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IN VIVO-INHIBITION OF SEROTONIN SYNTHESIS IN MOUSE BRAIN BY β-PHENYLETHYLHYDRAZINE, AN INHIBITOR OF MONOAMINE OXIDASE

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Abstract—β-Phenylethylhydrazine inhibits the decarboxylation of 5-hydroxytryptophan in mouse brain, both *in vitro* and *in vivo*. It is from twenty to fifty times more potent an inhibitor of monoamine oxidase than of 5-hydroxytryptophan decarboxylase. Despite a demonstrable inhibition of decarboxylase by about 50 per cent, even lethal doses of the drug do not decrease the overall rate of accumulation of serotonin in mouse brain from endogenous precursors after inhibition of monoamine oxidase.

INTRODUCTION

5-HYDROXYTRYPTOPHAN (5-HTP) decarboxylase is implicated in the biosynthesis of serotonin.[1] This enzyme has been shown to require pyridoxal-5-phosphate,[1–5] and to be inhibited by agents which react with carbonyl groups.[1, 4, 5] For example, Weissbach *et al.*,[5] found that the conversion to serotonin of 5-HTP administered to intact mice was diminished by pretreatment with semicarbazide. Therefore, the possibility was investigated that the carbonyl reagent and potent inhibitor of monoamine oxidase (MAO), β-phenylethylhydrazine[6] (phenelzine), could also inhibit the decarboxylation of 5-HTP *in vivo*.

EXPERIMENTAL

Female mice of the Manor Farms M-1 strain (Manor Farms, Staatsburg, N.Y.), weighing 18–22 g, were used. Phenelzine sulfate is a product of our laboratories. *trans*-Phenyl*cyclo*propylamine (tranylcypromine) hemisulfate was provided through the courtesy of Dr. Glenn Ullyot of Smith Kline and French Laboratories. The other chemicals used were obtained from commercial sources; pyridoxal hydrochloride, 4-deoxypyridoxine hydrochloride and DL-5-hydroxytryptophan from Nutritional Biochemicals Corp. (Cleveland, Ohio); pyridoxal-5-phosphate (CfP) from Calbiochem (Los Angeles, Cal.); and harmaline hydrochloride from L. Light and Co., Ltd., Colnbrook, Bucks, England.

For measurement of serotonin formation in mouse brain, whole brains were removed at precisely timed intervals after the intravenous injection of DL-5-HTP, 25 mg/kg, and frozen immediately in dry ice and acetone. Two pooled brains were weighed, homogenized in 0·1 N HCl, and the serotonin content was measured, as described previously.[6]

MAO-activity was measured by disappearance of tyramine. After precipitation of protein with zinc sulfate, the tyramine in an aliquot of the supernatant solution was

measured, without prior extraction, with the reagent, 1-nitroso-2-napthol, as described by Zile and Lardy.[7]

The *in vitro*-measurement of 5-HTP decarboxylase activity was accomplished by spectrophotofluorimetric measurement of serotonin (5-HT) formation from 5-HTP in 0·05 M sodium phosphate, pH 8·0, containing 0·04 M sodium fluoride and 0·0001 M tranylcypromine. Homogenates of whole brain were prepared directly in the above medium at ice-water temperature; the final concentration of tissue was usually 125 mg/ml. The last addition, before incubation at 37 °C in air for 30 min, was that of 5-HTP, in an amount sufficient to produce a concentration of 0·001 M. The formation of 5-HT was linear for 1 hr.

Inhibition of the metabolism of 5-*HTP* in vivo

Non-hydrazine MAO-inhibitors. The conversion of injected 5-HTP to serotonin was measured, after brief pretreatment with non-hydrazine MAO-inhibitors, in order to determine the maximum formation of serotonin in the absence of decarboxylase inhibition (Table 1). Since there is little accumulation of serotonin in the brain of

TABLE 1. CONVERSION OF 5-HTP* TO SEROTONIN IN MICE PRE-TREATED WITH NON-HYDRAZINE INHIBITORS OF MAO

MAO-inhibitor	Dose mg/kg (i.p.)	Serotonin (μg/g) Base level (no 5-HTP)	Serotonin (μg/g) 5 min after 5-HTP	Increase (μg/g)
None	—	0·67	0·87	0·20
L-Amphetamine	50	0·76	1·10	0·34
L-Amphetamine	100	0·68	1·05	0·37
Harmaline	2·5	0·76	1·28	0·52
Harmaline	5·0	0·81	1·87	1·06
Harmaline	5·0	0·85	1·77	0·92
Harmaline	10·0	0·86	2·22	1·36
Tranylcypromine	3·6	0·61	1·75	1·14
Tranylcypromine	7·2	0·67	1·83	1·16

* DL-5-HTP (25 mg/kg, i.v.), given 10 min after inhibitor, and brains taken 5 min later.

normal mice after the injection of 5-HTP, such an experiment would not distinguish between marked inhibition of the decarboxylase and a slight MAO-inhibition. L-Amphetamine, a low potency MAO-inhibitor, produced little accumulation of serotonin, whereas the more potent inhibitors, harmaline and tranylcypromine, caused the formation of approximately 1·2 μg of serotonin per gram of wet whole mouse brain per 5 min.

Phenelzine. It has been reported that the ultimate level of serotonin reached after injection of phenelzine is related directly to dosage.[6] In earlier work, mice injected subcutaneously with phenelzine (16, 48, 81 mg/kg) were treated with 5-HTP after 3 hr, when serotonin levels had reached the maximum for each dose of phenelzine. It was found that the formation of additional 5-HT, at 2 and 5 min after injection of 5-HTP, was less at the higher doses of phenelzine than at 16 mg/kg. Table 2 illustrates a similar inhibition of the conversion of 5-HTP to 5-HT. In these experiments the

effects of the three doses of inhibitor were compared within 30 minutes after the injection of phenelzine, at which time endogenous levels of serotonin were equivalent.*

Again, as in the earlier experiments, the formation of serotonin was less in the brain of mice receiving the higher doses of phenelzine. The difference was emphasized when 5-HT was measured 20 min after the dose of 5-HTP and 30 min after the dose of phenelzine.

TABLE 2. CONVERSION OF INJECTED 5-HTP TO SEROTONIN IN MOUSE BRAIN 15 AND 30 MIN AFTER VARIOUS DOSES OF PHENELZINE

Time after phenelzine (min)	Dose of phenelzine (mg/kg, i.v.)	Base level (μg/g)	Level of serotonin 5 min after 5-HTP* (μg/g)	Level of serotonin 20 min after 5-HTP† (μg/g)
15	16	0·76	0·89 ± 0·01	—
	48	0·80	0·68	—
	81	0·70	0·31	—
30	16	0·89	0·81 ± 0·11	2·11 ± 0·11
	48	0·91	0·53 ± 0·07	1·50 ± 0·20
	81	0·89	0·29 ± 0·01	1·17 ± 0·03

* DL-5-HTP (25 mg/kg, i.v.), given 10 or 25 min after phenelzine, and brains taken 5 min later.
† 5-HTP, given 10 min after phenelzine, and brains taken 20 min later.

TABLE 3. CONVERSION OF INJECTED 5-HTP TO 5-HT IN MOUSE BRAIN AT VARIOUS TIMES AFTER THE SAME DOSE OF PHENELZINE

Time after phenelzine (hr)	Serotonin increase after 5-HTP (μg/g)	
	After 5 min	After 20 min
¼	0·89 ± 0·01	—
½	0·81 ± 0·11	2·11 ± 0·11
3	0·56 ± 0·02	0·66 ± 0·10
24	0·39 ± 0·05	0·83 ± 0·12

Dose of phenelzine: 16 mg/kg/i.v.
Dose of DL-5-HTP: 25 mg/kg/i.v.

When the conversion of 5-HTP injected intravenously, 15 min, 30 min, 3 hr and 24 hr after the low dose of phenelzine (16 mg/kg), is considered (Table 3), there appears to be inhibition at 3 and 24 hr, relative to the production of 5-HT at 15 and 30 min. The activity at 24 hr was not stimulated by an injection of pyridoxal. MAO-activity appeared to remain completely inhibited for 3 hr and nearly so for 24 hr.

In vitro *assay after injection of phenelzine* in vivo. Normally, in crude homogenates of mouse brain, serotonin was formed at the rate of 0·53 ± 0·01 μmoles per gram of wet weight of tissue per hour at 37 °C. Injection of doses of phenelzine at 10 min, 3 hr and 24 hr prior to removal of the brains resulted in the definite inhibitions described

* Although the ultimate level of serotonin reached after injection of phenelzine is dose-dependent, the rate of accumulation is relatively independent of the dose.[6]

in Table 4. Although normal activity was not appreciably stimulated (Table 5) by pyridoxal-5-phosphate, phenelzine-inhibited decarboxylase was stimulated by the addition of the coenzyme (0·0006 M). However, neither dilution of the homogenate nor a five-fold increase in substrate concentration reversed the inhibition. This contrasts with the study by Smith[8] of α-methyldopa as an inhibitor of the same enzyme, in which this amino acid was reported to interact with both the substrate and the coenzyme.

TABLE 4. 5-HTP DECARBOXYLASE ACTIVITY *in vitro* IN MOUSE BRAIN AFTER INJECTIONS OF PHENELZINE

Time after dose	Dose of phenelzine (mg/kg/i.v.) 16	48	81
	*Specific Activity**		
10 min	0·46 ± 0·00	0·31 ± 0·01	0·27 ± 0·03
3 hr	0·41	0·43 ± 0·04	0·25 ± 0·01
24 hr	0·42 ± 0·08	0·40	0·31 ± 0·07

* μMoles of 5-HT formed/g of wet tissue/hr. The mean of ten determinations was 0·53 ± 0·01 for normal mouse brain.

TABLE 5. EFFECT OF PYRIDOXAL-5-PHOSPHATE ON 5-HTP DECARBOXYLASE ACTIVITY IN HOMOGENATES OF MOUSE BRAIN

(I) Homogenization with tranylcypromine, as in Experimental		(II) Homogenization with 5×10^{-5} M phenelzine replacing tranylcypromine		(III) Phenelzine, 48 mg/kg, injected i.v. 10 min prior to homogenization, as in Experimental	
Addition	Sp. act.	Addition	Sp. act.*	Addition	Sp. act.
None	0·53	none	0·53	none	0·24
Pyr 1	0·54	Pyr 1	0·75	Pyr 1	0·43
Pyr 1 – Phen.	0·56	Pyr 2	0·71		

Pyr 1 = pyridoxal-5-phosphate, 6×10^{-4} M.
Pyr 2 = pyridoxal-5-phosphate, 6×10^{-5} M.
Phen. = phenelzine 2×10^{-4} M.
* μMoles of serotonin formed/g per hr. The activity under "None" in section II was slightly higher than would be expected by reference to Fig. 1.

Inhibition of 5-HTP decarboxylase in mouse brain in vitro *by phenelzine*

The results in Fig. 1 show that phenelzine inhibited 5-HTP decarboxylase *in vitro* by 50 per cent when added to homogenates at a concentration of $1{\cdot}5 \times 10^{-4}$ M. Consequently, it is a less potent inhibitor of the decarboxylase than is α-methyldopa[9] and about 20–50 times more potent an inhibitor of MAO than of 5-HTP decarboxylase. The effect of pyridoxal-5-phosphate on this inhibition *in vitro* proved to be somewhat complicated. In some experiments it not only counteracted the phenelzine inhibition, but stimulated activity to levels higher than "normal". This effect is illustrated by experiments in Section II of Table 5. This finding was not consistent when tranylcypromine was used to inhibit destruction of formed serotonin, as described in Experimental; as shown in Section II of Table 5, phenelzine (5×10^{-5} M) was substituted

in the homogenizing medium to serve that function. Pyridoxal-5-phosphate was added after placing the homogenates in incubation flasks.

Effect of decarboxylase-inhibiting doses of phenelzine on rate of serotonin accumulation from endogenous precursors

Fig. 2 describes the rate of serotonin accumulation in mouse brain after high, even lethal,[6] doses of phenelzine. The line is a reference tracing of the rate of serotonin accumulation after lower doses of rapidly acting MAO-inhibitors.[6] The limits describe the range of values encountered. No inhibition of this overall rate of serotonin accumulation from endogenous precursors is indicated. Apparently, the rate of 5-HTP decarboxylation does not limit this rate of accumulation.

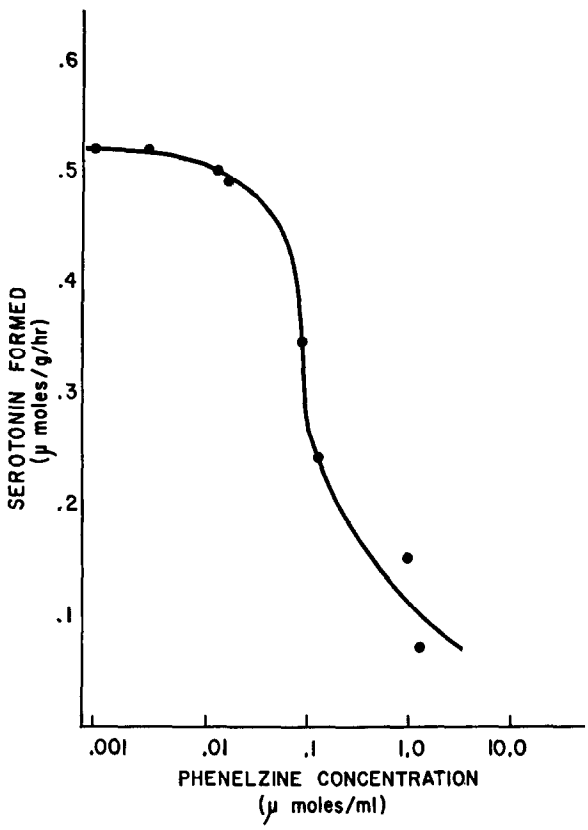

FIG. 1. Inhibition of 5-HTP Decarboxylase *in vitro* by phenelzine. Tissue concentration was 125 mg of fresh whole mouse brain per ml.

DISCUSSION

As noted above in the section concerned with Table 1, the *in vivo* experiments would not distinguish between a low degree of MAO-inhibition and a high degree of inhibition of the decarboxylase. If any of the treatments were to result in reduced penetration of 5-HTP into the brain, this too would have the same result, i.e. a lower

level of serotonin after injection of 5-HTP. However, significant inhibition of the decarboxylase after treatment *in vivo* is demonstrable by assay *in vitro*. Using the method described by Weissbach[10], in which the proteins were removed from brain homogenates and the total fluorescence was measured in the remaining solution, an attempt was made to determine whether the MAO-inhibitors reduced penetration of 5-HTP into mouse brain. The equivalent of approximately 4–5 μg of 5-HTP per gram was found 5 min after injection of 5-HTP, whether or not harmaline or phenelzine had been given 10 min earlier, a finding which shows that the MAO-inhibitors probably did not reduce the transport of 5-HTP into brain.

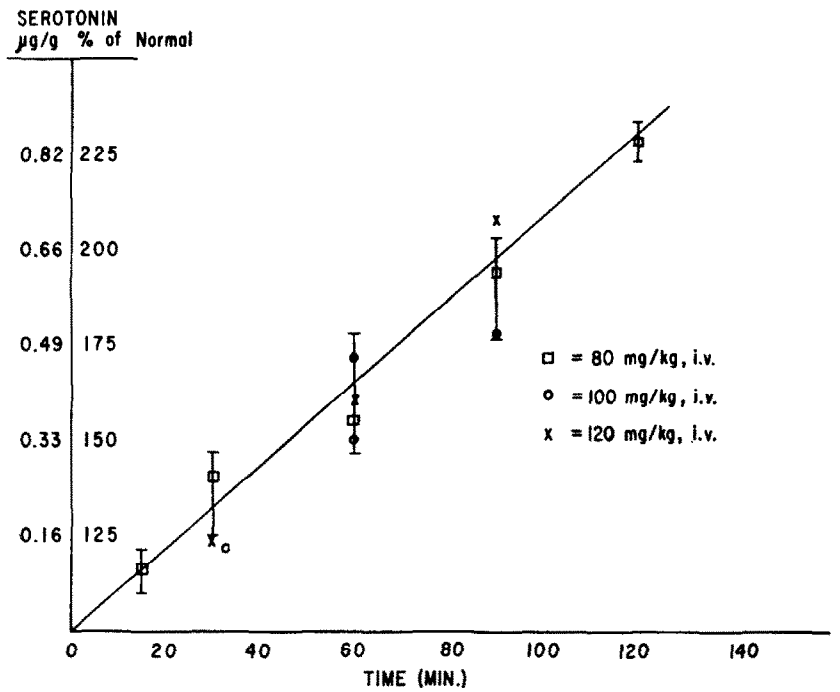

FIG. 2. Accumulation of serotonin in mouse brain after high doses of phenelzine.

The inhibition of the decarboxylase was not always greatest when the concentration of phenelzine was at its maximum, i.e. within 5 min after injection of the drug,* a circumstance which suggests that more than direct interaction of phenelzine with the coenzyme of 5-HTP decarboxylase may be involved. McCormick and Snell[12] reported that β-phenyl*iso*propylhydrazine is capable of inhibiting pyridoxal kinase of brain *in vivo*. Thus, although the initial inhibitions observed here may be attributable to a direct effect upon the pyridoxal-5-phosphate associated with the decarboxylase, reduced synthesis of the coenzyme eventually may be an important factor. The reduced accumulation of serotonin after 5-HTP injected 24 hr after the low dose of phenelzine (16 mg/kg), then, may be a result of reduced synthesis of the coenzyme.

* An assay procedure for phenelzine and its distribution was reported in Ref. 11.

This would not necessarily be revealed by determinations of 5-HTP decarboxylase *in vitro*, since in homogenates that enzyme might have access to pyridoxal-5-phosphate which had previously been associated with other systems. Of course, the reduced accumulation may also reflect some reversal of MAO-inhibition.

Although phenylacetic acid is a major metabolite of phenelzine,[13] and phenylacetic acid has been reported to inhibit 5-HTP decarboxylase,[14] such inhibition is not considered to be a major factor, since the parent hydrazine is approximately forty times more potent as an inhibitor of the decarboxylase than is the catabolite.

The stimulation of decarboxylase activity in the presence of both phenelzine and coenzyme is reminiscent of the reports of Gonnard[15, 16] concerning the stimulatory effects of the *iso*nicotinylhydrazone of pyridoxal-5-phosphate upon the decarboxylation of dopa and on a transaminase. It may also be related to the stimulation of 5-HTP decarboxylation by pyridoxal-5-phosphate, observed in the experiments of Smith[9] and of Price and West[17], in which a hydrazine derivative was used to inhibit MAO, and the lack of such a stimulation in the experiments of others.[1, 2, 18]

With doses of phenelzine demonstrated to inhibit the decarboxylation of 5-HTP *in vivo* by approximately 50 per cent, no effect on rate of accumulation of serotonin in mouse brain after inhibition of MAO was demonstrated. (The inhibitions might have been even greater, since the time intervals at which accumulated serotonin was measured after the injection of 5-HTP might not reflect maximum *rate* of synthesis.) If the rate of accumulation of serotonin in mouse brain after inhibition of MAO (0·43 μg/g per hr[6]) can serve as an approximation of the rate of serotonin synthesis (in brain) from endogenous precursors*, and if the rate of the decarboxylation step may be approximated from our experiments (about 93 μg/g per hr *in vitro* or 14·5 μg/g per hr based on 5 min *in vivo*), then in order to inhibit the overall rate of serotonin synthesis in brain by inhibition of the decarboxylation, it would be necessary to suppress the latter enzyme by at least 95 per cent, perhaps over 99 per cent. Thus, this would not appear to be a strategic point at which to attempt the control of serotonin synthesis.† Yet α-methyldopa, an inhibitor of 5-HTP decarboxylase,[19] has shown effects in man which have been attributed to reduced synthesis of amines;[20] and the levels of serotonin in the brain, in mice, have been reduced with this compound.[9] In the human experiments the inhibition was estimated to be 50–80 per cent, while in the mice nearly complete inhibition was achieved. However, there is no evidence as yet that α-methyldopa can decrease the turnover of serotonin in brain at levels of inhibition which might be useful for practical control of amine synthesis. The possibility has not been excluded that the amino acid may act at another site as well, e.g. tryptophan hydroxylase. It is interesting that recent evidence[21, 22] points to amine-release as an additional mechanism for the reduction by α-methyldopa of the levels of amine, at least with respect to brain norepinephrine.

* Since MAO-inhibitors may act to inhibit the release of amines, as well as their catabolism,[23] the accumulation could be independent of either 5-HTP decarboxylase or MAO. However, it would seem that this would more likely influence the equilibrium level of serotonin rather than the rate of accumulation.

† This suggestion has also been made by Dr. Sidney Udenfriend in formal talks.

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