THE ROLE OF GABA TRANSAMINASE INHIBITORS AND THE STRIATONIGRAL PATHWAY IN THE METHAMPHETAMINE-INDUCED DEPRESSION OF NEOSTRIATAL TYROSINE HYDROXYLASE

by

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ABSTRACT

The apparent Vmax of neostriatal tyrosine hydroxylase (TH) is decreased by subacute administration of methamphetamine (METH). It was previously postulated that this depression is mediated through GABAergic feedback inhibition of the nigral dopaminergic neurons via the striatonigral (SN) pathway. This hypothesis was tested by evaluating the effect of concurrent administration of a GABA transaminase inhibitor on the METH-induced depression of TH. Three GABA-T inhibitors were studied: γ-acetylenic GABA (γAG), aminooxyacetic acid (AOAA), and ethamolamine-O-sulfate (EOS). All three agents antagonize the METH effect; dose response curves were developed for γAG and AOAA. Control studies showed that hypothermia was not an essential component of this ability to antagonize METH.

The SN pathway was lesioned by three techniques to test the hypothesis that this tract is essential for the METH-induced depression of TH. The kainic acid lesion of the neostriatum destroys cell bodies by an "excitotoxic" action, thus eliminating the SN pathway. The second and third lesions, a wire knife lesion and an electrothermic (ET) lesion, interrupted the SN pathway in the crus cerebri just anterior to the substan-

tia nigra. None of the lesions prevented the METH-induced depression of TH, which indicates that the SN path is not an essential component of the METH effect. Agents known to block the METH effect were evaluated in kainic acid lesioned rats. Haloperidol (3 mg/kg) still protected both lesioned and control striata while γAG (15 mg/kg) protected only the control striata. This dose of γAG did not antagonize the METH-induced TH depression in the kainic acid lesioned striatum. Another dissimilarity between the action of the two antagonists of the METH effect was noted. METH caused the kainic acid lesioned rats to rotate ipsilateral to the lesioned side; both antagonists slowed the rotational rate but haloperidol reversed the direction of rotation while γAG did not.

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PART ONE

ORIENTATION

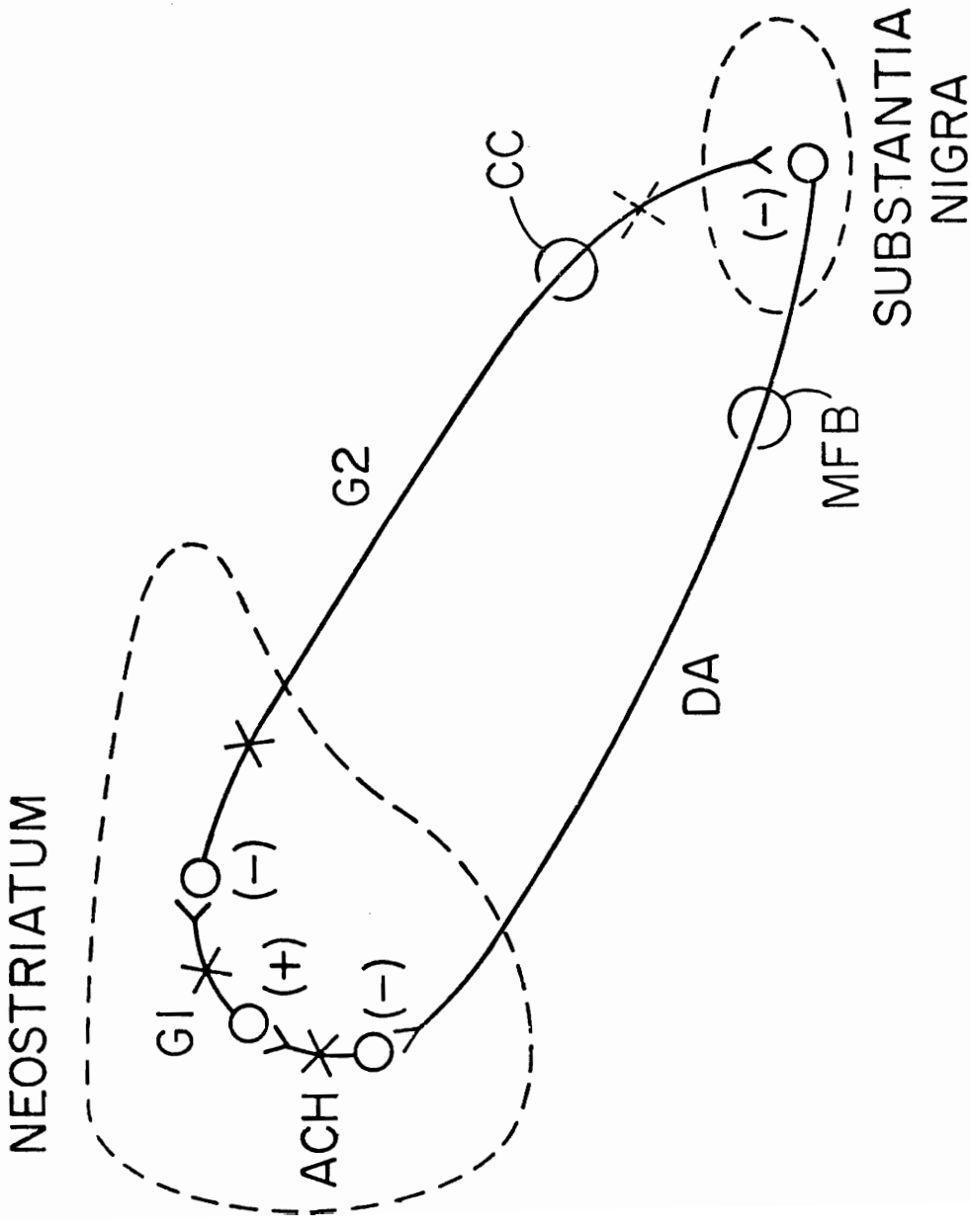
Background Information

The goal of this research has been to develop a better understanding of neurochemical changes observed in an animal model of subacute amphetamine intoxication. This work is divided into two parts: First, the evaluation of effects of GABA transaminase inhibitors on changes of neostriatal tyrosine hydroxylase (TH) activity following repeated doses of methamphetamine; secondly, a test of the hypothesis that a specific striatonigral pathway is the source of feedback inhibition which arises in the presence of methamphetamine. It is hoped that the results of these two studies will serve to enhance the understanding of homeostatic regulation of neuronal interaction and the mechanism of methamphetamine toxicity.

The relationship between the two nuclei studied here is depicted in Fig. 1. A similar model was first presented by Racagni et al.[1] Of course the true nature of nigral and neostriatal circuitry is much more complex and still controversial. Other neuronal types are also involved, including enkephalinergic striatal interneurons, substance P striatonigral neurons and possible recurrent collateralizations, as well as inputs and outputs of both nuclei involving other areas

FIG. 1 The two long pathways are the dopaminergic nigrostriatal pathway (DA) and the GABAergic striatonigral pathway (G2). Neostriatal interneurons shown are cholinergic (ACH) and GABAergic (G1). Excitatory (+) and inhibitory (-) actions are indicated. Kainic acid lesions are shown by solid crosses; the site of mechanical and electrothermic lesions is indicated by a dashed cross. Median forebrain bundle (MFB), crus cerebri (CC).

FIGURE 1



of the brain such as cerebral cortex, ventrolateral thalamus, dorsal and medial raphe, locus coeruleus etc. A much more complex circuit pattern has been published[2]. The primary value of this model is that it is logically consistent with well known effects of pharmacological agents on the firing rate of nigral dopaminergic cells and pharmacologically-induced changes in turnover of GABA, dopamine and acetylcholine in the neostriatum and in the substantia nigra. The major disadvantage of such models is that they are sometimes taken too seriously and therefore tend to codify major misconceptions about the functional relationships which we attempt to understand better.

To help orient the reader, a brief schematic is presented to demonstrate that the model in Fig. 1 could function as a negative feedback system under the influence of methamphetamine.

1) Methamphetamine releases dopamine onto the cholinergic interneuron (ACH).

2) The cholinergic interneuron is strongly inhibited, resulting in a decreased excitatory input to the GABAergic interneuron (GI).

3) The striatonigral projection, represented as G2, loses inhibition and becomes more active.

4) Increased activity of G2 leads to increased

inhibition of the dopaminergic cell body in the nigra.

It can be seen that the initial effect was increased stimulation of dopamine receptors on target cells in the nigra which led to decreased dopamine cell firing, a negative feedback.

The sites of lesions performed in Part II are also indicated in figure 1. The kainic acid lesion destroys striatal interneurons ACH and G1 as well as the efferent pathway, G2. The mechanical and electrothermic lesions of the crus cerebri interrupt the striatonigral pathway, G2, just rostral to the nigra.

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PART TWO

BLOCKADE OF METHAMPHETAMINE-INDUCED DEPRESSION OF TYROSINE HYDROXYLASE BY GABA TRANSAMINASE INHIBITORS

Introduction

Repeated, toxic doses of methamphetamine cause a decrease in the apparent Vmax of tyrosine hydroxylase (TH) in the neostriatum and substantia nigra of rats 36 hours after the first dose[7]. Since this type of methamphetamine exposure may represent a relevant animal model for predicting the effects of human parenteral abuse of amphetamine-like drugs, further understanding of the neurochemical basis of the methamphetamine-induced decrease in TH activity might help to explain certain aspects of their clinical toxicity.

Buening and Gibb[1] originally proposed that the continuous presence of methamphetamine caused the striatonigral pathway to impose a prolonged negative feedback onto the nigral dopaminergic neurons, which in turn led to a sustained inhibition of electrical activity in the nigrostriatal pathway. Because electrophysiological studies have demonstrated such an acute effect of amphetamine[2], it was logical to extend this observation to neurochemical studies in a chronic abuse model.

Prolonged preganglionic stimulation of adrenal medullary cells leads to an increase in adrenal TH activity whereas elimination of the preganglionic input

consistently causes a small depression of TH activity[15,16]. Therefore, the decrease in neostriatal TH activity observed in methamphetamine-treated rats could also be due to a decrease in excitatory drive. Alternatively, an increase in inhibitory drive to nigral dopamine neurons might induce an enzyme change similar to that seen in the decentralized adrenal medulla.

The effect of methamphetamine on neostriatal TH activity is blocked by neuroleptics[1] and by α-methyl-p-tyrosine[8], suggesting that catecholamine synthesis, release and receptor activation are necessary for methamphetamine-induced depression of TH. Because GABAergic neurons are thought to be involved in the striatonigral pathway[12], agents which elevated GABA levels were studied for possible effects on the methamphetamine model. Those results show that several GABA transaminase inhibitors block the ability of methamphetamine to reduce the apparent Vmax of TH. From this we conclude that the GABAergic systems involved in the regulation of nigral dopaminergic neurons can be made to antagonize the effects of amphetamine-like agents.

Methods

Male Sprague-Dawley rats (180 to 250 grams) were housed under 12-hour light-dark cycles, fed a standard diet ad libitum and allowed free access to water. Meth-

amphetamine was dissolved in 0.9% NaCl to a concentration of 10 mg/ml. The animals received methamphetamine (10 mg/kg, s.c.) at 6 p.m., midnight, 6 a.m., 12 noon and 6 p.m. Thirty-six hours after the initial injection, rats were sacrificed by decapitation and brains were removed and placed on an ice-cold surface. The neostriata were removed, frozen on dry ice and assayed for TH activity within 3 days. TH activity was measured by the method of Nagatsu et al.[10], with saturating concentrations of the pteridine cofactor, $DMPH_4$.

Appropriate doses of a GABA transaminase (GABA-T) inhibitor, aminoosyacetic acid (AOAA) or γ-acetylenic GABA (gAG), were administered intraperitoneally, concurrently with methamphetamine. Ethanolamine-O-sulfate (EOS), another GABA-T inhibitor, 400 µg, was dissolved in 10 µl of artificial cerebral spinal fluid, adjusted to pH 7.4 and injected stereotaxically under ether anesthesia in a single dose into the lateral ventricle, 2-6 hours before starting the subacute methamphetamine regimen. Control rats for the EOS experiment received intraventricular 10 µl injections of artificial CSF.

In experiments requiring temperature regulation, rectal temperatures were monitored periodically and rats were warmed as needed to keep body temperature between 36° and 38° C.

γAG was donated by Merrell International Research Center, EOS was purchased from Aldrich Chemical Company and AOAA was acquired from Sigma Chemical Company.

Results

AOAA inhibited methamphetamine-induced depression of TH in a dose dependent manner (Fig. 1). The ED_{50} was about 7 mg/kg and the maximal response was achieved at 20 mg/kg. Neither low nor high doses (5 and 40 mg/kg) of AOAA, given alone every 6 hours for 5 total doses, affected the apparent Vmax of TH.

Rectal temperatures, measured 24 hours after the first injection, indicated that methamphetamine produced hyperthermia, and AOAA (20 mg/kg) produced hypothermia even with concurrent methamphetamine administration (Fig. 2). Another report showing that alterations in environmental temperature cause changes in adrenal TH activity[15], suggested that hypothermia alone caused by AOAA might antagonize the effect of methamphetamine on neostriatal enzyme activity. Therefore, body temperature was controlled to within 36° to 38° C during administration of methamphetamine and AOAA in separate experiments. There was no significant difference in TH activity between normothermic and hypothermic rats (Fig. 3) suggesting that hypothermia alone did not account for the effect of AOAA on methamphetamine-in-

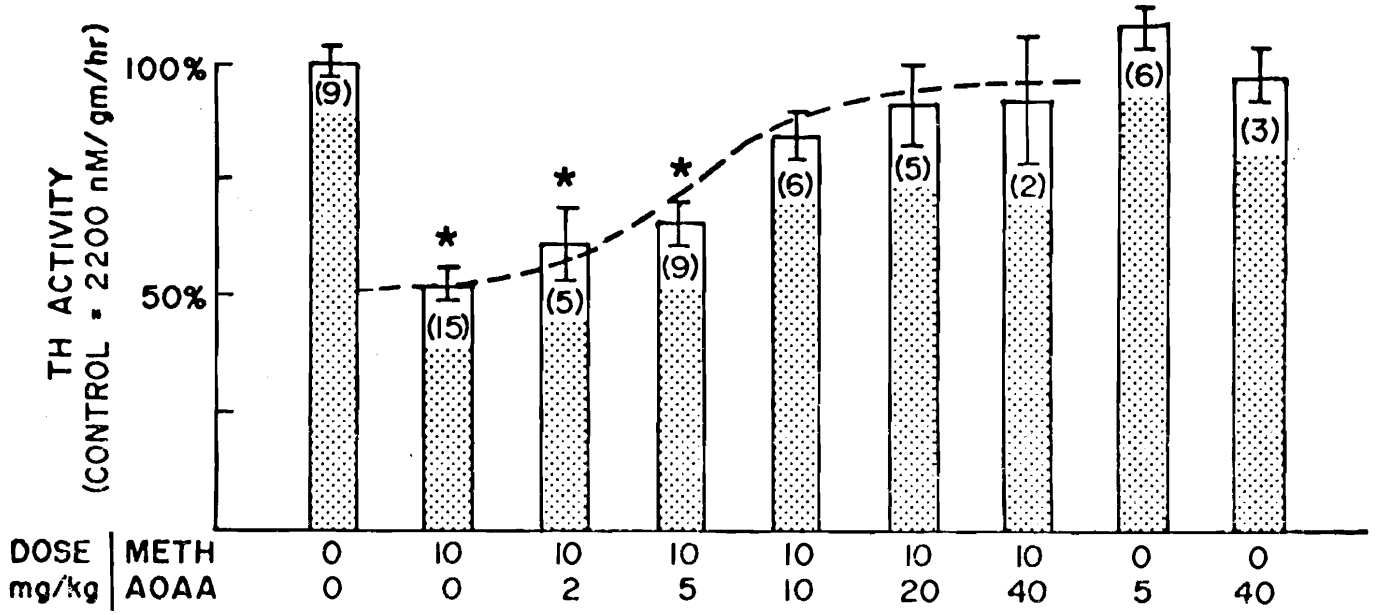


Fig. 1 Neostriatal TH activity with varying combinations of AOAA and methamphetamine. Both drugs were given simultaneously every 6 hours for total doses as described in text. Numbers in parenthesis refer to the number of animals per group. (*) $p < 0.01$ compared to saline control.

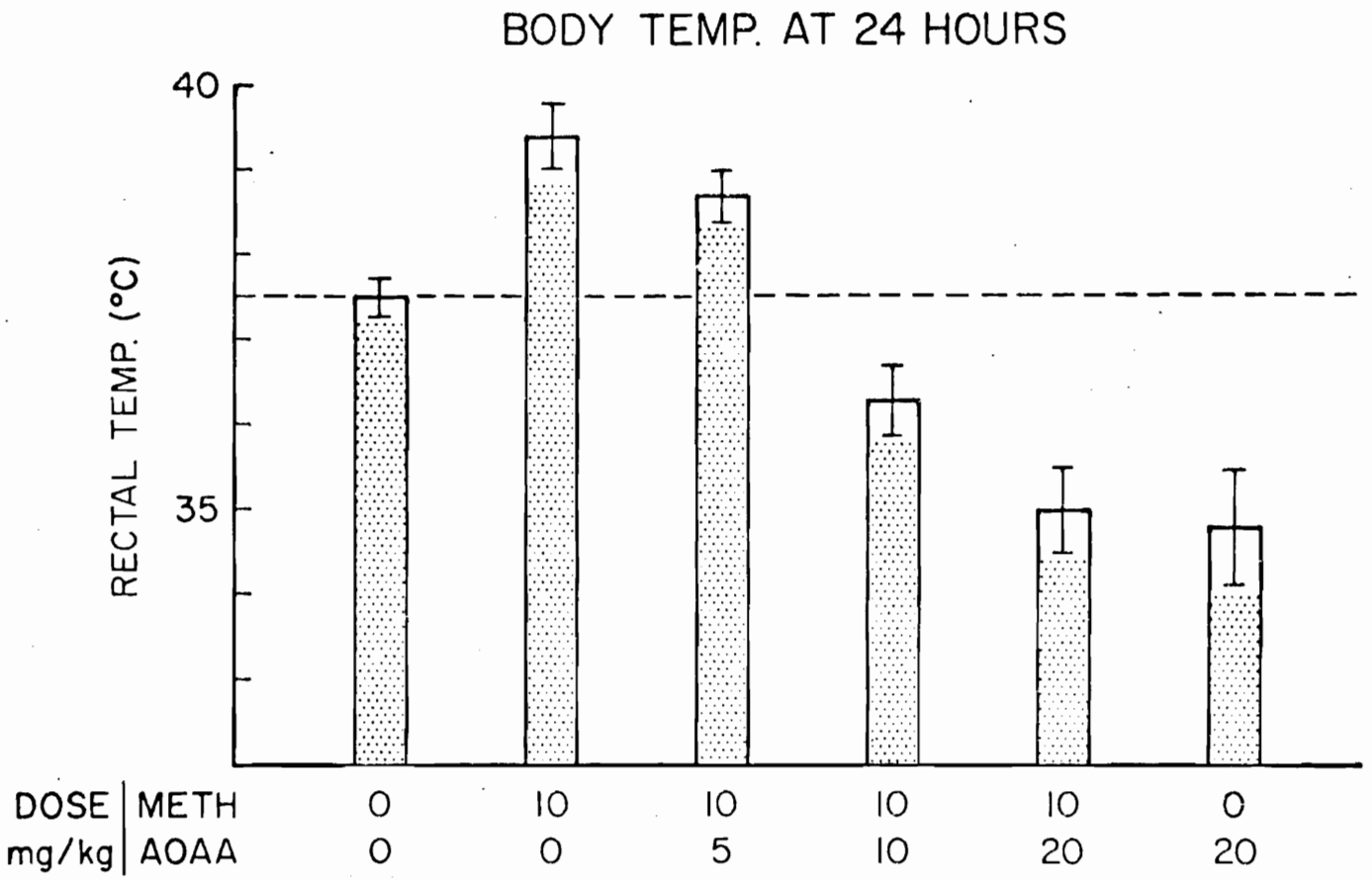
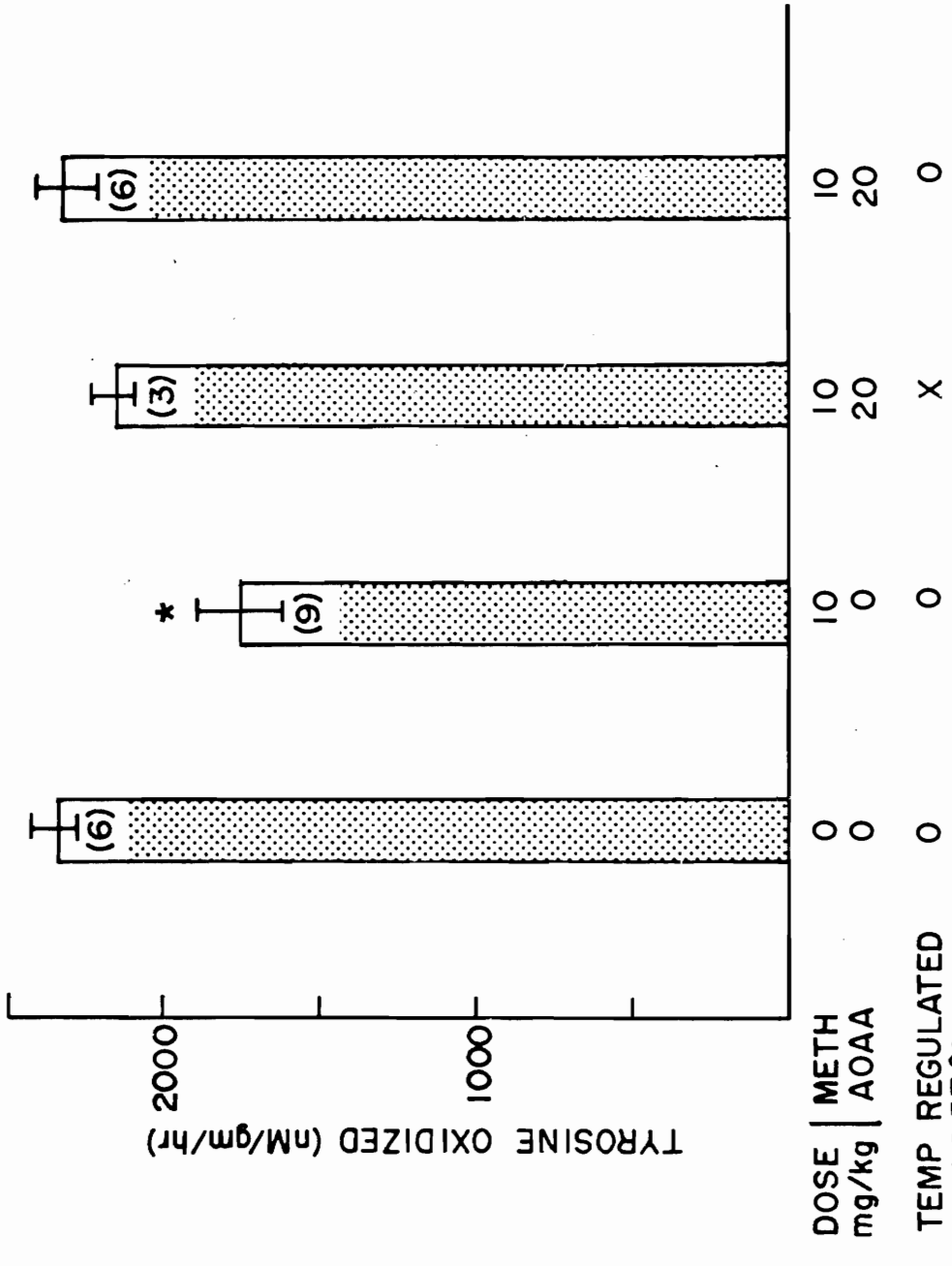


FIG. 2 Rectal temperature prior to the fifth dose of drugs; see the legend of Fig. 1 for dosage schedule.

FIG. 3 Effect of temperature control on methamphetamine-induced neostriatal TH depression. Numbers in parenthesis refer to the number of animals per group. (*) $p < 0.01$ compared to saline control.

FIGURE 3


TYROSINE OXIDIZED (nM/gm/hr)
2000
1000
(6)
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*
(9)
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DOSE | METH 0 0 10 0 10 20 10 20
mg/kg | AOAA 0 0 0 X 0
TEMP REGULATED AT 37°C

duced TH depression.

A second GABA-T inhibitor, γAG, was also evaluated for its effect on methamphetamine-induced TH depression. Concurrent administration of 15 mg/kg of γAG significantly antagonized and 40 mg/kg completely antagonized the TH depression; 1 and 5 mg/kg of γAG were ineffective (Fig. 4).

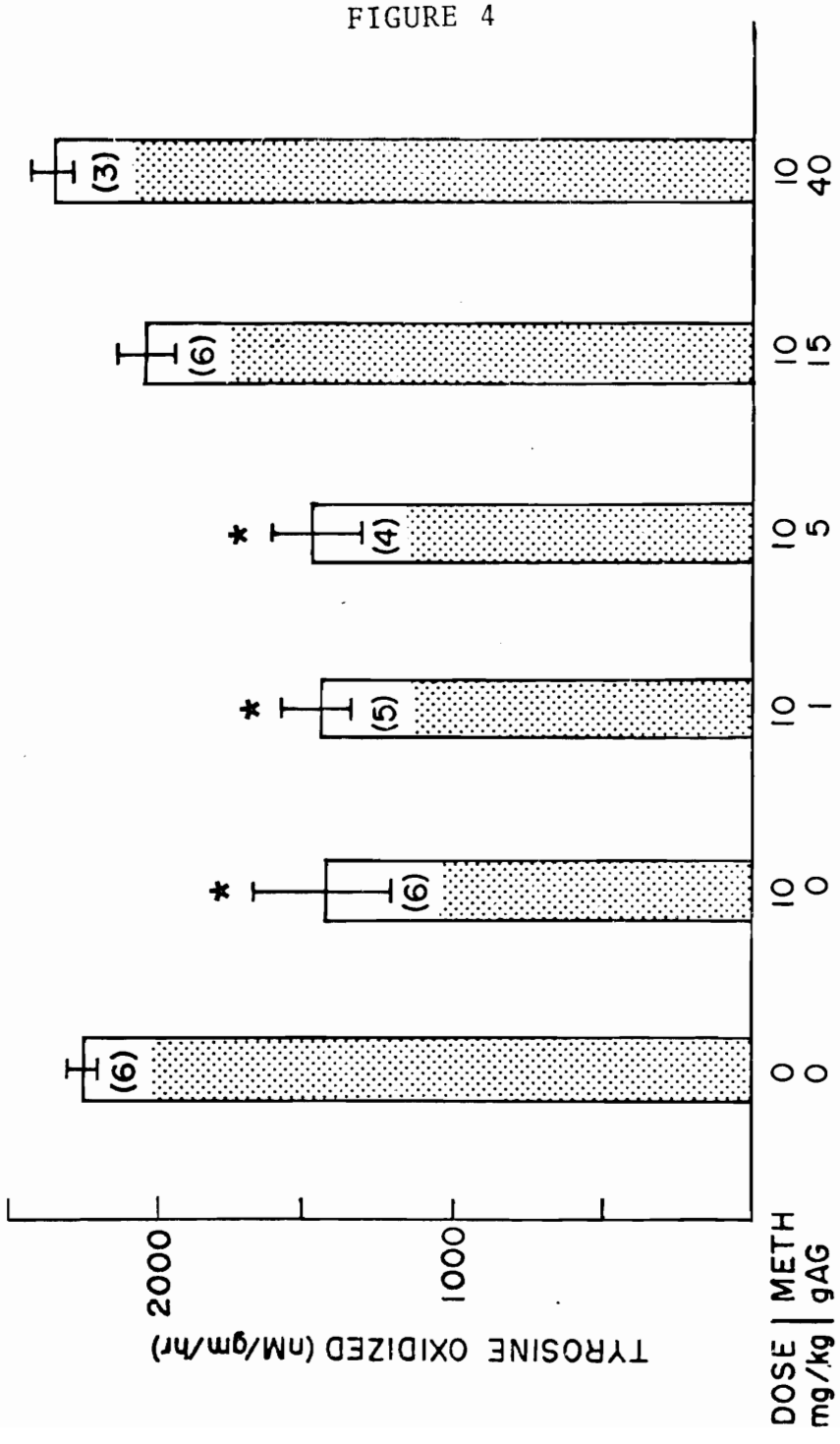
A single intraventricular administration of 400 μg of EOS completely reversed the effect of methamphetamine on TH, whereas EOS alone had no effect (Fig. 5).

At maximal doses of each GABA-T inhibitor, general motor activity was obviously reduced and the rats exhibited somewhat reduced stereotypic behavior. This sedation alone, however, did not appear to be responsible for the antagonism of the methamphetamine-induced TH depression because the concurrent administration of pentobarbital (25 mg/kg) had no effect on the TH depression despite a greater level of sedation than that produced by maximal doses of any of the GABA-T inhibitors.

A drug from another class, the benzodiazepines, which are thought to facilitate GABA-actions[3], was evaluated to see whether it too could block the action of methamphetamine on neostriatal TH. Diazepam (0.3 to 6.0 mg/kg), given concurrently with the methamphe-

FIG. 4 Neostriatal TH activity with increasing doses of gAG. Drugs were administered as described in the legend of Fig. 1. Numbers in parenthesis refer to the number of animals per group. (*) $p < 0.01$ compared to saline control.

FIGURE 4


TYROSINE OXIDIZED (nM/gm/hr)
2000
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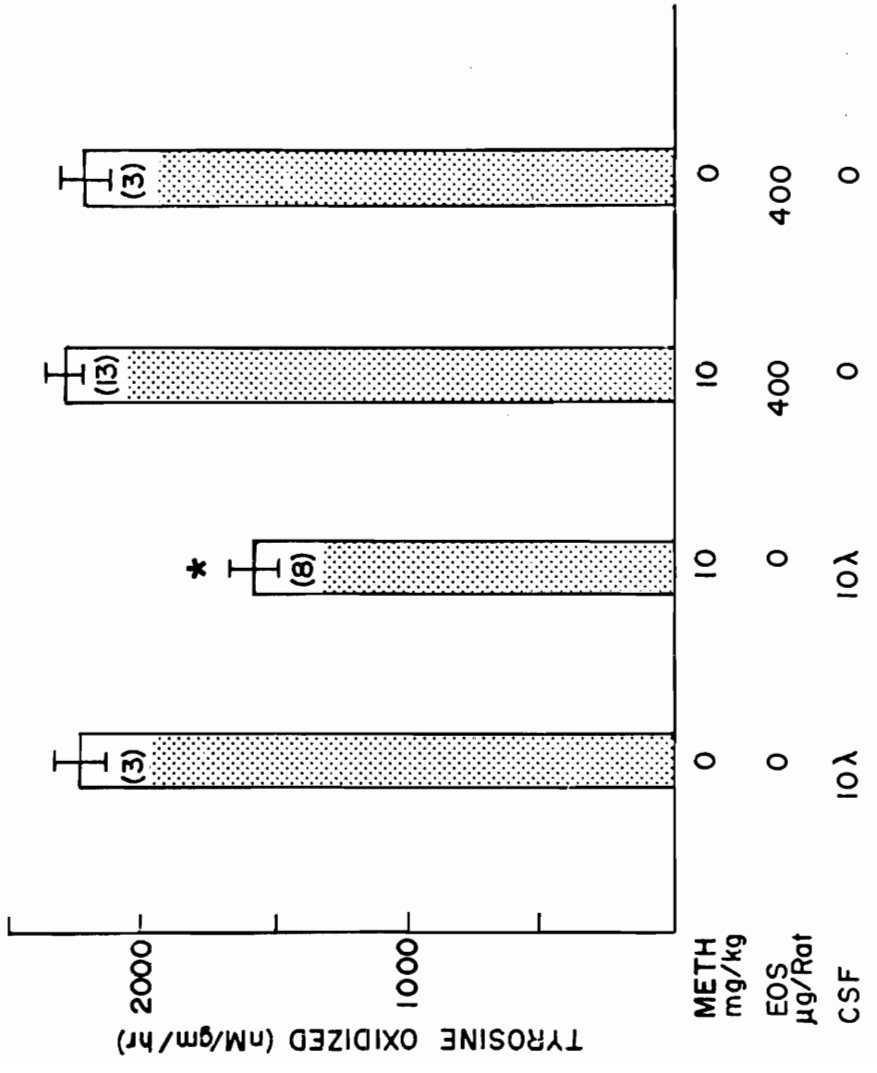
DOSE | METH
mg/kg | gAG
0 0
10 0
10 1
10 5
10 15
10 40

FIG. 5 Neostriatal TH activity with EOS, given only once, combined with the methamphetamine schedule described in the legend of Fig. 1. Numbers in parenthesis refer to the number of animals per group. (*) $p < 0.01$ compared to control.

FIGURE 5


TYROSINE OXIDIZED (nM/gm/hr)
2000
1000
(3)
(8)
*
(13)
(3)
METH mg/kg
EOS µg/Rat
CSF
0
10
10
0
0
0
10λ
0
400
10λ
0
400

tamine, did not block neostriatal TH depression

Discussion

The maximal doses of AOAA[17,19], gAG[6] and EOS[4] used in these experiments have been demonstrated to increase GABA levels several-fold. Although AOAA and gAG can also inhibit glutamic acid decarboxylase (GAD) to an important degree, EOS is considerably more selective, causing little GAD inhibition *in vitro*[4]. This indicates that a functional increase in GABA levels is responsible for antagonizing the effect of the amphetamine on TH.

Since a range of diazepam doses failed to antagonize the action of methamphetamine on neostriatal TH, it appears that GABA-T inhibitors may be able to uniquely activate GABAergic systems to counteract the methamphetamine effect. It is possible, however, that a well defined GABAmimetic such as muscimol[9] could exert an antagonism similar to that seen with GABA-T inhibitors.

Our hypothesis was that methamphetamine decreases neostriatal TH activity primarily by decreasing the firing rate of the nigral dopamine cells and that the GABA-T inhibitors act by blocking the methamphetamine-induced suppression of these cells. The research of other investigators supports the observations that

GABA-T inhibitors antagonize the effects of methamphetamine. Walters et al.[18] have shown that AOAA and the GABA agonist, muscimol, can antagonize electrophysiological effects of amphetamine. These workers evaluated the effect of concurrent AOAA treatment on amphetamine-induced suppression of the firing of neurons in the zona compacta of the substantia nigra and showed that AOAA (50 mg/kg, i.p.) will antagonize this effect of amphetamine at doses comparable to those used in this study.

The primary site of action of the GABA-T inhibitors in antagonizing the suppression of neostriatal TH could be in the striatum or in the nigra. A decrease of inhibitory input to the nigral dopaminergic cells could begin with striatal GABAergic interneurons suppressing the inhibitory striatonigral pathway[13]; however, it has been shown that this pathway need not be intact for methamphetamine to depress TH activity in the neostriatum[5]. It appears, then, that the GABA-T inhibitors may be acting primarily in the substantia nigra, where GABAergic interneurons may reside[11].

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PART THREE

THE EFFECT OF STRIATONIGRAL PATHWAY LESIONS ON METHAMPHETAMINE-INDUCED DEPRESSION OF TYROSINE HYDROXYLASE

Introduction

The abuse of amphetamine and related drugs frequently results in personality changes that essentially mimic certain forms of psychotic behavior. High dose parenteral abuse, referred to as "speeding," often produces a state similar to paranoid schizophrenia[18]. Biochemical changes in the brain that occur during such an exposure to amphetamine may be linked to the underlying causes of non-drug related psychopathology. Our goal has been to determine the mechanism for biochemical changes that are observed in an animal model of parenteral abuse, specifically changes in tyrosine hydroxylase (TH) activity seen in the nigro-striatal dopaminergic system.

Several models have been used to demonstrate changes in the apparent Vmax of TH in the nigrostriatal system following amphetamine or methamphetamine intoxication[12,7,11]. The model developed by Koda and Gibb[12] was used in the experiments reported here; this model consists of five parenteral (s.c.) doses of methamphetamine over a 24 hour period, followed by measurement of neostriatal TH, 36 hours after the first dose. Methamphetamine-induced TH depression in this model can be blocked by neuroleptics[2] or GABA transaminase inhibi-

tors[9].

Studies on the regulation of TH in the adrenal medulla have shown that increased preganglionic nerve activity, presumably excitatory to chromaffin cells, causes an increase in adrenal TH[20]. Electrophysiological studies of the dopaminergic cells of the pars compacta of the substantia nigra have demonstrated that amphetamine greatly decreases the firing rate of these neurons. Haloperidol alone only slightly increased the firing rate, but completely inhibited the amphetamine effect[5]. These two pieces of information led Buening and Gibb[2] to propose that the continuous suppression of dopamine cell firing by methamphetamine would lead to the converse of the situation in the adrenal medulla mentioned previously, i.e., decreased excitation of the nigral dopamine neurons would lead to a decrease in the synthesis of TH. Kogan et al.[13] then demonstrated that continuous inhibition of the nigral DA cells caused by the continuous action of methamphetamine resulted in decreased TH activity in cell bodies in the nigra. They then postulated that less TH was transported by axoplasmic flow to the neostriatum, and that this explained the consequent decrease in tyrosine hydroxylase in the neostriatum.

Thus it has been proposed that the primary effect

of methamphetamine is to turn on the striatonigral negative feedback pathway which leads to the decrease of neostriatal TH. This is consistent with the observation that the striatonigral pathway mediates the amphetamine-induced depression of nigral dopaminergic cell firing[3,4]; therefore, if this descending inhibitory pathway were also the mediator of methampetamine-induced TH depression, the elimination of this tract would block the TH depression on the lesioned side. This hypothesis was tested in the present report by a kainic acid lesion in the neostriatum, which should destroy interneurons and efferent tracts[17], and also by mechanical lesions of the striatonigral pathway just rostral to the substantia nigra.

Materials

Tritiated tyrosine (L-3', 5', ^{3}H-tyrosine) was purchased from Amersham Searle and glutamic acid (L-1, ^{14}C-glutamate) from New England Nuclear. All other chemicals were acquired from Sigma Chemical. γ-Acetylenic GABA was generously supplied by Merrill International Research Center and the haloperidol was donated by McNeil Laboratories. Methamphetamine sulfate was obtained from the National Institute of Drug Abuse.

Methods

Kainic acid lesions of the neostriatum were made on male Sprague-Dawley rats weighing between 180 and 210 g. The rats were anesthetized with ether, the dorsal cranium was exposed, the midline was estimated from the medial suture of each rat, and the interaural line, as defined by the ear bars, was used as the anterior-posterior zero. Lesion locations were described according to the König and Klippel atlas[14].

Kainic acid was dissolved in artificial cerebral spinal fluid to a concentration of 3 mg/ml, degassed and the pH was adjusted to 7.4. Coordinates for the injection site were anterior 7.9 mm, lateral 2.6 mm and ventral 0.5 mm. A 30-gauge cannula that had an electrolytically tapered, blunt point (Hamilton) was lowered into the caudate and 0.5µl of the kainic acid solution was injected over a 1 minute period; the cannula was withdrawn after another 2 minutes, the burr hole was filled with bone wax and the skin was sutured.

Wire-knife lesions of the crus cerebri were made with a 25-gauge cannula, bent slightly at its end, which contained a 33-gauge wire that could be extended 2.8 mm from the guide cannula at an angle of 45° to the vertical axis, the same angle of the crus cerebri at the level of anterior 3.3 mm. Rats (240 to 280 g)

were anesthetized with Nembutal, placed in a stereotaxic holder and the cranium exposed. The cannula tip was lowered to a position just above the crus cerebri, anterior 3.3 mm, lateral 4.4 mm and ventral -0.4 mm. The wire was then extended and withdrawn every 100 μm as the crannula was lowered another 2.2 mm, which effectively created a rectangular lesion encompassing all but the most medial 1.5 mm of the crus cerebri. Histological evaluation of the extent of the lesion was made for each rat.

The electrothermic lesions of the crus cerebri were essentially the same as that reported by Gale et al.[8]; spherical lesion of approximately 1 mm was made that was centered at anterior 3.3 mm, lateral 2.5 mm and ventral 215 mm.

Five injections of methamphetamine (10 mg/kg, s.c.) were administered, once every 6 hours beginning at 6 p.m. Rats were sacrificed 36 hours after the first injection, the brain was removed, placed on ice and sectioned with a coronal razor cut through the optic chiasm. Right and left neostriata were removed from the rostral block and frozen on dry ice. In crus cerebri lesioned rats the caudal block was placed in an aluminum foil cup (2.5 cm X 3 cm deep), surrounded with OCT compound (Tissue Tec) and immersed in a pentane-

dry ice mixture for 15 seconds. These blocks were mounted in a microtome-cryostat and 10 micron sections were cut through the lesion area for histological verification. When GAD was to be measured in the nigra, sectioning was continued and 200 µm sections were taken through the complete extent of the nigra. These sections were lyophylized and the right and left substantia nigra reticulata samples were dissected, with the aid of a dissecting microscope, from the region of the emergence of the third cranial nerve. The sample size ranged from 10 to 20 µg dry weight.

Tyrosine hydroxylase was measured by the method of Nagatsu et al.[15], which is a tritium displacement assay. Tissues were homogenized in 30 volumes of 0.2% Triton X - 100, incubated in a solution containing sodium acetate (0.2 M), Fe++ (10^{-3} M), mercaptoethanol (0.1 M) and tyrosine (10^{-4} M, 0.4 µCi); pH of the reaction mixture was 6.0.

Glutamate decarboxylase (GAD) was measured by a modification of the procedure of Albers and Brady[1]. Lyophilized tissue was weighed, homogenized in 30 µl of 0.1% bovine serum albumin, 0.2% Triton X-100 and dithiothreotol (4×10^{-3} M) and centrifuged 10 min. at 3000xg in the homogenizer. Ten µl of the supernatant was used in the GAD assay which was run in duplicate.

The reaction mixture contained potassium phosphate buffer (38 mM), sodium glutamate (12 mM), 1-L-^{14}C glutamic acid, (0.1 μCi), pyridoxal phosphate (0.2 mM) and KCl (1.0 mM); the pH of the final reaction mixture was 6.5. The CO_2 trapping tube was identical to the reaction tube (6 mm X 50 mm) and contained a piece of filter paper (8 X 15 mm) wetted with 50 μl of hyamine hydroxide (1 M in methanol). The reaction tube and trap were joined with a 5 cm piece of rubber tubing, secured together with a rubber band and incubated at 37° for 60 min. The reaction was stopped by injection of 100 μl of 5 N H_2SO_4 through the rubber connecting tube, into the reaction tube. The tubes were again incubated for 30 minutes at 37° C in a horizontal position to complete trapping; with the filter paper present, trapping was complete by 15 minutes. The filter paper was transferred to a 4 ml scintillation vial (Beckman Biovial) and the trapping tube was washed with 4 ml of cocktail. To calculate the enzyme activity, the CO_2 trapping efficiency was assumed to be 100%.

Student's t test was used to evaluate all comparisons except in the kainic acid lesion experiment, where the Sequential Studentized Range test was used.

Results

Following the injection of kainic acid into the

neostriatum, rats exhibited barrel-rolling seizure activity shortly after regaining consciousness and were anorexic and adipsic for about 2 days after making the lesion. However, by 12 days mortality was only 20% and most of the rats had gained 10 to 20 grams in body weight.

After the 12-day recovery period, the methamphetamine regimen was begun. Preliminary studies demonstrated that neostriatal TH activity in CSF-injected control rats was not different from that in rats with uninjected striata whether rats were treated with methamphetamine or saline; therefore, the uninjected contralateral side was used as a control throughout the kainic acid lesion study. Figure 1 shows that methamphetamine causes a 50% depression in TH on the unlesioned side; however, the kainate lesion did not prevent the tyrosine hydroxylase (TH) depression in methamphetamine-treated rats; in this case TH was decreased to only 34% of control. Thus the lesion itself, which should have eliminated the striatonigral pathway, did not diminish the TH depression caused by methamphetamine.

GABA transaminase inhibitors such as γAG have been shown to block the methamphetamine-induced depression of neostriatal tyrosine hydroxylase[9]. This

FIG. 1 Neostriatal TH activity in kainic acid lesioned striata and contralateral, unlesioned striata. Twelve days after the lesion methamphetamine (10 mg/kg, s.c.) was given every 6 hours for 5 doses; γAG and haloperidol were given i.p. on the same schedule. TH activity was measured 36 hours after the first injection. Statistical comparisons were made only between treatments from the same side, i.e., kainic acid-lesioned methamphetamine treated versus kainic acid-lesioned saline treated.

(*) $p < 0.05$ compared to saline control

(▲) $p < 0.05$ compared to methamphetamine treated

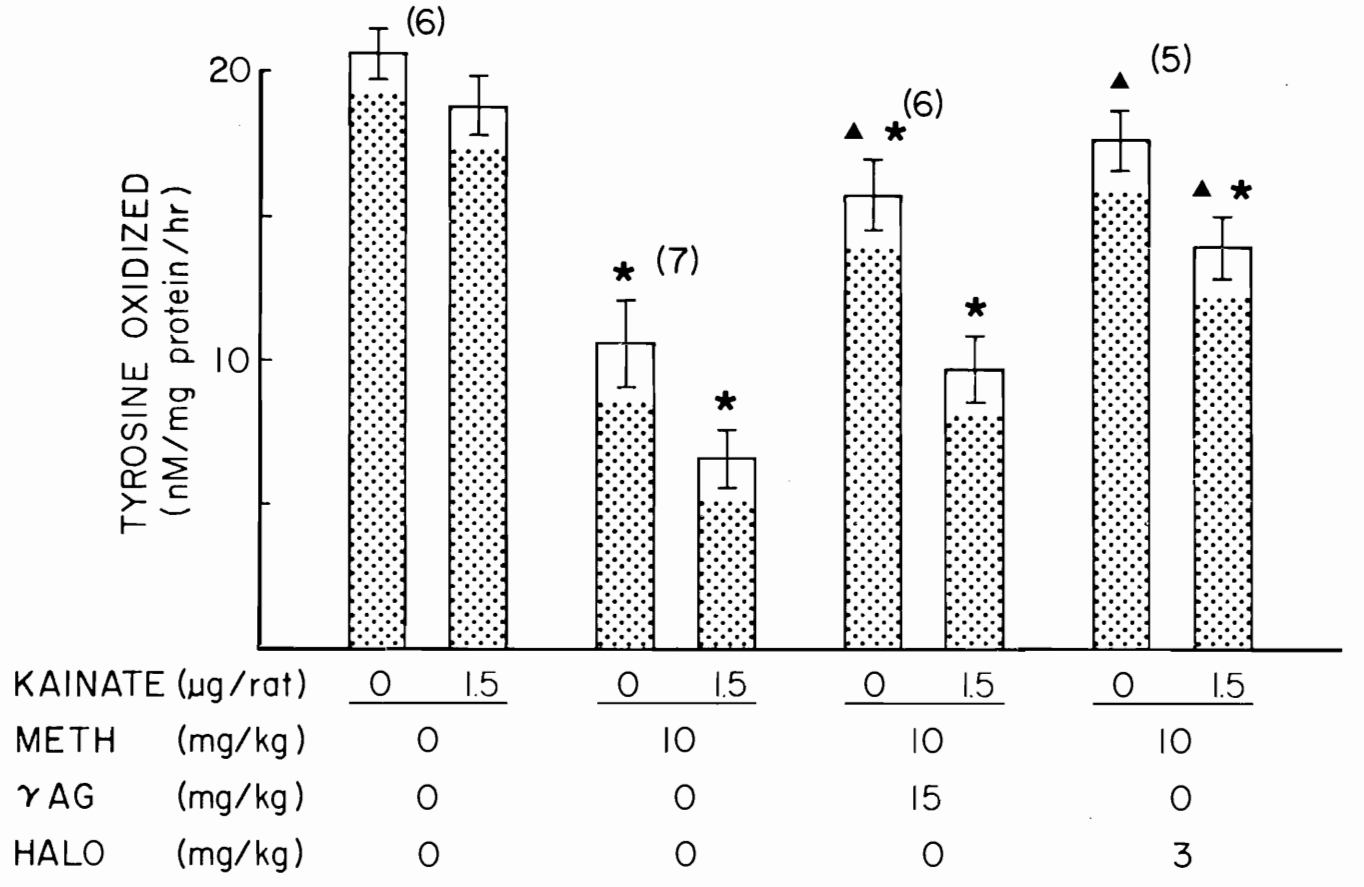

TYROSINE OXIDIZED
(nM/mg protein/hr)
20
10
(6)
(7)
(6)
(5)
*
▲
KAINATE (µg/rat) 0 1.5 0 1.5 0 1.5 0 1.5
METH (mg/kg) 0 10 10 10
γAG (mg/kg) 0 0 15 0
HALO (mg/kg) 0 0 0 3

FIGURE 1

protective ability of γAG appears to have been diminished by the kainic acid lesion because the TH depression of the lesioned side was not significantly antagonized by γAG at 15 mg/kg, whereas this same dose significantly antagonized the depression of TH in the contralateral unlesioned side when compared to contralateral unlesioned striatum of rats receiving only methamphetamine. Thus, the lesion appears to have decreased the ability of the GABAergic systems that interact with the dopamine cells to respond to the effects of a GABA-T inhibitor. The dose of γAG used in these experiments has been shown to be about an ED90 for antagonism of the methamphetamine effect on neostriatal TH[9], therefore, it is possible that a higher dose of γAG could exert some antagonism to this action of methamphetamine.

Haloperidol has also been shown to protect against the methamphetamine-induced TH depression[2]. The dose of haloperidol used here significantly blocks TH depression in both lesioned and non-lesioned neostriata. Thus, this effect of haloperidol is not altered by the kainic acid lesion, at least at the dosage level chosen for this experiment.

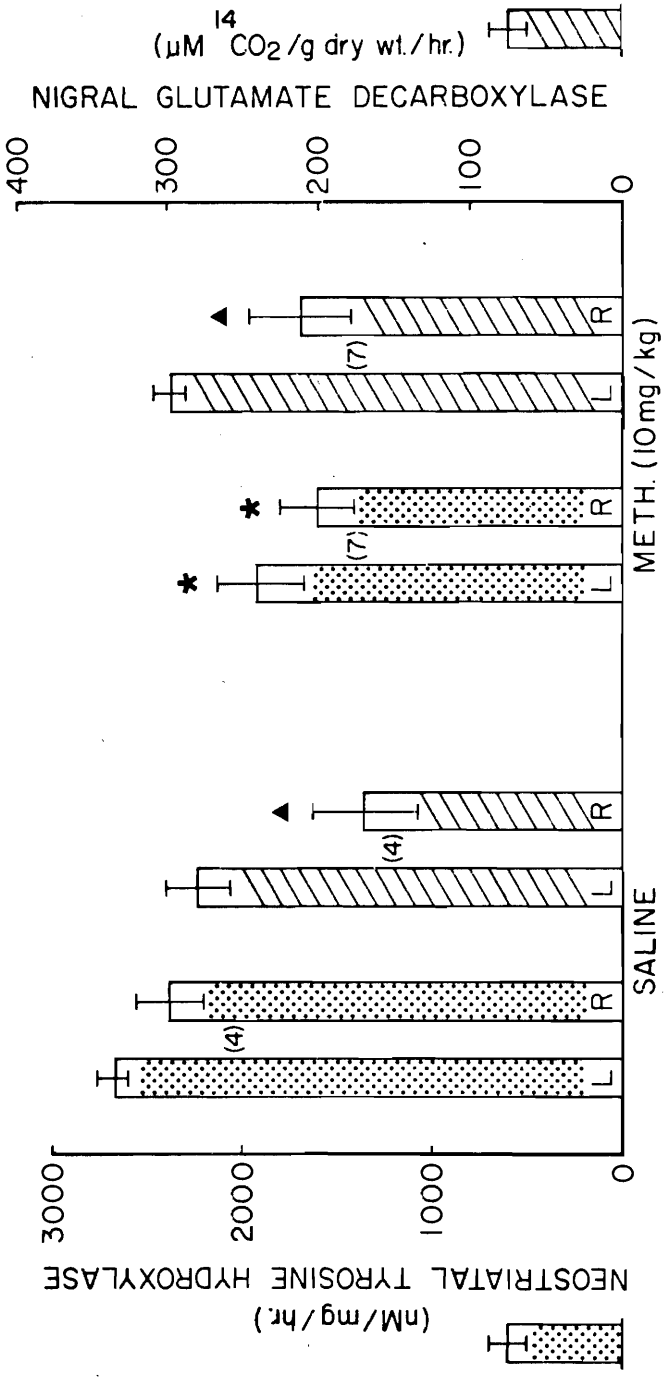
The kainic acid lesion produces an asymmetrical motor behavior which is presumed to be related to un-

equal dopaminergic cell activity between the lesioned and unlesioned nigrostriatal system[6]. Rotational behavior was evaluated in each treatment group by observing the direction of rotation and relative intensity of the turning during the 10-20 minute period following the first injection of methamphetamine. Ten minutes after injection, rats that were given methamphetamine (10 mg/kg s.c.) displayed ipsilateral rotation at 6 to 9 turns per minutes; saline treated rats showed little spontaneous rotation. Animals receiving γAG and methamphetamine turned ipsilaterally at a slower rate than those receiving methamphetamine alone. Haloperidol alone (not shown in Fig. 1) caused no rotation but when given in conjunction with methamphetamine caused a contralateral rotation, at a rate comparable to the group treated with γAG and methamphetamine.

Wire-knife lesions also failed to block depression of TH produced by methamphetamine (Fig. 2). GAD activity in the ipsilateral nigra decreased 36% indicating that there was significant destruction of the striatonigral pathway. Other investigators have reported that all GABAergic projections from the striatal-pallidal complex may account for 70 - 80% of nigral GAD[19,10]. Our lesions left from 100 to 200 microns

FIG. 2 Neostriatal TH activity (dotted bars) and nigral GAD activity (crosshatched bars) in rats with wire knife lesions of the crus cerebri on the right side. Eleven days after the lesion methamphetamine was given as described in the legend of Fig. 1.
(*) $p < 0.01$ compared to ipsilateral control
(▲) $p < 0.05$ compared to contralateral nigra with same treatment

FIGURE 2


(μM $^{14}CO_2$/g dry wt./hr.)
NIGRAL GLUTAMATE DECARBOXYLASE
400
300
200
100
0
METH. (10mg/kg)
SALINE
R
L
(7)
(4)
*
3000
2000
1000
0
NEOSTRIATAL TYROSINE HYDROXYLASE
(nM/mg/hr.)

of the most medial aspect of the crus cerebri intact with the intention to avoid damaging the median forebrain bundle (MFB). Because the MFB contains the nigrostriatal tract, it was thought that any damage to the MFB might interfere with the interpretation of the methamphetamine-induced changes in neostriatal TH.

The GAD level in the control nigra was not changed by methamphetamine treatment (Fig. 2). When GAD was measured in the neostriatum over a 20-day period it was found to be unchanged, although TH activity was significantly depressed throughout the entire observation period (unpublished observation).

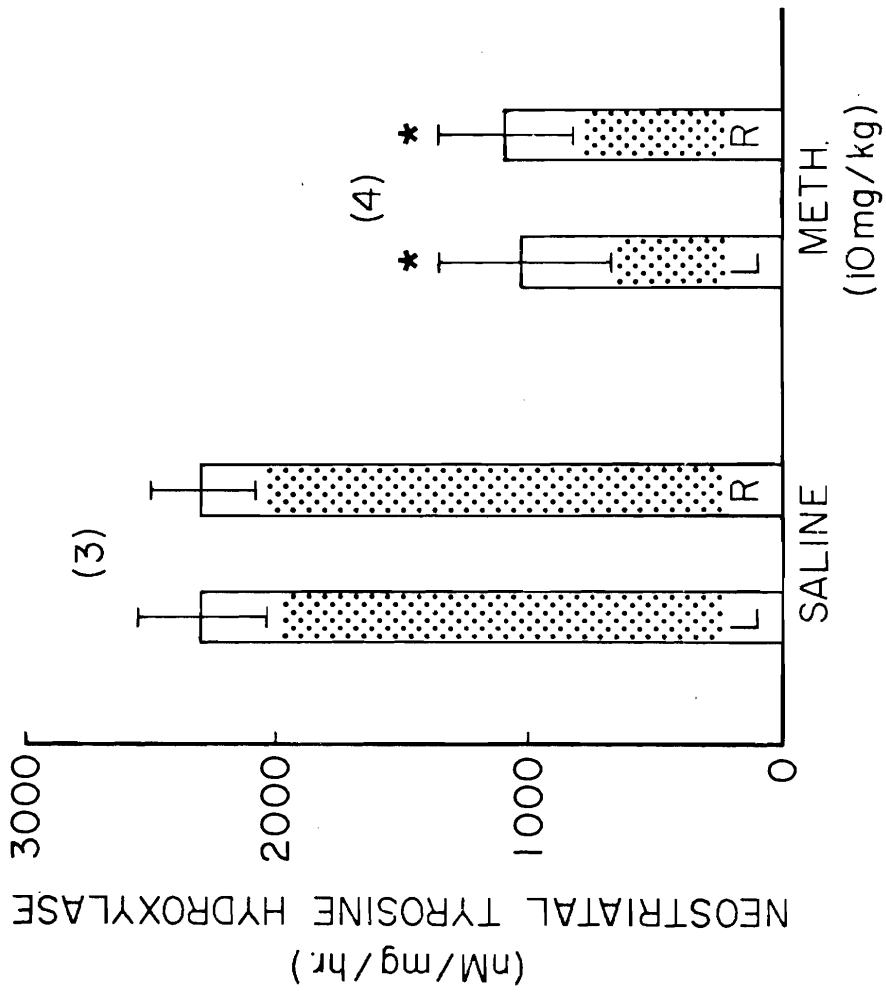
The electrothermic lesion also failed to antagonize TH depression in the lesioned side (Fig. 3); this is the same result seen with the wire-knife lesion, which is expected since both lesions should create similar damage to the striatonigral tract.

Discussion

The original hypothesis set forth to explain the methamphetamine-induced depression of neostriatal TH can be considered in two aspects. First, chronic exposure to methamphetamine causes a decrease in the firing rate of the nigral dopaminergic cells via the striatonigral negative feedback pathway. Secondly, this decrease in dopaminergic cell activity reduces the rate

FIG. 3 Neostriatal TH activity in electrothermic lesioned rats. Thirteen days after the lesion in the right crus cerebri, methamphetamine was given as described in the legend of Fig. 1. (*) $p < 0.05$ compared to saline ipsilateral control.

FIGURE 3


NEOSTRIATAL TYROSINE HYDROXYLASE
(nM/mg/hr.)
3000
2000
1000
0
SALINE
METH.
(10 mg/kg)
L
R
L
R
(3)
(4)
*
*

of synthesis of TH; consequently, less TH is transported to the neostriatum by axoplasmic flow resulting in a decrease in neostriatal TH.

The first aspect of this hypothesis was not suported by the experiments reported here. According to this hypothesis all three types of lesions should have attenuated or eliminated feedback inhibition from the neostriatum to the substantia nigra; however, none of the lesions blocked the methamphetamine-induced depression in TH. This result was consistently obtained in duplications of these experiments. We conclude from these observations that a different mechanism than the postulated striatonigral negative feedback must account for the decrease of striatal TH activity. Although the striatonigral pathway certainly must inhibit these dopaminergic cells at lower doses of methamphetamine, another negative feedback system must be operating at this high dose of methamphetamine. It should be noted that the threshold dose of methamphetamine for TH depression is 6 to 8 mg/kg [12], whereas a considerably lower dose, 1.6 mg/kg, was used by Bunney and Aghajanian [3] to demonstrate the action of the striatonigral tract in the amphetamine silencing of dopamine neurons.

It, therefore, appears that alternative neuronal systems exist which can produce feedback inhibition of

dopamine neurons in the absence of the striatonigral pathway and which are probably normally active with high doses of methamphetamine. One possible alternative might be that local circuits in the striatum composed of interneurons exert a presyaptic inhibition on dopaminergic boutons; however, the kainic acid lesion study eliminates this possibility. Other alternatives remain to explain the source of inhibition of TH activity, such as dopaminergic autoreceptors in the nigra and the striatum, local interneuronal circuits in the nigra or afferent nigral inhibition from other sources such as the raphe. Another possibility remains which may be unrelated to classical neuronal inhibition; it is possible that there is direct damage of the nigrostriatal dopaminergic terminals by a toxic action of methamphetamine.

Other investigators have evaluated the regulation of dopamine metabolism in the kainate-lesioned striata. One study indicated that haloperidol-induced activation of TH, seen as a decrease in Km for the pteridine cofactor, does require an intact striatum[21]; this shows that striatal interneurons and/or the striatonigral pathway are essential for neuroleptic-induced activation of TH. But another report showed that dopamine turnover is still increased by haloperidol in the kai-

nic acid lesioned striatum[22]. Thus it appears that pathways involved in methamphetamine-induced depression of neostriatal TH activity are not the same as those required for haloperidol to activate TH. However, both the ability of haloperidol to increase dopamine turnover, and the ability of haloperidol to block methamphetamine changes in neostriatal TH activity (Fig. 1) seem to work independently of the neurons destroyed with the kainic acid lesion, perhaps through the same pathway. In contrast to the lack of effect of the kainic acid lesion on the protective ability of haloperidol, the protective ability of the GABA-T inhibitor, γAG, appears to have been compromised by the lesion (Fig. 1); therefore, the striatonigral pathway and/or striatal interneurons must play a role in the ability of γAG to antagonize the methamphetamine-induced depression of TH.

Since nigral GAD activity was unchanged with methamphetamine treatment, methamphetamine may be acting specifically on nigral dopamine cells instead of causing a general depression of many enzymes. Following the subacute methamphetamine regime, neither GAD nor choline acetyl transferase in the neostriatum are changed significantly during a 20-day period; throughout this time neostriatal TH is significantly depressed

(unpublished observations). This observation also argues against a generalized depression of enzyme levels caused by a nonspecific toxic action of methamphetamine. Thus it appears that if there is destruction of neostriatal boutons, the dopaminergic nigrostriatal fibers may be specifically affected.

The apparent decrease in responsiveness of the nigrostriatal system to the effects of γAG in the presence of methamphetamine indicates that the GABAergic projection to the nigral dopamine cells plays a role in suppressing neostriatal TH changes; the construction of a complete dose response curve would elucidate whether there is a change in potency and/or a change in efficacy. If haloperidol was to act predominately at presynaptic sites at this high dose of 3 mg/kg, one would expect that little difference in responsiveness would be brought about by the kainic acid lesion, as is suggested by the results in Fig. 1.

There were interesting differences in rotational behavior seen with the combinations of methamphetamine with γAG or haloperidol. The combination of methamphetamine and γAG resulted in ipsilateral rotation while haloperidol used in combination with methamphetamine caused a contralateral rotation. Since only haloperidol offered significant protection to neostriatal TH

on the lesioned side, the ability to reverse rotational behavior elicited by methamphetamine in kainic acid lesioned neostriata may correlate with the ability to antagonize neostriatal TH depression. If this is so, one might be able to use higher doses of γAG, for example, which could produce some protection against neostriatal TH depression on the lesioned side and at the same time reverse the rotational behavior caused by methamphetamine.

The decrease in nigral GAD seen in the wire knife experiment is less than that reported by others. The study of Spano et al.[19], who showed a 70% decrease in GAD following an electrothermic lesion, differed from our experimental design in two significant respects. First, the lesion apparently included the lateral margin of the MFB whereas it was our intention to avoid this fiber bundle. Secondly, in their study only 4 days elapsed between the lesion and measurement of the enzyme while we waited 13 days. Gale et al.[8] found that GABA levels were depressed as much as 70% 7 days after crus cerebri lesion but another group reported that nigral GABA levels were decreased only 55% 12 days after wire knife lesions of the crus cerebri[16]. This indicates that the time of GAD measurement after the lesion of the crus cerebri may be important and that

GAD may increase with time.

The second aspect of this hypothesis, i.e., decreased synthesis and transport leading to decreased neostriatal TH, is currently being investigated in this laboratory.

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