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Enhanced Analgesic Response to Morphine in Adult Rats Exposed to Morphine Prenatally[1]

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KIRBY, M. L., S. E. DEROSSETT AND S. G. HOLTZMAN. *Enhanced analgesic response to morphine in adult rats exposed to morphine prenatally*. PHARMAC. BIOCHEM. BEHAV. 17(6) 1161–1164, 1982.—Morphine exposure during development has been shown to produce fetal tolerance to morphine as measured by spontaneous activity only if a particular injection schedule is used. The present study was undertaken to compare the morphine-induced analgesic response in adult offspring of rats which had been injected during the last half of gestation on schedules known to produce fetal tolerance (5 mg/kg morphine at 6 hour intervals) versus a schedule known not to produce fetal tolerance (10 mg/kg morphine at 12 hour intervals). At 30 days postnatally the offspring of animals injected on these 2 schedules show no changes in their responsiveness to the analgetic effect of morphine as determined in the hot-plate test. The present study shows that adult offspring of mothers injected with 20 mg/kg/day of morphine in four divided doses on days 12–20 of gestation have an enhanced analgetic response to morphine in the tail-flick test. In contrast, offspring of mothers injected during the same period of gestation with 20 mg/kg/day of morphine in two divided doses respond to the analgetic effect of morphine in the same manner as the offspring of saline-treated mothers. These results show that the schedule for prenatal morphine administration can play a role in the behavioral effects of morphine in adulthood.

Morphine Tail flick test Analgesia Rats

THERE have been several reports of altered analgetic responsiveness to opiates as determined in the hot-plate test in offspring of animals exposed to opiates prior to or during gestation. Friedler [3,4] treated female mice as well as rats of either sex for 5 consecutive days with very high doses of morphine. The animals were bred 5 days after the last morphine injection and the adult offspring were found to be tolerant to the analgetic effect of morphine. Morphine administration to rats during early [9] or late gestation [5,10] produces tolerance to the analgetic effect of opiates in the offspring that can persist for as long as 11 weeks postnatally. Zimmerman *et al.* [12] treated neonatal rats with morphine during the prepuberal period and found that these animals were tolerant to the analgetic effect of morphine at 80 days of age.

More recently, Zagon and McLaughlin [11] have shown that adult rats exposed to methadone perinatally had increased baseline hot-plate latencies and increased analgetic responses to acute methadone administration at 120 days of age.

In a previous paper, we reported that different morphine injection schedules in pregnant rats produced different effects in fetal responsiveness to morphine [7]. For example, 5 mg/kg of morphine delivered 4 times daily on days 12–20 of gestation caused tolerance to morphine-induced suppression of activity in the fetus. On the other hand, maternal administration of the same total daily dose of morphine in 2 injections rather than 4 did not produce tolerance in the fetuses. We also found that the 30 day old offspring of these animals suffered no changes in their responsiveness to the analgetic effect of morphine in the hot-plate test.

It is of interest to compare the morphine responsiveness of adult animals known to be tolerant to morphine during late gestation with animals exposed to the same daily dose of morphine but known not to be tolerant to morphine. Since the morphine injection schedule coincided with a period of very intense development of the spinal cord including spinal cord reflexes [8] we have used the tail-flick for analgesia because it involves a polysynaptic reflex considered to be mediated within the spinal cord. This test would give an

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assessment of morphine-induced changes in a specified area of the nervous system caused by morphine exposure during development.

METHOD

Pregnant Wistar rats were obtained from Harlan-Sprague Dawley (Indianapolis) on the 6th day of gestation. The rats were weighed and divided into 4 groups with the mean weight of each group approximately the same. All of the rats were placed in wire-bottomed metabolism cages so that food intake could be measured. The cages were located in one room with 12-hour light/dark cycle and constant temperature and humidity. The animals were weighed daily and all groups were fed powdered lab chow ad lib until the 12th day of gestation.

Injections

Beginning on the 12th day of gestation three of the four groups were injected every 6 hours as follows. Group 1 (n=4) received 1 ml of physiological saline. Groups 2 (n=6) and 3 (n=8) received 20 mg/kg/24-hour period of morphine. In group 2, 5 mg/kg of the morphine was injected every 6 hours while in group 3, 10 mg/kg of the morphine was injected at 12-hour intervals with saline being injected at intervening 6-hour intervals. In this manner both groups received an identical amount of morphine and four injections in every 24-hour period; however, group 2 received 5 mg/kg of morphine in every injection, while group 3 received 10 mg/kg of morphine in every other injection. Group 4 (n=4) was not injected but was subjected to pair-feeding. Injections and pair-feeding were suspended after the second injection on day 21 of gestation and the dams moved into nesting cages in preparation for parturition.

Feeding Schedule

Groups 1–3 were fed ad lib. Each day the food intake of the injected animals was measured. The mean amount of food intake for animals injected with 5 mg/kg four times daily was determined and that amount made available to each animal in group 4 for the subsequent day. In this way group 4 was pair-fed with animals receiving 5 mg/kg of morphine four times daily.

Litter weights and mortality of the neonatal pups were noted. Twenty-four hours following parturition each litter was standardized at 8–9 pups and was cross-fostered by a dam which had not been exposed to any drugs during gestation. The pups were mixed within each group such that pups in any one postnatal litter were derived from a minimum of 3 dams. The day of parturition was designated day 0. The litters were weaned on postnatal day 21 and, on day 30, the pups were separated according to sex and marked according to treatment group. Beginning at postnatal day 60, the offspring were tested for analgesic responsiveness to morphine.

Tail Flick Analgesia

Each group was tested in random order for analgesia following subcutaneous administration of 10 mg/kg of morphine. Approximately one week later, the same animals were retested in same order with 5 mg/kg of morphine. Testing was always conducted between 10 a.m. and 2 p.m.; the investigators who performed the tests were "blind" to the treatment group of the rats.

During testing, radiant heat from a 20 V, high amperage light bulb (GE 100T81 1SC) was focused on the lower third of the rat's tail with an ellipsoidal reflector (02.REM 011, Z291μ6783, 143 mm B; Optical Industries, Inc.). Illumination of the bulb started a timer; when the rat flicked its tail a photocell located beneath the tail was exposed to the light, resulting in the bulb being turned off and the timer being stopped.

Baseline reaction times were determined for each rat. A voltage drop of 5.3±0.05 V across the element of the bulb produced control latencies ranging from 1.37 to 3.50 seconds. Each rat was then given the appropriate dose of morphine and was returned to its home cage. The time course of the analgesic effect of morphine was determined by retesting each animal at 20 minute intervals. To minimize tissue damage to the tail, a cutoff time of 6 seconds was used and a different portion of the tail was used during each determination.

Analgesia is expressed as percent of maximum possible effect [2] and was computed as follows:

$$\%\text{MPE} = \frac{\text{Actual Change (postdrug latency} - \text{predrug latency)}}{\text{Possible Change (cut-off time} - \text{predrug latency)}} \times 100$$

Drugs

Morphine sulfate was dissolved in 0.9% saline and was administered SC in a volume of 1.0 ml/kg of body weight. All doses are expressed in terms of the free base.

Data Analyses

Data from tail-flick tests were evaluated by means of multiple *t*-tests. Areas under the curve were computed by performing trapezoidal integrations on the data [2]. Weight differences were evaluated using analysis of variance. Perinatal mortality was compared using Chi square analysis.

RESULTS

The morphine-injected dams consumed 20–30% less lab chow than the saline-injected controls. The decrease in food intake was the same in both groups of morphine-injected animals. Even though the pair-fed animals were technically pair-fed with the M5×4 animals, actual food intake was the same across the three groups: M10×2, M5×4, and pair-fed. Thus, the pair-fed animals had their food intake restricted to 70–80% of their normal intake during days 13–21 of gestation. Body weight gain from days 12 to 21 of gestation in both groups of morphine-injected and pair-fed dams was significantly less (40–50%) than saline-injected animals ($p<0.001$). Litter size in all groups was 10–12 pups/dam and was not affected by treatment. Birth weight of the pups of the morphine-injected and pair-fed dams did not differ from each other but was less than that of the offspring of saline-injected dams (6.0–6.4 g/pup in the morphine-treated and pair-fed groups vs 7.6 g/pup in the saline-injected group). Although this represents a decrease of about 17%, the difference was not statistically significant by one-way analysis of variance. Neonatal mortality was elevated significantly ($p<0.005$) in the morphine-injected groups (31–50%) compared to the saline-injected and pair-fed offspring (3–5%). All deaths occurred within the first 48 hours following parturition.

Tail-Flick Analgesia

No differences were apparent in the baseline tail-flick

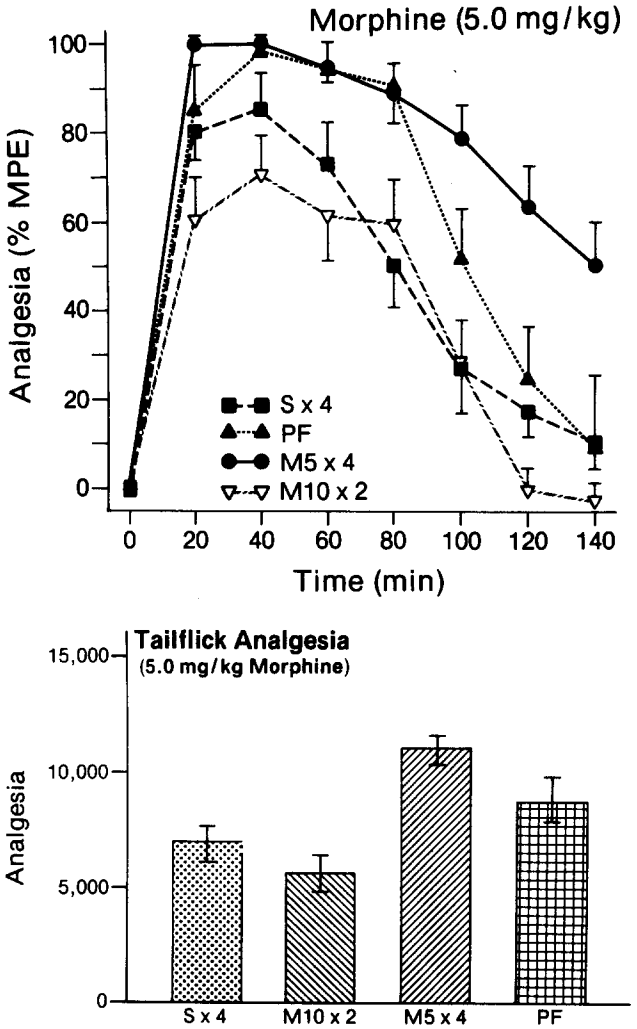

FIG. 1. Top: Time course of the analgesic effect of 5.0 mg/kg of morphine as determined by the tail-flick test every twenty minutes after administration. Each point is the mean for the group±SEM; the SEM is not shown where it is less than the radius of the point. (S×4, n=16; M10×2, n=18; M5×4, n=16; PF, n=9.) Bottom: The analgesic effect of 5.0 mg/kg of morphine as determined by the tail-flick test. Each bar represents the mean area bounded by the analgesia curve for that group±SEM. M5×4 is significantly different from both S×4 and M10×2 at $p<0.01$.

$$\%\text{MPE}=\frac{\text{Actual Change (postdrug latency} - \text{predrug latency)}}{\text{Possible Change (cut-off time} - \text{predrug latency)}}\times 100$$

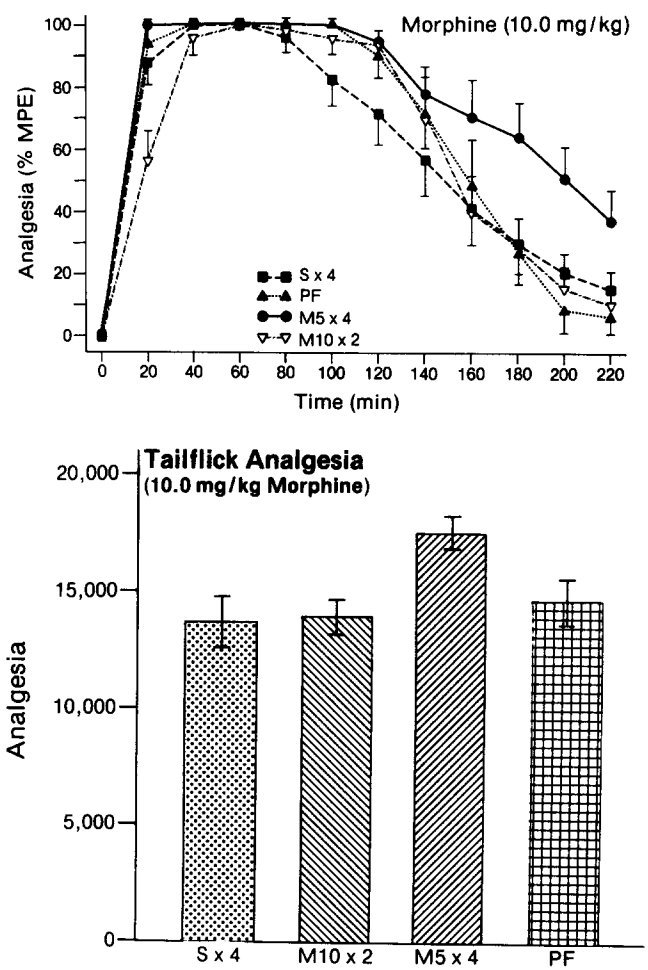

FIG. 2. Top: Time course of the analgesic effect of 10.0 mg/kg of morphine as determined by the tail-flick test every twenty minutes after administration. Each point is the mean for the group ± SEM; the SEM is not shown where it is less than the radius of the point. (S×4, n=15; M10×2, n=18; M5×4, n=14; PF, n=9.) Bottom: The analgesic effect of 10 mg/kg of morphine as determined by the tail-flick test. Each bar represents the mean area bounded by the analgesia curve ± SEM. M5×4 is significantly different from both S×4 and M10×2 at $p<0.01$ and from PF at $p<0.05$.

$$\%\text{MPE}=\frac{\text{Actual Change (postdrug latency} - \text{predrug latency)}}{\text{Possible Change (cut-off time} - \text{predrug latency)}}\times 100$$

latencies among the animals of the four treatment groups. Group control latencies prior to the administration of 10 mg/kg of morphine ranged from 1.80–2.04 sec. Latencies prior to the administration of 5 mg/kg of morphine ranged from 1.96–2.29 sec.

Following injection of 5 mg/kg, the offspring in the M5×4 group showed significantly earlier onset and longer duration of analgesia than offspring of the other groups. At 20 minutes post-injection 14/16 animals in the M5×4 group were 100% analgetic while 6/18, 8/16, and 5/9 were 100% analgetic in the M10×2, S×4, and PF groups respectively ($p<0.005$). At 140 minutes post-injection, 4/16 animals in the M5×4 group were still 100% analgetic while none of the animals in any other group were 100% analgetic ($p<0.001$) (Fig. 1 top). The mean percent analgesia in the M5×4 group was 50% at 140 minutes postinjection while in the other groups it was between 0 and 10%. Thus, the area under the curve for offspring of the M5×4 group is significantly elevated (Fig. 1 bottom). Analgesia in the M10×2 offspring appears the same as in saline-injected offspring.

Administration of 10 mg/kg causes an even more pronounced hyperanalgetic response in the M5×4 offspring. Analgesia reaches 100% in all M5×4 animals tested by 20 minutes postinjection ($p<0.001$). At the last postinjection time point analgesia remains at about 35% while analgesia in

the other 3 groups is 5–15% ($p<0.005$) (Fig. 2 top). Again the total area of analgesia is elevated in the M5×4 group while the M10×2 and PF groups are similar to saline-injected controls.

DISCUSSION

These results show that adult offspring of animals exposed prenatally to 5 mg/kg of morphine four times daily are hypersensitive to the analgetic effect of morphine. On the other hand, offspring of animals exposed to the same total daily dose but in 2 injections rather than 4 react normally to morphine-induced analgesia.

We have previously shown that fetuses exposed to 5 mg/kg morphine four times daily are tolerant to the depressant effect of morphine on fetal activity [7]. This is in contrast to fetuses exposed to 10 mg/kg of morphine twice daily which showed no tolerance to this effect of morphine. These results suggest that the injection schedule may be as important as the total daily dose of morphine in producing behavioral changes in responsiveness to morphine in adulthood. We do not mean to suggest that prenatal tolerance necessarily leads to postnatal hypersensitivity, merely that the two phenomena have been observed to occur in the same group of animals in our experiments. We should make it clear that we are not talking about the exact same animals, but rather the same treatment conditions. Despite the fact that only the M5×4 fetuses show tolerance to morphine, both M5×4 and M10×2 animals are dependent on morphine and show withdrawal symptoms in the form of hyperactivity and increased neonatal mortality when morphine is withdrawn.

Even though the M5×4 offspring show altered responsiveness to morphine both in utero and as adults while the M10×2 offspring respond normally to morphine, both groups have abnormal spinal opiate receptor numbers (Kirby, submitted for publication). During the prenatal period both groups have decreased receptor numbers while as adults the opiate receptor population is above normal. Thus there is no apparent relationship between total spinal opiate receptor population and behavioral responsiveness to morphine.

O'Callaghan and Holtzman [9] used an M10×2 injection schedule from gestation days 5 to 12. The 3–5 week old offspring showed tolerance to morphine in hot-plate testing. In the present study the M10×2 group received the same amount of morphine twice daily as the animals in O'Callaghan and Holtzman's study. However, the injections were initiated on gestation day 12 and continued to day 21. The offspring demonstrate normal responsiveness to morphine both at 30 and 60 days postnatally. Thus, the period during which the developing organism is exposed to morphine in utero may be a crucial determinant of the magnitude and persistence of tolerance to the analgetic effect of morphine in the adult animal. However, any comparison of this type of experiment must be considered cautiously because of variables, such as animal strain, breeding and shipping arrangements, etc., all of which may contribute to differences in results from one lab to another.

Most studies using morphine perinatally have shown offspring to be tolerant rather than hypersensitive [5]. Indeed, even in adult animals tolerance has been shown to last up to a year following chronic morphine administration and withdrawal [1]. Only the study of Zagon and McLaughlin [11] has shown hypersensitivity in adult offspring. In that study rats were treated perinatally with methadone. Since morphine given 4 times daily appears to mimic the effect of methadone in this situation, we postulate that the changes in behavioral responses seen in the M5×4 offspring are related to the constant presence of morphine which must be attained in fetal plasma and central nervous system following morphine injection every 6 hours. This is supported by the fact that the half-life of morphine in fetal tissue is about 3 hours [6].

The M5×4 animals have been shown in a previous study to respond normally to morphine on postnatal day 30 using hot-plate testing. The altered response to morphine shown at day 60 was demonstrated using tail-flick testing. Since different measures were used to assess morphine-induced analgesia at days 30 and 60, procedural differences cannot be ruled out in accounting for the different results. Thus, in future studies it may be necessary to use the same measure of analgesia throughout postnatal testing. In addition, more than one test of analgesia is needed to more completely describe morphine's effects.

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