MODULATION OF BIOGENIC AMINE ENZYMES

by

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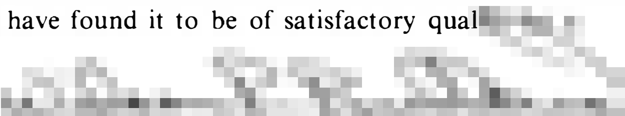

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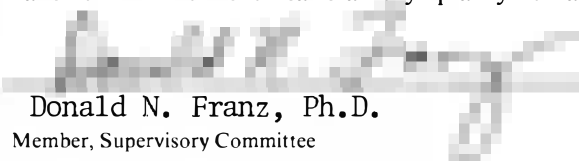

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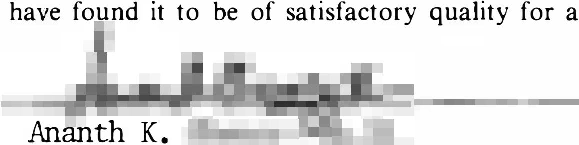

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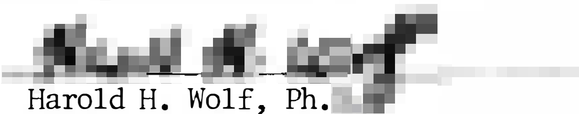

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ABSTRACT

Acute administration of haloperidol to rats causes a marked decrease in the Km of neostriatal tyrosine hydroxylase for the pteridine cofactor, $6MPH_4$, with no change in Vmax. This effect is dependent on the pH of the assay mixture. It occurs at pH 6.5 but not at pH 6.0, the pH optimum for TH. With phosphorylating conditions at pH 6.5, the haloperidol-induced activation is no longer observed, and the kinetics of TH are the same as those from control rats. At pH values of 6.0, 6.3 and 6.5, a significant decrease in Vmax occurs, with increasing pH, while no significant change in Km for the cofactor is observed for TH from control rats. However, when phosphorylating conditions are employed, a marked increase in Km for the cofactor is observed while only a slight decrease in Vmax is seen, with increasing pH, for the control enzyme at the three pH values tested.

The kinetic characteristics of neostriatal tyrosine hydroxylase (TH) activity were then determined after rats were given large, repeated doses of methamphetamine. The Vmax of the enzyme was markedly decreased after methamphetamine, but no change in the Km for pteridine cofactors nor for the substrate, tyrosine, was detected. The decrease in Vmax with methamphetamine was independent of the phosphorylated state of the enzyme.

The effect of propranolol in blocking the methamphetamine-induced depression of tryptophan hydroxylase was then investigated. Acute administration of methamphetamine produced a marked decrease in tryptophan hydroxylase activity in all serotonergic nerve terminal regions of rat brain examined. This decrease was antagonized in a regionally selective manner by propranolol. Almost complete blockade occurred in regions that are commonly implicated in schizophrenia, mesolimbic areas, neostriatum, and hypothalamus; whereas in other regions examined, only partial or no blockade occurred. In contrast, haloperidol did not produce a similar antagonism. The results are discussed in the context of the efficacy of large doses of propranolol in some schizophrenic patients.

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PART ONE

ORIENTATION

This thesis is comprised of three parts that may appear to be different but do, in fact, have a common theme: i.e. the effect of centrally acting drugs on the activity of enzymes that are rate-limiting in the biosynthesis of certain neurotransmitters. This includes drugs that can induce a schizophrenia-like syndrome as well as drugs that are used clinically in the treatment of schizophrenia.

The first part involves a study of the mechanism by which haloperidol, a commonly used antipsychotic drug, produces activation of tyrosine hydroxylase, the rate-limiting enzyme in the biosynthesis of dopamine. It includes a systematic kinetic study of the interaction between the *in vitro* effect of the hydrogen ion concentration, the phosphorylated state of the enzyme and the *in vivo* induction of tyrosine hydroxylase activation by haloperidol. The relevance of this study lies in understanding the mechanisms by which haloperidol produces an increase in tyrosine hydroxylase activity which controls the synthesis of dopamine. This may provide an insight into the clinical effects seen with haloperidol. The second part involves a kinetic study of the effect of methamphetamine in lowering the activity of tyrosine hydroxylase, which has been observed with chronic treatment. The possibility of a change in the affinity of the enzyme for the substrate or the cofactor, as well as alteration in the maximum velocity of the enzyme, was investigated. In addition, the influence of the phosphorylated state of the enzyme on the above parameters was also examined. Finally, a time-course of kinetic changes

in tyrosine hydroxylase activity following chronic treatment of rats with methamphetamine was characterized.

The third and last part attempts to provide a biochemical rationale for the efficacy of large doses of propranolol in schizophrenia. An animal model was utilized in which rats were treated with methamphetamine to produce stereotyped behaviors that may be a correlate of the psychotic state in humans. This behavior in animals is associated with a decrease in the activity of tryptophan hydroxylase in different regions of the rat brain. The effect of propranolol, which has recently been shown to bind the 5-hydroxytryptamine binding sites, in preventing the depression in tryptophan hydroxylase activity was investigated and the results were correlated with its clinical efficacy.

PART TWO

IN VITRO EFFECTS OF pH AND PHOSPHORYLATION ON NEOSTRIATAL TYROSINE HYDROXYLASE FROM CONTROL AND HALOPERIDOL-TREATED RATS

Introduction

Modulation of the activity of tyrosine hydroxylase (TH), the rate-limiting enzyme in catecholamine biosynthesis (1), by hydrogen ion concentration and phosphorylating systems has become the focus of increasing attention in recent years. The activity of TH from rat brain and adrenal medulla is sensitive to pH (2,3) as well as to phosphorylation (3-10). Various antipsychotic drugs also produce a change in the activity of the enzyme (11,12). For example, acute doses of haloperidol produce a marked increase in the affinity of TH for the pteridine cofactor with no effect on the Km for the substrate (11). We have recently shown that this effect of haloperidol is pH-dependent (13). It occurs at higher pH values but not at lower values. It is possible that haloperidol, as well as other drugs, may produce their effect by phosphorylation of the enzyme. In an attempt to understand the relationship between pH, phosphorylation and the haloperidol-induced effects on the regulation of TH, we have examined the kinetic characteristics of rat neostriatal TH with regard to the pteridine cofactor.

Methods

Male Sprague-Dawley rats, weighing 150-250 gm, were housed three per cage in a temperature-controlled room (26°) with a 12-hr light-dark cycle. Food and water were offered ad libitum. Rats were injected with haloperidol (1 mg/kg, i.p.) which was dissolved in 1%

lactic acid. Control rats were injected with the lactic acid solution. Control and treated rats were sacrificed by decapitation one hour after injection. Rat neostriata were rapidly dissected out, wrapped in parafilm and placed on dry ice, and then stored at -70°C until assayed.

All steps for preparing the enzyme were performed at 0-5°C and TH activity was determined by the method of Nagatsu et al. (14). The neostriata were homogenized in 0.8 ml of 0.2 M sodium acetate buffer, containing 0.2% Triton X-100 and centrifuged at 27,000 X g for 15 min. at 4°C. The desired pH was adjusted at 37°C with glacial acetic acid. The supernatant fractions of neostriata from two rats were combined and 50-μl aliquots were assayed in a total volume of 100 μl of incubation medium, containing: 1 mM ferrous ammonium sulfate, 100 mM of mercaptoethanol, 0.2 M sodium acetate pH 6.0, 6.3 or 6.5, 0.1 mM tyrosine, 0.4 μCi purified 3,5-ditritiotyrosine, and varying concentrations of 2-amino-6-hydroxy-6-methyltetrahydropterin ($6MPH_4$, Calbiochem). When phosphorylating conditions were employed, the incubation mixture (100 μl) also contained, in addition to the above, the following components (expressed as final concentrations): 12.5 mM of magnesium acetate, 12.5 mM of sodium fluoride, 0.5 mM of theophylline, 0.075 mM of EGTA, 0.312 mM of ATP, and 0.0725 mM of 3', 5'-cAMP (5). Control samples from the same homogenate were assayed under normal and phosphorylating conditions at pH values of 6.0, 6.3 and 6.5.

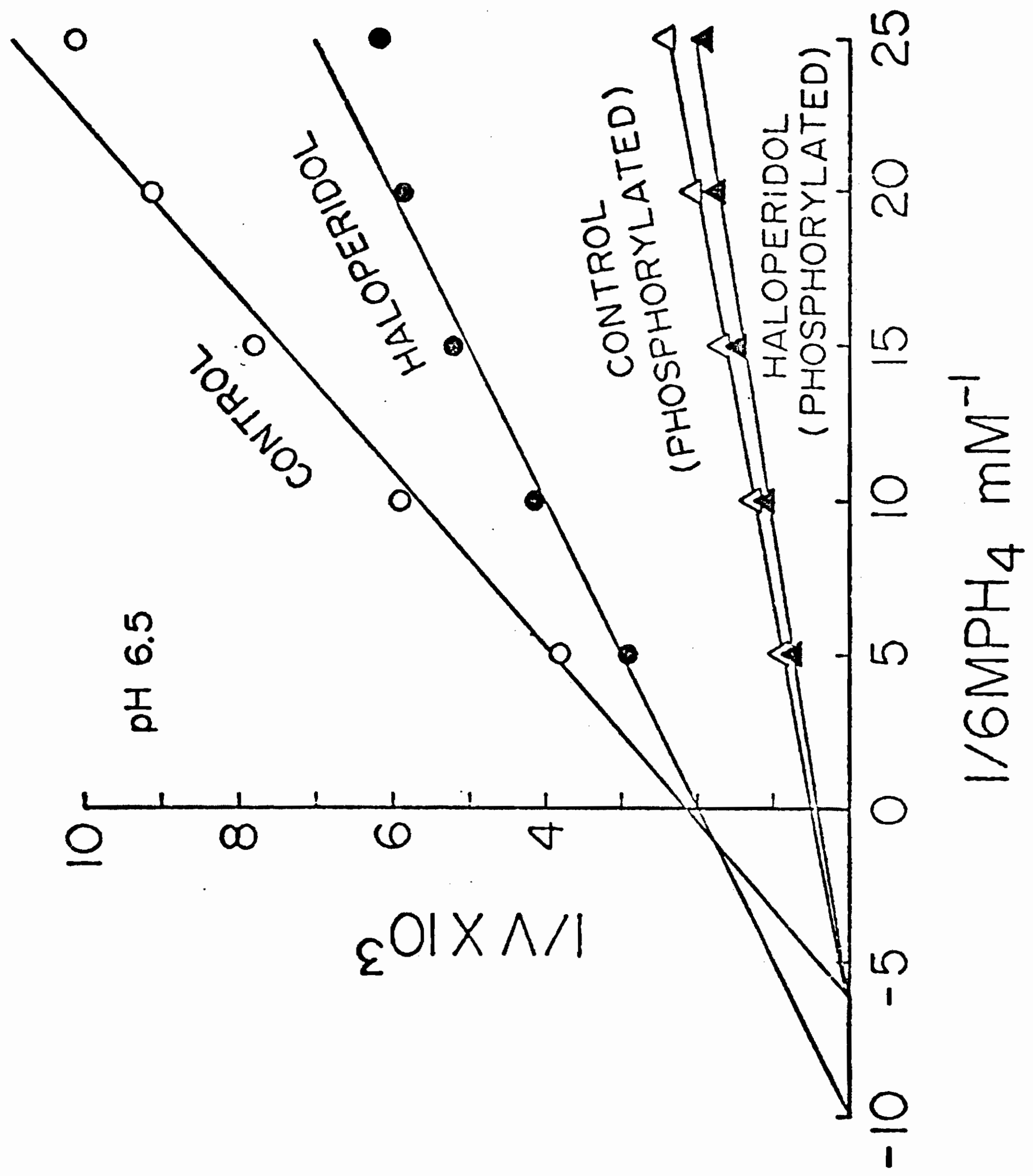
The incubation was carried out at 37°C for 15 min. and terminated by the addition of 100 μl of 10% trichloroacetic acid. The

acidified incubation medium was then placed on a 0.5 x 2.0 cm Dowex 50-H^+ column and washed with 1.8 ml of distilled water. After addition of 15 ml of scintillation fluid, which contained 7.6 g of 2,5-diphenyloxazol in 1 liter toluene-Triton X-100 (2:1), the tritiated water produced was counted in a Packard liquid scintillation counter.

Results

Neostriatal tyrosine hydroxylase activity from control and haloperidol-treated rats was assayed at the following final pH values, 6.0, 6.3 and 6.5, with and without phosphorylating conditions. Lineweaver-Burk plots were obtained by varying the concentration of the cofactor, $6MPH_4$, and measuring the initial velocity of tyrosine oxidizied. The activity of TH may be variably influenced by different cofactors and assay systems (15,16). In this study the cofactor was held constant and the effects of pH and phosphorylation were determined. As reported previously by other investigators (11), at pH 6.5 haloperidol did not change the apparent Vmax but caused a marked decrease in the Km of the enzyme for the pteridine cofactor (Fig. 1). However, when TH activity from control and haloperidol-treated rats was assayed under phosphorylating conditions at pH 6.5, an increase in Vmax but no change in Km for the enzyme from control rats occurred. In the haloperidol-treated rats an increase in Vmax and an increase in the Km for $6MPH_4$ was observed with phosphorylating conditions. This Km was not significantly different from the Km of the enzyme from control animals.

Fig. 1 The effect of phosphorylating conditions on the apparent Km for $6MPH_4$ and Vmax of neostriatal tyrosine hydroxylase from control and haloperidol treated rats at pH 6.5. The velocities are expressed in nanomoles of tyrosine oxidized/gm/hr.


pH 6.5
CONTROL
HALOPERIDOL
CONTROL
(PHOSPHORYLATED)
HALOPERIDOL
(PHOSPHORYLATED)
1/V X 10^3
10
8
6
4
-10
-5
0
5
10
15
20
25
1/6MPH4 mM^-1

It should be noted that at pH 6.5 the enzyme activity was greatly diminished relative to values obtained at optimum pH conditions (5.7 - 6.0) and the activation produced by phosphorylation increased the Vmax by more than 3-fold, to a value approximating that of the nonphosphorylated TH measured at the *in vitro* pH optimum.

The kinetic parameters for the control and haloperidol-treated rat neostriatal TH at three pH values, with and without phosphorylating conditions, is presented in Table 1. At pH 6.3, neostriatal TH from haloperidol-treated rats showed no significant activation and the Vmax and Km values were not significantly different from control values. Furthermore, when the enzyme from the two groups was phosphorylated, a similar increase in Vmax and a slight decrease in Km occurred in neostriatal TH from both control and haloperidol-treated rats. In addition, at pH 6.0, no significant difference was observed in the kinetics of TH from control and haloperidol-treated rats.

The kinetics of the phosphorylated enzyme with respect to the cofactor were then studied at the pH optimum, 6.0. As reported previously by other investigators (5-7), we observed a significant decrease in Km but no change in Vmax (Table 1). The effects of pH variation on the kinetics of the enzyme from control rats assayed under normal and phosphorylating conditions were analyzed by comparative Lineweaver-Burk plots (Fig. 2).

The kinetics of control and control-phosphorylated enzyme differed markedly. With increasing pH, a sharp decrease in Vmax but no significant change in Km was observed for control TH (Fig. 2a).

Table 1

Effects of pH and phosphorylation on apparent Km for $6MPH_4$ and Vmax of neostriatal tyrosine hydroxylase from control and haloperidol-treated rats.

		Control[ϕ]	Haloperidol	Control Phos-phorylated	Haloperidol Phos-phorylated
pH 6.0	Km*	0.15 ± 0.02	0.15 ± 0.02	0.08 ± 0.005	----
	Vm†	2430 ± 168	2499 ± 162	2638 ± 78	----
pH 6.3	Km	0.17 ± 0.01	0.15 ± 0.02	0.12 ± 0.01	0.11 ± 0.02
	Vm	1387 ± 165	1151 ± 131	2155 ± 142	2440 ± 60
pH 6.5	Km	0.18 ± 0.01	0.10 ± 0.003	0.17 ± 0.01	0.18 ± 0.01
	Vm	508 ± 10	522 ± 24	1931 ± 210	2281 ± 110

The results are expressed as the means ± SEM from at least four separate enzyme preparations each of which were obtained from pooled striata from 2 rats and assayed in duplicate.

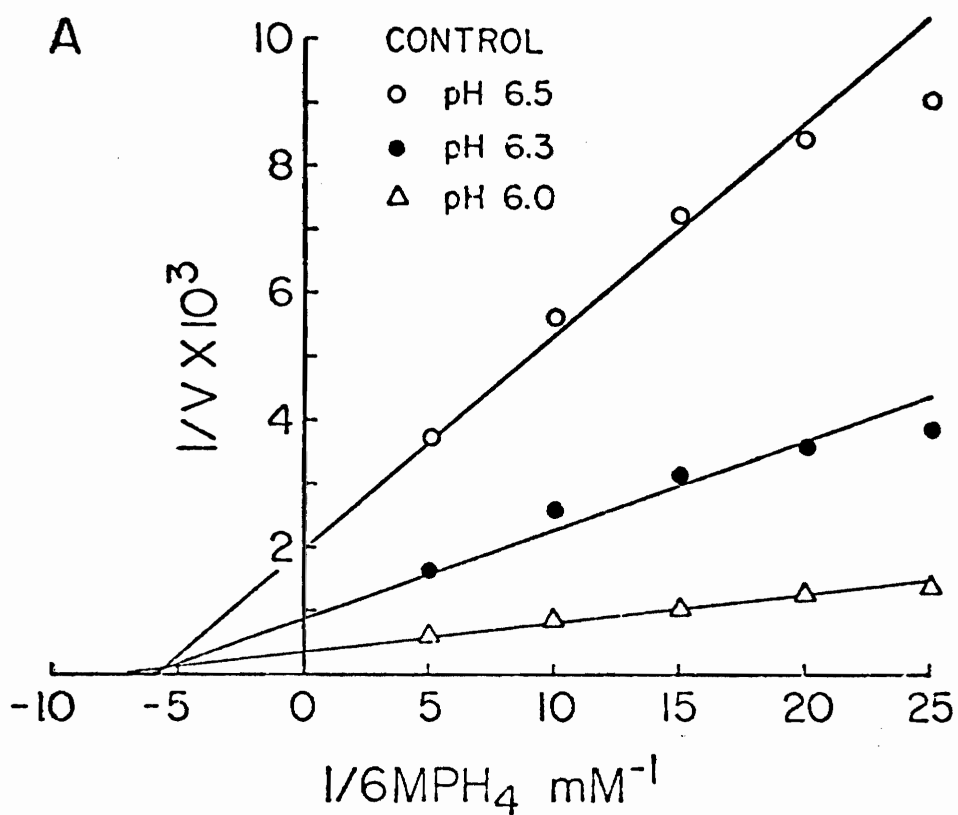
* expressed in mM

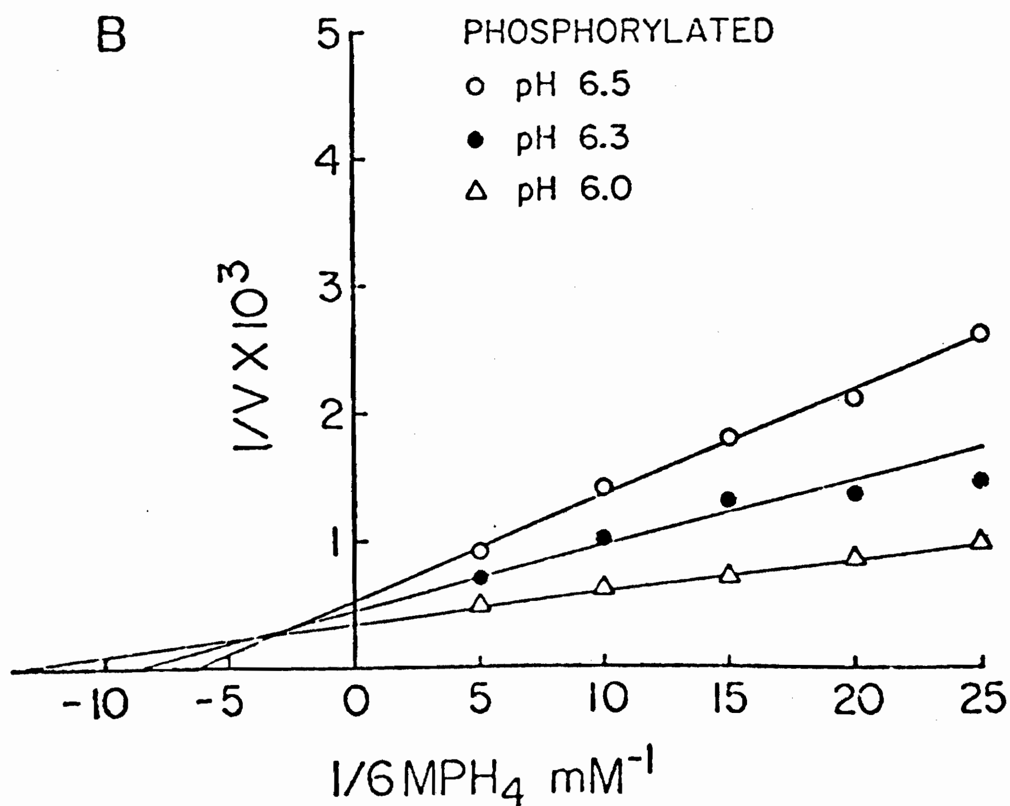
† expressed in nmoles tyrosine oxidized/gm/hr

ϕ The kinetic behavior of TH from lactic acid controls were not significantly different than saline-injected rats.

Appropriate comparisons of values in the table were made and significance determined by Student's t-test. When the SEM's did not overlap, the values were found to be significant, $p<0.05$.

Fig. 2 The effect of pH on the apparent Km for $6MPH_4$ and Vmax of control and phosphorylated neostriatal tyrosine hydroxylase. The velocities are expressed in nanomoles of tyrosine oxidized/gm/hr.


A
CONTROL
pH 6.5
pH 6.3
pH 6.0
1/V X 10^3
10
8
6
4
2
-10
-5
0
5
10
15
20
25
1/6MPH4 mM^-1


B
PHOSPHORYLATED
pH 6.5
pH 6.3
pH 6.0
1/V X 10^3
5
4
3
2
1
-10
-5
0
5
10
15
20
25
1/6MPH4 mM^-1

With phosphorylating conditions, the Km of the enzyme for the cofactor markedly increased, and a slight decrease in the Vmax was observed with increasing pH values (Fig. 2a).

Discussion

The present studies indicate that the kinetic behavior of tyrosine hydroxylase, with respect to the pteridine cofactor, is variably influenced by the pH of the assay mixture and the phosphorylated state of the enzyme. The affinity of TH for the pteridine cofactor is significantly enhanced following acute treatment of rats with haloperidol (11). This haloperidol-induced effect is, however, dependent on the pH of the assay medium (13), and can only be observed at the higher pH range, but not at the *in vitro* pH optimum for TH, 6.0. In fact, no significant activation by haloperidol treatment was observed at pH 6.3 (Table 1). When measured above pH 6.3 the TH activity was significantly diminished (2,3), and may indicate relative inactivation of the enzyme. By lowering the pH of the assay mixture to 6.0, the activity of TH is increased severalfold compared to its activity at pH 6.5. This suggests that haloperidol produces activation of a relatively inhibited enzyme. It is recognized that one cannot conclusively state whether the hydrogen ion concentration is acting directly on the enzyme itself or whether another component of the assay system is affected which may indirectly influence the apparent enzyme activity. The haloperidol-induced activation of TH is relatively small compared to the *in vitro* maximally activated enzyme. The importance of this activation of TH through a decrease in

Km of the enzyme for the pteridine cofactor is not well understood at this time, but may, in fact, be much greater under physiological conditions than under conditions employed in the *in vitro* assay.

When phosphorylating conditions were employed at pH 6.5, similar kinetics were observed for TH from control and haloperidol-treated rats. The haloperidol-induced activation was no longer evident and the increased affinity of TH for the pteridine cofactor was reversed i.e., the Km returned to the control value (Fig. 1). For control TH, at pH 6.5, activation due to phosphorylation was accomplished by a change in Vmax only. In contrast, with phosphorylating conditions at the same pH, the kinetics of the enzyme from haloperidol-treated rats showed an increase in Km for the cofactor in addition to an increase in Vmax, to values similar to those observed for the phosphorylated enzyme from control rats. The marked difference in the kinetics of TH from haloperidol-treated rats on the one hand and TH from control and haloperidol-treated rats assayed under phosphorylating conditions, on the other, does not support the argument that haloperidol is producing its effect through phosphorylation of TH. In fact, it suggests that other mechanisms may be involved.

The effect of pH on the kinetics of the control and control-phosphorylated enzyme with respect to the pteridine cofactor was then assessed by varying the pH of the assay mixture. The Vmax of control TH decreased markedly with increasing pH values, which is consistent with the narrow pH optimum of the enzyme (2); but the affinity of TH for the cofactor did not change significantly (Fig. 2a). An increase in Vmax without a change in Km of the enzyme may reflect the

conversion of inactive enzyme molecules to catalytically active components (8).

When the kinetics of TH are compared in control or phosphorylating conditions at varying pH values, some interesting observations emerge. Whereas the Vmax of the control enzyme decreases dramatically with increasing pH, the Vmax of phosphorylated enzyme is not markedly altered by a change in pH but remains relatively high (Table 1). In contrast, the Km for cofactor of the control enzyme did not change significantly, but the Km of the phosphorylated TH increased twofold by increasing the pH from 6.0 to 6.5.

The significance of these findings may be better appreciated if one considers that pH and phosphorylation, in addition to other factors such as nerve stimulation (9,17) inhibition by dopamine and norepinephrine and anions (18,19), may be influencing TH activity under physiological conditions. Thus tyrosine hydroxylase may exhibit a great deal of flexibility with multiple mechanisms for modulation of catecholamine synthesis rates in response to a variety of complex conditions which may be operating on the enzyme _in vivo_.

Finally, conflicting reports have appeared concerning activation of TH with phosphorylation. In some cases a decrease in Km (4-7) has been reported while in others activation is manifested by a change in Vmax (8,10). Our observations may help resolve some of the controversy in the literature concerning the effects of cAMP on the kinetics of TH with respect to the pteridine cofactor.

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PART THREE

EFFECTS OF METHAMPHETAMINE ON KINETIC CHARACTERISTICS OF NEOSTRIATAL TYROSINE HYDROXYLASE

Introduction

It has been reported previously from our laboratory that methamphetamine in large doses depresses tyrosine hydroxylase (TH) in the corpus striatum (1,2) and in the substantia nigra (3). This decrease in enzyme activity was prevented by concomitant administration of neuroleptic agents (2). The mechanism(s) by which methamphetamine depresses neostriatal TH activity has not been elucidated. Such a depression of enzyme activity could be attributed to several factors such as a decrease in affinity of the enzyme for the cofactor or substrate, activation of inhibitors, conversion of active components into inactive molecules, or to a decrease in the amount of enzyme present. The affinity for the pteridine cofactor of neostriatal TH is increased by a number of factors, including neuroleptic agents (4), phosphorylation and gel filtration (5), and polyanions (6).

In this study, the kinetic characteristics of rat neostriatal tyrosine hydroxylase were examined after chronic treatment of rats with methamphetamine. The effects of employing a phosphorylating system *in vitro* on the kinetics of the enzyme were also explored. The results suggest that the decrease in TH activity cannot be attributed to a change in Km for the cofactor or substrate but, rather, to a decrease in the maximal velocity.

Methods

Male Sprague-Dawley rats weighing 150-250 gm were housed three per cage in a temperature-controlled room (26°C) with a 12-hr light-dark cycle. Food and water were offered ad libitum.

Rats were injected every 6 hr with methamphetamine (15 mg/kg, s.c.) and were sacrificed by decapitation at varying intervals after initiating treatment. Rat neostriata were removed, placed on dry ice, and assayed within 24 hr. All steps for preparing the enzyme were performed at 0-5°C. TH activity was determined by the method of Nagatsu et al. (7). The neostriata were homogenized in 0.8 ml of 0.2 M sodium acetate, pH 6.0, containing 0.2% Triton X-100 and were centrifuged at 27,000 xg for 15 min. at 4°C. The supernatants were assayed in a 100-μl incubation medium containing 1 mM of ferrous ammonium sulfate, 100 mM of mercaptoethanol, 0.2 M of sodium acetate, pH 6.0, 0.1 mM of tyrosine, 0.5 μCi of purified 3,5-ditritiotyrosine, and varying concentrations of 2-amino-4-hydroxy-6,7-dimethyltetrahydropterin ($DMPH_4$, Aldrich) or 2-amino-4-hydroxy-6-methyltetrahydropterin ($6MPH_4$, Calbiochem).

When phosphorylating conditions were employed, the final concentration of the following components were 12.5 mM of magnesium acetate, 12.5 mM of sodium fluoride, 0.5 mM of theophylline, 75 μM of EGTA, 312.5 μM of ATP, and 62.5 μM of 3', 5',-cAMP (8). In determining the kinetic constants with respect to tyrosine, concentrations of 0.02 to 0.1 mM were used with $6MPH_4$ (0.2 mM) or $DMPH_4$ (2mM).

The incubation was carried out at 37°C for 15 min. and terminated by the addition of 100 μl of 10% trichloroacetic acid. The acidified

incubation medium was then placed on a 0.5 x 2.0 cm Dowex 50-H^+ column and washed with 1.8 ml of distilled water. After the addition of 15 ml of scintillation fluid, which contained 7.6 g of 2,4 diphenyloxazole in 1 liter of toluene-Triton X-100 (2:1), the radioactivity of the tritiated water produced was counted in a Packard liquid scintillation counter.

Results

The neostriatal TH activity in rats treated with repeated doses of methamphetamine is reported in Figure 1A. Double-reciprocal plots were obtained at various concentrations of the cofactor, $DMPH_4$, against the initial velocity of tyrosine oxidized. Methamphetamine did not significantly change the apparent Km for cofactor, but it decreased the apparent Vmax of the enzyme from 2730 ± 272 to 1218 ± 387 nmoles tyrosine oxidized/gm tissue/hr. The possible alteration by methamphetamine of the affinity of the enzyme for its substrate was also determined. Kinetic properties of neostriatal TH from normal rats and from those receiving methamphetamine are compared in Table 1. Enzyme activity was measured in the presence of either $DMPH_4$ (2 mM) or $6MPH_4$ (0.2 mM). Treatment of rats with methamphetamine did not significantly alter the apparent Km of TH for the substrate, tyrosine. The apparent Vmax was decreased to 47% of control when $6MPH_4$ was used as cofactor and 37% of control when $DMPH_4$ was used as cofactor. This decrease is similar to the depression of enzyme activity observed in Figure 1A.

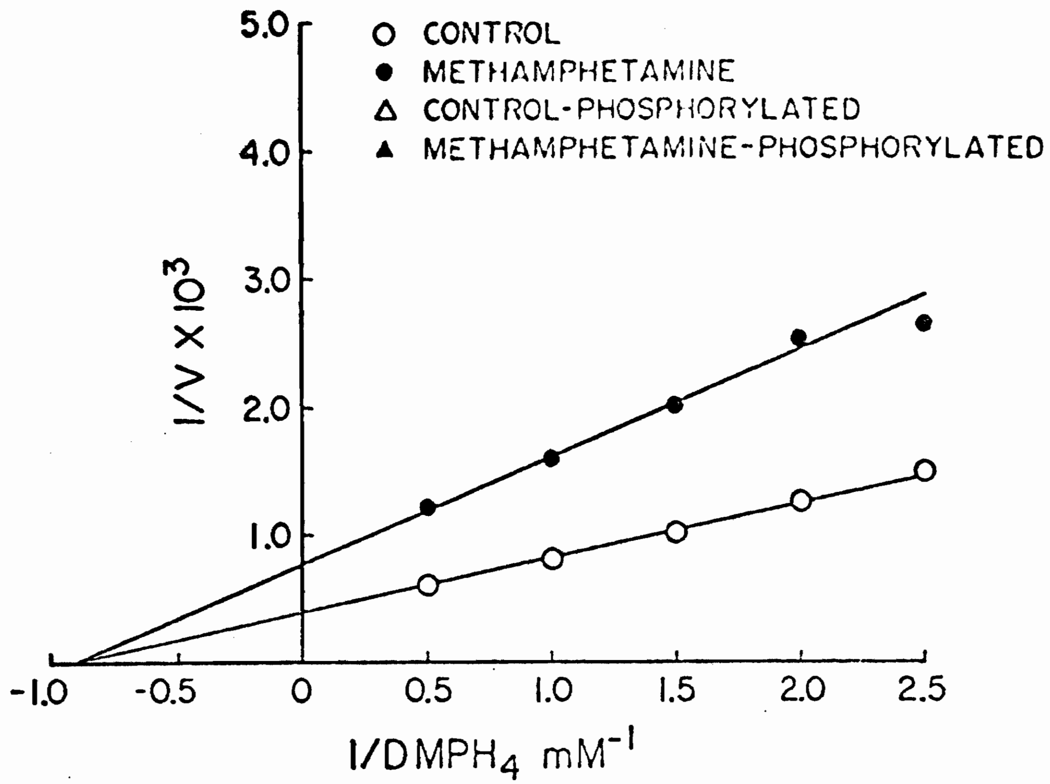
At optimal pH conditions, phosphorylation of TH increases the affinity of the enzyme for the cofactor, while the initial velocity of the enzyme is unaltered (5,9). The influence of methamphetamine on the kinetic characteristics of neostriatal TH when an _in vitro_ phosphorylating system was present in the incubation medium is shown in Figure 1B. The apparent Km was decreased to the same degree, both in the neostriatum from methamphetamine-treated rats as well as from the saline control rats. The apparent Vmax was depressed by methamphetamine to the same extent when the phosphorylating system was present or absent.

We have previously reported (3) that the neostriatal dopamine levels were elevated within 6 hrs. after the initial dose of methamphetamine. The possibility that the drug in the initial stages of treatment was changing the Km of the enzyme for cofactor and, thus, increasing the synthesis of dopamine, was explored. The data presented in Table 2 indicate that there was no significant change in the apparent Km during the 36-hr. period when the Km and Vmax were monitored, while a gradual decrease in Vmax was observed over the same period of time.

Discussion

Neostriatal TH activity is depressed by large, repeated doses of methamphetamine (1,2,3,10). This depression of enzyme activity could theoretically be attributed to an altered affinity of the enzyme for the cofactor or substrate or to a decreased Vmax. When the enzyme activity was measured with varying concentrations of cofactor, the

Fig. 1 Effect of methamphetamine on kinetics of neostriatal tyrosine hydroxylase. Double-reciprocol plots of initial velocity of tyrosine hydroxylase against various concentrations of either $DMPH_4$ or $6MPH_4$ in the presence of tyrosine (0.1 mM). The values shown are means of separate determinations on five different groups of rats. Fig. 1B also shows the effect of phosphorylating conditions on the kinetic properties of tyrosine hydroxylase. The conditions for phosphorylation experiments are described in the text. The velocities are expressed in nmoles of tyrosine oxidized/gm/hr.


A
O CONTROL
● METHAMPHETAMINE
△ CONTROL-PHOSPHORYLATED
▲ METHAMPHETAMINE-PHOSPHORYLATED
5.0
4.0
3.0
2.0
1.0
1/V X 10³
-1.0
-0.5
0
0.5
1.0
1.5
2.0
2.5
1/DMPH₄ mM⁻¹

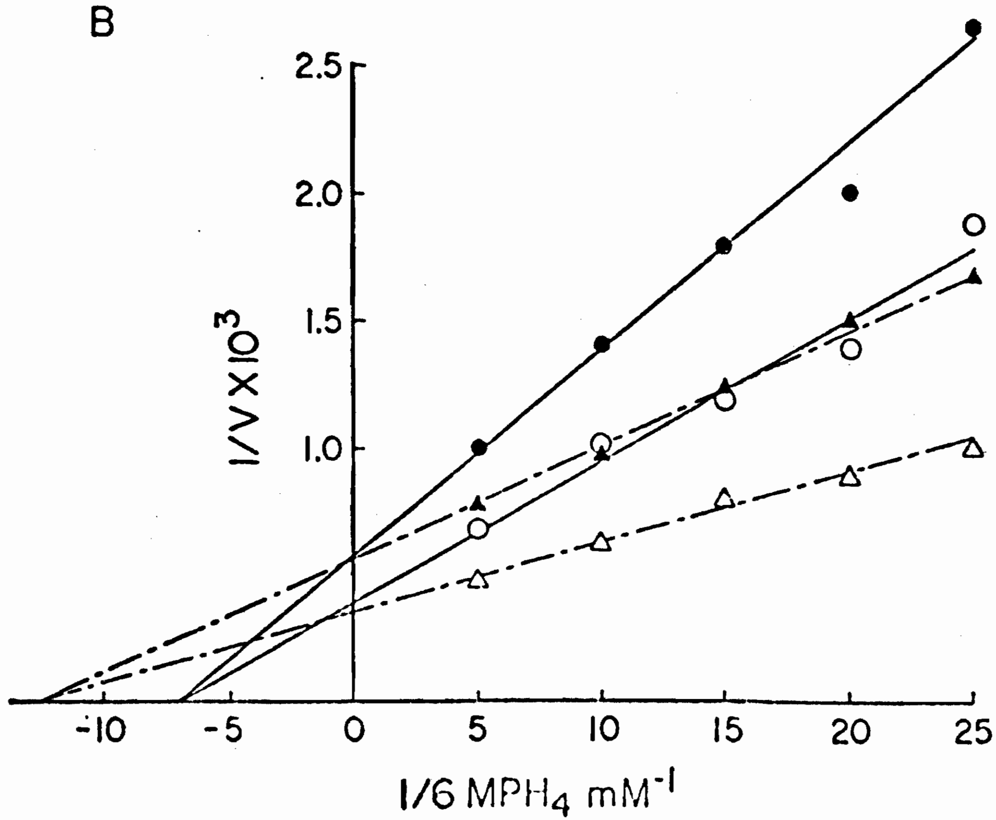

B
2.5
2.0
1.5
1.0
1/V X 10³
-10
-5
0
5
10
15
20
25
1/6 MPH₄ mM⁻¹

Table 1

Kinetic Properties of Neostriatal Tyrosine Hydroxylase in Rats Receiving Methamphetamine

	Km for Tyrosine (mM)			
Cofactor	Control	METH	Control	METH
$DMPH_4$ (2 mM)	0.10 ± 0.005	0.085 ± 0.008	3167 ± 769	1501 ± 168
$6MPH_4$ (0.2 mM)	0.022± 0.002	0.023 ± 0.005	1547 ± 43	569 ± 37

Methamphetamine (15 mg/kg, s.c.) was administered every 6 hrs for 30 hrs and animals were sacrificed 35 hr after the first injection. The values given are means and standard error of the mean for 4 or more rats whose neostriata were analyzed separately. Enzyme activity was measured in the presence of either $DMPH_4$ or $6MPH_4$. Kinetic constants with respect to tyrosine were determined using tyrosine concentrations of 0.02 to 0.1 mM. The velocities are expressed in nmoles of tyrosine oxidized/gm/hr.

Table 2

Time Response of Methamphetamine on Kinetics of Rat Neostriatal Tyrosine Hydroxylase

Hours of Methamphetamine Treatment	0	2	4	6	9	12	24	36
Km $DMPH_4$ (nM)	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25
Vmax	3125	3225	2941	2985	2439	2381	1219	1266

The figures depict a representative experiment wherein the Km for $DMPH_4$ and the Vmax were monitored for increasing periods of time after methamphetamine treatment. One dose of methamphetamine (15 mg/kg, s.c.) was administered in animals sacrificed at 2, 4 and 6 hrs, while two doses were administered to animals sacrificed 9 and 12 hrs. Animals sacrificed at 24 and 35 hrs received four and five doses of methamphetamine respectively. Vmax is expressed in nmoles tyrosine oxidized/gm tissue/hr.

apparent Km for the cofactor was not affected (Fig. 1). In addition, the affinity of TH for the substrate did not change with treatment of rats with methamphetamine (Table 1). A similar depression in the Vmax of TH was observed when both cofactor and substrate concentrations were varied in normal and methamphetamine-treated rats.

Lovenberg et al. (8) found that addition of a phosphorylating system in vitro to the assay medium reduced the apparent Km for cofactor of solubilized TH from rat neostriata. When an in vitro phosphorylating system was added to the assay medium for the measurement of neostriatal TH activity from normal and methamphetamine-treated rats, the apparent Km was altered by phosphorylation, but methamphetamine had no effect on the apparent Km for the cofactor. The apparent Vmax was altered to the same degree by methamphetamine with or without a phosphorylating system in the incubation medium (Fig. 1B).

The exact mechanism by which methamphetamine is causing a decrease in TH activity cannot be conclusively defined at this time. Several mechanisms may provide an explanation for the decrease in TH activity following treatment of rats with methamphetamine. Examples include our hypothesis proposed earlier (2), by which methamphetamine-released dopamine in the neostriatum results in an intense activation of the striatonigral neuronal feedback loop to the substantia nigra which would cause a decreased synthesis of TH in the cell bodies of the nigrostriatal pathway located in the substantia nigra. However, results obtained recently (11) in our laboratory with lesion studies, in addition to electrophysiological data reported by Groves et al. (12) and Bunney et al. (13), suggest that the initial site of action

of methamphetamine may be the release of dopamine from the dendrites of dopaminergic neurons in the nigra. The released dopamine would then act on autoreceptors located on cell bodies or on dendrites in the nigra to depress the firing rate of the dopaminergic cells which would result in a decrease in neostriatal TH activity 18-36 hrs later, as we previously observed (3). Another mechanism that may explain the data reported in this paper involves the activation of presynaptic receptors. Carlsson and associates (14) suggested that dopamine activates "autoreceptors" on the nerve terminals, which decreases TH activity in the nerve terminal. Presumably, the neuroleptic agents would prevent this effect by blocking the presynaptic "autoreceptors."

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PART FOUR

PROPRANOLOL DIFFERENTIALLY BLOCKS THE METHAMPHETAMINE-INDUCED DEPRESSION OF TRYPTOPHAN HYDROXYLASE: IMPLICATIONS OF USE IN SCHIZOPHRENIA

In recent years, large doses of propranolol have been reported to be beneficial in the treatment of schizophrenia (1). In addition to their membrane stabilizing and β-blocking activity, recent evidence indicates that propranolol and related β-blockers have 5-hydroxytryptamine (5HT) receptor antagonist activity. It has been shown that propranolol has high affinity for 5HT binding sites (2), blocks 5HT-induced hyperactivity in rats (3), and antagonizes the action of 5HT in the superior cervical ganglion of cats (4). High doses of methamphetamine or amphetamine can produce symptoms similar to paranoid schizophrenia in humans (5). In animals, methamphetamine or amphetamine produces a stereotypic behavior which is thought by some to be an animal correlate of human schizophrenia (6). Acute (7) and chronic (8) administration of methamphetamine produces large decreases in the activity of tryptophan hydroxylase (TPH), the rate-limiting enzyme in the biosynthesis of 5HT (9), in serotonergic nerve terminal regions of the rat brain. In addition, methamphetamine (10) and amphetamine (11) decrease the levels of brain 5HT and 5-hydroxyindoleacetic acid (5HIAA). Furthermore, clinical reports indicate that increased urinary excretion of 5HIAA by some schizophrenic patients, is reduced upon treatment with propranolol (12). Thus it has been proposed that a biochemical aberration of the serotonergic system may be involved in schizophrenia (13). In this study we report that propranolol blocks the methamphetamine-induced depression of TPH in a regionally selective manner.

Male Sprague-Dawley rats (Sasco, Omaha) weighing 200 to 300 g were used in these experiments. Rats were administered either meth-

amphetamine (10 mg/kg, s.c.), d,l-propranolol (40 mg/kg, i.p.), haloperidol (0.5 mg/kg, i.p.) or methamphetamine with either of the two antagonists concurrently. Control rats were injected with saline. Rats were sacrificed by decapitation 3 hours after drug injection. Experiments were designed so that all rats were sacrificed between 11 a.m. and 2 p.m. to avoid any significant variation in TPH activity due to diurnal rhythm. After decapitation, rat brains were rapidly removed and the following regions dissected on ice: nucleus accumbens, olfactory tubercle, neostriatum, hypothalamus, cerebral cortex, hippocampus, spinal cord, and raphe nuclei. All brain tissues were stored at -70°C until assayed. Tryptophan hydroxylase activity was measured by a $^{14}CO_2$ trapping method (14), as described by Hotchkiss et al. (8).

The basal level of activity of tryptophan hydroxylase in the regions studied is shown in Table 1. TPH activity varied from one region to another. The cell body region, raphe nuclei, had enzyme activity that was several-fold higher than the terminal regions. Among the terminal regions, hypothalamus, hippocampus and spinal cord had much higher basal enzyme activity than the other regions. Following a single dose of methamphetamine (10 mg/kg), TPH activity was markedly depressed in all serotonergic nerve terminal regions assayed (Table 1). Reduction in enzyme activity varied from one region to another; TPH activities in the cortex, hippocampus, olfactory tubercle, and spinal cord were reduced to less than half of control values. However, enzyme activity in the serotonergic nerve cell bodies in the raphe nuclei were not affected (8). Concurrent administration of pro-

Table 1. Effect of drug treatment on tryptophan hydroxylase activity. Values for nucleus accumbens and olfactory tubercle expressed in nmoles tryptophan oxidized/mg protein/hr. (mean ± S.E.M.). All other values are in nmoles/gram tissue weight/hr. Values in brackets are percent of control.

	control	propranolol	meth-amphetamine	meth-amphetamine + propranolol	methamphetamine + haloperidol
N. Accumbens	0.22 ± 0.01	0.24 ± 0.02[a] (109%)	0.12 ± 0.02[b] (54%)	0.19 ± 0.02[a,d] (86%)	0.14 ± 0.01[b,c] (64%)
O. Tubercle	0.24 ± 0.02	0.26 ± 0.01[a] (108%)	0.08 ± 0.02[b] (33%)	0.18 ± 0.02[b,d] (75%)	0.12 ± 0.02[b,d] (50%)
Neostriatum	15.0 ± 1.2	18.0 ± 1.0[a] (120%)	9.0 ± 0.9[b] (60%)	13.8 ± 2.0[a,d] (92%)	9.4 ± 1.5[b,c] (63%)
Hypothalamus	55.0 ± 5.7	75.0 ± 8.0[a] (136%)	35.0 ± 2.8[b] (64%)	54.0 ± 4.0[a,d] (98%)	37.4 ± 2.9[b,c] (68%)
C. Cortex	19.0 ± 0.9	21.5 ± 0.8[a] (108%)	8.9 ± 1.4[b] (45%)	12.4 ± 1.1[b,d] (62%)	9.3 ± 1.0[b,c] (47%)
Hippocampus	36.0 ± 1.4	34.0 ± 1.4[a] (94%)	14.0 ± 1.7[b] (39%)	19.8 ± 1.6[b,d] (55%)	21.0 ± 1.4[b,d] (58%)
Spinal Cord	30.7 ± 1.3	23.0 ± 1.8[a] (75%)	13.9 ± 1.9[b] (45%)	16.6 ± 3.0[b,c] (54%)	20.6 ± 2.2[b,d] (67%)
Raphe	630 ± 62	675 ± 63[a] (107%)	508 ± 64[a] (81%)	517 ± 39[a] (82%)	

a not significantly different from control, $p>0.05$.

b significantly different from control, $p<0.05$.

c not significantly different from methamphetamine treatment, $p>0.05$.

d significantly different from methamphetamine treatment, $p<0.05$.

pranolol (40 mg/kg, i.p.) produced differential regional blockade of TPH depression induced by methamphetamine. Depression of enzyme activities in the mesolimbic (nucleus accumbens and olfactory tubercle) areas, neostriatum and hypothalamus was almost completely blocked by propranolol, whereas the activities in other regions were still markedly depressed. Propranolol alone had no significant effect on TPH activity, although a trend toward an increase was observed, especially in the hypothalamus (Table 1). To compare the effects of propranolol with a more commonly used antipsychotic drug, haloperidol (0.5 mg/kg, i.p.) was given concurrently with methamphetamine. Haloperidol produced partial blockade of the methamphetamine-induced depression of TPH in olfactory tubercle, hippocampus and spinal cord and no significant blockade in all other regions (Table 1). Haloperidol alone had no significant effect on enzyme activities.

These data indicate that propranolol produces significant blockade of the methamphetamine-induced depression of TPH. It is interesting to note that the extent of the blockade varies from one serotonergic terminal region to another. Furthermore, in the mesolimbic areas, neostriatum and hypothalamus, regions that are commonly implicated in schizophrenia (15), propranolol almost completely prevents the depression in TPH activity. In contrast, only partial protection against enzyme depression is observed in the hippocampus and cortex and no effect was found in spinal cord. However, unlike propranolol, the antipsychotic drug haloperidol in the dose used, did not significantly protect against enzyme depression in most re-

gions. Unlike the long latency of the antipsychotic drugs to produce their effect, propranolol provides rapid relief from clinical symptoms of schizophrenia (1). Thus, the acute effects of propranolol on TPH activity are compatible with its clinical effects.

High doses of propranolol were used in this study. Clinically, there is wide variation in the dosage of propranolol that is effective in schizophrenic patients, but doses of 3000 mg/day are not uncommon, and doses of more than 5000 mg/day have been reported (1). In fact, low doses do not appear to be effective in relieving schizophrenic symptoms (16).

We have previously reported (17) that methamphetamine produces a marked decrease in tyrosine hydroxylase activity only after chronic treatment. In contrast, a single injection of methamphetamine causes a marked decrease in TPH activity. Thus, it appears that the serotonergic system is more vulnerable to the action of methamphetamine. Furthermore, several reports (18) have demonstrated an interaction between the dopaminergic and serotonergic systems. Considering the complexity of the schizophrenic syndrome, this difference may help explain the clinical effectiveness of drugs such as propranolol and haloperidol which have diverse mechanisms, in the treatment of schizophrenia.

Our data complement previous reports (12) which suggest an involvement of the serotonergic system in schizophrenia. The present results demonstrate for the first time that propranolol blocks the methamphetamine-induced depression of TPH in a regionally selective

manner. These findings may provide a biochemical rationale for the efficacy of propranolol in schizophrenia. In addition, these results emphasize the regional differences in sensitivity of serotonergic nerve terminal regions to drug treatment and, hence, the potential importance of these differences to the response by the central nervous system, in the etiology of schizophrenia.

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