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Further Evidence that Amphetamines Produce Long-Lasting Dopamine Neurochemical Deficits by Destroying Dopamine Nerve Fibers

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Methamphetamine and amphetamine were continuously administered to rats for 3 days by means of subcutaneously implanted osmotic minipumps. The total daily dose of each drug was approximately 4 mg/day. Dopamine, norepinephrine and serotonin determinations two weeks later indicated that both amphetamines produced a selective striatal dopamine depletion. Anatomical studies indicated that this depletion was associated with striatal nerve fiber degeneration. To determine whether this fiber degeneration induced by amphetamines was dopaminergic, the long-lasting dopamine depletion produced by methamphetamine was antagonized with a-methyl-para-tyrosine. This prevented the appearance of nerve fiber degeneration after methamphetamine. These findings suggest that amphetamines produce a long-term striatal dopamine depletion by destroying striatal dopamine nerve fibers.

INTRODUCTION

Recently, there has been concern that chronic exposure to amphetamines may lead to irreversible damage of brain dopamine (DA) neurons. This concern has largely been prompted by the discovery that chronically administered amphetamines produce long-lasting reductions in striatal DA content[9-12, 14-16,20], uptake[16,19,20] and metabolites[12,18]. Also, amphetamines have been found to cause long-term decreases in striatal tyrosine hydroxylase activity[2-6]. Numerous investigators[6,7,10-12] have postulated that amphetamines induce these persistent presynaptic DA deficits by destroying DA fibers. Direct morphological evidence supporting this view, however, is still not complete.

Two approaches have been taken in an effort to document DA fiber degeneration after amphetamines. The first has made use of fluorescent histochemical techniques, the second of silver degeneration methods. Studies using the former approach have led to the discovery of swollen brightly fluorescent striatal axons following amphetamines[2,7-10]. This finding has been taken to indicate that amphetamines damage DA fibers. Whether this damage involves actual DA fiber destruction, however, is unclear. Studies using silver degeneration methods have revealed degenerating nerve fibers in the striatum of rats administered either high doses of methamphetamine[12] or a single dose of amphetamine in combination with iprindole[13]. Unfortunately, in the first of these studies[12], it could not be concluded with certainty that the degenerating fibers were dopaminergic since the high doses of methamphetamine used produced both DA and serotonin (5-HT) deficits[14]. Also, these high doses may have produced non-specific neurotoxic effects. The other degeneration study[13] demonstrated fiber degeneration after an amphetamine-iprindole treatment which produced a selective long-lasting DA depletion. While this study certainly indicated that amphetamine induced DA fiber degeneration in iprindole-treated rats, it left unanswered the question of whether amphetamines induce DA fiber degeneration in the absence of iprindole.

The purpose of this study was to determine whether amphetamines administered alone (without iprin-

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dole) and in lower doses than in previous studies produced a long-lasting DA depletion by destroying DA nerve fibers.

MATERIALS AND METHODS

Animals

Subjects for this study were male albino Sprague-Dawley rats (Holtzman Co., Madison, WI) weighing 250 (± 10) g at the time drug treatment was initiated. Rats were housed singly in wire-mesh cages with ad libitum access to food and water. Ambient temperature was 23 ± 1 °C. Fluorescent lighting was turned on at 06.00 h and off at 18.00 h.

Drug treatment

Methamphetamine and amphetamine were continuously administered by means of subcutaneously implanted ALZET osmotic minipumps, models 2001 and 2002. These pumps are specified by the manufacturer to release their contents at a rate of 1.0 and 0.5 ^1/h for 7 and 14 days, respectively. Pumps loaded with the appropriate drug solution were implanted subcutaneously under light ether anesthesia. After the desired treatment duration, the pumps were removed, again under light ether anesthesia. Because of expense, only experimental animals were implanted with pumps. Control rats did, however, undergo the same minor surgical procedures.

In experiment 1, methamphetamine was administered at various total daily doses for various periods of time. Each daily dose and duration was tested in a group of 6 rats. Rats were killed two weeks after drug treatment for neurochemical assay. Specific daily doses tested were 1, 2 and 4 mg. The 1 mg/day dose was administered for 3, 6 and 12 days; the 2 mg/day dose for 1.5, 3 and 6 days and the 4 mg/day dose for 0.75, 0.5 and 3 days. Daily dose was calculated by multiplying the rate of release (in /zl/h) by the concentration of the drug solution (in fig/fd) loaded into the pump and then multiplying this number by 24. Pumps were filled with D-methamphetamine hydrochloride dissolved in sterile physiological saline at the desired concentrations (50, 100 and 200 wg/ld). The drug dose was expressed as the base. When amphetamine was tested at a dose of 4 mg/day it was necessary to implant two 2001 pumps containing D-amphetamine sulfate at a concentration of 100 $\mu g/\mu l$ into each rat because amphetamine sulfate is less soluble in saline than methamphetamine hydrochloride.

In experiment 2, methamphetamine and amphetamine were administered continuously at a dose of approximately 4 mg/day for 3 consecutive days. Only the 4 mg/day dose was used since experiment 1 showed this to be the only dose of methamphetamine that produced long-term neurochemical effects. Ten rats received methamphetamine and another 10 received amphetamine. Of the surviving rats, 5 were used for confirmatory neurochemical assays and 3 for histology.

In experiment 3, a-methyl-para-tyrosine (AMT) was administered subcutaneously every 12 h for the duration of the 3-day methamphetamine regimen. AMT was administered at a dose of 150 mg/kg of the methyl ester hydrochloride salt. For this experiment, 10 rats were administered methamphetamine and saline, 10 methamphetamine and AMT, 10 AMT alone and 10 saline alone. Again, 5 rats from each group were used for neurochemical assays and 3 for histology.

Monoamine level determinations

Two weeks after drug treatment, rats were killed for DA, 5-HT and norepinephrine (NE) level determinations by cation-exchange liquid chromatography coupled with electrochemical detection as detailed elsewhere[14]. This long post-drug survival period was used in an attempt to assess toxic rather than pharmacologic drug effects. Monoamines were measured in striatal, hippocampal and rest of brain samples.

Dissection

The method for isolating the striatum, hippocampus and rest of brain has been described in detail previously[11].

Fiber degeneration studies

Rats for studies of nerve fiber degeneration using the Fink-Heimer method[3] (Procedure I) were killed one day after drug treatment. This short survival period was used since successful demonstration of degenerating rat nigrostriatal DA fibers with the Fink-Heimer method is most likely after a short survival period[12]. Fink-Heimer sections through the striatum were evaluated for the presence or absence of fiber

degeneration by an experienced observer unaware of the various treatment conditions.

Statistics

Significance of differences between group means was evaluated with a two-tailed Student's z-test or Dunnett's test.

Materials

Osmotic minipumps were purchased from the ALZA Co., Palo Alto, CA. D-Amphetamine sulfate was obtained from the Sigma Chemical Co., St. Louis, MO and D-methamphetamine hydrochloride from the National Institute of Drug Abuse. DA-hydrochloride, NE-hydrochloride and serotonin creatinine sulfate were all purchased from Sigma Chemical Co., St. Louis, MO.

RESULTS

Experiment 1

Methamphetamine continuously administered at a dose of approximately 4 mg/day for 3 consecutive days produced a significant striatal DA depletion two weeks later (Fig. 1). This same daily dose administered for shorter periods of time (1.5 or 0.75 days) did not reduce striatal DA. Lower daily methamphetamine doses (2.0 and 1.0 mg/day) administered for longer periods of time (6 and 12 days, respectively) also failed to significantly reduce striatal DA content (Fig. 1).

Amphetamine continuously administered at a dose of approximately 4 mg/day for 3 days also produced a significant striatal DA depletion two weeks later. Levels of DA in control rats and amphetamine-treated rats were 10.9 ± 0.7 and 7.9 ± 0.5/zg/g (P< 0.05).

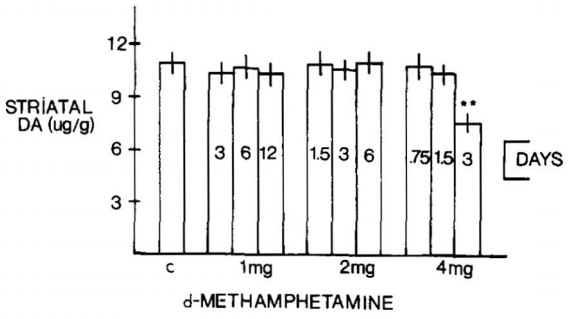

Fig. 1. Striatal DA content two weeks after continuous administration of various daily doses of D-methamphetamine for various periods of time.

To assess the selectivity of DA-depleting action of the amphetamines, 5-HT and NE levels were measured in the striatum and hippocampus. Neither striatal nor hippocampal 5-HT level was altered by either continuous methamphetamine or amphetamine (Table I). NE content of the hippocampus was also not affected by the amphetamines (Table I).

Experiment 2

To determine whether the long-lasting selective DA depletion induced by the amphetamines was related to DA fiber degeneration, the striatum of rats treated in parallel with those showing DA deficits was examined for evidence of fiber degeneration. Fiber degeneration was found both in rats continuously administered methamphetamine and in those administered amphetamine (Fig. 2). Fiber degeneration was present in every rat administered either of the amphetamines.

TABLE I

Selectivity of the long-lasting striatal DA depletion induced by continuously administered amphetamines

Both D-methamphetamine and D-amphetamine were administered at a dose of 4 mg/day for 3 consecutive days. Values represent the mean ± S.E.M. expressed in/zg/g tissue (n = 5). Rats were killed two weeks after drug treatment.

Treatment	Monoamine level two weeks later			
	Striatal		Hippocampal	
	DA	5-HT	NE	5-HT
Control	11.5 ±0.5	0.44 ± 0.03	0.42 ± 0.03	0.44 ± 0.03
D-Methamphetamine	8.1 ±0.8*	0.42 ± 0.03	0.43 ± 0.04	0.51 ± 0.04
D-Amphetamine	7.5 ± 0.8*	0.43 ± 0.03	0.40 ± 0.03	0.49 ± 0.04

* P<0.05.

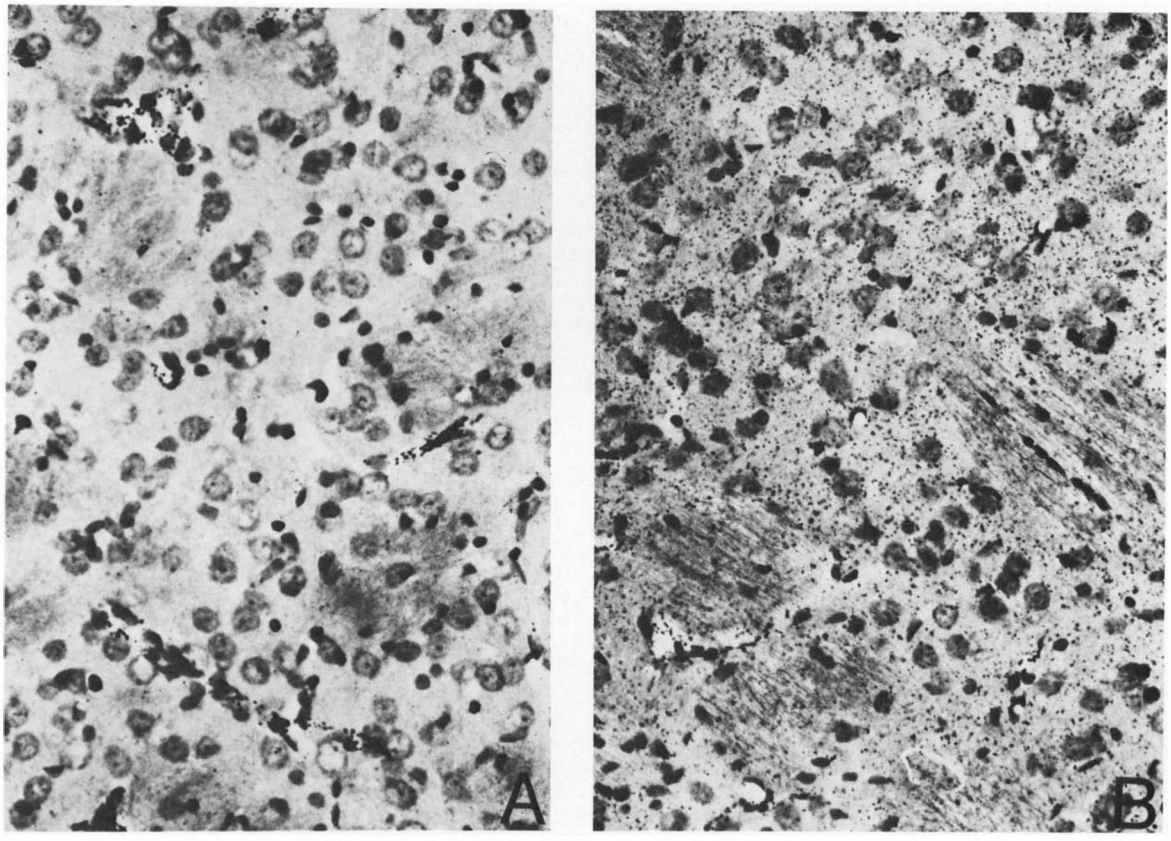

Fig. 2. Silver-stained sections through the striatum of: A, a control rat; and B, a rat continuously administered 4 mg/day D-methamphetamine for 3 days. Note fine granular degeneration in D-methamphetamine rat. Fink-Heimer method (Procedure I). Sections counter-stained with cresyl violet.

Experiment 3

To ascertain whether the striatal fiber degeneration induced by the amphetamines was dopaminergic, the long-lasting DA depletion produced by continuous methamphetamine was blocked with AMT. This was done by administering AMT subcutaneously at a dose of 150 mg/kg approximately every 12 h for the duration of the 3-day methamphetamine regimen (Table II). No evidence of fiber degeneration was found in the striatum of any of the 3 rats administered methamphetamine in combination with AMT. Fiber degeneration was present, however, in the striatum of rats administered only methamphetamine.

DISCUSSION

This study sought to determine whether amphetamines given alone (without iprindole) and in lower doses than in past studies[11,12,14] produced long-lasting DA deficits by destroying DA fibers. It has been found that a 4 mg/day dose of methamphetamine given continuously for 3 days produces a long-lasting depletion of striatal DA (Fig. 1). Since the rats used

TABLE II

Blockade of long-lasting striatal DA depletion induced by continuous D-methamphetamine by a-methyl-para-tyrosine (AMT)

AMT (150 mg/kg) was administered subcutaneously approximately every 12 h throughout the 3-day methamphetamine regimen. Rats were killed two weeks after the administration of drugs and the long-term effects of the treatment regimen were measured.

Treatment	n	Striatal DA (pg/g)
Saline	5	12.1 ± 0.9
Methamphetamine	5	9.4 ± 0.8*
Methamphetamine + AMT	5	11.9 ±0.9
AMT	5	11.9 ± 0.9

*P<0.05

in this study weighed approximately 250 g, the 4 mg/day dose is equivalent to approximately a 16 mg/kg/day dose. This daily dose is almost 10 times lower than that used in our previous studies[12-14]. Thus, by administering methamphetamine continuously rather than by means of repeated injections, it has been possible to substantially lower the dose of methamphetamine required to produce a long-term DA deficit without resorting to the use of iprindole.

The 4 mg/day dose of continuously infused methamphetamine did not produce a persistent DA depletion when administered for only 0.75 or 1.5 days. This would seem to be at odds with the conclusion of several recent reports that a 12-18 h exposure to amphetamine is sufficient to induce prolonged DA deficits[5,18]. This apparent discrepancy, however, can be reasonably explained by postulating that when methamphetamine is given continuously a certain amount of time is needed before the drug attains DA neurotoxic levels.

At first, we were surprised to find that continuously administered methamphetamine did not produce a long-term 5-HT depletion in either the striatum or hippocampus, two brain regions known to be quite sensitive to the 5-HT toxic effect of repeated high doses of methamphetamine[6-14]. We then realized that our method of continuous drug delivery might be responsible for this interesting difference. The slow continuous infusion of methamphetamine may facilitate metabolic conversion of methamphetamine into amphetamine, which several recent studies indicate does not produce long-term 5-HT deficits[11-16-18]. That methamphetamine is in fact metabolized into amphetamine in rats has been demonstrated in two previous studies[1-8].

Having found that continuous methamphetamine administration produced a selective long-lasting DA depletion, this finding was extended to amphetamine. Like methamphetamine, amphetamine continuously administered at a dose of 4 mg/day for 3 days produced an approximately 30% decrease in striatal DA content two weeks later. In agreement with previous reports[11-16-18], amphetamine did not affect either 5-HT or NE levels. Thus both amphetamine and methamphetamine administered continuously induce a selective long-lasting depletion of striatal DA.

To determine whether amphetamines produced a long-lasting DA depletion by destroying DA fibers, the striata of rats treated identically to those showing a DA deficit were examined for evidence of nerve fiber degeneration. Fine granular argyrophilic debris indicative of fiber degeneration was found in all rats continuously administered either of the amphetamines. Given that this fiber degeneration is occurring in rats known to have a selective long-lasting DA depletion, it seems reasonable to surmise that the degenerating striatal DA fibers are dopaminergic.

To evaluate this hypothesis, the long-lasting DA depletion produced by methamphetamine was blocked with AMT. This drug has been shown to antagonize not only the pharmacologic, but also the long-lasting DA neurotoxic effect of amphetamines[5-6]. It would be anticipated that if the degenerating fibers are dopaminergic, blocking the long-lasting DA depletion induced by methamphetamine with AMT should prevent the appearance of fiber degeneration after methamphetamine. No evidence of fiber degeneration was found in any of the rats administered AMT in combination with methamphetamine. The fact that preventing DA synthesis and depleting DA blocks the long-lasting DA depletion shows that the mechanism of degeneration involves DA, but does not prove that the cells seen to degenerate are dopaminergic. However, these results combined with others that show that steady-state levels of DA are down, the number of reuptake sites are decreased, tyrosine hydroxylase is down and the granular debris is seen in areas known to contain substantial amounts of dopaminergic fibers, strongly support the contention that the degeneration induced by continuous methamphetamine administration is dopaminergic[12].

The present findings extend earlier reports that amphetamine may be toxic to striatal DA nerve fibers. Some of these reports[2-7-10] were based largely on results obtained with fluorescence histochemical methods which are unable to directly demonstrate nerve fiber degeneration. Because of this, Elison et al.[2] prudently interpreted the presence of swollen, brightly fluorescent axons in amphetamine-treated animals as being indicative of DA fiber damage, but left the exact nature of this damage unspecified. The present results indicate that this damage involves actual DA fiber destruction. This study also extends our previous reports of nerve fiber degeneration af-

ter either high doses of methamphetamine[12] or a single dose of amphetamine in iprindole-treated rats[13], since the present results indicate that lower doses of amphetamines also induce DA fiber degeneration and that this fiber degeneration is induced by amphetamines in the absence of iprindole.

In summary, this study has presented combined morphological and pharmacological evidence of striatal DA nerve fiber degeneration after the continuous administration of both methamphetamine and amphetamine. This DA fiber destruction would appear to underlie the prolonged DA neurochemical changes reported after chronic exposure to amphetamines. How amphetamines destroy DA fibers remains to be elucidated. It may be that amphetamines are metabolized into DA neurotoxic compounds or that they destroy DA fibers by means of excessive activation. Whether abuse of amphetamines and related stimulants leads to DA fiber degeneration in man remains to be ascertained.

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