Biochemical Pharmacology, Vol. 21, pp. 1501–1510. Pergamon Press, 1972. Printed in Great Britain.

p-CHLOROAMPHETAMINE—INHIBITION OF CEREBRAL TRYPTOPHAN HYDROXYLASE*†

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(*Received* 22 *October* 1971; *accepted* 10 *December* 1971)

Abstract—Earlier experiments have suggested that the simultaneous decrease in the levels of 5-hydroxytryptamine (5HT) and 5-hydroxyindole acetic acid (5HIAA) in brain following the administration of *p*-chloroamphetamine and *p*-chloromethamphetamine may be the consequence of an inhibition of the synthesis of cerebral 5HT. In the present investigations, the effects of these drugs have been examined on the activity of cerebral tryptophan hydroxylase. The addition *in vitro* of either *p*-chloroamphetamine or *p*-chloromethamphetamine does not reduce the activity of tryptophan hydroxylase isolated from brainstems of rats. Under similar conditions, *p*-chlorophenylalanine causes marked inhibition. However, when tryptophan hydroxylase was assayed in preparations obtained from brains of rats treated 16 hr previously with *p*-chloroamphetamine, a dose-related reduction in the activity of the enzyme was observed. Experiments involving various combinations of enzyme preparations from control rats and from rats pretreated with *p*-chloroamphetamine do not indicate the presence of an inhibitor in the preparations isolated from rats pretreated with the drug. Moreover, the reduction in enzyme activity was not removed by dialysis. Kinetic studies showed that the K_m values for tryptophan and $DMPH_4$ were the same for the enzyme isolated from control rats and from rats pretreated with *p*-chloroamphetamine. The reduction of cerebral 5HT and the decrease in the activity of tryptophan hydroxylase occur simultaneously; both effects are still present 6 days following a single dose of 10 mg/kg of *p*-chloroamphetamine. It is concluded that the inhibition of cerebral tryptophan hydroxylase by *p*-chloroamphetamine can satisfactorily explain the prolonged reduction in the levels of 5HT and 5HIAA in brain.

THE ADMINISTRATION of *p*-chloroamphetamine and *p*-chloromethamphetamine to rats causes a prolonged and simultaneous decrease in cerebral 5-hydroxytryptamine (5HT) and 5-hydroxyindole acetic acid (5HIAA).[1–8] Pletscher *et al.*[1] first suggested that the chlorinated amphetamines may release 5HT in a unique manner, bypassing monoamine oxidase (MAO). Later, it was proposed that an inhibition of MAO was responsible for the reduction in the levels of 5HIAA.[2,9,10] Still other investigators have explained the reduction of brain 5HT by mechanisms involving a release of 5HT or an inhibition of its reuptake or possibly by a combination of both of these mechanisms.[11–13]

A number of experiments *in vivo* from our laboratory have indicated that *p*-chloroamphetamine causes a reduction of cerebral 5HT and 5HIAA by an inhibition of the synthesis of cerebral 5HT. Moreover, our data *in vivo* suggested that the site of this inhibition is located at the hydroxylation step.[14] Our investigations have now

* Supported by United States Public Health Service Grant MH-11468.

† Presented in part before the Federation of American Societies for Experimental Biology, Chicago, Ill., 1971 (*Fedn Proc.* **30**, 1054 1971).

been extended to a direct measurement of the activity of cerebral tryptophan hydroxylase and its modification by *p*-chloroamphetamine. The results of these studies are presented in this paper.

METHODS

Assay of tryptophan hydroxylase. Tryptophan hydroxylase activity was assayed by a procedure involving essentially a combination of the methods of Lovenberg *et al.*[15] and Peters *et al.*[16]

Male Sprague–Dawley rats (Sprague–Dawley Company, Madison, Wis.), 180–220 g, were used in all experiments. The animals were sacrificed by decapitation; the brains were rapidly removed and placed on a chilled dish. The cerebral hemispheres and cerebellum were dissected free and discarded. The remaining tissue was homogenized in 3 vol. of ice-cold 0·05 M tris–acetate buffer (pH 7·4); the homogenate was centrifuged at 30,000 *g* for 20 min. An aliquot of the supernatant, equivalent to 100 mg of tissue (4 mg of protein), was added to an incubation mixture containing [^{14}C]L-tryptophan (0·08 mM), 2-amino-4-hydroxy-6,7-dimethyl-5,6,7,8-tetrahydropteridine ($DMPH_4$) (0·8 mM), pargyline hydrochloride (0·33 mM), 5HT (0·14 mM), $Fe(NH_4)_2(SO_4)_2$ (0·08 mM), and 2-mercaptoethanol (45 mM) in a final volume of 0·63 ml. After incubation at 37° in an atmosphere of air for 1 hr, the reaction was stopped by the addition of 0·5 ml of 10% (w/v) trichloroacetic acid. The mixture was centrifuged at 30,000 *g* for 10 min.

The [^{14}C]5HT formed from [^{14}C]L-tryptophan was isolated by ion exchange chromatography using Amberlite CG-50 as follows. The supernatant solutions from the incubations were added to 3 ml of 0·5 M Na–K phosphate buffer (pH 7) containing 0·2% ascorbic acid and 0·2% EDTA. Following centrifugation, the samples were poured over columns (6 × 25 mm) of Amberlite CG-50 (H^+ form, previously washed with water and soaked in 0·5 M phosphate buffer). After two washes with 50 ml of water, [^{14}C]5HT was eluted from the columns with 6 ml of 4 N acetic acid. A 3-ml aliquot of this fraction was added to 10 ml of Bray's scintillation fluid[17] and the sample was counted in a Nuclear Chicago Mark I liquid scintillation counter. Recovery of added [^{14}C]5HT was 82–89 per cent. All data were corrected for counting efficiency by external standardization. The activity of tryptophan hydroxylase is expressed as nanocuries of [^{14}C]5HT formed per gram of wet brain per hour.

In some experiments, the reaction mixture was incubated in the presence of additional L-amino acid decarboxylase enzyme. The original mixture was incubated for 1 hr as described above, and the samples were transferred to an ice bath. After the addition of 0·5 ml of 1 M tris–acetate buffer (pH 9), 2·3 μmoles of DL-5-hydroxytryptophan (5HTP) and 3 units of purified 5HTP decarboxylase (isolated from guinea pig kidney according to the method of Clark *et al.*[18]), the mixture was incubated for an additional 20 min in an atmosphere of nitrogen. This reaction was terminated by the addition of 1 ml of 10% trichloroacetic acid and [^{14}C]5HT was isolated and estimated as previously described. A comparison of the amount of [^{14}C]5HT formed with and without the addition of the decarboxylase preparation showed that an excess of this enzyme was normally present in the reaction mixture. Consequently, all of the [^{14}C]5HTP formed during the incubation was converted to [^{14}C]5HT. It was thus valid to use the rate of formation of [^{14}C]5HT from [^{14}C]L-tryptophan

as an estimate of the activity of tryptophan hydroxylase; and purified decarboxylase was not routinely added.

In our preparations, most of the tryptophan hydroxylase activity was localized in the 30,000 *g* supernatant. Moreover, in agreement with previous reports,[15,16] enzyme activity was markedly stimulated (5- to 10-fold) by the addition of $DMPH_4$. The formation of [^{14}C]5HT in incubations with enzyme from control rats and from rats treated with *p*-chloroamphetamine was linear with time of incubations up to 60 min and with protein concentration over a range of at least 0·5–4 mg of protein/sample.

The possible presence of [^{14}C]-tryptamine in the acetic acid eluate was examined as follows. After the addition of 0·5 ml of 2 M K_2HPO_4 for each ml of eluate, the solution was adjusted to pH 10 by the addition of NaOH. The sample was then extracted twice with an equal volume of benzene, and each benzene fraction was washed with an equal volume of 1·3 M phosphate buffer (pH 10). In this way, tryptamine is almost completely separated from 5HT; the partition coefficients under these conditions are 3·4 and 0·13 respectively. An analysis by this procedure showed that less than 4 per cent of the radioactivity in the acid eluate could be tryptamine.

Determination of endogenous compounds. The spectrophotofluorometric method of Bogdanski *et al.*[19] was used for the assay of 5HT in whole brain. Protein analyses were carried out according to the method of Lowry *et al.*[20]

Radioactive materials and drugs. L-(methylene)[^{14}C]-tryptophan (54–56 mc/m-mole) was obtained from Amersham–Searle (Arlington Heights, Ill.); the amino acid was purified by passing the material over a column of Amberlite CG-50 (6 × 40 mm). This procedure reduced the blank in the standard assay to about 100–200 counts/min. Non-labeled L-tryptophan was added to reduce the specific activity of L-tryptophan to 10 mc/m-mole.

The following compounds were purchased from Sigma Chemical Company (St. Louis, Mo.): 5-hydroxyindole-3-acetic acid and 5-hydroxytryptamine creatinine sulfate. *p*-Chlorophenylalanine (ethyl ester HCl) was purchased from the Aldrich Chemical Company (Milwaukee, Wis.); *p*-chloroamphetamine was initially donated by Eli Lilly (Indianapolis, Ind.) and, when available, purchased from Regis Chemical Company (Chicago, Ill.). Also purchased from Regis were $DMPH_4$ and *p*-chloromethamphetamine. Pargyline was donated by Abbott Laboratories (North Chicago, Ill.), and SKF 525-A was provided by Smith, Kline & French Labs. (Philadelphia, Pa.). Amberlite CG-50 (H^+ form, 100–200 mesh) was purchased from Mallinckrodt (St. Louis, Mo.). All other chemicals were reagent quality.

RESULTS

Effect of the addition in vitro *of* p-*chloroamphetamine on the activity of cerebral tryptophan hydroxylase.* The addition *in vitro* of *p*-chloroamphetamine in concentrations up to 10^{-3} M failed to influence the formation of [^{14}C]5HT from [^{14}C]-tryptophan. Under similar conditions, the addition of *p*-chlorophenylalanine (5×10^{-4} M) caused a 65 per cent reduction in the activity of tryptophan hydroxylase. Moreover, preincubation of the enzyme preparation for 30 min in the presence of *p*-chloroamphetamine (10^{-4} M) did not result in an inhibition of the enzyme when hydroxylase activity was subsequently determined. In other experiments, the concentrations of tryptophan and $DMPH_4$ were varied over a 10-fold range and, in each case, the addition *in vitro* of *p*-chloroamphetamine did not reduce the formation of [^{14}C]5HT.

Effect of pretreatment of rats with p*-chloroamphetamine on the activity of cerebral tryptophan hydroxylase assayed* in vitro. *p*-Chloroamphetamine was administered intraperitoneally to rats 16 hr prior to sacrifice. Tryptophan hydroxylase activity was then measured according to the procedure described in Methods. When tryptophan hydroxylase activity was assayed in these preparations, a dose-related reduction in activity of the enzyme was observed (Table 1).

TABLE 1. EFFECT OF THE INTRAPERITONEAL ADMINISTRATION OF *p*-CHLOROAMPHETAMINE ON THE ACTIVITY OF TRYPTOPHAN HYDROXYLASE

Dose (mg/kg*)	Tryptophan hydroxylase activity†	
	nc [^{14}C]5HT formed/g/hr ± S. E.	Per cent inhibition
0 (5)	31·4 ± 2·9	0
2 (4)	25·2 ± 0·4	20
5 (7)	21·0 ± 1·2	33
7·5 (7)	18·0 ± 2·2	43
10 (7)	12·5 ± 1·2	60

* *p*-Chloroamphetamine was administered, i.p., 16 hr prior to sacrifice. The number of animals is indicated in parentheses.

† Tryptophan hydroxylase activity was measured according to the procedure described in Methods.

In other experiments, preparations from control rats and from rats pretreated with *p*-chloroamphetamine were divided and assayed as described in Methods, except that the substrate was either [^{14}C]L-tryptophan (0·08 mM) or [^{14}C]DL-5HTP (0·1 mM). The amount of [^{14}C]5HT formed was determined. In preparations obtained from rats pretreated with *p*-chloroamphetamine, the usual reduction in the formation of [^{14}C]5HT from [^{14}C]tryptophan was observed. However, when [^{14}C]5HTP was the precursor, the amount of [^{14}C]5HT formed was the same in preparations obtained from control rats and from animals pretreated with *p*-chloroamphetamine (Table 2).

Experiments with *p*-chloromethamphetamine showed that this drug also reduces tryptophan hydroxylase only after its administration *in vivo* (Table 3).

TABLE 2. EFFECT OF PRETREATMENT WITH *p*-CHLOROAMPHETAMINE ON THE FORMATION *in vitro* OF 5HT FROM DIFFERENT SUBSTRATES*

Treatment	Enzyme activity (nc [^{14}C]5HT/g brain/hr)†	
	[^{14}C]-tryptophan	[^{14}C]5HTP
Control	91·8	96·2
p-Chloroamphetamine	41·8	90·0

* Ten mg/kg, i.p., 16 hr before sacrifice.

† Enzyme activity was assayed in the presence of [^{14}C]-tryptophan (0·08 mM) or [^{14}C]5HTP (0·1 mM) and other reactants as described in Methods. The values presented are the mean of triplicate determinations.

TABLE 3. EFFECT OF *p*-CHLOROMETHAMPHETAMINE ON THE ACTIVITY OF TRYPTOPHAN HYDROXYLASE

	Tryptophan hydroxylase activity (nc [^{14}C]5HT/g brain/hr ± S. E.)
Experiment I*	
Control	20·6
p-Chloromethamphetamine (1×10^{-4} M)	25·0
Experiment II†	
Control	45·9 ± 7·3
p-Chloromethamphetamine (10 mg/kg, i.p.)	28·1 ± 2·3

* *p*-Chloromethamphetamine (1×10^{-4} M) was added to a typical incubation mixture. The values reported are the mean of triplicate determinations and are representative of two separate experiments.

† *p*-Chloromethamphetamine was administered 16 hr prior to sacrifice. The values represent the mean of five animals.

Studies on the mechanism of the inhibition in vivo *of tryptophan hydroxylase by* p-*chloroamphetamine.* A number of experiments were conducted to elucidate the mechanism of action of the apparent selective inhibition *in vivo* of cerebral tryptophan hydroxylase by *p*-chloroamphetamine. The possibility was considered that the inhibition *in vivo* could be mediated through the formation *in vivo* of a metabolite of *p*-chloroamphetamine. Experiments involving various combinations of boiled and

TABLE 4. CROSS-OVER EXPERIMENTS WITH ENZYME PREPARATIONS FROM CONTROL RATS AND FROM RATS TREATED WITH *p*-CHLOROAMPHETAMINE

Active preparations	Heat-inactivated preparations*		Tryptophan hydroxylase activity‡ (nc [^{14}C]5HT/g brain/hr)
	Control	Treated†	
Control			25·3§
Control		+	54·5
Control	+		52·6
Treated†			14·2
Treated	+		34·2
Treated		+	32·7

* Aliquots of enzyme preparations were placed in a boiling water bath for 5 min. Following centrifugation, 0·2 ml of the supernatant was added to the samples as indicated. Boiled preparations had no hydroxylase activity when assayed alone.

† Rats were treated with *p*-chloroamphetamine (10 mg/kg, i.p.) 16 hr prior to sacrifice.

‡ Tryptophan hydroxylase activity was assayed as described in Methods, except that the total volume was increased to 0·9 ml; the concentration of the reactants remained the same.

§ The values presented are the mean of triplicate determinations and are representative of two separate experiments.

TABLE 5. EFFECT OF SKF 525-A ON THE ACTION OF *p*-CHLOROAMPHETAMINE ON TRYPTOPHAN HYDROXYLASE ACTIVITY

Treatment*	Tryptophan hydroxylase activity† (nc [^{14}C]5HT/g/hr ± S. E.)
Saline (6)	96·8 ± 3·3
Saline + *p*-chloroamphetamine (6)	49·8 ± 4·4
SKF 525-A (6)	62·9 ± 9·6
SKF 525-A + *p*-chloroamphetamine (8)	27·8 ± 3·4

* Rats were treated with SKF 525-A (40 mg/kg, i.p.) or saline 30 min prior to the administration of *p*-chloroamphetamine (10 mg/kg, i.p.) or saline. Animals were sacrificed 6 hr after *p*-chloroamphetamine. The figures in parentheses indicate the number of animals.

† Tryptophan hydroxylase activity in brain stem was assayed according to Methods.

fresh preparations from treated and control rats did not indicate the presence of a heat-stable inhibitor in the brains of rats pretreated with *p*-chloroamphetamine. Thus, the addition of boiled supernatants from treated rats did not reduce the hydroxylating ability of control preparations (Table 4). To the contrary, a heat-stable factor which enhances enzyme activity was present to the same effect in preparations from brains of control and treated rats. Pretreatment of rats with SKF 525-A, an inhibitor of hepatic drug metabolism, did not prevent the decrease in tryptophan hydroxylase activity caused by the administration of *p*-chloroamphetamine (Table 5).

TABLE 6. EFFECT OF DIALYSIS ON THE INHIBITION OF TRYPTOPHAN HYDROXYLASE CAUSED BY *p*-CHLOROAMPHETAMINE

	Tryptophan hydroxylase activity* (nc [^{14}C]5HT formed/mg protein/hr)	
	Control	*p*-Chloroamphetamine
Experiment I		
Nondialyzed†	2·97	1·35
Dialyzed‡	0·63	0·34
Nondialyzed§	0·84	
Experiment II		
Nondialyzed†	2·01	
Dialyzed‡	0·59	0·24
Nondialyzed§	0·79	0·32

* The initial incubation was followed by an incubation in the presence of added decarboxylase enzyme as described in Methods.

† Prepared just prior to assay.

‡ Enzyme preparations from control rats and from those treated with *p*-chloroamphetamine (10 mg/kg, i.p., 16 hr prior to sacrifice) were dialyzed overnight against 0·05 M pH 7·4 tris–acetate buffer containing 0·1 mM 2-mercaptoethanol.

§ The enzyme preparation was allowed to stand overnight at 5°.

In other experiments, enzyme preparations from non-treated rats and from rats pretreated with *p*-chloroamphetamine (10 mg/kg, i.p., 16 hr before sacrifice) were dialyzed overnight against 0·05 M tris-acetate buffer (pH 7·4 containing 0·1 mM 2-mercaptoethanol). The inhibition caused by *p*-chloroamphetamine was not reversed by dialysis of the enzyme preparation (Table 6). Apparently, the observed loss of enzyme activity with dialysis is merely due to instability of either the enzyme or an unknown cofactor, because a similar decrease in activity occurred when samples were allowed to stand overnight at 5°. A similar reduction in hydroxylase activity by dialysis has been observed by Gal *et al.*[21] although other investigators have previously reported that dialysis stimulated the activity of this enzyme.[22]

Effect of pretreatment with p*-chloroamphetamine on the* K_m *for tryptophan and for DMPH*$_4$. Tryptophan hydroxylase activity in enzyme preparations from control rats and rats pretreated with *p*-chloroamphetamine (10 mg/kg, i.p.) was determined in the presence of various concentrations of tryptophan (0·15–0·75 mM) or $DMPH_4$ (0·09–0·75 mM) and other reactants as described in Methods. K_m values were calculated from the extrapolated intercept on the ordinate of a double-reciprocal plot of [^{14}C]5HT formed vs. concentration of substrate. These kinetic studies showed that the apparent K_m values for tryptophan ($3{\cdot}0 \times 10^{-4}$ M) and $DMPH_4$ ($1{\cdot}5 \times 10^{-4}$ M) were the same in preparations from control and treated rats.

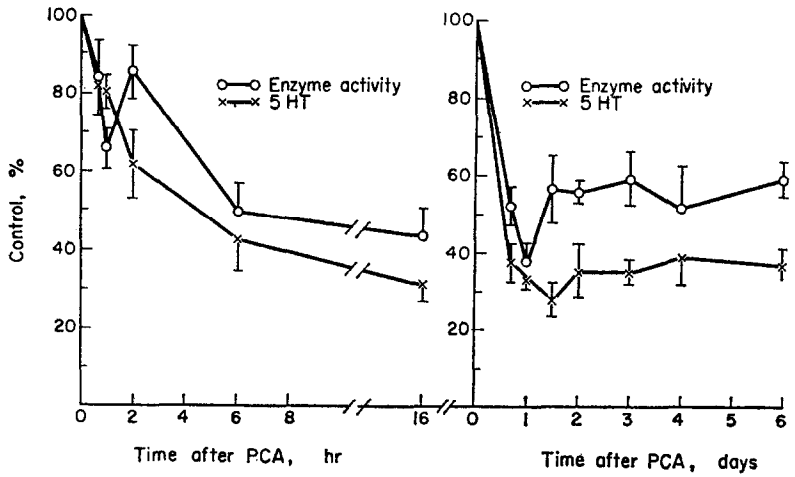

FIG. 1. Level of 5HT (×——×) and tryptophan hydroxylase activity (○——○) in rat brainstem following the administration of *p*-chloroamphetamine (PCA). Rats were sacrificed at various times after the i.p. administration of 10 mg/kg of *p*-chloroamphetamine. The enzyme activity and the level of 5HT were measured in brainstem as described in Methods. The results are expressed as a percentage of the control values. Each point is the mean of four to six rats. Vertical bars represent standard error of the mean.

Relationship between inhibition of tryptophan hydroxylase and reduction of brain 5HT. *p*-Chloroamphetamine (10 mg/kg, i.p.) was administered to rats. The animals were sacrificed at various times after the administration of the drug and the level of 5HT and the activity of tryptophan hydroxylase in brainstem were determined as described under Methods. During the early hours after the administration of the drug, the activity of tryptophan hydroxylase fluctuated (Fig. 1), suggesting two phases

of inhibition—an initial transient reduction with partial recovery by 2 hr, followed by an irreversible inactivation which persists and leads to a progressive decrease in the activity of the enzyme. The inhibition of tryptophan hydroxylase was maximal 1 day following the administration of the drug and the enzyme activity remained reduced (40–50 per cent of control value) for at least 6 days. Similarly, the levels of 5HT were maximally reduced between 1 and 1·5 days after the administration of the drug and remained at a level equal to 35 per cent of the control value for at least 6 days (Fig. 1).

DISCUSSION

The initial observations by Pletscher *et al.*[1] of a simultaneous and long-lasting depletion of cerebral 5HT and 5HIAA following the administration of chlorinated amphetamines have been repeatedly confirmed.[2–8] The results of our investigations *in vivo* on the mechanism of action of these drugs indicated that an inhibition of the synthesis of cerebral 5HT could be responsible for these biochemical changes.[14]

Results of the present investigations demonstrate that the addition *in vitro* of *p*-chloroamphetamine or *p*-chloromethamphetamine does not inhibit cerebral tryptophan hydroxylase. Under similar assay conditions, *p*-chlorophenylalanine clearly causes marked inhibition. These data are in agreement with those of Pletscher *et al.*[23] However, when tryptophan hydroxylase was assayed in preparations obtained from brains of rats previously treated with *p*-chloroamphetamine or *p*-chloromethamphetamine, the activity of the enzyme was reduced to 30–50 per cent of the activity measured in the brains of control rats. Interestingly, hepatic tryptophan hydroxylase is not reduced after the intraperitoneal administration of *p*-chloromethamphetamine[1] or *p*-chloroamphetamine.[3] The findings that intestinal 5HT is not decreased by systemic administration of *p*-chloromethamphetamine[1] or *p*-chloroamphetamine* are in agreement with these results. It thus appears that the chlorinated amphetamines are specific inhibitors of the cerebral enzyme.

The relatively high K_m observed for tryptophan (3×10^{-4} M) indicates that the over-all rate of synthesis of cerebral 5HT may be partially dependent upon the availability of the substrate.[24] It is thus pertinent that *p*-chloroamphetamine, in doses which reduce the endogenous level of cerebral 5HT, does not change the concentration of cerebral tryptophan.[14]

The possibility was considered that the inhibition *in vivo* could be mediated through a metabolite formed from *p*-chloroamphetamine. However, the addition of heat-inactivated homogenates of brains from rats treated with the drug did not reduce the hydroxylating ability of an active preparation prepared from brains of control animals. To the contrary, boiled homogenates prepared both from rats treated with *p*-chloroamphetamine and from control rats actually stimulated hydroxylase activity to the same extent. Moreover, the treatment of rats with SKF 525-A, a drug which inhibits the hepatic metabolism of many drugs including the oxidative deamination of amphetamine,[25] did not prevent the inhibitory action of *p*-chloroamphetamine. These data suggest that the formation *in vivo* of a reversible inhibitor of tryptophan hydroxylase does not occur. This conclusion is further supported by the observation that the inhibition caused by *p*-chloroamphetamine was not removed by dialysis. Kinetic studies with enzyme preparations from control rats and from rats treated

* E. Sanders-Bush, J. A. Bushing and F. Sulser, unpublished observations.

with *p*-chloroamphetamine demonstrated that the apparent K_m values for tryptophan and $DMPH_4$ are not changed by treatment with *p*-chloroamphetamine, suggesting that the drug may have reduced the amount of active enzyme without having altered its properties.

A comparison of the time course of the decline in the level of cerebral 5HT and the reduction of cerebral tryptophan hydroxylase following *p*-chloroamphetamine did not provide unequivocal evidence that the decrease in the activity of tryptophan hydroxylase is solely responsible for the decrease in cerebral 5HT. At early times after the administration of the drug, the rate of decline in enzyme activity and in the level of 5HT was not precisely related. Thus, other mechanisms in addition to an inhibition of tryptophan hydroxylase may be involved in the initial decrease in cerebral 5HT. It is noteworthy that both enzyme activity and levels of 5HT were still markedly reduced 6 days following the administration of *p*-chloroamphetamine.

In several respects, the inhibition of tryptophan hydroxylase by *p*-chloroamphetamine is similar to that caused by *p*-chlorophenylalanine. The effect of both drugs is long lasting and irreversible. Moreover, the irreversible inactivation following the administration *in vivo* of either *p*-chloroamphetamine or *p*-chlorophenylalanine cannot be demonstrated by the addition *in vitro* of these drugs to a preparation of cerebral tryptophan hydroxylase. A reversible competitive inhibition of the hydroxylase enzyme, however, does occur after the addition *in vitro* of *p*-chlorophenylalanine.[22] Dissimilarities in the effect *in vivo* of the two drugs are also evident. For example, a kinetic analysis indicated that *p*-chloroamphetamine does not alter the interaction of the substrate or cofactor with the active site, while the apparent K_m of tryptophan for the enzyme isolated from rats treated with *p*-chlorophenylalanine is greater than that for the enzyme isolated from control animals.[26] Moreover, pretreatment with *p*-chlorophenylalanine reduces hepatic hydroxylation of tryptophan and phenylalanine,[27] whereas treatment with *p*-chloroamphetamine causes a specific reduction of cerebral tryptophan hydroxylase.

In conclusion, the results of the present investigation demonstrate that *p*-chloroamphetamine can cause an irreversible inactivation of cerebral tryptophan hydroxylase. The precise mechanism of enzyme inactivation by the drug in *vivo* remains to be elucidated. While not precluding other possible mechanisms or combinations of proposed mechanisms, the inhibition of cerebral tryptophan hydroxylase by *p*-chloroamphetamine can satisfactorily explain the simultaneous, prolonged reduction in the cerebral levels of 5HT and its major metabolite, 5HIAA.

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