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# SEROTONIN SYNTHESIS WITH RAT BRAIN SYNAPTOSOMES

## EFFECTS OF SEROTONIN AND MONOAMINEOXIDASE INHIBITORS

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**Abstract**—Synthesis of serotonin from tryptophan was measured in synaptosomes isolated from rat brainstem and diencephalon. Incubation with exogenous seroton adds only to small decreases in the rate of serotonin synthesis. Of several monoamine oxidase inhibitors examined only pheniprazine appeared to inhibit serotonin synthesis. This effect of pheniprazine was probably not mediated by monoamine oxidase inhibition and secondary feed-back inhibition by elevated levels of end-product. It is suggested that pheniprazine can inhibit the formation of both serotonin and catecholamines at the aromatic amino acid decarboxylase step.

TRYPTOPHAN-5-MONOOXYGENASE was first detected in mammalian brain homogenates by Grahame-Smith[1] and later found to be associated with isolated nerve endings.[2] Synaptosomes catalyze the hydroxylation of tryptophan (Try)‡ to 5-hydroxytryptophan (5HTP) and the subsequent decarboxylation to serotonin (5HT) with practically no accumulation of 5HTP, suggesting that the tryptophan-5-monooxygenase step is the rate limiting reaction in the biosynthesis of 5HT.[3] Although the oxygenase is believed to require a pteridine cofactor,[2,3] the formation of 5HT can occur in synaptosomes without the addition of exogenous cofactor.[2,3] Apparently synaptosomes contain adequate amounts of endogenous cofactor as well as the enzyme system(s) necessary to regenerate it.

Based on evidence from *in vivo* experiments it has been suggested that the biosynthesis of serotonin may be regulated by a mechanism of negative feed-back inhibition.[4] Since the 5HT level in synaptosomes can be increased by the addition of exogenous 5HT to the incubation medium[5] and since the degradation of 5HT can be decreased by inhibition of monoamine oxidase (MAO), these approaches were utilized to study the effects of increased 5HT concentrations upon the synthesis of 5HT from Try in isolated nerve endings of the rat brain.

### MATERIALS AND METHODS

*Materials.* L-[1-$^{14}$C]-tryptophan (Try), L-[3,5-$^{3}$H]-tyrosine (Tyr), L-[1-$^{14}$C]-tyrosine and [$^{3}$H-(G)]-serotonin binoxalate were obtained from New England Nuclear

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‡ Abbreviations used: Try: L-tryptophan, Tyr: L-tyrosine, 5HTP: L-5-hydroxytryptophan, 5HIAA: 5-hydroxy-3-indoleacetic acid, 5HT: Serotonin, MAO: Monoamineoxidase, L-DOPA: L-dihydroxyphenylalanine.

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Company, Boston, Mass. and L-[$^3$H-(G)]-tryptophan from Amersham/Searle Corp., Sowers Grove, Ill. The purity of the radioactive Try was confirmed by paper chromatography.[6] L-[3,5-$^3$H]-tyrosine and L-[1-$^{14}$C]-tyrosine were purified by column chromatography as previously described.[7] All amino acids were lyophilized, and then resuspended in an aliquot of the incubation medium immediately before use. Pargyline–HCl (Eutonyl) was donated by Abbott Laboratories, No. Chicago, Ill., tranylcypromine-sulfate (Parnate) by Smith, Kline & French Labs., Philadelphia, Pa., and pheniprazine-HCl (Catron) by Lakeside Laboratories, Milwaukee, Wisc.

*Preparation of the tissue fractions.* Young male Sprague–Dawley rats, weighing 180–230 g, were killed by decapitation and the brains were removed. The brain-stem and diencephalon were used for the preparation of a crude mitochondrial fraction ($P_2$)[8] and purified nerve endings (synaptosomes) were obtained by sucrose density gradient centrifugation.[9] Protein in the tissue preparations was estimated by the method of Lowry *et al.* using bovine serum albumin as standard.[10]

*Estimation of tryptophan-5-monooxygenase activity.* Enzyme activity was estimated by two methods. The method of Ichiyama *et al.*[3] takes advantage of the greater affinity of L-aromatic amino acid decarboxylase for 5-hydroxytryptophan (5HTP) than for Try. The radioactive $^{14}CO_2$ evolved from L-[1-$^{14}$C]-Try is preferentially formed by decarboxylation of L-[1-$^{14}$C]-5HTP, the reaction product of tryptophan-5-monooxygenase.[3] Portions of the tissue preparations, containing 1–2 mg of protein in crude $P_2$ fractions or 0·3–1·0 mg of protein in synaptosomal fractions were resuspended in an incubation medium containing (mM): NaCl, 120; KCl, 5; Na-phosphate buffer at pH 7·45, 20; dextrose, 10; and sucrose, 40. In incubations with added serotonin (5HT), 1 mM Na-ascorbate and 1 mM EDTA were added to prevent oxidation of the amine. The initial concentrations of L-[1-$^{14}$C]-Try (sp. act. 12 $\mu$c/m-mole) in each experiment are given in the legends of the tables and figures. Reactions were carried out in a final volume of 0·25 ml in a metabolic shaking bath at 37°. Unless stated otherwise, the reactions were stopped after 20 min by the addition of 1 ml of 1 N perchloric acid containing 2% (w/v) EDTA, and the $^{14}CO_2$ thus displaced was captured[3] and counted by scintillation spectrometry. "Blank" values were obtained by incubating the complete reaction mixture at 0°. Addition of 10 mM 5HT or 10 mM pheniprazine had no effect on the recovery of $^{14}CO_2$.

In the second assay method, 5HT and 5-hydroxyindolacetic acid (5HIAA) were separated from Try by column chromatography.[11] Crude $P_2$ pellets, resuspended in 1·5 ml of the incubation medium described above were incubated with L-[$^3$H-(G)]-Try. After 40 min of incubation at 37° in the water bath the reactions were stopped by the addition of ice cold 0·1 N HCl containing 2% (w/v) ascorbic acid. Also 0·6 $\mu$g each of unlabeled 5HIAA, HT and 5HTP were added as carriers. Protein was removed by centrifugation at 30,000 *g* for 10 min. The resulting pellets were resuspended in 3·0 ml of the HCl–ascorbic acid solution, recentrifuged and the pellets were discarded. After the addition of a drop of 2% (w/v) EDTA the pH of the combined supernatant extracts was adjusted to 6·9 with 1 N NaOH, and the tissue extracts were poured over Amberlite-CG-50 ($H^+$-form) at pH 6·1.[12] The 5HT adhering to the column was then eluted with 2 ml of 3·5 N HCl and 0·2 ml was removed for liquid scintillation counting.[13] In some experiments the Amberlite eluate was extracted with benzene to remove any tryptamine present[14] to measure the amount of direct decarboxylation of Try. Essentially no radioactivity was found to be benzene extract-

able, while authentic tryptamine was readily extractable. The pH of the Amberlite effluent was adjusted to 1·5 with 1·0 N HCl and passed through a Sephadex G-15 column[15] and the effluent of the Sephadex column was poured over a column of Dowex-50 (200–400 mesh, $H^+$ form). Labeled 5HIAA, retained by the Sephadex column was eluted with 0·02 N $NH_4OH$ and extracted into butylacetate[16] and its radioactivity was measured.[13] The [$^3$H] radioactivity in the butylacetate extract migrated on a thin layer chromatogram[17] in a single peak at an $R_f$ identical to that of authentic 5HIAA. The amino acids adhering to the Dowex column were eluted with 0·5 N $NH_4OH$. This eluate was lyophilized and the residue was taken up in 2 ml $H_2O$. In some qualitative experiments 5HTP and Try were separated by paper chromatography.[6]

*Determination of the formation of* [$^3$*H*]-*5HIAA from* L-(*G*)]-*Try by isolated synaptosomes.* Purified synaptosomes were incubated together with 0·5 $\mu$c L-[$^3$H-(G)]-Try at an initial concentration of 20 $\mu$M in 0·5 ml of the incubation medium described above. Incubation was done at 37° in the metabolic bath for 30 min. The reaction was stopped with 0·1 N HCl containing 2% (w/v) ascorbic acid and 0·6 $\mu$g of unlabeled 5HIAA was added as carrier. After removal of the denatured tissue protein by centrifugation, [$^3$H]-5HIAA was isolated by chromatography on Sephadex G-15 columns as already described.[15]

*Estimation of the hydroxylation of tyrosine by crude mitochondria.* Estimation of the formation of L-DOPA from L-[3,5,-$^3$H]-tyrosine was done by the method of Nagatsu *et al.*,[18] modified for use with synaptosomes.[19] Incubations were done for 20 min at 37° in a medium containing (mM): NaCl, 120; KCl, 5; Na-phosphate buffer at pH 6·7, 15; dextrose, 10; sucrose, 40; and L-[3,5-$^3$H]-tyrosine, 10 $\mu$M. Incubation volume was 0·25 ml. Tritiated water formed by hydroxylation of tyrosine was then separated from other reaction products by column chromatography and counted.[19]

*Estimation of the formation of catecholamines from* L-[1-$^{14}$C]-*tyrosine by crude mitochondria.* In this assay, L-[1-$^{14}$C]-tyrosine was incubated with the crude $P_2$ pellet and the $^{14}CO_2$ formed was counted. The incubation conditions were the same as described above for the tritium displacement assay of tyrosine hydroxylation, except that L-[1-$^{14}$C]-tyrosine was used as the substrate. This method, as the assay of Ichiyama *et al.*[3] depends on the greater affinity of L-aromatic amino acid decarboxylase for the hydroxylated amino acids, dopa and 5HTP. The amount of radioactive $^{14}CO_2$ formed was proportional to the decarboxylation of dopa, which was formed by hydroxylation of tyrosine. The details of this assay will be described elsewhere.[20]

*Estimation of the accumulation of* L-[$^3$H]-(G)]-Try *or* [$^3$*H*-(*G*)]-*5HT by crude mitochondria.* All incubations were carried out with $P_2$ fractions resuspended in the incubation medium described above for the determination of tryptophan-5-monooxygenase. Usually, 1–2 mg of protein were incubated together with L-[$^3$H-(G)]-Try in a final volume of 0·5 ml for 5 min in a shaker bath at 37°. Samples with [$^3$H-(G)]-5HT were incubated for 10 min. Reactions were stopped by adding 5 ml of an ice cold 0·16 M NaCl in 10 mM Na-phosphate buffer at pH 7·45. The samples were immediately poured over moistened cellulose Millipore filters (25 mm dia., 0·64 $\mu$ pore size) which were washed twice with 5 ml of the buffered saline solution. Blank values were obtained by incubating the complete reactions mixture at 0°. The filters were then removed, dried under an infrared lamp, and the radioactivity was determined.[13]

## RESULTS

*Characteristics of the enzymatic formation of $^{14}CO_2$ from L-[$^{14}C$]-tryptophan by crude mitochondria and synaptosomes.* The formation of $^{14}CO_2$ from L-[1-$^{14}$C]-Try increased linearly with the amount of $P_2$ protein added (Fig. 1a) and increased with time (Fig. 1b). The apparent $K_m$ ($\pm$ S.E.M.) value for Try was $4{\cdot}5 \pm 0{\cdot}3 \times 10^{-6}$ M ($N = 7$), (Fig. 2) under the present incubation conditions. The $V_{max}$ value ($\pm$ S.E.M.) was $1{\cdot}55 \pm 0{\cdot}13$ pmole/min/mg protein, determined in different crude mitochondrial preparations ($N = 7$).

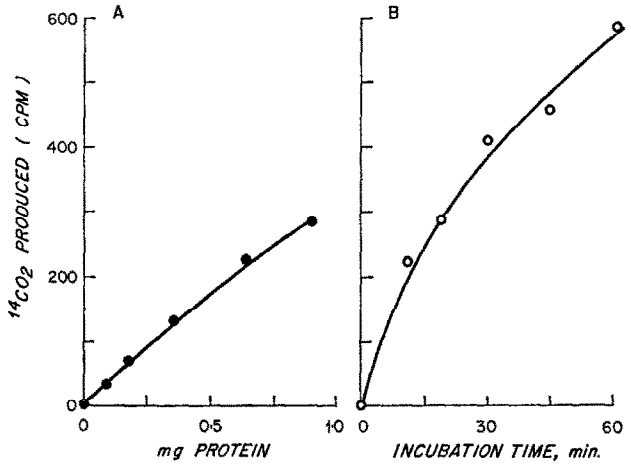


FIG. 1. Formation of $^{14}CO_2$ from L-[1-$^{14}$C]-tryptophan by crude mitochondria from rat brain-stem and diencephalon. Reactions were carried out with 2·5 $\mu$M of L-[1-$^{14}$C]-tryptophan (12 mc/mM) as substrate. (A) Various amounts of a $P_2$ preparation were incubated for 2 min. (B) Reactions were carried out for the time periods indicated. Nonenzymatic formation of $^{14}CO_2$ was 45 $\pm$ 16 counts/min (S.E.M., $N = 3$) and was subtracted from each value.

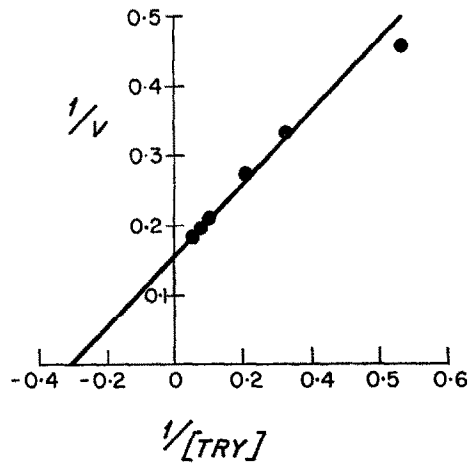


FIG. 2. Effect of different concentrations of tryptophan on the enzymatic formation of $^{14}CO_2$ from L-[1-$^{14}$C]-Try. Synaptosomes (1·48 mg) were incubated with different concentrations of L-[1-$^{14}$C]-Try under conditions detailed in Methods. Data were calculated as l/velocity ($V$ = pmoles $^{14}CO_2$/min) vs. l/Try (M $\times$ $10^{-6}$).

*Effect of exogenous 5HT on the formation of $^{14}CO_2$ from* L-[1-$^{14}$C]*-tryptophan by crude mitochondria and synaptosomes.* The addition of exogenous 5HT to the incubation medium decreased the rate of formation of $^{14}CO_2$ by nerve ending particles (Fig. 3) and by $P_2$ fractions, although the amount of inhibition by 5HT was small, even at very high concentrations of exogenous 5HT (Fig. 3). The same effect of 5HT could be observed with $P_2$ fractions in the presence of 12·5 $\mu$ Mnialamide, a concentration reported to inhibit the degradation of 5HT by MAO, but not the uptake of the exogenous amine.[21] Nialamide alone had no effect on the rate of 5HT synthesis (Table 1) and did not alter the inhibition of $^{14}CO_2$ formation by $P_2$ fractions incubated in the presence of 5HT. In another experiment, tryptamine was added to the incubation medium, and had no effect on the formation of 5HT in the concentrations examined (0·1–10 $\mu$M) (Fig. 3).

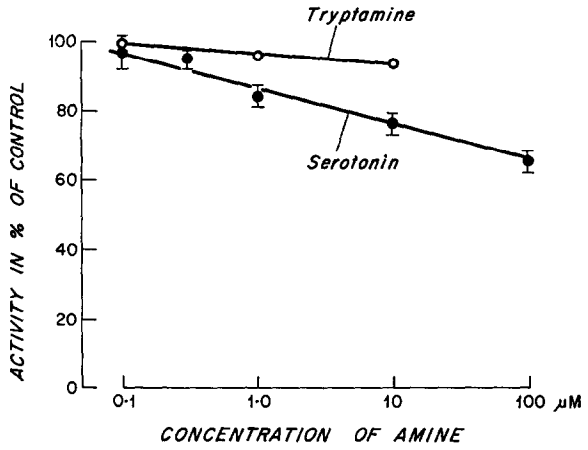


FIG. 3. Effect of 5HT and tryptamine on the formation of $^{14}CO_2$ by synaptosomes. Initial concentration of L-[1-$^{14}$C]-Try was 10 $\mu$M. Results are given as a percentage of the control values ± S.E.M. ($N$ = 3). Formation of $^{14}CO_2$ in control incubations was 1·19 pmoles/min. ● Serotonin, ○ Tryptamine.

*Effect of MAO inhibition on the deamination of the newly synthesized 5HT by isolated synaptosomes.* In order to determine whether MAO inhibitors had an action on the synaptosomal deamination of newly synthesized serotonin, the accumulation of [$^3$H]-5HIAA was measured after a 30-min incubation with [$^3$H]-Try. Pargyline, tranylcypromine and pheniprazine each decreased the formation of [$^3$H]-5HIAA at concentrations of 0·1 mM (Table 2).

*Effect of MAO inhibitors on the formation of $^{14}CO_2$ from* L-[1-$^{14}$C] *tryptophan by crude mitochondria and synaptosomes.* Of several substances with known inhibitory action on MAO, only pheniprazine had an effect on the formation of $CO_2$ by crude mitochondria and synaptosomes (Table 2). Kinetic analysis of the effect of this drug on the formation of radioactive $^{14}CO_2$ from L-[1-$^{14}$C]-Try by the method of Dixon,[22] indicated non-competitive inhibition to Try with a $K_i$ value of 35 $\mu$M (Fig. 4). The inhibition by pheniprazine appeared to be reversible (Table 3).

To evaluate further the inhibition of the biosynthesis of 5HT by pheniprazine, crude $P_2$ preparations were incubated with L-[$^3$H-(G)]-Try and the radioactive metabolites

TABLE 1. EFFECT OF MAO INHIBITORS ON THE FORMATION OF $^{14}CO_2$ BY CRUDE MITOCHONDRIA (EXPERIMENT A) AND BY SYNAPTOSOMES (EXPERIMENT B)

| Drug | Concentration ($\mu$M) | Activity (% Control ± S.E.M.) |
|---|---|---|
| *Experiment A* | | |
| Control | 0 | 100·0 ± 1·6 |
| Iproniazid | 100 | 98·4 ± 3·1 |
| Isoniazid | 100 | 100·1 ± 1·4 |
| Nialamide | 10 | 102·2 ± 1·4 |
| | 50 | 100·3 ± 4·4 |
| Pheniprazine | 5 | 91·6 ± 1·4 |
| | 10 | 81·9 ± 2·6 |
| | 50 | 42·8 ± 1·9 |
| *Experiment B* | | |
| Control | 0 | 100·0 ± 3·4 |
| Pargyline | 100 | 101·0 ± 3·0 |
| Tranylcypromine | 100 | 88·9 ± 2·9 |

L-[1-$^{14}$C]-Try concentration was initially 6 $\mu$M in experiment A and 10 $\mu$M in experiment B. Results are expressed as percentage of control values ± S.E.M. ($N$ = 3). Formation of $^{14}CO_2$ in control incubations was 1·3 pmoles/min in experiment A and 1·2 pmoles/min in experiment B.

of the indolamine pathway were isolated. The addition of the drug led to the inhibition of MAO by pheniprazine but also led to a decreased accumulation of radioactive 5HT (Table 4). When the amino acid fraction of the samples incubated with pheniprazine was subjected to paper chromatography to separate Try from 5HTP a slight accumulation of radioactivity was observed at an $R_f$ value identical to that of authentic 5HTP.

*Effect of pheniprazine on the formation of dopa and catecholamines from tyrosine by crude mitochondria.* If pheniprazine inhibited the formation of 5HT at the decarboxylase step, then the formation of catecholamines should also have been inhibited. Experiments were therefore done to determine whether pheniprazine inhibited the synthesis of catecholamines. Using the tritium displacement assay already described to examine the hydroxylation of tyrosine, pheniprazine (0·1 mM) added to the

TABLE 2. EFFECT OF MAO INHIBITION ON THE DEAMINATION OF NEWLY SYNTHESIZED [$^3$H]-5HT BY ISOLATED SYNATPSOMES

| Drug | [$^3$H]-5HIAA formed % of control ± S.E.M. |
|---|---|
| Control | 100 ± 4·0 |
| Pheniprazine 0·1 mM | 32·6 ± 4·2 |
| Tranylcycpromine 0·1 mM | 35·2 ± 1·5 |
| Pargyline 0·1 mM | 44·7 ± 1·5 |

Isolated nerve ending particles were incubated with L-[$^3$H-(G)]-Try at an initial concentration 20 $\mu$M, and the amount of [$^3$H]-5HIAA formed was measured as described in Methods. Results are expressed as a percentage of control values ± S.E.M. ($N$ = 3). Formation of [$^3$H]-5HIAA in control incubations was 3·1 pmoles/min.

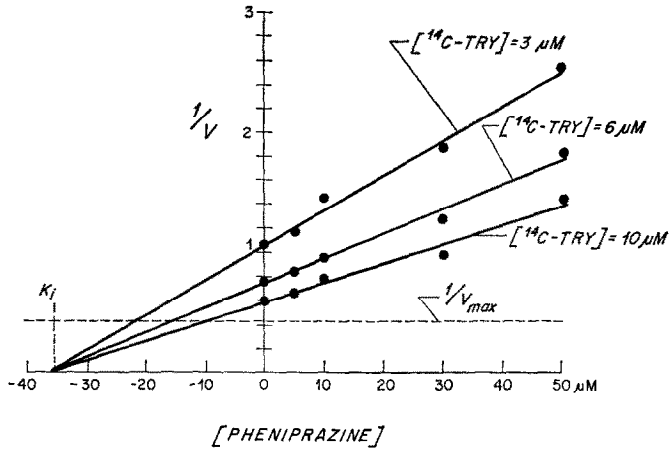


FIG. 4. Effect of pheniprazine on the formation of $^{14}CO_2$ from L-[1-$^{14}$C]-Try by crude mitochondria. Resuspended crude mitochondrial fractions were incubated for 20 min with L-[1-$^{14}$C]-Try (3–6 or 10 $\mu$M) and with or without pheniprazine (5–50 $\mu$M). Data were calculated as 1/velocity ($V$ = pmoles $^{14}CO_2$/min) vs. pheniprazine concentration.

TABLE 3. EFFECT OF PREINCUBATION WITH PHENIPRAZINE ON THE FORMATION OF $^{14}CO_2$ BY CRUDE MITOCHONDRIA

| Additions | | |
|---|---|---|
| Preincubation | Incubation | Activity % of control ± S.E.M. |
| None | None | 100·0 ± 1·6 |
| None | Pheniprazine (0·1 mM) | 45·0 ± 2·3 |
| Pheniprazine (0·1mM) | None | 81·0 ± 1·7 |
| Pheniprazine (0·1mM) | Pheniprazine (0·1 mM) | 29·7 ± 1·4 |

$P_2$ fractions were resuspended in the incubation medium used for the determination of tryptophan-5-monooxygenase and preincubated at 37° in 1 ml incubation volume with or without pheniprazine 0·1 mM. After 10 min, 10 ml of ice cold 0·32 M sucrose was added, the $P_2$ fractions were recentrifuged and the supernatants were discarded. The tissue pellets were then resuspended in the incubation medium and assays for tryptophan-5-monooxygenase activity as described in Methods. Results are expressed as a percentage of control (± S.E.M.) ($N$ = 3).

TABLE 4. EFFECT OF PHENIPRAZINE ON THE FORMATION OF [$^3$H]-5HT AND [$^3$H]-5HIAA FROM L-[$^3$H-(G)]-TRYPTOPHAN BY A CRUDE MITOCHONDRIAL-SYNAPTOSOMAL FRACTION

| Incubation conditions | Product | Product formed (pmoles ± S.E.M.) |
|---|---|---|
| Control | [$^3$H]-5HT | 10·59 ± 4·33 |
| | [$^3$H]-5HIAA | 195·48 ± 26·70 |
| | total | 206·07 (100%) |
| Pheniprazine $10^{-4}$ M | [$^3$H]-5HT | 8·75 ± 4·54 |
| | [$^3$H]-5HIAA | 65·67 ± 8·74 |
| | total | 74·42 (36%) |

L-[$^3$H-(G)]-Try, 10 $\mu$M, (0·5 $\mu$c) was incubated with $P_2$ fractions for 40 min and [$^3$H]-5HT and [$^3$H]-5HIAA were separated by column chromatography as described in Methods. Recovery for [$^3$H]-5HT was 53 per cent and for [$^3$H]-5HIAA, 51 per cent. Results are expressed as pmoles formed in 40 min incubation ($N$ = 3).

incubation medium did not decrease the rate of formation of DOPA (Table 5). In other experiments, the effect of pheniprazine on the decarboxylation of DOPA newly synthesized from L-[1-$^{14}$C]-Tyr was observed (Table 5). While pheniprazine did not inhibit the formation of DOPA, the subsequent decarboxylation of DOPA was inhibited. Hence catecholamines formation from L-[1-$^{14}$C]-tyrosine was inhibited to the same extent as the formation of 5HT from L-[1-$^{14}$C]-Try.

TABLE 5. EFFECT OF PHENIPRAZINE ON THE FORMATION OF DOPA, DOPAMINE, AND 5HT BY A CRUDE MITOCHONDRIAL-SYNAPTOSOMAL FRACTION

| | Formation of | | |
|---|---|---|---|
| | DOPA | Catecholamines (% of control ± S.E.M.) | Serotonin |
| Control | 100 ± 2·3 | 100 ± 2·0 | 100 ± 0·4 |
| Pheniprazine (0·1 mM) | 119 ± 7·7 | 34·5 ± 2·6 | 26·9 ± 1·6 |

Formation of DOPA from tyrosine was measured by a tritium displacement method, and synthesis of catecholamines from tyrosine was measured by counting $^{14}CO_2$ formed from L-[1-$^{14}$C]-tyrosine. 5HT synthesis was estimated by measuring the formation of $^{14}CO_2$ from L-[1-$^{14}$C]-Try. Results are expressed as a percentage of control values ± S.E.M. ($N = 3$).

*Effect of pheniprazine and 5HT on the initial accumulation of* L-[$^3$*H-(G)*]*-Tryptophan by crude mitochondria.* To determine whether the drugs which are found to decrease the formation of serotonin could exert this influence by inhibiting the accumulation of Try, "initial" uptake of L-[$^3$H-(G)]-Try was measured. Neither compound changed the accumulation of Try significantly (Table 6).

TABLE 6. EFFECT OF PHENIPRAZINE AND 5HT ON THE INITIAL ACCUMULATION OF L-[$^3$H-(G)]-TRY BY A CRUDE MITOCHONDRIA

| | Accumulation of L-[$^3$H-(G)]-Try ± S.E.M. (nmoles/min) | % Control ± S.E.M. |
|---|---|---|
| Control | 0·778 ± 0·023 | 100·0 ± 2·9 |
| Pheniprazine (0·1 mM) | 0·746 ± 0·009 | 95·9 ± 1·0 |
| Serotonin (0·1 mM) | 0·790 ± 0·040 | 101·5 ± 5·0 |

Results are expressed as nmoles taken up per min and as a percentage of control values ± S.E.M. ($N = 3$). Initial concentration of L-[$^3$H-(G)]-Try was 0·4 mM.

*Accumulation of* [$^3$*H-(G)*]*-5HT by crude mitochondria under the incubation conditions for tryptophan-5-monooxygenase.* Since the conclusions presented in this communication are based on the assumption that 5HT is accumulated by synaptosomes uptake of [$^3$H-(G)]-5HT was confirmed under the incubation conditions for tryptophan-5-monooxygenase (Table 7). Nialamide (12·5 $\mu$M) was added to prevent deamination of 5HT.

TABLE 7. ACCUMULATION OF [$^3$H-(G)]-5HT BY CRUDE MITOCHONDRIAL FRACTIONS

| [$^3$H]-5HT ($\mu$M) | Accumulation of [$^3$H]-5HT (pmoles/min/mg protein) |
|---|---|
| 0·19 | 3·15 ± 0·15 |
| 0·96 | 10·51 ± 0·61 |
| 1·92 | 15·59 ± 0·71 |

Uptake of [$^3$H-(G)]-5HT by $P_2$ fractions was determined with the Millipore filter technique described in Methods. Results are expressed as pmoles taken up/min/mg protein (± S.E.M.) ($N = 3$).

## DISCUSSION

The formation of serotonin by isolated synaptosomes and by crude mitochondrial preparations containing nerve ending particles depends on enzymatic and transport activities. Tryptophan has to be taken up through the synaptosomal membrane by an uptake system which exhibits several characteristics of facilitated diffusion.[23] Serotonin is then synthesized from Try by two reactions, the hydroxylation to 5HTP and the subsequent decarboxylation of 5HTP to 5HT.[3] The hydroxylation of Try is believed to be the rate-limiting step in the formation of 5HT, and it has been shown that, in crude $P_2$ preparations, the activity of the aromatic amino acid decarboxylase exceeds by 100-fold the activity of the tryptophan-5-monooxygenase.[24]

In the present study the effect of various drugs on the synthesis of 5HT was examined with an assay system which is based on the different affinities of substrates for aromatic amino acid decarboxylase.[3] The experimental conditions described by Ichiyama *et al.*,[3] were slightly changed in the present experiments. The incubations were carried out at pH 7·45 in an incubation medium which had a high content of $Na^+$ to facilitate the accumulation of exogenous amines.[5,21] Thus uptake of 5HT did occur under the present incubation conditions (Table 7). The apparent $K_m$ for Try was 4·5 $\mu$M, a value about four times lower than reported by Ichiyama *et al.*[3] who examined crude $P_2$ fractions in a medium containing 100 mM Tris buffer at pH 8·2. This difference in the apparent $K_m$ value is probably due to the differences in the animal species and incubation media used. Only small differences in the $V_{max}$ values were obtained when seven different crude mitochondrial preparations were examined, suggesting that despite the potential variations introduced by the preparative procedure, $P_2$ fractions can be obtained with a reproducible capacity to synthesize 5HT.

The metabolism of the newly synthesized 5HT in synaptosomes seemed to be very rapid *in vitro*, with 90 per cent of the 5HT deaminated within 40 min of incubation (Table 4). This deamination was probably catalyzed by intrasynaptosomal MAO since most of the extrasynaptosomal mitochondrial enzyme had been removed by density gradient centrifugation.[25]

It has been suggested that the biosynthesis of serotonin may be controlled by a mechanism of negative feed-back inhibition.[4] Evidence for this regulatory mechanism was obtained by studies of the effects of inhibitors of MAO on the synthesis of [$^3$H]-5HT from intracisternally-injected [$^3$H]-Try.[4] In these experiments, a significant negative correlation between [$^3$H]-5HT synthesis and endogenous 5HT levels was

reported. Therefore it was of interest to see whether such a feed-back mechanism might also occur *in vitro* in synaptosomes, which possess many of the metabolic activities of intact presynaptic terminals but have no electrophysiological activity. Synthesis of 5HT was therefore measured in synaptosomes, and the effect of 5HT on its own rate of synthesis was examined.

Concentrations of 5HT up to 0·1 mM did not decrease the rate of 5HT synthesis by more than 30 per cent (Fig. 3). Thus, either the intrasynaptosomal levels of 5HT were not raised adequately by uptake to inhibit synthesis, or else the proposed feed-back mechanism is of only minor importance in isolated nerve endings. The velocity and affinity of the 5HT uptake by synaptosomes have been reported to be of the same order of magnitude as for catecholamines.[21] In other experiments in which the effect of catecholamines on the hydroxylation of tyrosine in synaptosomes was measured, synthesis of DOPA was inhibited about 50 per cent by the addition of catecholamines at concentrations of 0·5–1·0 $\mu$M, indicating that a mechanism of feed-back regulation of a pteridine cofactor dependent aromatic amino acid oxygenase can be detected in isolated synaptosomes.[19] It was therefore concluded that in synaptosomes synthesis of 5HT from Try is not regulated by elevated levels of end-product.

The synthesis of 5HT by isolated nerve ending particles was inhibited by pheniprazine (Table 1). No effect of pheniprazine on the uptake of Try into crude $P_2$ fractions was observed (Table 6). If the accumulation of Try into a mixed population of isolated nerve endings resembles the uptake by 5HT synthesizing synaptosomes, then the results suggest that pheniprazine and 5HT did not decrease the availability of Try at sites of 5HT synthesis. Furthermore, the inhibition of 5HT synthesis by pheniprazine did not appear to be correlated to the ability of the drug to inhibit MAO which could lead to increased accumulation of end-product, since other MAO inhibitors also decreased the deamination of 5HT synthesized *in vitro* (Table 2) without affecting the rate of 5HT synthesis (Table 1). The inhibition by pheniprazine was therefore likely to occur at one of the enzymatic steps involved in 5HT synthesis. It has been already reported that pheniprazine can inhibit the decarboxylation of 5HTP in brain slices.[26] Evidence for a direct inhibition of the synthesis of 5HT from [$^3$H]-Try was obtained when it was found that pheniprazine decreased the accumulation of both radioactive 5HT and 5HIAA (Table 4) and probably increased the formation of labeled 5HTP as well.

In order to obtain more information on the action of pheniprazine, its effects on the synthesis of catecholamines were also examined. If pheniprazine inhibited the L-aromatic amino acid decarboxylase required for 5HT synthesis then the drug should also inhibit the formation of catecholamines as the same decarboxylase is involved in both pathways. Here the hydroxylation of tyrosine could be measured directly with a tritium displacement assay. Pheniprazine did not inhibit the formation of DOPA, but decreased the decarboxylation of the newly synthesized radioactive DOPA (Table 5). These findings strengthen the conclusion that pheniprazine can inhibit L-aromatic amino acid decarboxylase enough to inhibit the synthesis of 5HT and catecholamines *in vitro*. Although it is difficult to extrapolate from the effect of drugs *in vitro* to their action *in vivo* it seems possible that treatment with pheniprazine *in vivo* could lead to an inhibition of aromatic amino acid decarboxylase in addition to its effects on MAO.

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