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Abstract

Methamphetamine (MA) toxicity in aggregated mice was studied by varying the number of mice and the proportion of MA treated mice kept in the same confined space. The lethality was measured 24 h after intraperitoneal injections of MA at doses ranging from 10 to 100 mg/kg. MA lethality, over a wide dose range (15 to 50 mg/kg), was higher in aggregated mice than in those maintained in isolation. The greater the proportion of MA-treated mice in aggregation was, the higher the MA lethality was. In aggregations of 10 mice, MA was lethal at lower doses than in aggregations of 5 mice. These results indicate that the lethality of MA is influenced by confinement and aggregation.

KEYWORDS: methamphetamine, mortality, aggregation

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Effects of Aggregation on Methamphetamine Toxicity in Mice

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Methamphetamine (MA) toxicity in aggregated mice was studied by varying the number of mice and the proportion of MA treated mice kept in the same confined space. The lethality was measured 24 h after intraperitoneal injections of MA at doses ranging from 10 to 100 mg/kg. MA lethality, over a wide dose range (15 to 50 mg/kg), was higher in aggregated mice than in those maintained in isolation. The greater the proportion of MA-treated mice in aggregation was, the higher the MA lethality was. In aggregations of 10 mice, MA was lethal at lower doses than in aggregations of 5 mice. These results indicate that the lethality of MA is influenced by confinement and aggregation.

Key words : methamphetamine, mortality, aggregation

Since amphetamine-induced psychosis in man was reported by Connell (1), behavioral studies on abnormalities induced by amphetamine have been performed in rats, cats, dogs, guinea pigs and man (2, 3).

Gunn and Gurd (4) reported a marked increase in the lethality of amphetamine in aggregated animals, as compared with that in animals maintained in isolation. Cohen and Lal (5) reported that an increase in the toxicity of amphetamine in aggregated animals was related to factors such as experimental temperature, sound, illumination, strain of mice and sex. The influence of aggregation of mice on amphetamine toxicity was investigated by Wang, *et al.* (6), with a result indicating the augmentation of toxicity in groups containing greater numbers of dosed animals.

The purpose of this study was to determine whether the toxicity of methamphetamine (MA) might vary with the confinement of animals and with the ratio of untreated to treated animals in aggregation.

Materials and Methods

Animals. A total of 1240 male ddY mice weighing 20 to 25 g were used in this experiment. Immediately after arrival at the laboratory, animals were housed in groups of 20 each in plastic cages (30×35×17 cm) and supplied with food and water *ad libitum*.

Animal-Grouping. The animals were divided into the following experimental groups: Group I, one mouse dosed with MA was housed together with 4 untreated mice (1MA+4U); Group II, 2 mice dosed with MA were housed together with 3

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untreated mice (2MA+3U); Group III, 5 mice dosed with MA were housed together (5MA); Group IV, one mouse dosed with MA was housed together with 9 untreated mice (1MA+9U); Group V, 5 mice dosed with MA were housed together with 5 untreated mice (5MA+5U); Group VI, 10 mice dosed with MA were housed together (10MA); and Group VII, mice dosed with MA were housed singly (1MA).

Drug. d-Methamphetamine hydrochloride (Philopon) was dissolved in physiological saline and injected intraperitoneally in a volume of 0.1 ml per 10 g of body weight. Drug injections were made between 8:30 and 9:00 *A.M.*

Lethality Observations. Lethality was determined in each group from the numbers of animals which died 0.5, 1, 2, 4, 8 and 24 h after the injection of the drug. The room temperature was kept at 23±1°C during all experiments. Throughout the observation of lethality and behavioral signs of toxicity, mice in Groups I to VI were housed in plastic cages with a floor area of 16×10 cm and a depth of 13.5 cm, while smaller plastic cages measuring 5.4×8.2×13.5 cm were used for those in Group VII.

Statistics. The Fisher exact probability test (7) was employed to determine statistical significance of differences.

Results

Toxicity of MA in 5 Aggregated Mice. The dose-lethality curves of MA in mice in Groups I, II, III and VII (isolation control) are shown in Fig. 1. Table 1 shows levels of significance in intergroup comparisons of MA lethality to mice in aggregations of 5 mice. The degree of MA lethality at dose levels of 15-70 mg/kg was in the order of Group III(5MA) > Group VII(1MA) > Group II(2-MA+3U) and Group I(1MA+4U). Group III(5MA) displayed significantly higher lethality than Group VII (the control group of singly housed animals, 1MA) ($p<0.05$), Group I(1MA+4U) ($p<0.02$) and Group II (2MA+3U) ($p<0.02$), at a dose level of 50 mg/kg. At a dose of 70 mg/kg, Group III(5MA) also showed a higher lethality than Group I(1MA+4U) ($p<0.02$) and Group II (2MA+3U) ($p<0.02$). In all these groups, the animals died within about 4 h of an injection of 30-70 mg/kg or within 30 min of an injection of 100 mg/kg.

There was a remarkable difference in behavioral changes between the control group of isolated mice and the groups of aggregated mice, with changes being particularly pronounced in MA-dosed animals. Salivation, lacrimation, sweating and ruffled hair were common in both the control group and groups of aggregated animals. In the low-dose groups of aggregated mice, marked hyperactivity with violent running and jumping

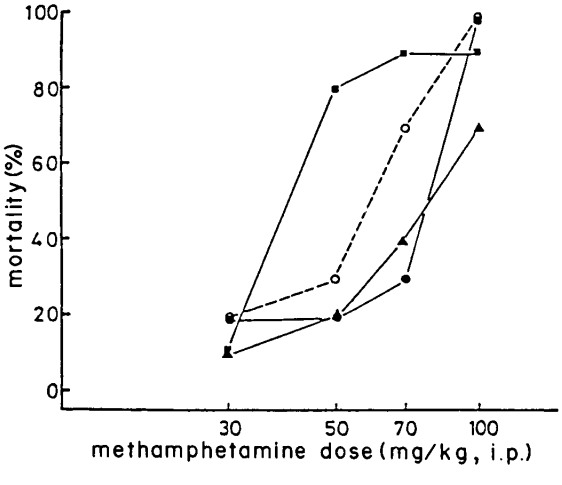

Fig. 1 Methamphetamine (MA) lethality in isolated and aggregated mice, determined 24 h after intraperitoneal injection of drug: experiments with 5 mice. Each point was obtained from the number of dead mice/10 mice. ○---○ Mortality in group VII in which mice dosed with MA were housed singly; ●——● mortality in group I in which one mouse dosed with MA was housed together with 4 untreated mice; ▲——▲ mortality in group II in which 2 mice dosed with MA were housed together with 3 untreated mice; ■——■ mortality in group III in which 5 mice dosed with MA were housed together.

Table 1 Levels of significance between two independent groups on methamphetamine (MA) lethality in 5 aggregated mice[a]

Dose of MA (mg/kg)[b]	Group[c]	Level of significance between groups			
		VII(1MA)	I(1MA+4U)	II(2MA+3U)	III(5MA)
50	VII(1MA)	—	NS[d]	NS	$p<0.05$
	I(1MA+4U)		—	NS	$p<0.02$
	II(2MA+3U)			—	$p<0.02$
	III(5MA)				—
70	VII(1MA)	—	$p<0.1$	NS	NS
	I(1MA+4U)		—	NS	$p<0.02$
	II(2MA+3U)			—	$p<0.05$
	III(5MA)				—

a: p values were calculated by the Fisher exact probability test.
b: Injected intraperitoneally.
c: Group VII, mice dosed with MA were housed singly (1MA); group I, one mouse dosed with MA was housed with 4 untreated mice (1MA+4U); group II, 2 mice dosed with MA were housed together with 3 untreated mice (2MA+3U); group III, 5 mice dosed with MA were housed together (5MA).
d: NS, no significance.

was evident, *i.e.*, the animals exhibited excitatory behavior such as squeaking, gnawing, sniffing, licking or biting the cage, and attacking languid mice. Dead mice were mostly found with the ears bitten off, and even with the head and viscera plucked or eaten off. Animals of the high-dosed groups in aggregation developed tremors immediatety after injection, followed by the onset of convulsions leading to death. Isolated animals receiving high doses similarly developed tremors that shifted to convulsions. Gnawing, sniffing, pivoting on the hindlegs, walking backwards, licking the cage, preening and grooming were conspicuous with animals receiving the drug at dose levels other than 100 mg/kg. Attacks of MA-treated mice on untreated mice were noticeable, especially on the ears of mice in Groups I (1MA+4U) and II(2MA+3U). However, there was little or no fighting among the MA-treated mice in Group II. Isolated mice in Group VII showed slight hypoactivity and frequently crouched with a curved back.

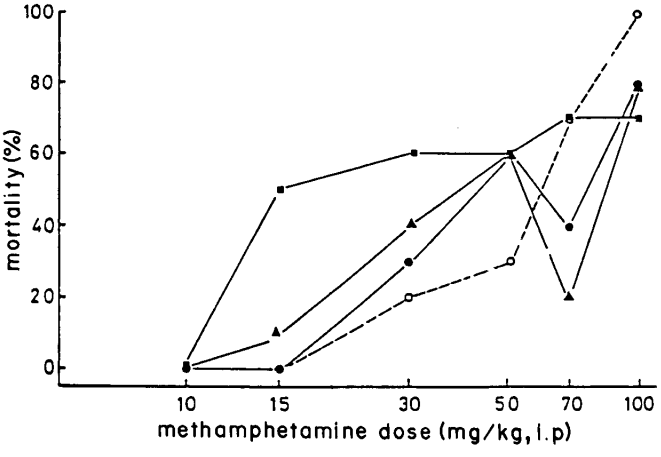

Fig. 2 Methamphetamine (MA) lethality in isolated and aggregated mice, determined 24 h after intraperitoneal injection of drug: experiments with 10 mice. Each point was obtained from the number of dead mice/10 mice. ○---○ Mortality in group VII in which mice dosed with MA were housed singly; ●——● mortality in group IV in which one mouse dosed with MA was housed together with 9 untreated mice; ▲——▲ mortality in group V in which 5 mice dosed with MA were housed together with 5 untreated mice; ■——■ mortality in group VI in which 10 mice dosed with MA were housed together.

Table 2 Levels of significance between two independent groups on methamphetamine (MA) lethality in 10 aggregated mice[a]

Dose of MA (mg/kg)[b]	Group[c]	Level of significance between groups			
		VII(1MA)	IV(1MA+9U)	V(5MA+5U)	VI(10MA)
15	VII(1MA)	—	NS[d]	NS	$p < 0.02$
	IV(1MA+9U)		—	NS	$p < 0.02$
	V(5MA+5U)			—	NS
	VI(10MA)				—
30	VII(1MA)	—	NS	NS	$p < 0.10$
	IV(1MA+9U)		—	NS	NS
	V(5MA+5U)			—	NS
	VI(10MA)				—
50	VII(1MA)	—	NS	NS	NS
	IV(1MA+9U)		—	NS	NS
	V(5MA+5U)			—	NS
	VI(10MA)				—
70	VII(1MA)	—	NS	$p < 0.05$	NS
	IV(1MA+9U)		—	NS	NS
	V(5MA+5U)			—	$p < 0.05$
	VI(10MA)				—

a: p values were calculated by the Fisher exact probability test.
b: Injected intraperitoneally.
c: Group VII, mice dosed with MA were housed singly (1MA); group IV, one mouse dosed with MA was housed with 9 untreated mice (1MA+9U); group V, 5 mice dosed with MA were housed together with 5 untreated mice (5MA+5U); group VI, 10 mice dosed with MA were housed together (10MA).
d: NS, no significance.

Toxicity of MA in 10 Aggregated Mice. The data from mice in Groups IV, V, VI and VII (control) are shown in Fig. 2. Table 2 shows levels of significance in intergroup comparisons of MA lethality to mice in aggregations of 10. As shown in the figure, the degree of lethality at dose levels of 15-50 mg/kg was in the order of Group VI (10MA) > Group V(5MA+5U) > Group IV (1MA+9U) > Group VII(1MA). Group VI (10MA) showed a significantly higher lethality than Group VII(1MA) and Group IV (1MA+9U) at a dose of 15 mg/kg ($p < 0.02$, respectively), and had a tendency to show higher lethality than Group VII(1MA) at a dose level of 30 mg/kg. With a dose of 70 mg/kg, however, the lethality in Group V(5MA+5U) was lower than that in the control group of isolated mice (1MA) and Group VI(10MA) ($p < 0.05$, respectively). Deaths occurred within 4 h of injection of 15 to 70 mg/kg or within 30 min of the administration of 100 mg/kg.

Discussion

These experiments demonstrated higher MA lethality over a wide dose range from 15 to 50 mg/kg in groups of aggregated mice, as compared with mice maintained in isolation. The greater the number of MA-treated mice in aggregation was, the higher the lethality of MA was, and the lethality of MA to mice in aggregations of 10 was higher at lower doses than in aggregations of 5 mice. Chance (8) showed that the toxicity of amphetamine in aggregated mice was increased by decreasing the floor area per mouse (confinement), and Land and Larson (9) observed a marked increase in amphet-

amine toxicity when the number of mice per cage (aggregation) was increased from 5 to 10. Thus their observations indicate that the toxicity of amphetamine in aggregated animals involves both confinement and aggregation. The high lethality of MA at low doses in aggregated mice might be attributed to the confinement and the aggregation in the present experiment. Therefore, our results indicate that the lethality of MA may not be dependent upon inherent drug toxicity, but might be the result of various stresses caused by excitatory behavior of treated animals, such as jumping, running, squeaking and fighting. It is most probable that emotional stress influences the aggregation effect.

A difference in the lethality of MA at a dose of 70 mg/kg was observed in Group I (1MA+4U) of 5 aggregated mice and in Group V(5MA+5U) of 10 aggregated mice, as compared with the isolated control groups (1MA). The dose-lethality curve of MA in Group IV(1MA+9U) and Group V(5MA+5U) was found to be nonlinear and was biphasic for low and high doses in mice in aggregations of 10. Moore (10) previously reported that the lethality of amphetamine in aggregated mice progressively increased at low doses and decreased at high doses. The same phenomenon in amphetamine lethality was also reported by Gardocki *et al.* (11), George and Wolf (12) and Wang *et al.* (6). According to these authors, the lethal effect of the high dose reflects not simply the toxicity of the compound, but rather the pharmacological activation of the drug by aggregation. No precise explanation of MA lethality at a dose level of 70 mg/kg can presently be made. In a high dose of 100 mg/kg of MA, a high lethality of over 75% was observed in all groups of 5 and 10 aggregated mice, indicating that the lethal effect by a high dose reflects the toxicity of the drug.

It would be reasonable to conclude, therefore, that animals dosed with MA are more liable to be influenced by various stresses, and that the lethal effect of the compound is enhanced by aggregation as well as by emotional stress.

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