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Antagonism of Cocaine, Amphetamine, and Methamphetamine Toxicity

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DERLET, R. W., T. E. ALBERTSON AND P. RICE. *Antagonism of cocaine, amphetamine, and methamphetamine toxicity.* PHARMACOL BIOCHEM BEHAV 36(4) 745–749, 1990.—The effect of diazepam, haloperidol, MK-801, and propranolol in antagonizing behavioral symptoms induced by lethal doses of cocaine, amphetamine, and methamphetamine were studied in a rat model. Animals were first pretreated IP with potential antagonists, diazepam (2, 5, and 10 mg/kg), haloperidol (5, 10, and 20 mg/kg), propranolol (5, 10, and 20 mg/kg), MK-801 (0.5, 1.0, and 2.5 mg/kg), and then were challenged IP with cocaine (70 mg/kg) (LD_{85}), *d*-amphetamine (75 mg/kg) (LD_{100}), and methamphetamine (100 mg/kg) (LD_{90}). Diazepam, at all doses, provided significant protection against cocaine- ($p \leq 0.01$) and methamphetamine- ($p \leq 0.05$) induced seizures and produced a dose-dependent effect against amphetamine-induced seizures. MK-801, at all doses, reduced seizures in all groups ($p \leq 0.01$). Propranolol altered the incidence of methamphetamine-induced seizures. Significant protection against cocaine-induced death was afforded by diazepam ($p \leq 0.01$) and propranolol ($p \leq 0.05$). Significant protection against amphetamine-induced death was provided by haloperidol (all doses, $p \leq 0.1$), MK-801 (all doses, $p \leq 0.1$), and propranolol (10 and 20 mg/kg, $p \leq 0.1$). No agent reduced the incidence of methamphetamine- (50 or 100 mg/kg) induced death. The failure of *d*-amphetamine antagonists to protect against methamphetamine-induced toxicity and death suggest that different mechanisms of toxicity may exist between these drugs.

Amphetamine Cocaine Diazepam Haloperidol Methamphetamine MK-801 Propranolol

COCAINE, amphetamine, and methamphetamine are commonly abused stimulant drugs, which produce a wide variety of serious medical complications (4,11). Many of the medical complications induced by each of these drugs are clinically similar or undistinguishable regardless of the drug used to produce toxicity (8,18). The similarity of clinical symptoms produced has been explained by shared mechanisms of toxicity. However, recent work suggests that the mechanisms of death induced by cocaine may differ from amphetamine, as not all agents which antagonize cocaine toxicity effectively antagonize amphetamine (6,7). Conversely, additional reports show that some agents which antagonize amphetamine toxicity are ineffective against cocaine toxicity. While many studies have defined antagonists to the acute toxicity of cocaine and amphetamine, very little work has examined approaches to acute methamphetamine toxicity in lethal dose models. This laboratory has utilized an animal model of acute toxicity from cocaine, amphetamine, or methamphetamine to determine the protective characteristics of the benzodiazepine antagonist, diazepam, the dopamine receptor antagonist, haloperidol, the NMDA antagonist, MK-801, and the beta-adrenergic antagonist, propranolol.

METHOD

Male Sprague-Dawley rats weighing between 200 and 300 grams were used in these experiments, and cared for following the guidelines of our animal control committee that approved the experimental protocol. Rats were kept under 12-hour light/dark cycles and had ad lib access to food and water. All agents were administered intraperitoneally (IP).

Toxic doses of cocaine (70 mg/kg), and *d*-amphetamine (75 mg/kg) with (LD_{85} and LD_{100} respectively) were selected based on prior work in this laboratory (6,7). Methamphetamine was administered in doses of (12.5, 25, 50, 75, and 100 mg/kg). Twenty animals were tested at each dose. Methamphetamine 100 mg/kg (LD_{100}) was selected for comparison studies with cocaine and *d*-amphetamine. *d*-Amphetamine sulfate, cocaine hydrochloride, and methamphetamine hydrochloride were obtained from the Sigma Chemical Company (St Louis, MO). These drugs were dissolved in saline and administered in a volume of 2 ml/kg.

Test animals received diazepam (2, 5, or 10 mg/kg), haloperidol (5, 10 or 20 mg/kg), MK-801 [(+)-5-methyl-10,11-dihydro-5H-dibenzo [a,d]-cyclohepten-5,10-imine (0.5, 1.0, 2.5 mg/kg)], or propranolol (5, 10, or 20 mg/kg) (IP) 30 minutes prior to challenge by each stimulant drug. Ten animals were tested at each dose. Diazepam was obtained from Hoffmann-La Roche, Inc. (Nutley, NJ). Haloperidol and propranolol were obtained from Sigma Chemical Company, and MK-801 was obtained from Merck Sharpe and Dohme (Bluebell, PA).

All test agents were dissolved in dimethylsulfoxide (DMSO, Sigma Chemical Company) 1 ml/kg. This dose of DMSO was also used as the vehicle control. Control animals (n) received DMSO 30 minutes prior to challenge with cocaine (n = 40), amphetamine

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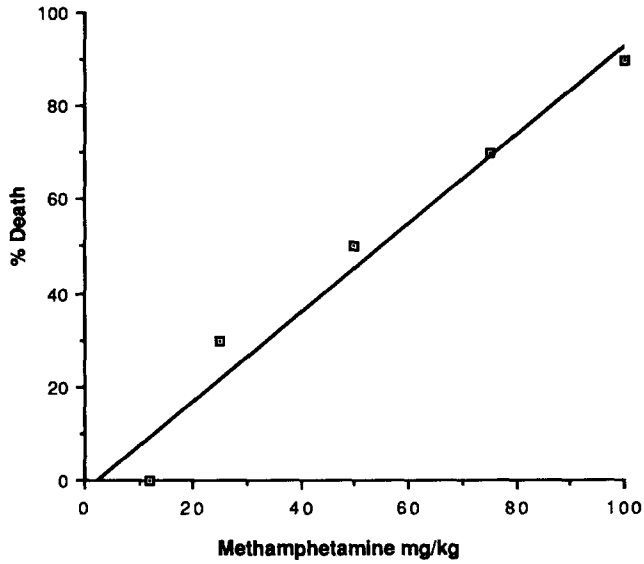

FIG. 1. The incidence of total death induced by methamphetamine for five doses. Twenty animals were tested at each dose.

(n = 40), and methamphetamine (n = 30). The doses of test drugs were selected to be consistent with previously established dose ranges in rats (5–7). All animals were individually housed. The condition of each animal was evaluated prior to test drug administration, after test drug, and after stimulant drug. Parameters evaluated included sedation, muscle tone, gait ataxia, and the presence of a righting reflex (spontaneous ability of the rodent to upright itself when placed on its back). After administration of stimulant drugs, animals were observed for four hours, then checked daily for one week.

In a second set of experiments, 10 animals were each pretreated with DMSO vehicle 1 ml/kg, diazepam 5 mg/kg, haloperidol 10 mg/kg, MK-801 2.5 mg/kg, or propranolol 10 mg/kg and then challenged with an LD_{50} of methamphetamine (50 mg/kg).

In a third set of experiments, 12 animals were chronically implanted with cortical electrodes using methods previously described (5). After two weeks to recover, animals were then pretreated with either MK-801 (2.5 mg/kg) or DMSO (1 ml/kg) 30 minutes before challenge with either cocaine (70 mg/kg), amphetamine (75 mg/kg) or methamphetamine (100 mg/kg). EEG and behavior were observed for two hours.

Data is reported overall as a percent of either seizures or death for each group. Mean time in minutes to seizure and death was calculated to include standard error. Animals dying after four hours were considered "late" deaths and categorized separately in time calculations. Time comparisons were performed using the Students' *t*-test or one-way ANOVA. If significant effect was detected by ANOVA, Dunnett's test was used to compare individual means to the control values. The chi-squared test was used to determine overall significance of the incidence of seizures and deaths between experimental treatments and controls.

RESULTS

The dose-response curve for death of animals receiving methamphetamine is depicted in Fig. 1. At doses of 50 mg/kg and above, the rats became agitated at five minutes after receiving methamphetamine. Piloerection and generalized fine body tremor occurred, and unless animals died, this agitation and tremor lasted between 60 and 120 minutes. Seizures occurred intermittently and lasted between 30 and 60 seconds. A variable number of animals at each dose that survived to four hours post injection, were found dead 12 to 48 hours later (Table 1).

TABLE 1

METHAMPHETAMINE: ACUTE AND LATE DEATHS

Dose (mg/kg)	Acute Death*	Late Death†	Total Death (%)
12	0/20	0/20	0/20 (0)
25	2/20	4/20	6/20 (30)
50	5/20	5/20	10/20 (50)
75	4/20	10/20	14/20 (70)
100	14/20	4/20	18/20 (90)

*Acute Death = 0–4 hours; †Late Death = 4–48 hours.

Table 2 illustrates the effect of diazepam, haloperidol, MK-801, and propranolol on the incidence of death induced by cocaine, *d*-amphetamine, or methamphetamine. Cocaine induced death in 85% of vehicle control animals in 8.9 ± 0.5 minutes respectively. Significant protection against death was effected by diazepam (2, 5, and 10 mg/kg) and propranolol (10 and 20 mg/kg). Amphetamine (75 mg/kg) induced death in 100% of control vehicle-pretreated animals in a mean time of 55.4 ± 2.4 minutes. Haloperidol, MK-801, and propranolol provided significant protection against amphetamine-induced death. Methamphetamine (100 mg/kg) induced acute death in 70% of pretreated vehicle control animals in a mean time of 21.4 ± 4.7 minutes. In additional animals, death occurred 4 to 48 hours after methamphetamine injection. These animals (late deaths) would appear to have recovered from the acute toxic doses of methamphetamines and have normal cage behavior between two hours and four hours, after which time observation was intermittent. Late deaths accounted for 20% of these animals, having died between 4 and 48 hours following injection of methamphetamine. None of the drugs tested reduced the incidence of death from this dose of methamphetamine, and did not significantly change time to death or number of late deaths.

The effect of test drugs on seizures is presented in Table 3. Diazepam and MK-801, at all doses tested, provided significant protection against cocaine-induced seizures. Haloperidol had a significant effect against cocaine-induced seizures only at the highest dose tested, 20 mg/kg. Amphetamine-induced seizures occurred in 95% of vehicle controls in 12.6 ± 1.0 minutes, and seizure incidence significantly was decreased by pretreatment with diazepam and MK-801. However, other drugs tested did not alter time to seizure or incidence. The incidence of methamphetamine-induced seizures seen with pretreatment of vehicle (70%) occurred in 14.8 ± 3.3 minutes and significantly decreased with diazepam, propranolol, and MK-801, but not with haloperidol.

Because all drugs failed to antagonize methamphetamine- (100 mg/kg) induced death, a lower dose of methamphetamine (50 mg/kg) was selected for further studies. The incidence of death in animals pretreated with diazepam, haloperidol, MK-801, and propranolol was not significantly different from controls (Table 4).

Table 5 demonstrates that MK-801 protected animals from EEG evidence of stimulant-induced afterdischarges and convulsions. Of interest, cortical spiking, which was seen with cocaine and amphetamine prior to afterdischarges and seizures, was not seen prior to methamphetamine-induced afterdischarges.

TABLE 2

INCIDENCE OF DEATH

Pretreatment:		% Death Cocaine (70 mg/kg)	Amphetamine[1] (75 mg/kg)	Methamphetamine (100 mg/kg)
Vehicle:		85	100	90
Diazepam	2 mg/kg	10†	80	90
	5 mg/kg	0†	100	80
	10 mg/kg	0†	90	90
Haloperidol	5 mg/kg	60	60†	70
	10 mg/kg	70	50†	80
	20 mg/kg	60	40†	80
MK-801	0.5 mg/kg	70	20†	90
	1.0 mg/kg	90	0†	90
	2.5 mg/kg	90	0†	90
Propranolol	5 mg/kg	80	90	80
	10 mg/kg	50*	70*	70
	20 mg/kg	50*	40†	80

[1]Data on amphetamine with diazepam, haloperidol, and propranolol previously reported (7).

*$p \leq 0.05$; †$p \leq 0.01$.

DISCUSSION

Cocaine, amphetamine, and methamphetamine have been described as having similar neuropharmacologic effects. Studies have shown that humans or trained laboratory animals cannot discriminate the difference between the subjective effects of cocaine and amphetamine (8,18). Certain pharmacologic agents (for example, prazosin) antagonize behavioral effects of both cocaine and amphetamine (21). In addition, the clinical central nervous system (CNS) toxicity seen in patients who present to emergency departments appears to be similar (4,10). However, on a molecular and cellular level, differences in site specificity and excitatory pathways stimulated by each of these compounds have been described (15, 16, 20). This study, using lethal doses of cocaine and *d*-amphetamine, has clearly demonstrated differences in the effect of specific pharmacologic agents to antagonize toxicity. The current study also suggests that mechanisms leading to methamphetamine toxicity differ from those of cocaine and *d*-amphetamine.

The failure of *d*-amphetamine antagonists to protect methamphetamine-intoxicated animals was unexpected. Both pharmacologically and clinically, the toxicity of these two agents would appear to be even more closely related than *d*-amphetamine and cocaine (22). Several hypotheses may account for the differences between *d*-amphetamine and methamphetamine seen in this study. The biodistribution of each into the CNS may differ. Subtle differences in receptor sites could result in stimulation of different final CNS pathways. The half-life of *d*-amphetamine compared to methamphetamine may differ in the rat. The pharmacologic agents tested may have been ineffective against methamphetamine because of pharmacologic mismatching in which the prolonged toxic half-life of methamphetamine is longer than the protective effects of tested agents. Against this argument is the failure of test agents to protect against methamphetamine even at a lower dose (LD_{50}). Furthermore, animals pretreated with either vehicle or test agent, for example MK-801, die too quickly after receiving methamphetamine to consider a second or third injection of MK-801. Metham-

TABLE 3

INCIDENCE OF SEIZURES

Vehicle		% Seizures Cocaine 96	Amphetamine 95	Methamphetamine 70
Diazepam	2 mg/kg	0†	70	0*
	5 mg/kg	0†	40†	0*
	10 mg/kg	0†	10†	0*
Haloperidol	5 mg/kg	90	90	50
	10 mg/kg	70	80	70
	20 mg/kg	60*	80	100
MK-801	0.5 mg/kg	40†	0†	0*
	1.0 mg/kg	30†	0†	0*
	2.5 mg/kg	0†	0†	0*
Propranolol	5 mg/kg	80	100	20†
	10 mg/kg	70	100	20†
	20 mg/kg	80	70	50

*$p \leq 0.05$; †$p \leq 0.01$.

TABLE 4

METHAMPHETAMINE (50 mg/kg)

Pretreatment:		N	Death Acute	Late	Total Death	%
Vehicle:		24	8	6	14/24	(42)
Diazepam	5 mg/kg	10	3/10	2/10	5/10	(50)
Haloperidol	10 mg/kg	10	3/10	2/10	5/10	(50)
MK-801	2.5 mg/kg	10	4/10	0	4/10	(40)
Propranolol	10 mg/kg	10	0	5/10	5/10	(50)

N = number of animals.

TABLE 5

EFFECT OF MK-801 ON STIMULANT-INDUCED EEG ACTIVITY

			Post Treatment Effects§		
N*	Treatment 1†	Treatment 2‡	Cortical Spikes	EEG AD	Behavior Seizure
2	MK-801	Cocaine	+	0	0
2	Vehicle	Cocaine	+	+	+
2	MK-801	Amphetamine	+	0	0
2	Vehicle	Amphetamine	+	+	+
2	MK-801	Methamphetamine	+	0	0
2	Vehicle	Methamphetamine	0	+	+

*N = Number of animals.

†MK-801, 2.5 mg/kg, IP or 1 cc/kg dimethylsulfoxide IP, 30 minutes before Treatment 2.

‡Cocaine (70 mg/kg IP), amphetamine (75 mg/kg IP), and methamphetamine (100 mg/kg IP).

§Cortical spikes were defined as EEG spikes 2–3 times baseline amplitude, after discharge (AD) was rapid, sustained spikes greater than 1 spike/sec with amplitude of 2–3 baseline, and behavioral seizures were observed convulsions.

phetamine passes the blood-brain barrier more rapidly than amphetamine (12). In addition, the distribution sites of methamphetamine may differ from amphetamine, as well as specific mechanisms of action in toxic and lethal doses.

Diazepam was highly efficacious in preventing cocaine-induced seizures and death. The antagonistic effects of diazepam at lower doses has been reported and is presumably due to enhancement of gamma-aminobutyric acid- (GABA) induced inhibitory pathways in the CNS (5). Our finding that higher doses of diazepam also antagonize cocaine toxicity provides additional evidence of these properties. The failure of diazepam to antagonize amphetamine-induced death suggests that death may involve a different mechanism. Although the incidence of death was not decreased, the incidence of seizures was decreased by diazepam, suggesting that seizures are not necessary for death to occur from toxic doses of amphetamine. The effect of diazepam against methamphetamine-induced seizures and death was similar to the effect against *d*-amphetamine protection against seizures, but not against death.

Haloperidol is an effective antagonist against *d*-amphetamine toxicity. Although haloperidol has not been shown to reduce the incidence of seizures, the incidence of amphetamine-induced death in haloperidol-pretreated animals is significantly decreased. Haloperidol also antagonizes cocaine toxicity to a lesser degree. In sharp contrast, haloperidol has no significant effect in decreasing methamphetamine toxicity. The antagonistic effects of haloperidol presumably occur through blocking the D_2 receptor, which is hyperstimulated in both cocaine and amphetamine toxicities (19). Haloperidol has previously been shown to antagonize acute toxicity (3), as well as behavioral abnormalities induced by *d*-amphetamine (1). The failure of methamphetamine to be antagonized by haloperidol in this study would suggest that additional neurotransmitters or direct peripheral effects contribute significantly to high-dose methamphetamine toxicity. Our finding is in contrast to that of Witkin *et al.* who found that haloperidol (1 mg/kg) decreased the incidence of methamphetamine death (23). It is difficult to compare this finding to our study, because Witkin *et al.* used a much lower dose of methamphetamine, 12.5 mg/kg, a different rat strain (Fisher 344), and based conclusions on only six animals.

Highly significant antagonism against amphetamine-induced death by MK-801 was demonstrated in this study. Since the mechanism of action of MK-801 is believed to be through the antagonism of N-methyl-D-aspartate (NMDA) excitatory amino acid receptors (2), our finding would suggest that significant *d*-amphetamine toxicity occurs through this mechanism. In contrast, methamphetamine-induced death was unaffected by MK-801. This difference in the effect of MK-801 against two pharmacologically similar stimulants has profound implications on the mechanisms of lethality of each.

This contrast further supports evidence from prior studies, showing that certain antagonists which provide protection from one stimulant fail to protect against the other. However, in order to explain these differences through underlying physiologic and pharmacologic mechanisms, additional studies are needed using different types of models. MK-801 did abolish EEG evidence of seizures and behavioral convulsions in methamphetamine-intoxicated animals, but did not affect the incidence of death. Similarly, despite the ability of MK-801 to decrease cocaine-induced seizures, the incidence of death was unaffected; therefore, these seizures are not necessary for cocaine-induced death to occur. In other seizure models, MK-801 has been demonstrated to have selective antagonist properties as shown through anticonvulsant actions against lithium-pilocarpine and amygdala kindled models, but not a pilocarpine model alone (9,13).

Propranolol had a significant effect in decreasing the incidence of death in both cocaine- and amphetamine-treated animals. The current study is consistent with recent work demonstrating the efficacy of propranolol in protecting mice challenged with a lethal dose of cocaine (17). When compared to other antagonists, the efficacy of propranolol was less than that of diazepam against cocaine, and less of either haloperidol or MK-801 against amphetamine. Propranolol antagonizes beta-1 and beta-2 stimulation at both central and peripheral adrenoceptors (14). The efficacy of propranolol against cocaine and amphetamine has been attributed to the importance of β-adrenoceptor stimulation in the mechanism of either amphetamine- or cocaine-induced death. In contrast, methamphetamine toxicity was unaffected by pretreatment with propranolol. Some of the toxic effects of methamphetamine do occur through hyperstimulation of beta-receptors (24). The lack of β-blockers to block methamphetamine toxicity suggests additional mechanisms are also important in its toxicity.

In conclusion, 1) MK-801 is a highly efficacious antagonist against a lethal dose of *d*-amphetamine. 2) MK-801 provides highly significant protection against convulsions induced by cocaine, amphetamine, or methamphetamine. 3) Diazepam provides significant protection to cocaine toxicity, but not against amphetamine or methamphetamine. 4) Propranolol affords some protection against cocaine and amphetamine toxicity. 5) The failure of amphetamine antagonists to protect against methamphetamine-induced death suggests differing mechanisms of toxicity exists between these drugs or that pharmacologic mismatching occurs.

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