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Analgesia and Hyperreactivity Following Morphine Microinjection into Mouse Brain[1]

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CRISWELL, H. E. *Analgesia and hyperreactivity following morphine microinjection into mouse brain.* PHARMAC. BIOCHEM. BEHAV. 4(1) 23–26, 1976. – Analgesia and a paradoxical hyperreactivity to stimuli of sudden onset have recently been reported following the microinjection of morphine into the periacqueductal gray matter of rats. These effects have not been systematically investigated in other species. In the present study, both analgesia and hyperreactivity were observed as dose dependent effects of morphine microinjection into the periacqueductal gray matter of several strains of mice. Analgesia alone was produced by low doses of morphine while at higher doses analgesia was accompanied by hyperreactivity. Strain differences were noted with B6D2F$_1$ mice being more susceptible to the hyperreactivity following morphine than BALB/c mice.

Morphine Analgesia Hyperreactivity Periacqueductal gray Microinjection

THE injection of microgram quantities of morphine into the periacqueductal gray matter (PAG) of the rat [3,4] rabbit, [2] or monkey [11], brain produces analgesia similar to that resulting from systemic administration of morphine. Significant analgesia results from the central administration of as little as three μg of morphine. When larger doses are administered to rats, analgesia is accompanied by a paradoxical hyperreactivity to stimuli of sudden onset. Thus, while a rat which has received a high dose of morphine microinjected into the PAG will not respond to 55 degree stimulation on a hot-plate, he will respond violently to electric shock at a level which previously elicited little or no response [4].

While several authors have described hyperreactivity in the rat following central morphine administration, [3, 4, 12] hyperreactivity has not been systematically investigated in other species. There is some suggestion that a similar response occurs in the rabbit where some authors have reported a flight response following high doses of morphine injected into the central gray [2] while others have not noted this response following a similar procedure [15]. Hyperreactivity was not observed following the microinjection of morphine into the PAG of the monkey brain [11].

In the present study, the production of analgesia and hyperreactivity by morphine injection into the mouse brain was examined as a function of dose, site and strain.

EXPERIMENT 1

Method

Animals. Forty mice from a local colony derived from International Cancer Institute (ICR) stock were divided into 4 groups of 5 males and 5 females. Each group of 10 animals received a different dose of morphine sulfate dissolved in 1 μl of normal saline. As this strain of mice is heterogeneous and not generally available, 30 BALB/c females and 10 B6D2F$_1$ males received an intermediate or high dose of morphine to determine whether hyperreactivity could be observed in generally available homogeneous strains of mice.

Procedure. The microinjection of 0, 7.5, 15, or 30 μg of morphine sulfate dissolved in 1 μl of saline was accomplished by a method modified from that previously used for the placement of stereotaxic lesions in the mouse [1]. A 30 ga cannula was placed into a 22 ga cannula with both ends of the inner cannula extending beyond the outer one and crimped with needle nosed pliers to prevent slippage, with 2.6 mm of the inner cannula extended from the lower end of the outer cannula and approximately 10 mm extended beyond the upper end of the outer cannula. The cannulae were then press fit into a hole drilled in a 5 mm cube of Plexiglas so that the 2.6 mm length of 30 ga cannula extended below the Plexiglas cube. By pressing the exposed tip of the 30 ga cannula through the skull into the brain, until the Plexiglas stop rested upon the skull, chemicals can be microinjected to a 2.5 mm below the surface of the brain and perpendicular to the skull, which is approximately 0.1 mm thick. A 20 μl Hamilton syringe was filled with the solution to be injected and the solution was led to the injection apparatus by a length of PE-10 tubing.

Each animal was etherized and a 10 mm incision made at midline exposing the skull. Bregma is often a poor landmark in the mouse as skull size and the position of the sutures vary with age and between strains [14]. The brain, however, is much less variable and due to the extreme

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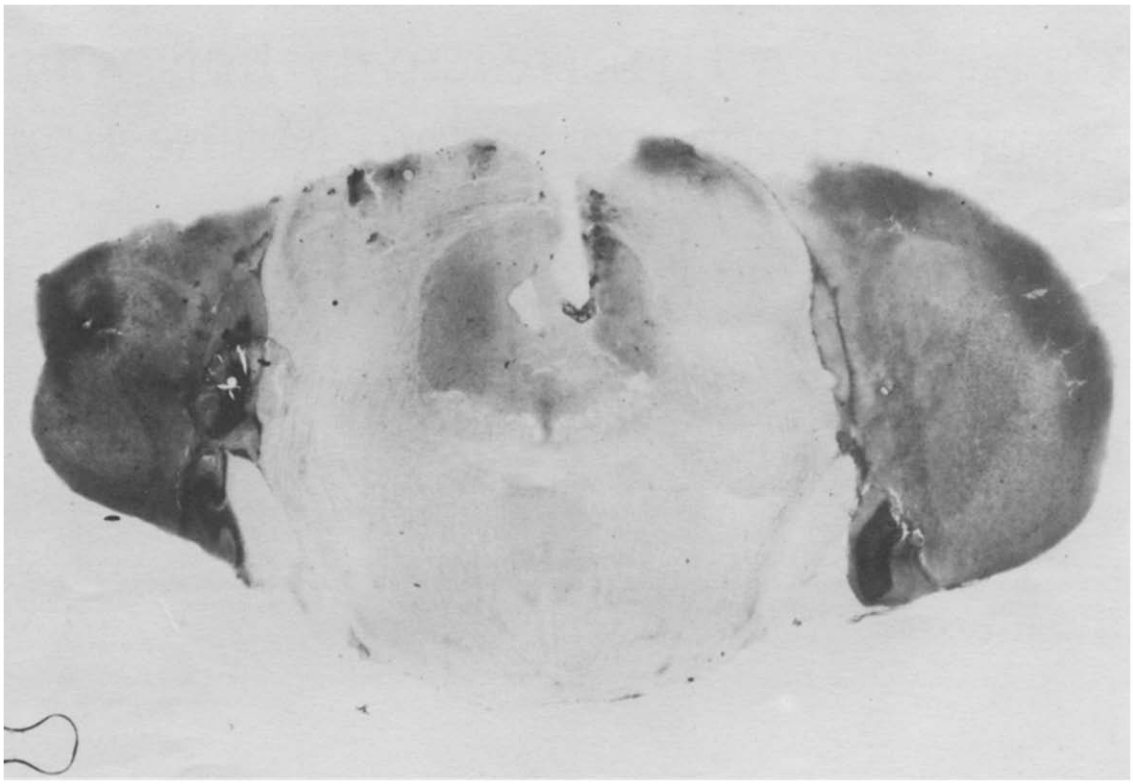

FIG. 1. Photograph of a 48 μ stained section of mouse brain showing the injection site in the PAG which produced hyperreactivity at the lowest dose of morphine (7.5 μg).

thinness of the mouse skull, the superior and inferior colliculi may be visualized through the skull just posterior to Lambda and can be used as stereotaxic landmarks. In the present study, the injection apparatus was held by the outer cannula and the inner cannula was pushed through the skull at a point on midline and at the junction of the inferior and superior colliculi. The cannula was lowered until the Plexiglas stop rested on the surface of the skull. At that point, the cannula extended into the periacqueductal gray matter at the level of the Raphe nucleus. A photograph of a typical injection site is shown in Fig. 1. One microliter of solution was injected and the cannula withdrawn after a 5 sec delay. The scalp incision was closed with a suture and the animal was placed in an observation cage. The entire etherization and microinjection procedure takes approximately 3 min and the effects of the ether dissipate within 2 to 3 min after the injection.

Each animal was tested for analgesia at 5 min and 30 min postinjection by the hot-plate method. The analgesia score consisted of the number of seconds elapsing before the animal lifted and either shock or mouthed a forepaw from a 55°C hot plate. Any animal not responding within 15 sec was removed to prevent tissue damage.

Hyperreactivity was tested after a method previously reported for rats [3] by periodically tapping on the observation cage. Hyperreactive animals responded with a vigorous running and jumping response with a duration of at least 2 sec which often ended in a tonic seizure.

Animals remaining alive after 1 hr were sacrificed by overdose of chloroform. Their calvaria were then removed and placed in acetate buffered Formalin for at least 24 hr. The brains were then dissected out and 48 μ frozen sections were cut and stained with cresyl violet to verify the site of the injection. Only animals which received injections into the PAG were included in the analysis.

Results

There were no differences between the sexes for either the number of animals from which hyperreactivity was recorded ($\chi^2 = 4.55$, $df = 3$; $p>0.05$) or for the mean level of analgesia as measured by the hot-plate method, $F(3,16) = 1.16$, $p>0.05$, as a function of dose. There also was no difference between analgesic level measured at 5 min as opposed to 30 min, $F(1,32) = 1.78$, $p>0.05$, and there was no interaction between the time of measurement of analgesia and sex, $F(1,32) = 1.0$, $p>0.05$, or dose, $F(3,32) = 1.32$, $p>0.05$. The dose response curves for these two variables were therefore averaged across sex for the hyperreactivity and across both sex and time for the level of analgesia. This curve is presented in Fig. 2.

There was an overall effect of dose upon the number of animals from which hyperreactivity was recorded ($\chi^2 = 21.53$, $df = 3$; $p<0.01$) with the group which received 30 μg of morphine showing a significantly higher incidence of hyperreactivity than that receiving 15 μg ($\chi^2 = 5.39$, $df = 1$; $p<0.05$). The group which received 15 μg did not show a significantly greater incidence of hyperreactivity than that which received 7.5 μg ($\chi^2 = 2.4$, $df = 1$; $p>0.05$) but did show a significantly greater incidence than did the group which received no morphine ($\chi^2 = 5.0$, $df = 1$; $p<0.05$). The group which received 7.5 μg did not show a signifi-

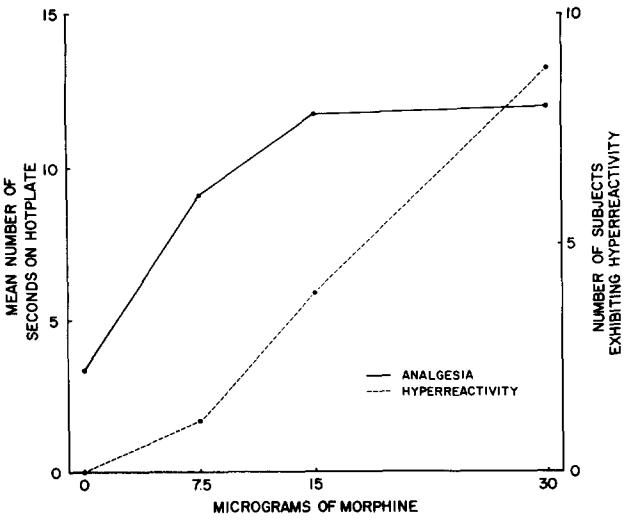

FIG. 2. Dose response curves for analgesia as measured in seconds required for a response to 55° stimulation on the hot plate (left margin) and hyperreactivity as measured by the number of animals in each group of 10 which demonstrated hyperreactivity (right margin).

cantly greater incidence of hyperreactivity than the saline control group ($\chi^2 = 1.05$, $df = 1$; $p > 0.05$).

The latency of onset of hyperreactivity was similar for all cases tested with a mean of 6.6 min and a range of 2–15 min. The one animal which became hyperreactive at the lowest dose did so with a latency of 5 min while the range for each of the other two groups was 2–15 min. Latencies of less than 2 min could not be measured due to the effects of the ether anesthesia.

An overall effect of dose upon level of analgesia is also shown in Fig. 2, $F(3,16) = 9.73$, $p < 0.01$. It was expected that increased doses of morphine would produce stronger analgesic effects and one tailed t-tests indicated that the group which received 7.5 μg showed significantly more analgesia than the saline control ($t = 5.12$, $df = 38$; $p < 0.01$). The group which received 15 μg was significantly more analgesic than that which received 7.5 μg ($t = 1.87$, $df = 38$; $p < 0.05$) but the group which received the 30 μg dose was not significantly more analgesic than the 15 μg group ($t = 0.19$, $df = 38$; $p < 0.05$).

A high degree of analgesia was produced in some animals even at the lowest (7.5 μg) dose as is indicated by the number of animals reaching the 15 sec maximum hot plate reaction time. While the range of reaction times for the saline control group was from 1–8 sec, that of the 7.5 μg group was from 5–15 sec with 5 animals reaching the 15 sec maximum. The range of the 15 μg group was 3–15 sec with 8 of the 10 animals remaining on the hot plate for the maximum allotted time. The range for the 30 μg group was 5–15 sec with 7 of the 10 animals reaching the 15 sec maximum.

A significant proportion of the animals in the 15 and 30 μg groups were hyperreactive at the time of analgesia testing and the low end of the ranges of analgesia scores represent animals which were hyperreactive at the time of testing. The analgesia scores of these animals may have been artifactually lowered through their violent responses to seemingly neutral stimuli [4,12]. The scores for these groups should, therefore, be considered conservative estimates of the true analgesic level.

One mouse which received 30 μg of morphine died following tonic seizure activity 37 min after the injection. All others were alive at the time of sacrifice 1 hr post-injection.

Histological verification of the injection sites did not reveal any systematic variation in either the site of the injection or the amount of tissue damage at the injection site between groups which received differing doses. All were within the periacqueductal gray and within 1 mm (A.P.) of the level of the dorsal Raphe nucleus.

EXPERIMENT 2

To determine whether the hyperreactivity produced by morphine was due to a local action or to diffusion into the nearby ventricle, 10 ICR mice were injected with 30 μg of morphine into the ventricle at the level of the injections in Experiment 1. Testing was identical to that in Experiment 1.

Results

None of the 10 animals showed hyperreactivity. In fact, all were hypoactive and would not move unless strongly stimulated. All 10 animals remained on the hot plate for the 15 sec maximum allowed time.

EXPERIMENT 3

The locally raised ICR mice are a heterogeneous strain and not generally available. Therefore, 30 BALB/c females and 10 $B6D2F_1$ males which can be obtained through The Jackson Laboratories, were tested with the intermediate or high dose of morphine to determine whether hyperreactivity could be observed in generally available homogeneous strains of mice.

Results

Six of 10 $B6D2F_1$ mice showed hyperreactivity following the microinjection of 15 μg of morphine in the PAG while 0 of 20 BALB/c mice showed hyperreactivity at that dose. Following these results, 10 additional BALB/c mice were tested at the 30 μg level, 8 of these 10 animals showed hyperreactivity. Analgesia was not tested in these animals. The $B6DF_1$ mice were significantly more likely to show hyperreactivity following the intermediate dose of morphine ($\chi^2 = 9.02$, $df = 1$; $p < 0.01$). Death during a tonic seizure occurred in 4 of the $B6D2F_1$ animals and in 4 of the BALB/c animals which received the 30 μg dose of morphine. Time of death ranged from 20–55 min and the remaining animals were sacrificed 1 hr after injection.

DISCUSSION

Both analgesia and hyperreactivity were shown to be dose dependent effects of morphine microinjection into the PAG of mouse brain. Figure 2 illustrates that the dose response relationship differs for the two effects with hyperreactivity requiring a higher dose than analgesia. This is consistent with the more recent rat data which also showed both hyperreactivity and analgesia at higher doses of morphine with analgesia alone at lower doses [4,12]. The lack of hyperreactivity following ventricular morphine

suggests that diffusion of morphine into the nearby ventricle is not necessary for the occurrence of hyperreactivity. The increased analgesia observed in these animals when compared to animals which received microinjections into the PAG is, probably, a measurement artifact. Previous authors have noted that it is extremely difficult to measure analgesia in hyperreactive animals [4,12] and some false positive reactions to the hot plate were undoubtedly recorded from the active group.

The elicitation of hyperreactivity in the mouse indicates that the phenomenon is not a species specific response peculiar to the rat. The previous equivocal results in the rabbit [15] and negative results in the monkey [11] suggest that hyperreactivity may not, however, be produced in all species following centrally administered morphine. The lack of clearly demonstrated hyperreactivity in other species may also be explained by differences in sensitivity to morphine hyperreactivity between species. The within species differences in sensitivity to hyperreactivity observed in the present study suggest the possibility of even larger between species differences and larger doses may produce hyperreactivity in other species. The methodology of observing hyperreactivity may also be of importance as the response to morphine is one of reactivity rather than simple activity and animals tested in the absence of environmental stimulation would not be expected to show the response. Just this result has been found for excitation following systemic injection of morphine in mice. High doses of morphine produce a syndrome in the mouse which is similar if not identical to the hyperreactivity produced by injection of morphine into the PAG of rats and mice. Excitation occurred only when the animal was placed in a novel environment during testing. Mice placed in familiar environments with limited stimulation became hypoactive rather than hyperreactive [10]. Animals in the present study were placed in an observation cage which differed from their home cage and stimulation in the form of a tap on the cage was applied periodically. The amount of stimulation necessary to initiate an episode of hyperreactivity following centrally administered morphine does not appear to be great as the author has observed hyperreactivity in both rats and mice following a sudden movement or the sound of a footstep when animals were in their home cage. That such stimulation was effective suggests that most studies where analgesia is tested should supply ample stimulation for hyperreactivity to be observed. The response is violent and can be noted even in a restrained animal where it appears as convulsive movements.

While there is some doubt regarding the anatomical specificity of morphine induced analgesia [2], the PAG appears to be intimately involved. Analgesia has been produced by the injection of morphine into the PAG of rats [2, 3, 4, 12] and monkeys [11]. Both cholinergic [9] and electrical [5, 7, 8] stimulation of the PAG have also been shown to produce analgesia. Furthermore, cross tolerance between electrical and morphine stimulation has been shown and both are blocked by a narcotic antagonist [7]. Changes in levels of putative neurotransmitter substances which are specific to painful stimulation have also been found in the PAG and these changes were blocked by pretreatment with morphine in an analgesic dose [13]. Generally, morphine is without strong analgesic effect when injected into other structures such as the postromedial thalamus, midbrain teticular system, hippocampus, lateral geniculate body, tectum, hippocampus caudate nucleus, cortex or septum [15]. Some investigators have, however, noted analgesia following the microinjection of morphine into the posterior hypothalamus [3] and preoptic area [6].

While analgesia may be obtained from sites along the full length of the central gray matter, hyperreactivity appears to be limited to the area surrounding the cerebral acqueduct [3,4] and other sites were not investigated in the present study. Clearly, a thorough mapping of the brain sites from which analgesia and hyperreactivity can be elicited by morphine injection is in order. The strain differences shown in the present study and species differences suggested by previous results may account for the present lack of consistent data.

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