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Effect of Lethal Doses of Morphine on Brain Amines in Isolated and Aggregated Mice

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MOHRLAND, J. S. AND A. L. CRAIGMILL. *Effect of lethal doses of morphine on brain amines in isolated and aggregated mice.* PHARMAC. BIOCHEM. BEHAV. 12(2) 313–315, 1980.—Brain levels of norepinephrine, dopamine and serotonin were measured in isolated and aggregated mice after lethal doses of morphine. Although morphine significantly lowered all three brain monoamines there were no significant differences between isolated and aggregated mice. Measurements of brain morphine concentrations also failed to demonstrate any differences which could account for the difference in lethality observed in isolated and aggregated mice.

Morphine lethality Aggregation Catecholamines Serotonin

LETHALITY from amphetamine administration may be increased markedly if mice are housed in groups (aggregated) rather than housed alone (isolated) [4]. Heat exhaustion has been suggested as the mechanism of death in aggregated mice after amphetamine administration [6]. In addition, differences in brain levels of amphetamine [5] and/or catecholamines [18] between isolated and aggregated mice have been invoked as explanation for the difference in lethality.

Morphine has also been shown to be more lethal to aggregated mice than to isolated mice [1, 7, 16, 20]. Aggregated mice are hyperthermic after morphine administration, however, the degree of hyperthermia is not sufficient to explain the lethality [16]. The present investigation was undertaken to determine whether differences in brain levels of morphine and/or biogenic amines could account for the increased mortality observed in aggregated mice after morphine administration.

METHOD

Male Swiss Webster mice (20–25 g) were housed for at least three days prior to use in groups of 8 in 25×18×20 cm wire mesh cages at 22 ± 1°C. Twenty-four hr before treatment the mice were housed in groups of 24 in 45×45×22 cm metal cages at 29 ± 1°C. This temperature was chosen since the aggregation effect on morphine toxicity has been shown to be maximum at 29°C [16]. The animals were exposed to a 12 hr light–12 hr dark schedule and food and water were available ad lib. All treatments were given 3 to 5 hr after the beginning of the light period.

Morphine sulphate in isotonic saline was administered intraperitoneally in a volume of 10 ml/kg body weight. The doses of morphine used at each environmental temperature represent dosage ranges near LD_{16}, LD_{50} and LD_{84} of both isolated and aggregated mice. Immeidately after drug injection the animals were placed in groups of 12 in circular wire mesh cages 22 cm in dia. and 18 cm high (aggregated) or individually in jars, 8 cm in dia. and 18 cm high (isolated).

Whole brain levels of norepinephrine, dopamine and serotonin were measured in isolated and aggregated mice 15 min after saline or morphine injection; the brains of at least 5 isolated mice and 5 of 12 aggregated mice (selected randomly) at each of three morphine doses were evaluated. Animals were killed by decapitation and brain amines were measured fluorometrically [12]. Whole brain concentrations of morphine were measured after morphine administration by a fluorometric method [13]. Brains were obtained at the time of death, except at the lowest morphine dose when they were taken at 15 and 30 min after morphine injection, since very few mice died following this dose. The data was analyzed with a 2 (housing condition) × 4 (dose) factorial analysis of variance employing unweighted means method.

RESULTS

Table 1 shows the effect of morphine on the brain levels of biogenic amines in isolated and aggregated mice. Administration of lethal doses of morphine at 29°C significantly lowered brain levels of norepinephrine, $F(3,29)=15.91$, $p<0.001$; dopamine, $F(3,29)=3.17$, $p<0.05$; and serotonin, $F(3,29)=5.25$, $p<0.01$. No interaction between dose and housing condition was found indicating that the morphine effect was consistent for both isolated and aggregated mice.

The brain concentrations of morphine (base) in isolated and aggregated mice after morphine administration at 29°C is shown in Table 2. Analysis of morphine concentrations indicated that there was an effect of dose, $F(3,34)=3.91$, $p<0.02$. Observation of Table 2 indicates that the trend was for increased morphine doses to increase morphine brain concentrations. Morphine brain concentrations were not significantly different in isolated and aggregated mice.

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TABLE 1

BRAIN LEVELS OF MONOAMINES IN ISOLATED AND AGGREGATED MICE AFTER LETHAL DOSES OF MORPHINE AT 29 ± 1°C

Dose (mg/kg)	Norepinephrine (μg/g)		Dopamine (μg/g)		Serotonin (μg/g)	
	Isolated	Aggregated	Isolated	Aggregated	Isolated	Aggregated
Saline	0.32 ± 0.01	0.34 ± 0.01	0.67 ± 0.03	0.69 ± 0.04	0.62 ± 0.01	0.63 ± 0.01
300	0.30 ± 0.01	0.30 ± 0.01	0.61 ± 0.04	0.63 ± 0.03	0.59 ± 0.02	0.62 ± 0.01
400	0.28 ± 0.01	0.27 ± 0.01	0.60 ± 0.02	0.59 ± 0.02	0.57 ± 0.01	0.59 ± 0.01
500	0.28 ± 0.01	0.26 ± 0.01	0.57 ± 0.03	0.60 ± 0.03	0.54 ± 0.01	0.56 ± 0.01

TABLE 2

BRAIN LEVELS OF MORPHIN (BASE) IN ISOLATED AND AGGREGATED MICE AT 29 ± 1°C

Isolated		Aggregated	
Dose (mg/kg)	Brain levels* (μg/g)	Dose (mg/kg)	Brain levels* (μg/g)
300†	10.1 ± 1.2	300†	12.6 ± 1.0
300‡	12.0 ± 0.8	300‡	13.6 ± 1.0
400	12.3 ± 0.6	400	12.4 ± 0.6
500	15.2 ± 1.0	500	14.3 ± 1.4

*Values are the mean ± SEM obtained from at least 5 mice/dose for each group.

†Animals killed at 15 min.

‡Animals killed at 30 min.

DISCUSSION

An increase in drug-induced lethality by aggregation was first demonstrated with amphetamine [4]. Brain catecholamines have been suggested as being responsible for the mediation of the aggregation effect on amphetamine toxicity since aggregated mice were found to have lower brain levels of catecholamines than isolated mice after amphetamine administration [14,18]. However, differences in the distribution, metabolism and/or excretion of amphetamine have also been suggested as a possible means by which the difference in toxicity between isolated and aggregated mice occurs [10].

More recently, the narcotic analgesics were demonstrated to produce greater lethality in aggregated than in isolated mice [1, 7, 16, 20]. The increased mortality in aggregated mice after morphine administration has been shown to be associated with both increased locomotor activity and body temperature [17]. Morphine's effect on locomotor activity and body temperature have been attributed to alterations of brain biogenic amines [3, 9, 11]. These findings suggest that a difference in brain biogenic amines or morphine concentration may account for the difference in locomotor activity, body temperature and lethality between isolated and aggregated after morphine administration.

Lethal doses of morphine did significantly lower brain levels of norepinephrine, dopamine and serotonin. However, the present investigation failed to find any significant differences in the brain levels of the monoamines between isolated and aggregated mice which might account for the difference in lethality observed in those animals. This result, along with evidence that pretreatment of mice with various compounds known to alter the synthesis or degradation of brain monoamines has no effect on the aggregation enhancement of morphine toxicity [7,20], suggests that changes in brain monoamines are not causally related to the aggregation effect on morphine's action in mice. Brain monoamine mediation of the aggregation enhancement of morphine toxicity cannot be excluded entirely, however, since the measurements of brain monoamine levels reflect the steady-state levels of the amines and it is possible that the dynamic aspects of brain monoamine metabolism were altered without effecting the net concentration of the amines [19].

There were also no significant differences found in the whole brain levels of morphine between isolated and aggregated mice. Thus, results of the present study demonstrated no differences between isolated and aggregated mice in whole brain levels of either monoamines or morphine at doses which produced significantly different degrees of lethality, thereby further differentiating the aggregation effect of morphine toxicity from that of amphetamine toxicity.

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