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Possible Mechanism for the Enhanced Lethality of Morphine in Aggregated Mice

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MOHRLAND, J. S. AND A. L. CRAIGMILL. *Possible mechanism for the enhanced lethality of morphine in aggregated mice*. PHARMAC. BIOCHEM. BEHAV. 13(3) 475–477, 1980.—Morphine was shown to be more lethal to aggregated mice than to isolated mice at an ambient temperature of 29°C but not at 19°C. After morphine administration at 29°C, convulsions were found to be associated with death, and a higher incidence of convulsions was observed in aggregated mice than in isolated mice. After morphine administration at 19°C, there was no clear association between convulsions and death, and there was no significant difference between the incidence of convulsions in isolated and aggregated mice. When tactile stress was induced upon morphine-treated isolated mice at 29°C, all of the mice convulsed and died. These results suggest that the greater lethality of morphine in aggregated mice is due to a greater incidence of convulsions which results from stress induced on one animal by the other.

Morphine lethality Aggregation Convulsions Stress

THE effect of housing density on drug action has been studied extensively with amphetamine since initial reports that amphetamine-induced mortality was increased if mice were put in groups (aggregated), rather than kept by themselves (isolated), after injection [2]. Morphine has also been shown to be more toxic to aggregated mice than isolated mice [1, 4, 9, 10]. Heat exhaustion has been suggested as the mechanism of death in aggregated mice after amphetamine administration [3]. Although morphine has been shown to produce hyperthermia in aggregated mice and hypothermia in isolated mice, the degree of hyperthermia in the aggregated animals was not sufficient to account for the greater lethality [9].

Morphine is known to lower the convulsive threshold of mice as measured by pentylenetetrazol-induced seizures [8,11]. In addition, there is some evidence to indicate that convulsions may be the cause of death in mice after lethal doses of morphine [5, 6, 12]. The present study was conducted to determine if a difference in the incidence of convulsions in isolated and aggregated mice might explain the difference in toxicity observed in those animals after morphine administration.

METHOD

All experiments were carried out using male Swiss Webster mice (20–25 g) which were housed for at least three days in groups of eight in 25×18×20 cm wire mesh cages at 22±1°C. Twenty-four hours before treatment, the mice were housed in groups of 24 in 45×45×22 cm metal cages at one of two environmental temperatures used in the experiment: 19 and 29±1°C. They were exposed to a 12 hr light-12 hr dark schedule, and food and water were available ad lib throughout the experiment. All treatments were given within three to five hours after the beginning of the light period.

Morphine sulfate, in isotonic saline, was administered intraperitoneally in a volume of 10 ml/kg body weight. Immediately after drug injection the animals were placed in groups of 12 in circular wire mesh cages, 22 cm in diameter and 18 cm high (aggregated) or individually in jars, 8 cm in diameter and 18 cm high (isolated).

The incidence of convulsions and mortality were visually monitored in both isolated and aggregated mice kept at 29±1°C after administration of 300 mg/kg of morphine sulfate and in mice kept at 19±1°C after administration of 400 mg/kg of morphine sulfate. The aggregation effect on morphine toxicity has been shown to be maximum if the animals are kept at an ambient temperature of 29°C after injection, whereas aggregation has no significant effect on morphine toxicity if the experiment is carried out at 19°C [9]. Thus, if convulsions are involved in the aggregation effect on morphine toxicity, one would expect to see a difference in the incidence of convulsions at 29°C, but not at 19°C. The doses used are near the LD_{50} at the respective environmental temperature. Thus, they are intended to be within an approximate equitoxic range. A convulsion was defined as clonic movements of all four limbs accompanied by loss of the righting reflex.

In a separate experiment with the ambient temperature at 29±1°C, the incidence of convulsions and mortality was determined in isolated mice subjected to stress after injection of 300 mg/kg of morphine sulfate. Stress was induced by giving continuous gentle pokes in the hindquarters of the mice with the blunt end of a spatula for five seconds every minute. Stress was administered for two consecutive hours after drug injection.

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TABLE 1

INCIDENCE OF CONVULSIONS AND DEATH IN ISOLATED AND AGGREGATED MICE AFTER MORPHINE SULFATE ADMINISTRATION

	Room temp. (°C)	Dose (mg/kg)	% of mice which convulsed	% of mice which died	% of dead which convulsed prior to death
Isolated*	29	300	34.5	24.1	100.0
Aggregated†	29	300	75.0§	75.0	88.9
Isolated‡	19	400	8.3	50.0	16.7
Aggregated‡	19	400	16.7	50.0	33.3

*N=29.
†N=24 (two groups of 12 mice per cage).
‡N=12 for each group.
§Significantly different from isolated value at 29°C, $p<0.01$.

The Chi-square test was used to test the results for significant differences.

RESULTS

Table 1 shows the incidence of convulsions and death in isolated and aggregated mice administered morphine sulfate at 29 and 19°C. Morphine produced a significantly greater lethality in aggregated mice than in isolated mice at 29°C. There was also a significantly greater incidence of convulsions in aggregated mice than in isolated mice at 29°C. All of the isolated mice and most of the aggregated mice at 29°C which died had convulsed prior to death. The clonic convulsions were approximately five seconds in duration and more than one convulsive episode was usually seen prior to death. If the clonic movements were followed by tonic extension of the hind legs, with the front legs extended but pulled in towards the abdomen, the animal died. All of the isolated mice and 12 of the 18 aggregated mice that died at 29°C did so in this manner. Four animals died in a semiconvulsive state, never recovering from the initial clonic convulsions, and two died without convulsions.

At 19°C, morphine produced the same degree of lethality in both isolated and aggregated mice. There was also no significant difference in the incidence of convulsions observed in isolated and aggregated mice at this temperature. In addition, there were substantially fewer convulsions observed in isolated and aggregated mice at 19°C than in isolated and aggregated mice at 29°C.

The effect of stress on the incidence of convulsions and death in isolated mice administered 300 mg/kg of morphine sulfate at 29°C is shown in Table 2. Less than 35% of the non-stressed mice showed convulsions after morphine, and only 24% died. On the other hand, if isolated mice were stressed after injection, all of the mice convulsed and died. The incidence of convulsions and death was significantly greater in stressed mice than in non-stressed mice ($p>0.005$).

TABLE 2

EFFECT OF STRESS ON THE INCIDENCE OF CONVULSIONS AND DEATH IN ISOLATED MICE ADMINISTERED 300 mg/kg OF MORPHINE SULFATE AT 29 ± 1°C

	% of mice which convulsed	% of mice which died	% of dead which convulsed prior to death
Non-stressed*	34.5	24.0	100.0
Stressed†	100.0‡	100.0‡	100.0

*N=29.
†N=10.
‡Significantly different from non-stressed value, $p<0.005$.

DISCUSSION

In the present study, aggregation affected morphine lethality at 29°C but not at 19°C, as previously reported [9]. At 29°C death in both isolated and aggregated mice after morphine administration was most often associated with convulsions. Most of these animals died in tonus in a manner resembling that previously described [12] which stated that death was not due to depression of the respiratory center, but was due instead to exhaustion and asphyxia from tetanic fixation of the respiratory muscles. There is also evidence, however, to suggest that death in the manner described above is due to circulatory collapse and not respiratory function [7].

Not all of the aggregated mice at 29°C died in tonus; a few remained in a semi-convulsive state (after the initial clonic convulsion) until death, which was presumably due to exhaustion. At 29°C convulsions preceded death in nearly all of the mice, but convulsions were not a criteria for death as two mice died in what appeared to be a depressed state without having convulsed.

Unlike at 29°C, at 19°C there was no clear association between convulsions and death in isolated and aggregated mice given toxic doses of morphine. At 19°C morphine-treated aggregated and isolated mice appeared depressed, and death may have been due to respiratory depression.

At 29°C a greater incidence of convulsions occurred in morphine-treated aggregated mice than in morphine-treated isolated mice. The convulsions and subsequent death in aggregated mice appeared to have been induced by stimulation from the other mice in the cage. A sudden movement by one mouse induced an increase in chaotic activity among the group, culminating in convulsions in some animals. A previous report [9] has shown that aggregated mice are significantly more active than isolated mice after morphine administration at 29°C. These findings suggest that the enhanced lethality of morphine in aggregated mice at 29°C is a result of a greater incidence of convulsions brought about by the

stimulation of one mouse by another during a state of hyperreactivity. This hypothesis is supported by the finding that at an ambient temperature (19°C) at which lethality does not differ in isolated and aggregated mice, there was also no significant difference in the incidence of convulsions between isolated and aggregated mice. Morphine has been shown to initially depress locomotor activity in isolated and aggregated mice at 19°C, and aggregated mice are not significantly more active than isolated mice [9].

The greater incidence of convulsions and subsequent deaths in morphine-treated aggregated mice at 29°C may have been due to stress induced by the other animals in the cage during a lowered convulsive threshold. If so, then inducing a similar stress on morphine-treated isolated mice at 29°C should increase the incidence of convulsions and death in these animals to that found for aggregated mice. When stress was induced on isolated mice after morphine administration at 29°C, all of the animals convulsed and died. The reason for the greater incidence of convulsions in stressed-isolated mice than in non-stressed-isolated mice is probably due to the duration of the stress. Stress was maintained in the surviving isolated mice for two hours after morphine injection, whereas in the aggregated situation the stress factor decreases markedly as the animals die.

In summary, the results of the present study show that death is most often associated with convulsions in isolated and aggregated mice at 29°C but not at 19°C. Furthermore, the results suggest that the greater degree of lethality observed in aggregated mice after morphine administration at 29°C is due to a greater incidence of convulsions which result from the stress induced on one animal by the other by tactile stimulation.

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