

Nerve terminal degeneration after a single injection of D-amphetamine in iprindole-treated rats: relation to selective long-lasting dopamine depletion

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A single injection of D-amphetamine has recently been shown to produce long-lasting dopamine (DA) deficits in rats pretreated with iprindole, an agent which interferes with the metabolism of amphetamine and prolongs its half-life. The basis for these persistent DA deficits has not been determined. The present results suggest that amphetamine produces prolonged DA depletions in iprindole-treated rats by destroying DA nerve terminals.

Under certain conditions D-amphetamine appears to be toxic to central dopamine (DA) neurons. When administered in high doses, amphetamine produces long-lasting decreases in DA content and uptake¹⁶. When lower amphetamine doses are used but administered continuously, similar DA deficits occur^{10,13} and striatal tyrosine hydroxylase activity is also reduced on a long-term basis¹. Recently, persistent DA deficits have been produced by a single injection of amphetamine in rats pretreated with iprindole^{5,15}. Iprindole interferes with the para hydroxylation of amphetamine and increases the half-life of amphetamine from 1 to approximately 4 h^{4,14}. Thus the continued presence of amphetamine in brain tissue, whether achieved by administering amphetamine in high doses, continuously or in combination with iprindole, induces prolonged DA deficits.

How amphetamine produces these long-lasting DA neurochemical deficits has not yet been determined. Studies employing fluorescent histochemical methods^{1,10} have suggested that amphetamine damages DA fibers. The nature of this damage, however, remains unclear. In a previous report¹¹, we presented evidence of striatal nerve terminal degeneration in rats administered high doses of methamphetamine. However, whether the degenerating terminals were

in fact dopaminergic could not be stated with certainty in that report for two reasons: (1) methamphetamine produced concomitant long-lasting DA and serotonin (5-HT) neurochemical deficits; and (2) methamphetamine was used in high doses. Thus there was potential for confounding DA terminal degeneration with 5-HT or some other type of terminal degeneration that might have been produced by the high doses of methamphetamine employed.

In the present study we have taken advantage of the recent discovery that long-lasting DA neurochemical deficits can be produced with a single dose of amphetamine by administering it in combination with iprindole in order to retard its metabolism^{4,5,14,15}. The aim of this study was to determine whether a single dose of amphetamine produces long-term DA chemical deficits by destroying DA nerve fibers.

Male albino Sprague-Dawley rats (Holtzman Co., Madison, WI) weighing approximately 250 g were used as subjects. Rats were housed individually in wiremesh cages with free access to food and water in a colony room where fluorescent lighting was automatically turned on at 06.00 h and off at 18.00 h. Ambient temperature was 22 ± 1 °C. In the first portion of this study groups of rats ($n = 5$) were adminis-

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tered saline, amphetamine alone, amphetamine in combination with iprindole or iprindole alone. In the second portion of this study, groups of rats were administered amphetamine and iprindole with or without amfonelic acid. D-Amphetamine sulfate and iprindole hydrochloride were dissolved in 0.9% sodium chloride at a concentration of 9.2 mg/kg and 10 mg/kg, respectively. Amphetamine and iprindole were administered concurrently. Amfonelic acid dissolved in a 9:1 (vol./vol.) mixture of propylene glycol and 2.5 N K_2CO_3 at a concentration of 5 mg/kg was administered immediately after amphetamine and iprindole. All drugs were administered intraperitoneally on a mg/kg basis. Rats for neurochemical studies were killed 2 weeks after drug treatment. Concentrations of DA, 5-HT and norepinephrine (NE) were determined by cation-exchange liquid chromatography coupled with electrochemical detection. DA and 5-HT measurements were performed using the method of Keller et al.⁷, as modified in this laboratory⁸. NE was assayed according to the method of Fenn et al.². Rats ($n = 3$ for each group) for degeneration studies were killed 3 days after drug treatment since silver impregnation of degenerating rat nigrostriatal DA terminals is best achieved after a short survival period^{6,11}. Nerve fiber degeneration studies were performed using the Fink-Heimer method³, as previously described¹¹. The Fink-Heimer method makes possible selective silver-impregnation of degenerating nerve fibers. Fink-Heimer sections were evaluated for the presence or absence of fiber degeneration by an experienced observer who was unaware of the treatment conditions.

As a first step, the long-lasting effect of amphetamine (9.2 mg/kg) on striatal DA content in rats pre-

treated with iprindole (10 mg/kg) was confirmed. In accord with previous reports^{5,15}, this amphetamine-iprindole treatment produced a long-lasting striatal DA depletion (Table I). This DA depletion was selective insofar as 5-HT levels in the striatum, as well as NE and 5-HT levels in the hippocampus, were not affected on a long-term basis by the same drug treatment (Table I).

Each of 3 rats treated identically to those showing a selective striatal DA depletion showed evidence of nerve terminal degeneration in the striatum (Fig. 1B). To determine whether the terminal degeneration found after amphetamine-iprindole treatment was dopaminergic, the DA depletion induced by this treatment was blocked with amfonelic acid. This prevented the appearance of striatal terminal degeneration after amphetamine-iprindole administration.

These results strongly suggest that amphetamine produces a long-lasting striatal DA depletion in iprindole-treated rats by destroying a fraction of striatal DA nerve terminals. That the terminal degeneration found after amphetamine-iprindole treatment is in fact dopaminergic is suggested by the following factors: (1) the terminal degeneration is present in rats known to have a selective long-lasting DA deficit (Table I); (2) the terminal degeneration is present in the striatum, a brain region richly innervated by DA nerve fibers⁹ known to be sensitive to the toxic effect of amphetamine^{5,10,13,16}; and (3) appearance of this terminal degeneration is prevented by amfonelic acid, a drug which selectively blocks DA deficits induced by amphetamines in iprindole-treated rats^{5,12,15}. These considerations do not rule out the possibility that the degeneration in Fig. 1 is of some

TABLE I

Selective long-lasting striatal DA depletion after D-amphetamine in iprindole-treated rats

Rats were administered D-amphetamine (9.2 mg/kg) immediately after iprindole (10 mg/kg). Rats were killed 2 weeks later and assayed for striatal DA and 5-HT and hippocampal NE and 5-HT. Values are the mean \pm S.E.M. ($n = 5$).

Treatment	Striatal		Hippocampal	
	DA (μ g/g)	5-HT (μ g/g)	NE (μ g/g)	5-HT (μ g/g)
Saline	12.9 \pm 0.6	0.44 \pm 0.03	0.41 \pm 0.03	0.45 \pm 0.03
D-Amphetamine	11.8 \pm 0.7	0.45 \pm 0.03	0.44 \pm 0.04	0.47 \pm 0.03
Iprindole	12.4 \pm 0.7	0.41 \pm 0.02	0.45 \pm 0.03	0.44 \pm 0.03
D-Amphetamine + iprindole	9.9 \pm 0.5*	0.42 \pm 0.03	0.39 \pm 0.03	0.42 \pm 0.03

* $P < 0.05$ Dunnett's test.

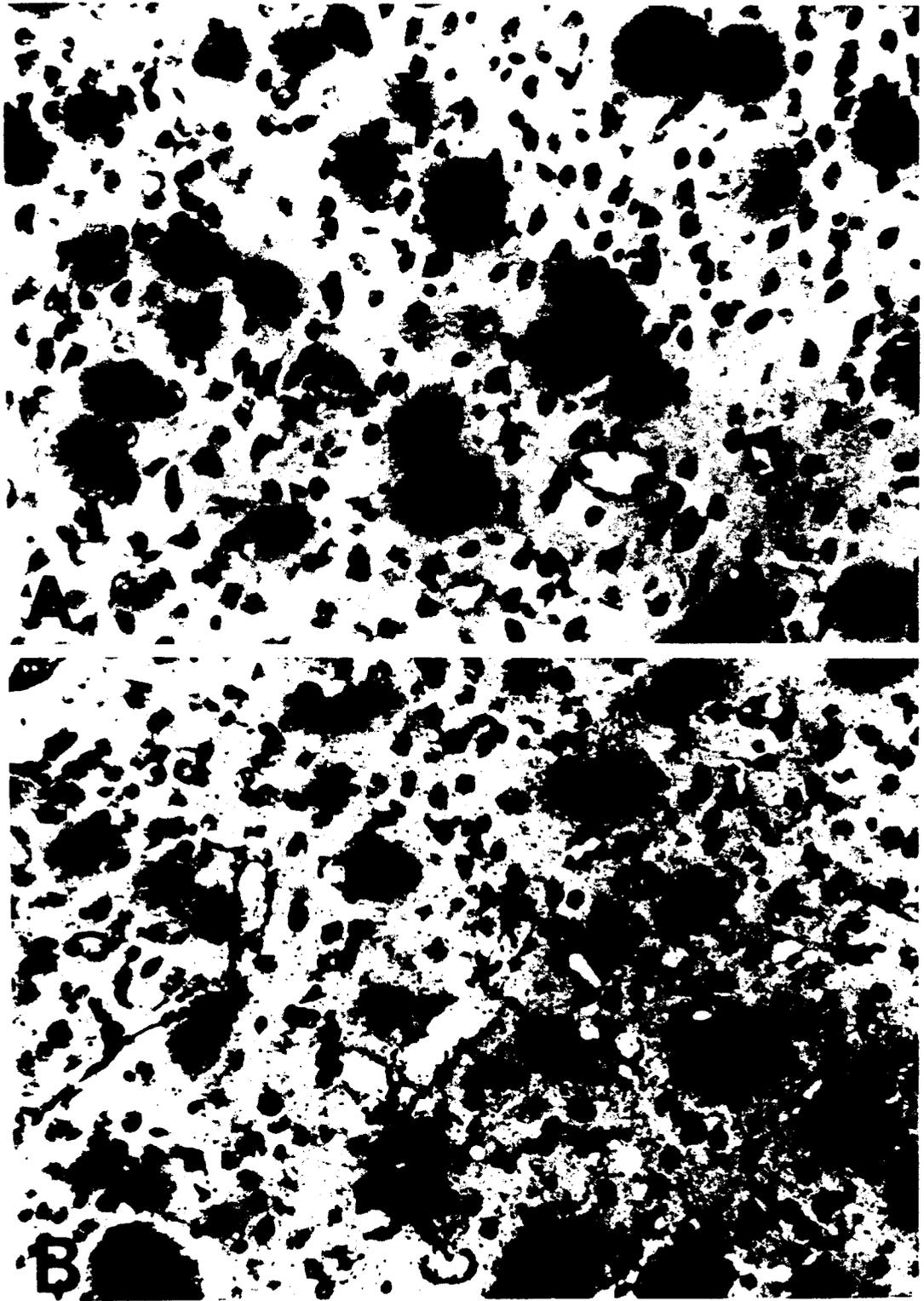


Fig. 1. Silver-stained sections counter-stained with cresyl violet through the striatum of: A, a control rat, and B, a rat administered amphetamine (9.2 mg/kg) and iprindole (10 mg/kg) 3 days previously. Fine granular argyrophillic debris indicative of nerve fiber degeneration is evident in the striatum of rat administered amphetamine in combination with iprindole.

nerve fiber system which is not dopaminergic but which is also affected by amphetamine-*iprindole* treatment and is also protected by amfonelic acid. This seems unlikely, however, given the apparent selectivity of both amphetamine-*iprindole* treatment (Table I) and amfonelic acid¹².

DA terminal destruction appears to be an effect produced by amphetamine (or one of its metabolites) itself rather than an effect uniquely produced by amphetamine-*iprindole* treatment. The strongest indication of this is that amphetamine alone also induces prolonged DA deficits when administered continuously^{10,13} or in high doses¹⁶. The primary action of *iprindole*, at least in the present context, seems to be to prolong the half-life of amphetamine^{4,14} so that an otherwise innocuous dose of amphetamine (9.2 mg/kg) produces DA toxic changes (Table I and Fig. 1).

This study extends previous reports that amphetamine damages striatal DA nerve terminals. As noted earlier, these reports^{1,10} suggested that amphetamine damaged DA nerve fibers, but the nature of this damage was unclear. The present finding of striatal terminal degeneration after amphetamine indicates that this damage involves frank DA neuronal destruction. This study is also in agreement with our previous study¹¹, in which evidence of striatal terminal degeneration was found after high doses of methamphetamine. Like amphetamine in this study, methamphetamine in that study produced a long-lasting striatal DA depletion which was correlated

with the presence of striatal nerve terminal degeneration. It seems reasonable to conclude, therefore, that both amphetamine and methamphetamine cause prolonged DA deficits by destroying DA terminals. Of note is that this toxic effect of amphetamines on DA neurons seems to be restricted to DA terminals since there appears to be no loss of DA cell bodies in the substantia nigra and ventral tegmental area^{10,11}.

Whether DA toxic changes of the type reported here occur in man as a result of amphetamine abuse is not known. Dose, frequency of administration and rate of amphetamine metabolism are some of the variables that may render man more or less sensitive to the DA neurotoxic effects of amphetamine. Future studies will have to ascertain whether amphetamines induce DA toxic changes in man.

In summary, through the use of combined pharmacological and morphological methods, this study has demonstrated that the long-lasting DA depletion produced by a single injection of amphetamine in *iprindole*-treated rats is due to partial DA nerve fiber destruction. The mechanism underlying DA fiber destruction by amphetamine remains to be elucidated.

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