can influence the accumulation of Try by nerve endings particles in mitochondrial fractions of rat brain.[4] *In vivo*, many amino acids compete for the same uptake site and conditions are more complex than in our simplified *in vitro* system. Nevertheless, administration of L-DOPA to rats has been found to lead to a decrease in the brain content of both Try and tyrosine[10] and L-DOPA in the presence of a decarboxylase inhibitor has been shown to inhibit the transport of 5HTP and Try into brain tissue.[11] Since amino acids enter the brain by a stereospecific transport mechanism which is inhibited by elevated levels of other amino acids administration of L-DOPA in therapeutic doses might lead to a decrease in the rate of transport of other amino acids into brain tissue.

L-DOPA, or dopamine formed from it during the incubation inhibited synthesis of 5HT with synaptosomes competitively to the substrate Try. The mechanisms of this inhibition of 5HT synthesis remains unknown, since L-DOPA can interfere with the activity of all the enzymes known to be involved in the biosynthesis of 5HT[12,13] and the assay system used in our experiments did not allow differentiation between inhibition of tryptophan-5-monooxygenase or L-aromatic amino acid decarboxylase. Inhibition of Try accumulation probably played a minor role in our synthesis experiments, since the concentrations of both substrate and L-DOPA used in our experiments were far below saturation of Try uptake.[8] Furthermore, L-3-methoxytyrosine, an equally potent inhibitor of Try accumulation was about 100 times less potent as an inhibitor of 5HT synthesis. Inhibition of Try accumulation might become more important with higher concentrations of L-DOPA and could contribute to the inhibition of 5HT synthesis.

Catechol compounds have been reported to inhibit soluble tryptophan-5-monooxygenase non-competitively to Try.[12] Catecholamines share the catechol group with L-DOPA, but they do not interfere with the accumulation of Try by synaptosomes and they can not serve as substrate for L-aromatic amino acid decarboxylase. They were used to determine whether catechol compounds could inhibit synaptosomal 5HT synthesis, although the inhibition by catecholamines is dependent on other factors, including accumulation of the amines into synaptosomes which synthesize 5HT, and possible compartmentalization of the amines within the nerve ending particles. Dopamine seemed to be a better inhibitor than *l*-noradrenaline. It seems, therefore, likely that compounds with a catechol group can inhibit synthesis of 5HT in nerve endings.

Another mechanism by which L-DOPA could inhibit 5HT synthesis might be its action as a substrate of L-aromatic amino acid decarboxylase for which it could compete with 5HTP formed from Try *in vitro*. Recently, Ichiyama *et al.*[7] suggested that in synaptosomes, tryptophan-5-monooxygenase and L-aromatic amino acid decarboxylase might be held together as an assembly which would facilitate the formation of 5HT from Try. Evidence for such an assembly was obtained in experiments with crude mitochondrial fractions of guinea pig brain. Large amounts of exogenous 5HTP inhibited the conversion of labeled Try to 5HT, but without any accumulation of labeled 5HTP. Evidently, exogenous 5HTP did not exchange with

that synthesized *in situ* from Try. Thus the two enzymes in synaptosomes exhibit different properties than in solution, and it seems likely that the kinetic properties of 5HT biosynthesis in synaptosomes with their complex organization might therefore differ from those of soluble enzymes.

Besides its effects on 5HT synthesis, L-DOPA or dopamine formed from L-DOPA during the incubation also seemed to interfere with the metabolism of 5HT synthesized *in vitro*. In control incubations, labeled 5HT formed from Try was rapidly deaminated, so that the major metabolite was 5HIAA. Addition of L-DOPA decreased the rate of synthesis and increased the deamination of 5HT synthesized *in vitro*. This finding is similar to previous observations that labeled 5HT, accumulated by slices[4] or synaptosomes[5] was more rapidly displaced in incubations with L-DOPA.

The present observations of the effects of L-DOPA on the synthesis and metabolism of 5HT might explain the decreased levels of 5HT and 5HIAA observed in patients treated with L-DOPA by inhibition of 5HT synthesis and by interference with 5HT storage. It remains unknown whether this metabolic effect of L-DOPA is of therapeutic value in the treatment of Parkinsonism or responsible for some of the side effects of this drug.

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