INFLUENCE OF AGGREGATION ON THE ACTION OF METH-AMPHETAMINE IN LOCOMOTOR ACTIVITY

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Influence of aggregation on the action of methamphetamine in general behavior was investigated in mice. The action of increasing the amount of movement by methamphetamine was significantly increased by aggregation compared to isolation of mice after administration of 5, 15 and 30 mg/kg, *i.p.*, suggesting the group toxicity of methamphetamine.

Keywords —methamphetamine toxicity; mortality; open-field activity; isolation; aggregation; mice

INTRODUCTION

A marked increase of lethality in animals in aggregation over the level in those in isolation was reported after administration of amphetamine, a central nervous stimulant.^{1,2)} Cohen *et al.*,³⁾ further pointed out an intimate relationship between the rise of toxicity to drugs of amphetamine group in aggregated animals on one hand and the rise of the degree of confinement, experimental temperature, sound, illumination, strain of mice and sex of mice on the other. Wang *et al.*,⁴⁾ furthermore, studied the influence of aggregation with non-amphetamine treated, sedated mice, demonstrating a rise of toxicity to drugs of the amphetamine group along with an increase in the proportion of amphetamine-treated animals among the aggregated animals. Gomita *et al.*,⁵⁾ on the other hand, also found a wide variation of toxicity to amphetamines according to the size of the group within the same space and the proportion of untreated animals in the group.

In the present study, in order to study the effect of the amount of exercise on the group toxicity to methamphetamine, the general behavior of the animals treated with methamphetamine in aggregation and isolation

was studied.

MATERIALS AND METHODS

Experiments were conducted on male ddY strain mice weighing 20–28 g (supplied from Kuroda Animal Farm). Immediately after the animals were brought to the laboratory, they were divided into groups according to body weight, followed by group housing in plastic cages measuring 30 × 35 × 17 cm each housing 20 mice. Food and water were supplied *ad libitum*. Animals were used for the experiment within 2–5 d. During the experiment, the animals were maintained in aggregation and isolation in stainless steel cages (15 × 16.5 × 13 cm).

For observation of general behavior, a revised open-field apparatus of Hall⁶⁾ was used. This apparatus was made of metal and consisted of a round floor with a diameter of 60 cm surrounded by walls 47 cm in height and a diameter of 80 cm at the upper edge, giving the overall appearance of a truncated cone. The inside was completely painted gray-white color. The floor was divided into 19 segments by lines. At 80 cm in height from the center of the floor, a white electric lamp of 100 W was placed for illumination.

The test drug, *d*-methamphetamine hydro-

chloride (Philopon) dissolved in distilled water was administered intraperitoneally at a dose of 0.1ml/10g after adjustment of the concentration between 8:30 and 9:00 a.m.

Influence of Aggregation on Methamphetamine Toxicity —One group of mice was administered with various doses of methamphetamine and kept in groups of 10 animals (the aggregation group). In the other group, the animals were individually isolated (the isolation group). The mortality was determined as a function of the time at 0.5, 1, 2, 4, 8 and 24 h after administration. The room temperature was kept at $23 \pm 1^\circ\text{C}$ in all the subsequent experiments. The data were statistically analyzed for significant differences using the Fisher exact probability test.⁷⁾ Unless otherwise specified, this method was used throughout the experiment.

Influence of Aggregation on Hyperactivity caused by Methamphetamine —Mice given various doses of methamphetamine were divided into aggregation groups and isolation groups. The general behavior was studied using the

open-field apparatus. The mouse was placed at the center of the floor of the apparatus and the frequency of crossing the dividing line on the

FIG. 1. 24 h Post-injection Toxicity of Methamphetamine in Isolated and Aggregated Mice

Each point represents 10 mice.

○-----○ isolation, ●-----● aggregation, a) $p < 0.05$.

FIG. 2. 30 and 100 min Post-injection Locomotor Activity of Methamphetamine at Doses of 0.2–5 mg/kg, i.p. in Isolated and Aggregated Mice

□ isolation, ■ aggregation, a) $p < 0.05$.

floor by the mouse in 1 min was determined and this was called ambulation. The frequency of rearing, preening, grooming, defecation and urination was also measured. An open-field test was performed on the day prior to administration and the animals were randomly divided into groups in order to minimize the difference in mean frequency of ambulation among the groups. In order to test the significance of difference between the groups, the Mann Whitney U test⁷⁾ was used.

Using 100 mice, 0.2, 0.5, 1, 2, and 5 mg/kg (first experiment-low doses) and 5, 10 and 30 mg/kg (second experiment-high doses) of methamphetamine were administered in one day for each dose level. General behavior was eval-

uated at 30, 100, 240 and 480 min after administration.

RESULTS

Influence of Aggregation on Methamphetamine Toxicity

Fig. 1 shows the mortality in 190 mice divided in groups of 10 animals each after administration of 5, 7, 10, 15, 20, 30, 50, 70 and 100 mg/kg methamphetamine. The mortality on administration of 7, 10, 15, 30, and 70 mg/kg methamphetamine was significantly higher in the aggregation group than in the isolation group ($p < 0.05$). The dose-mortality relationship did not exhibit a linear pattern, but was divided into 3 phases with divisions at 20 and 50 mg/kg. In

FIG. 3. 30 and 100 min Post-injection Locomotor Activity of Methamphetamine at Doses of 5–30 mg/kg, i.p. in Isolated and Aggregated Mice

□ isolation, ■ aggregation, a) $p < 0.05$.

the aggregation group, animals died mostly within 4 h after administration of 7–70 mg/kg methamphetamine, and within 30 min after administration of 100 mg/kg. After administration of the drug, most animals died within 8 h. In the isolation group, animals died within 30 min after the administration of 100 mg/kg.

Behaviors were markedly different between the aggregation group and the isolation group, especially in animals given methamphetamine in the aggregation group. In both groups, salivation, lacrimation, sweating and piloerection were noted. In animals in the aggregation group given lower dose of methamphetamine, hyperactivity with running and jumping, squeaking, gnawing, sniffing, licking and biting the cage,

fighting among each other and intense attacking of weakened mice was noted. The ears were bitten off, hair was pulled off and the head and internal organs of dead mice were occasionally eaten. Animals given higher dose developed tremors immediately after administration and died of respiratory paralysis following severe convulsions. Animals in the isolation group similarly died after exhibiting tremor and convulsion. In animals given doses other than 100 mg/kg, gnawing, sniffing, pivoting, walking back, biting the cage, preening and grooming were especially pronounced.

Influence of Aggregation on Hyperactivity caused by Methamphetamine

The results of the first experiment are shown

FIG. 4. 240 and 480 min Post-injection Locomotor Activity of Methamphetamine at Doses of 5–30 mg/kg, i.p. in Isolated and Aggregated Mice

□ isolation, ■ aggregation, a) $p < 0.05$.

in Fig. 2. The frequency of ambulation was significantly higher in the aggregation group than in isolation group in animals given 5 mg/kg 30 and 100 min later ($U=20, 21, p<0.05$). No remarkable changes were found at 240 and 480 min after administration in ambulation or any other behavior at any time.

The results of the second experiment are shown in Fig. 3 and 4. The frequency of ambulation was significantly higher in the aggregation group in animals given 5 mg/kg 30 and 100 min later ($U=23, 20, p<0.05$, Fig. 3). But a tendency of increase was shown at 240 min after the administration of 5 mg/kg (Fig. 4). Significant increases were shown in the aggregation group than in the isolation group 30 and 100 min after administration of 15 ($U=22, 21, p<0.05$) and 30 mg/kg ($U=20, 23, p<0.05$). Preening was also significantly increased in the aggregation group in comparison to the isolation group after administration of 15 mg/kg ($U=8, p<0.02$). No remarkable changes were seen in any other behavior.

DISCUSSION

The lethal action of methamphetamine was significantly increased in the aggregation group than in the isolation group of mice over the wide range of dosages used, indicating a group toxicity. The dose-mortality curve for methamphetamine in aggregated mice showed no linearity with a difference between low and high doses. These results are in agreement with the dose-mortality curve for amphetamine in aggregated mice reported by Gardocki *et al.*,⁸⁾ and may be dependent upon several different actions of methamphetamine at specific dosage ranges. The action of methamphetamine in increasing the amount of movement was also significantly higher in the aggregation group than in the isolation group. The action of methamphetamine thus changes markedly according to the condition of the animals during drug administration. The lethal action and action of increasing the amount of movement were intensified in the aggregation group compared to the isolation

group, indicating an effect caused by aggregation.

Many reports are available on the relationship between amphetamine toxicity and emotional stress. Stress is frequently reported to enhance the lethality of amphetamine in mice and rats.^{9,10)} And Stern *et al.*¹¹⁾ showed that chronically stressed rats survived longer following a high dose of amphetamine than non-stressed rats, but the amphetamine lethality was enhanced when a novel acute stress is paired with amphetamine administration. It therefore appears that animals given methamphetamine are readily influenced by stress loading, and the lethal action and action of increasing the amount of motion is augmented by aggregation and acute loading with emotional stress.

The action of increasing the amount of movement by methamphetamine was significantly increased more by aggregation compared to isolation of mice after administration of 5 and 30 mg/kg, suggesting an effect of general activity on group toxicity of methamphetamine.

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