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Effect of Morphine During Pregnancy and Lactation in Mice

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GANESAN, R. *Effect of morphine during pregnancy and lactation in mice.* PHARMACOL BIOCHEM BEHAV 45(2) 393–398, 1993. – The influence of pregnancy and lactation on morphine-induced hypothermia and hyperactivity was investigated in Rockland-Swiss mice. Pregnant mice were slower to recover from the hypothermic effect of morphine than nonpregnant controls. The greatest hypothermic response was seen in mice at day 18, the day before parturition. On the day after parturition, mice recovered faster from morphine-induced hypothermia than controls. During lactation, mice were again slower to recover from the morphine-induced hypothermia. Morphine-induced locomotor activity, however, was attenuated by both pregnancy and lactation. A further experiment compared the hypothermic effect of morphine in postpartum mice that were allowed to remain with pups and mice that were separated from pups. The enhanced hypothermia in the postpartum period was abolished by removing pups. This indicates that the altered response to morphine in the postpartum period was an effect of lactation rather than an aftereffect of pregnancy or parturition.

Morphine Hypothermia Hyperactivity Pregnancy Lactation Mouse

THERE are natural variations in the sensitivity to morphine and other opiates. Reactivity to morphine can be influenced, for example, by age (11), nutritional factors (13,20), and reproductive condition (2,5,14). Shifts in opiate sensitivity may be mediated by dispositional factors as well as functional differences.

The experiments reported below investigate the effect of pregnancy and lactation on the response to morphine on two variables, core body temperature and locomotor activity. Previous research indicated that pregnancy may enhance the effect of drugs by reducing liver metabolism (14,20). Hepatic microsomal preparations from pregnant rats showed an impaired metabolism of ethylmorphine (8) and other drugs (5). Accordingly, one behavioral effect of barbiturates, sleep time, was increased during the later stages of pregnancy (15,19).

More recent work indicated that lactation may influence the effect of morphine in a different manner. Haney and Miczek (10) showed that mice are less sensitive to the analgesic actions of morphine in the tail-flick assay during lactation as compared to virgin females. These authors did not find any systematic differences between virgin and lactating mice with regard to core body temperature. Kinsley and Bridges (16), also using the tail-flick assay, reported that parity affects morphine sensitivity during lactation. But, as a virgin or nulliparous control group was not included absolute effects of lactation could not be ascertained.

The studies reported here were undertaken to provide a more complete description of the influence of pregnancy and lactation on morphine-induced hypothermia and hyperlocomotion. The chosen dose of morphine, 20 mg/kg, reliably produces hypothermia (23) and hyperactivity (3,7) in the mouse. The two measures were monitored at different stages of pregnancy and lactation. A further experiment was also conducted to investigate whether changes occurring during lactation were a result of an aftereffect of pregnancy.

METHOD

Subjects and Housing

All experiments used female Rockland-Swiss (R-S) albino mice, bred in the mouse colony at SUNY, Albany. Prior to the experiment, they were housed in groups of six animals in polypropylene cages (28 × 18 × 13 cm) lined with wood shavings. The lighting in the laboratory was maintained on a 12 L : 12 D cycle (lights on at 6:00 a.m.) and ambient temperature was 70°(± 1) F.

Drugs

Morphine sulfate was purchased from Sigma (St Louis, MO) and dissolved in sterile saline for a concentration of 20 mg/ml. The dose of morphine used in all experiments was 20 mg/kg body weight, and it was administered via the SC route with a 0.25-ml syringe. All injections were administered between 10:00 and 11:00 a.m.

Body Temperature Measurements

Core body temperature was measured by means of a small animal probe (Yellow Springs Instruments, Yellow Springs, CO) inserted 2.5 cm into the rectum and held in place for 1 min. Body temperature measurements were taken every 30 min, starting 60 min before the injection, and until 240 min

after morphine administration. When pups were present (Experiments 2 and 3), they were removed from the dam's cage at the first temperature measurement and returned after the last.

Activity Measurements

Twenty-four hours prior to morphine treatment, experimental animals were housed in clear Plexiglas cages that were of the same dimensions as the home cage. These cages were placed on an Opto-Varimax C small-animal activity monitor (Columbus Instruments, Columbus, OH). Infrared photocell beams along both horizontal planes formed a 4 × 7 grid within the cage. The beams were 3.5 cm apart and 2 cm above the floor of the cage. A printer-counter (Columbus Instruments) in an adjacent room automatically counted and recorded the number of photobeam interruptions. Cumulative photobeam interruptions for each 30-min period for each animal were subject to statistical analysis in the experiments.

Procedure

Pregnancy. Female mice (80–110 days of age) were mated with Rockland-Swiss males for groups of pregnant females of approximately 10 subjects each. Each female was placed individually with a male and checked daily for the presence of the copulatory plug. The presence of a plug indicated that mating had occurred, and this was designated day 0 of pregnancy. Females were then isolated. For the temperature study, four stages of pregnancy were examined: days 6, 12, 16, and 18 (n = 9 or 10). For the activity study, days 12 and 18 were examined (n = 10 or 11). Parturition normally occurs on the night of day 18. All mice were retained after the experiment to ensure that pregnancy had taken place. Those that were not pregnant were omitted from the data analysis.

A further group of age-matched females (n = 10) that were not placed with males were included as nonpregnant controls for both the body temperature and activity experiments. These females were also isolated at the same time as pregnant mice.

Lactation. Female mice, 80–110 days of age, were mated with Rockland-Swiss males and allowed to give birth. When parturition had occurred (day 19 after the presence of the copulatory plug, designated day 0 of lactation), pups were culled to six per female. For the temperature study, four stages of lactation were examined: days 0, 5, 10, and 20 (n = 9 or 10). The investigation of locomotor activity used three groups of lactating females at days 0, 7, and 20 (n = 10–12). A separate group of age-matched nulliparous animals (n = 10–12) were also isolated to serve as controls in both studies.

Postpartum. A further experiment was conducted to investigate whether the differences observed during lactation were mediated by an aftereffect of pregnancy or the initiation of lactation. Three groups of female mice, 120–150 days of age, were included in this experiment. Mice from one group were isolated and did not come into contact with males (Group N, n = 12). Subjects from the other two groups were timed-mated with males. On day 18 of pregnancy, a hardware mesh (apertures of 1 cm^2) was placed on top of the cage floor. This allowed pups to drop through the mesh during parturition. On day 0 of lactation, these pups were removed and sacrificed. Females in Group P (n = 11) were each fostered six newborns from donor females and subjects in group NP (n = 12) did not receive any pups. Five days after parturition, the body temperature response to morphine was measured in all mice.

Statistical Analysis

Repeated-measures analyses of variance (ANOVAs) were used to analyze the results from all experiments. Significant interactions between groups and time were analyzed first with a series of simple main effects tests, followed by Tukey's tests for between-group differences at the appropriate time points.

RESULTS

Pregnancy

Mean body temperature for groups of mice at different stages of pregnancy is presented in Fig. 1 (upper panel). With increasing days of pregnancy, the hypothermia induced by morphine was lengthened. Mice at day 18 of pregnancy showed the slowest recovery from the hypothermic effects of morphine. A two-way ANOVA, with the factors of groups and days, was used to analyze the individual temperature values at each time point. This indicated a significant effect of time, $F(10, 430) = 400.32$, $p < 0.01$, but no effect of groups, $F(4, 430) = 2.04$, $p > 0.05$. The interaction was also significant, $F(40, 430) = 1.56$, $p < 0.05$. Simple main effects tests indicated that there was a significant effect of groups at all time points between 120 and 240 min, $F(4, 430) \geq 3.87$, $p < 0.05$. Further analyses indicated that mice at day 18 of pregnancy showed significantly greater hypothermia than mice at day 6 and nonpregnant controls from 120–240 min. Day-18 mice were also significantly more hypothermic than day-16 mice at 180 and 210 min. Mice at day 12 were significantly more hypothermic than day-6 mice and nonpregnant controls at 180 and 210 min.

Morphine increased locomotor activity in all subjects, and pregnant females showed a decreased level of activity relative to nonpregnant mice (Fig. 1, lower panel). Females at the later stages of pregnancy were less responsive to morphine's effects on locomotion than those at day 12 of pregnancy. However, pregnant mice also had a lower baseline activity level than nonpregnant mice. A two-way ANOVA indicated significant effects of groups, $F(2, 28) = 4.02$, $p < 0.05$, and time, $F(9, 252) = 10.07$, $p < 0.01$, but no interaction, $F(18, 252) = 1.21$, $p > 0.05$. This indicates that although morphine increased activity in nonpregnant females to a greater extent than pregnant females the controls' baseline activity level was also significantly higher. A subsequent Tukey's comparison indicated that nonpregnant animals showed a significantly greater locomotor activity than 18-day pregnant females. No other differences reached significance.

Lactation

Mean body temperature for groups of mice at different stages of lactation are presented in Fig. 2 (upper panel). Relative to the nulliparous control group, mice at day 0 of lactation were less sensitive to the hypothermic effects of morphine. At later stages of lactation (days 5, 10, and 20), mice displayed a greater hypothermia relative to the nonlactating controls. A two-way ANOVA performed on individual temperature values indicated significant effects of groups and time and a significant interaction, $F(40, 430) = 7.66$, $p < 0.01$. Further analyses indicated that the difference between groups was significant at 30 min and all subsequent time points. Posthoc tests showed that day-0 mice were significantly less hypothermic than subjects at days 5, 10, and 20 of lactation from 90 min onward. Day-0 mice were also significantly less hypothermic relative to nulliparous mice at 30, 60,

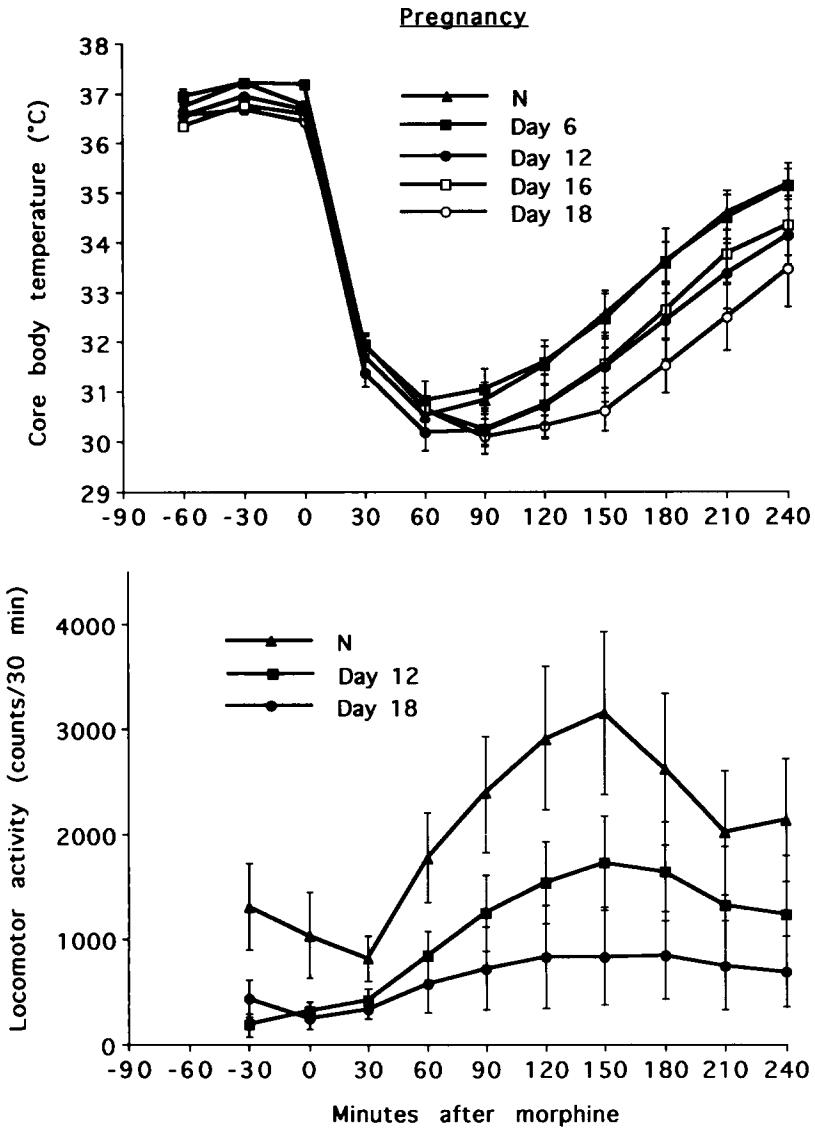

FIG. 1. The effect of morphine (20 mg/kg) at different stages of pregnancy on core body temperature (upper panel) and locomotor activity (lower panel). Data are presented as means ± SE. Core body temperature was examined in 5 groups: Nonpregnant controls (Group N) and mice at 4 stages of pregnancy (Days 6, 12, 16, and 18). Locomotor activity was examined in 3 groups: Nonpregnant controls (Group N) and mice at 2 stages of pregnancy (Days 12 and 18).

and 180–240 min. From 120 min onward, mice at days 5, 10, and 20 of lactation showed significantly greater hypothermia than nulliparous controls.

Lactating mice were less responsive to the locomotor stimulant effects of morphine relative to nonlactating mice (Fig. 2, lower panel). A greater degree of activity was seen in mice at day 0 postpartum than in mice at later stages of lactation, day 7 and day 20. A two-way ANOVA indicated that the effects of groups, $F(3, 42) = 9.54$, time, $F(9, 378) = 10.13$, and the interaction, $F(27, 378) = 4.02$, $p < 0.01$, were all significant. No differences were significant at the two time points before the injection except that at −30 min females at day 0 postpartum showed greater levels of activity than all other groups. At all time points between 90 and 240 min, females at days 7 and 20 of lactation showed less activity than all other groups. From 150–240 min, females at day 0 postpartum were also significantly less active than control females.

Postpartum

Mean body temperature for postparturient mice and controls is presented in Fig. 3. Mice with pups present (Group P) showed a greater hypothermic response to morphine than subjects without pups (Group NP) on day 5 after parturition. Mice from Group NP were comparable to those from Group N. A two-way ANOVA on individual body temperature values

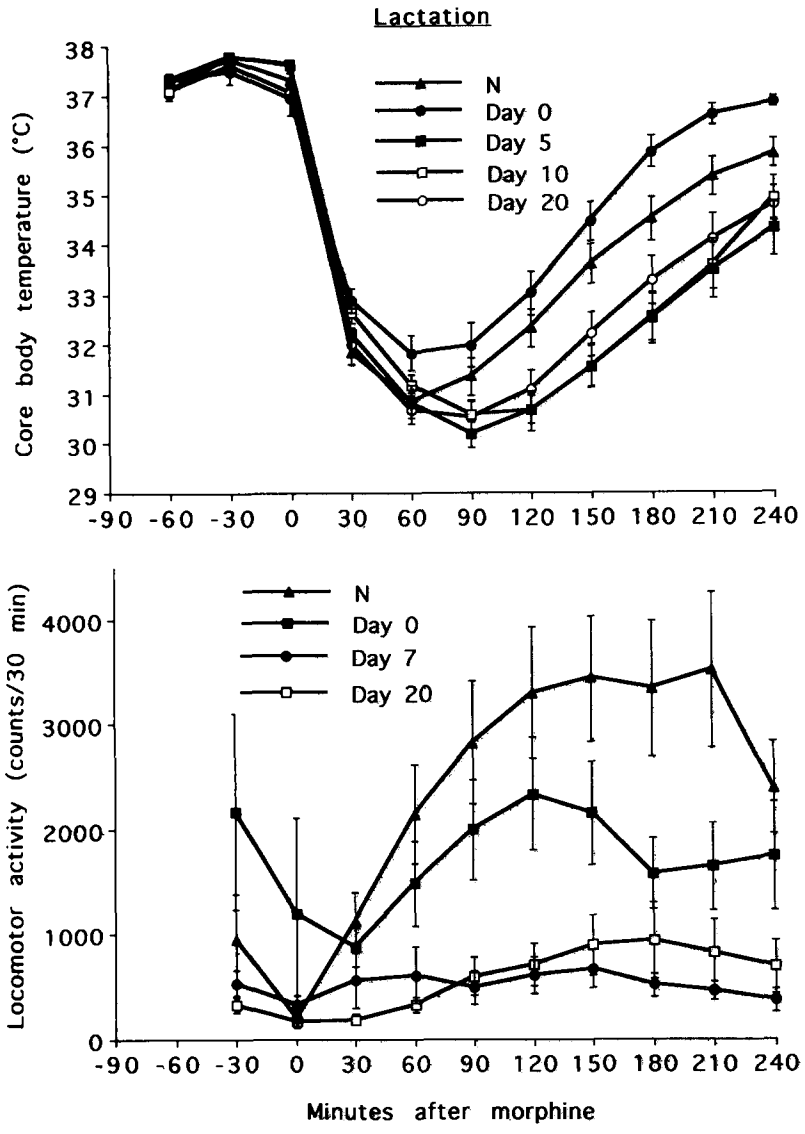

FIG. 2. The effect of morphine (20 mg/kg) at different stages of lactation on core body temperature (upper panel) and locomotor activity (lower panel). Data are presented as means ± SE. Core body temperature was examined in 5 groups: Nonpregnant, nonlactating controls (Group N) and mice at 4 stages of lactation (Days 0, 5, 10 and 20). Locomotor activity was examined in 4 groups: Nonpregnant, nonlactating controls (Group N) and mice at 3 stages of lactation (Days 0, 7 and 20).

indicated significant effects of groups and days and also of the interaction, $F(20, 320) = 9.72$, $p < 0.01$. Simple main effects tests indicated significant group effects at all times after 90 min, $F(2, 320) \geq 10.18$, $p < 0.01$. Tukey's tests at each time point revealed that Group P was significantly more hypothermic than both Groups NP and N from 90 min until the end of the experiment. In addition, there were differences between Groups NP and N at some time points. At 150, 180, and 210 min, mice in Group NP were significantly less hypothermic than those from Group N. These results indicate that the enhanced hypothermia in postparturient mice is a result of lactation rather than an aftereffect of pregnancy or parturition.

DISCUSSION

The studies reported above examined the effect of morphine on body temperature and locomotor activity during pregnancy and lactation in the mouse. During either reproductive condition, the hypothermic response to morphine at a dose of 20 mg/kg was exaggerated relative to virgin mice. However, the hyperactivity induced by morphine was attenuated by both pregnancy and lactation.

With increasing days of pregnancy, the hypothermic response to morphine was gradually enhanced. During early stages of pregnancy (day 6), the hypothermic response was no different from that in nonpregnant mice. The greatest effect

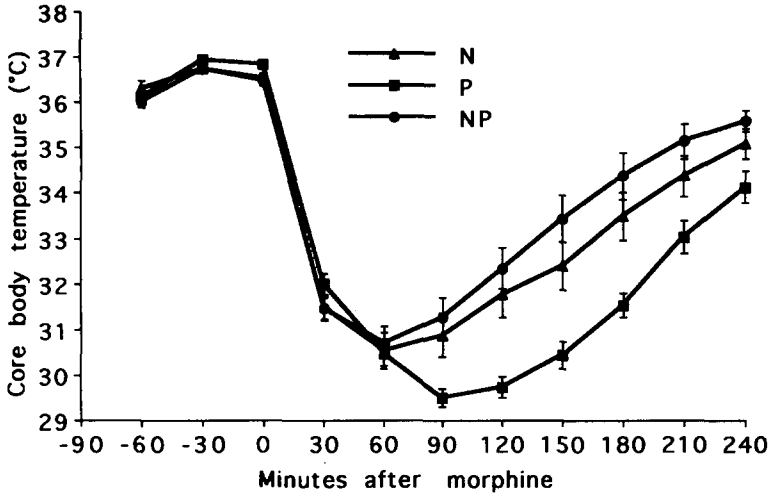

FIG. 3. The effect of morphine (20 mg/kg) in nonpregnant controls (Group N), and in two groups of mice at postpartum day 5. Data are presented as means ± SE. Mice in Group P were fostered pups soon after parturition, and mice in Group NP were not fostered pups.

of morphine on body temperature was found on the last day of pregnancy. However, on the day after parturition postpartum mice were significantly less susceptible to the hypothermic effects of morphine relative to virgin mice. With the onset of lactation, the hypothermic response was again enhanced until the start of the weaning stage (day 20).

A further study showed that the enhancement of hypothermia on day 5 postpartum was not due an aftereffect of pregnancy or parturition. When pups were removed at birth, females at day 5 postpartum were less hypothermic than postpartum females fostered pups. Indeed, at some time points postpartum mice that were not fostered pups showed a reduced hypothermic response in relation to virgin females. This is analogous to the finding in the previous experiment where mice at day 0 postpartum showed attenuated hypothermia in relation to control mice. Together, these results suggest that the enhanced susceptibility to morphine-induced hypothermia during pregnancy may rebound immediately at parturition and result in a reduced susceptibility. This resistance to morphine's effect may be sustained up to 5 days after parturition if lactation was not initiated.

It is possible that the observed exaggeration of the hypothermic response to morphine in this report was mediated by a reduction in speed of metabolism, which would result in the prolonged availability of the substrate. Estrogens and progestogens are known to interfere with the metabolic transformation and conjugation of drugs (12,21). Total increases in the level of ovarian steroids (17,18) may play a role in the enhanced hypothermic effect of morphine during pregnancy. However, estrogen levels are expected to be elevated on the morning after parturition (day 0 of lactation, the day of postpartum estrus) (4), when the present results showed a decrease in the susceptibility to the effects of morphine. Further, with the onset of lactation ovarian steroid levels normally decline to lower levels (4). The effect of lactation on morphine-induced hypothermia was at least as pronounced as the effect of pregnancy, and it is not possible to attribute this to increased steroid levels.

The pattern of morphine-induced hypothermia observed across pregnancy and lactation closely parallels the findings by other authors using a different measure, barbiturate sleeping time (15,19). The duration of anesthesia induced by barbiturates was gradually increased during pregnancy, but hexobarbital-induced sleep time 1 day after parturition was reduced to the level of nonpregnant rats. Neale and Parke (19) and Guarino et al. (8) related these effects to reductions in activity of the hepatic microsomal drug-metabolizing enzymes during pregnancy. The reduction in the activities of the microsomal enzymes was paralleled by a similar reduction in the liver content of cytochrome P-450. One possible mechanism for the reduction in cytochrome P-450 during pregnancy is through an increase in growth-promoting factors (19).

Although both reproductive states influenced the effect of morphine in the same direction with either dependent variable, generalizing from one stage to the next may not be warranted. While there have been reports of altered drug metabolism during pregnant relative to nonpregnant states (5,14,15,19), the effect of lactation has not been studied in detail. Such studies would be necessary before a conclusion can be reached with regard to lactation.

The effect of pregnancy and lactation on the locomotor response to morphine was the opposite of the body temperature response. Morphine induced stereotyped running in Rockland-Swiss mice, as in some other strains (1,3). During pregnancy and lactation, morphine-induced running was reduced relative to control females. This effect is interesting in relation to the hypothermic effect, which suggested that clearance of morphine is reduced by these reproductive states. Previous research (3,7) and dose-response studies conducted in this laboratory (unpublished) indicate that morphine induces an asymptotic running response that does not diminish at doses up to 80 mg/kg and above. It is unlikely, therefore, that the difference in the locomotor activity is due to changes in the relative effectiveness of morphine at the dose administered. Rather, the differences may be related to the overall decrease in locomotor activity observed during pregnancy and

lactation in rodents (24,25). However, as mice are less active during the light portion of the day it is possible that a floor effect could obscure the differences in baseline activity levels between the control and experimental groups. In the experiment examining lactation, for example, the baseline activity was not related to locomotor activity after morphine administration. On the other hand, the results obtained in pregnant mice and controls shows that animals differed in both baseline activity level as well as morphine-induced running and that the interaction between the effects of groups and time was not significant.

The influence of pregnancy and lactation on the hypothermic response to morphine, then, may be mediated by a different mechanism from the locomotor response. At this point, it is interesting to compare the current results with those of Haney and Miczek (10), who did not find systematic differences between lactating and nonlactating controls with respect to core body temperature at 20 min postinjection. Even though lower doses of morphine were used relative to the current study (1–10 mg/kg), it is possible that with continued monitoring of core body temperature differences would have been evident. With regard to the analgesic response, morphine was less effective in inducing analgesia in lactating mice relative to nonlactating controls at two doses (6 and 10 mg/kg) 20 min after injection and for 3 h after injection of one representative dose, 10 mg/kg. The results from the analgesia are difficult to reconcile with the finding that lactating mice show greater, not less, hypothermia in response to morphine compared to controls. This suggests that lactation influences analgesia through a different mechanism from hypothermia. However, in this study some of the mice assessed in the tail-flick analgesia were concurrently tested for aggressive behavior. It is possible that the results obtained reflected differences in fighting experience, which is known affect analgesia (22), rather than lactation.

Levels of morphine in the serum and brain were not measured as a part of this article. If clear evidence for delayed clearance was found in pregnant and lactating mice, this may be largely responsible for the observation that mice in these states show greater hypothermia in response to morphine. This is one issue that needs to be addressed in future work. Evidence for decelerated clearance, however, does not rule out other mechanisms that may play a role in the observed effects. The opioid system shows obvious differences in pregnant and lactating animals relative to controls whether assessed behaviorally (6) or physiologically (9). In addition, luteinizing hormone-releasing hormone has been shown to oppose the effects of morphine (2) and changes in this peptide during pregnancy and lactation may be important in determining the effects of morphine. A clearer way to address the influence of these factors separate from the peripheral metabolic effects of pregnancy or lactation would be to administer opiates directly to the brain. Both approaches will be used in future research.

REFERENCES

1. Belknap, J. K.; Noordewier, B.; Lame, M. Genetic dissociation of multiple morphine effects among C57BL/6J, DBA/2J and C3H/HeJ inbred mouse strains. Physiol. Behav. 46:69–74; 1989.
2. Berglund, L. A.; Simpkins, J. W. Luteinizing hormone-releasing hormone alters hypothalamic effects of morphine in the rat. Endocrinology 128:2799–2804; 1992.
3. Brase, D. A.; Loh, H. H.; Way, E. L. Comparison of the effects of morphine on locomotor activity, analgesia and primary and protracted physical dependent in six mouse strains. J. Pharm. Exp. Ther. 201(2):368–374; 1977.
4. Challis, J. R. G.; Heap, R. B.; Illingworth, D. V. Concentrations of oestrogen and progesterone in the plasma of non-pregnant, pregnant and lactating guinea-pigs. J. Endocrin. 51:333–345; 1971.
5. Creaven, P. J.; Parke, D. V. The effect of pregnancy on the microsomal metabolism of foreign compounds. Proc. 2nd Meet., Fed. Eur. Biochem. Soc. London: Academic Press; 1965: p. 88.
6. Gintzler, A. R.; Bohan, M. C. Pain thresholds are elevated during pseudopregnancy. Brain Res. 507:312–316; 1990.
7. Goldstein, A.; Sheehan, P. Tolerance to opioid narcotics. I. Tolerance to the "running fit" caused by levorphanol in the mouse. J. Pharm. Exp. Ther. 169(2):175–184; 1969.
8. Guarino, A. M.; Gram, T. E.; Schroeder, D. H.; Call, J. B.; Gillette, J. R. Alterations in kinetic constants for hepatic microsomal aniline hydroxylase and ethylmorphine n-demethylase associated with pregnancy in rats. J. Pharm. Exp. Ther. 168(2):224–228; 1969.
9. Hammer, Jr., R. P.; Bridges, R. S. Preoptic area opioids and opiate receptors increase during pregnancy and decrease during lactation. Brain Res. 420:48–56; 1986.
10. Haney, M.; Miczek, K. A. Morphine effects on maternal aggression, pup care and analgesia in mice. Psychopharm. 98:68–74; 1989.
11. Hoskins, B.; Burton, C. K.; Ho, I. K. Differences in morphine-induced antinoception and locomotor activity in mature adult and aged mice. Pharm. Biochem. Behav. 25:599–605; 1986.
12. Juchau, M. R.; Fouts, J. R. Effects of norethynodrel and progesterone on hepatic microsomal drug-metabolising enzyme systems. Biochem. Pharmacol. 15:891–898; 1966.
13. Kanarek, R. B.; White, E. S.; Biegen, M. T.; Marks-Kaufman, R. Dietary influences on morphine-induced analgesia in rats. Pharm. Biochem. Behav. 38:681–684; 1991.
14. Kato, R. Drug metabolism under pathological and abnormal physiological states in animals and man. Xenobiotica. 7:25–92; 1977.
15. King, J. E.; Becker, R. F. Sex differences in the response of rats to pentobarbital sodium. I. Males, nonpregnant females and pregnant females. Amer. J. Obstet. Gynecol. 86:856–864; 1963.
16. Kinsley, C. H.; Bridges, R. S. Parity-associated reductions in behavioral sensitivity to opiates. Biol. Reprod. 39:270–278; 1988.
17. McCormick, J. T.; Greenwald, G. S. Progesterone and oestradiol-17β concentrations in the peripheral plasma during pregnancy in the mouse. J. Endocrin. 62:101–107; 1974.
18. Murr, S. M.; Stabenfeldt, G. H.; Bradford, G. E. Plasma progesterone during pregnancy in the mouse. Endocrinology. 94:1209–1211; 1974.
19. Neale, M. G.; Parke, D. V. Effects of pregnancy on the metabolism of drugs in the rat and rabbit. Biochem. Pharm. 22:1451–1461; 1973.
20. Parke, D. V. The biochemistry of foreign compounds. Pergamon Press. 1968.
21. Quinn, G. P.; Axelrod, J.; Brodie, B. B. Species, strain and sex differences in metabolism of hexobarbitone, amidopyrine, antipyrine and aniline. Biochem. Pharmacol. 1:152–159; 1958.
22. Rodgers, R. J.; Randall, J. I. Environmentally induced analgesia: Situational factors, mechanisms and significance. In: Rodgers, R. J.; Cooper, S. J., eds. Endorphins, opiates and behavioral processes. John Wiley & Sons; 1988:107–142.
23. Rosow, C. E.; Miller, J. M.; Poulsen-Burke, J.; Cochin, J. Opiates and thermoregulation in mice. IV. Tolerance and cross-tolerance. J. Pharm. Exp. Ther. 223(4):702–707; 1982.
24. Slonaker, J. R. The effect of copulation, pregnancy, pseudopregnancy, and lactation on the voluntary activity and food consumption of the albino rat. Am. J. Physiol. 71:362–394; 1925.
25. Wang, G. H. The relation between the "spontaneous" activity and oestrous cycle in the white rat. Comp. Psychol. Monogr. Vol II(6):1–27; 1923.