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Effects of Prenatal Morphine Exposure on NK Cytotoxicity and Responsiveness to LPS in Rats

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SHAVIT, Y., E. COHEN, R. GAGIN, R. AVITSUR, Y. POLLAK, G. CHAIKIN, G. WOLF AND R. YIRMIYA. *Effects of prenatal morphine exposure on NK cytotoxicity and responsiveness to LPS in rats.* PHARMACOL BIOCHEM BEHAV **59**(4) 835–841, 1998.—Prenatal exposure to opiates can adversely affect fetal development, resulting in long-term growth retardation and impairments in physiological and behavioral functions. In the present study we studied long-term effects of prenatal morphine exposure on immune functions, including the activity of natural killer (NK) cells and the febrile and behavioral responses to lipopolysaccharide (LPS). Pregnant Fischer 344 rats were given increasing doses of morphine in slow release emulsion during gestational days 12–18. Control rats were injected with vehicle and were either pair fed to morphine rats or fed ad lib. Postnatal experiments were conducted when offspring were 10–12 weeks old. Compared to both control groups, rats prenatally exposed to morphine exhibited: 1) suppressed cytotoxic activity of NK cells; 2) reduced LPS-induced fever measured by a biotelemetric system; 3) reduced hyperalgesia measured by the hot-plate test at 30 min, and augmented hypoalgesia at 2–6 h post-LPS; 4) higher open-field activity in saline-treated animals, and more pronounced suppression of activity in LPS-injected animals; 5) LPS-induced reduction of food consumption, body weight, and social exploration, which did not differ from the reduction observed in control animals. These findings indicate that prenatal exposure to morphine induces long-term impairment of host–defense mechanisms, which may render the offspring more susceptible to infectious diseases. © 1998 Elsevier Science Inc.

Prenatal morphine Opiates Natural killer cell activity LPS Fever Sickness behavior Hyperalgesia and hypoalgesia Immunocompetence

OPIATES can adversely affect fetal development, as is evident from reports of reduced birth weight and length (24), smaller head size (6), and higher mortality rates (11,12) observed in neonates exposed to opiates during gestation. Long-term effects of intrauterine opiate exposure have also been reported. For example, growth retardation (16) and impaired cognitive potential (50) were observed in preschool and school age children. In rodents, enhanced self-administration of cocaine and heroine (35), impaired female reproductive behavior (45), and long-term changes in opiate receptors (10, 44,52) were reported following prenatal exposure to opiates.

We have recently shown that prenatal exposure to morphine produced long-term behavioral effects, including increased analgesic response to acute morphine administration (13), enhanced preference for sweet solutions (13), and higher preference for a place that had been associated with morphine in a classical conditioning paradigm (14). Because endogenous opiates are known to play a modulatory role in these behaviors, the findings suggest that prenatal exposure to morphine induces a long-term increase in the sensitivity and/or activity of endogenous opiate systems.

Exogenous and endogenous opiates have been shown to modulate immune functions, including, chemotaxis, macrophage and granulocyte activity, mast cell activation, lymphocyte proliferation and function, and natural killer (NK) cytotoxic activity [for review, see (40)]. Morphine administration is commonly associated with suppression of NK cell activity, as demonstrated in animal and human studies. This effect is probably mediated by central mechanisms, because morphine injected intracerebroventricularly (ICV) (39) or into the periaqueductal gray area (PAG) (49), suppressed NK activity at much lower doses than those required peripherally, and this suppression was blocked by opiate antagonists (39,49). Furthermore, opiates were shown to modulate the production of cytokines, induced by activation of the immune system. A useful model for demonstrating these effects has been the administration of the en-

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dotoxin lipopolysaccharide (LPS), a product of the cell wall of gram-negative bacteria, which induces the secretion of proinflammatory cytokines. For example, LPS-induced secretion of tumor necrosis factor-α (TNF-α) was inhibited by either in vivo (2) or in vitro (5) exposure to morphine, or Met-enkephalin (28). On the other hand, LPS-induced bioactivity of interleukin-1β (IL-1β) was potentiated by Met-enkephalin (28), and partially blocked by an opiate antagonist, naloxone (9).

Opiates can also alter neuroimmune interactions, including the febrile, neuroendocrine, and behavioral responses to immune challenges. Febrile responses to TNF-α or to interferon-α (IFN-α) were attenuated by pretreatment with naloxone (19,32). Naloxone effectively counteracted the inhibitory effect of IL-1β on pituitary release of luteinizing hormone (3). Finally, opiate antagonists prevented the suppression of exploratory behavior induced by IL-1 (41), and the analgesic effect of IFN-α (31), or LPS (51).

Because prenatal morphine has long-term effects on endogenous opiate systems, and because these systems modulate immune functions and neuroimmune interactions, it is hypothesized that in utero exposure to morphine may alter immune functions and responsiveness to immune challenges. This hypothesis was tested in this study by examining NK activity, and by observing febrile and behavioral responses induced by LPS in rats prenatally exposed to morphine.

METHOD

Prenatal Treatment

Nulliparous Fischer 344 female rats (Harlan Laboratories, Jerusalem), 10–12 weeks old, weighing 230–250 g, were maintained under standard laboratory conditions (23 ± 1°C; 12 L:12 D cycle, with lights on between 1930–0730 h). Food and water were always available (unless otherwise specified). To determine the day of estrus, females were daily placed with sexually vigorous studs for a brief observation. Estrus was determined by the occurrence of lordosis in response to the stud's sexual approach. Estrous females were housed with Fischer 344 males for approximately 16 h. The day of mating was considered day 0 of pregnancy. Females mated on the same day were housed in group cages until day 12 of pregnancy, after which they were separated into individual cages until parturition.

Morphine treatment began on day 12 of gestation, the day just preceding the emergence of opiate receptors in the rat brain (7). Pregnant dams were randomly assigned into three groups. Morphine-treated dams (group MR) received increasing doses of morphine HCl (Teva, Israel), 0.75, 1.5, 1.5, 3.0, 6.0, 12.0, and 12.0 mg/animal on days 12–18 of pregnancy. Morphine was dissolved in saline and the solution mixed with light white mineral oil (Sigma, Israel) and Arlacel-A (Sigma, Israel), in ratios of 8:6:1, respectively, to give a slow-release emulsion. Injections were administered subcutaneously (SC) at a volume of 1 ml. This protocol was developed in our laboratory, based on a previously described pilot study (13). Two control groups were employed in this study: animals of both control groups received a daily 1-ml injection of the vehicle emulsion; they were, however, either fed ad lib (group AL), or given restricted feeding, corresponding to the average food intake measured on the previous day in MR dams (group PF).

Litters were typically born on days 22–23 of pregnancy; they were culled to eight pups (with both sexes evenly represented, as possible) 24 h after birth, and fostered to drug-naive dams. At 3 weeks of age, offspring were weaned, housed in cages of three to four rats according to sex and prenatal treatment, and maintained under standard conditions.

Postnatal testing began when offspring were 10–12 weeks old. All the experiments described in this article were conducted on male rats, using one animal per litter per experimental condition. Consequently, each experiment was carried out in several batches separated by at least 1 month. Because the batches did not significantly differ, data of all batches were pooled and analyzed together.

Experiment 1—NK Cell Cytotoxicity

Rats of the three groups (n = 5 MR, 9 PF, and 10 AL) were anesthetized with halothane, their spleens removed, and dissociated into single cell suspensions. Cells were washed twice in phosphate-buffered saline (PBS), and their concentration adjusted to 10^7 cells/ml in complete RPMI medium (1640 RPMI medium supplemented with 15% heat-inactivated fetal bovine serum, 0.1 mg/ml gentamicin, 2 mM L-glutamine, 0.1 mM nonessential amino acids, and 1 mM sodium pyruvate). NK cytotoxicity was assessed using a standard 4-h chromium-51 release assay. YAC-1 cells were radioactively labeled by coincubation with 200 μCi chromium-51 (^{51}Cr) for 1 h at 37°C, following which the YAC-1 cells were washed twice in complete medium, and their concentration adjusted to 10^5 cells/ml. Splenocyte suspension (effector cells) (100 μl) was coincubated with 100 μl of a labeled YAC-1 cell suspension (target cells) in 96-well V-bottom plates, using the following effector:target (E:T) ratios: 100:1, 50:1, and 25:1. Plates were centrifuged (200 × g for 5 min), and placed in an incubator (37°C, 5% CO_2) for 4 h. Next, plates were centrifuged again (200 × g for 8 min), and samples of 100 μl of supernatant were recovered from each well. Level of radioactivity was determined for each sample, using a gamma-counter. Specific cytotoxicity was calculated as: [sample CPM − spontaneous CPM]/[maximal CPM − spontaneous CPM] × 100, where spontaneous release was determined in samples of YAC-1 cells alone, and maximal release was determined by adding 10 μl of Triton X into samples of YAC-1 cells.

Experiment 2—LPS-Induced Analgesia

Pain sensitivity was determined in male rats, prenatally exposed to either morphine or control treatments (n = 19 MR, 19 PF, and 15 AL), using the hot-plate test. The hot-plate apparatus consisted of 20 × 20 cm copper plate, maintained at 53 ± 1°C and surrounded by a 45 cm-high enclosure of clear Plexiglas. The plate was covered with an insulating cardboard and the rat placed on the cardboard for 30 s of adjustment. Following this, the cardboard was removed and the rat exposed to the heated surface. Hot plate latency (HPL) was defined as the latency to the first incidence of hind paw licking. Cutoff latency was set at 45 s to prevent tissue damage. Baseline HPL was determined for each animal, following which animals were injected intraperitoneally (IP) with a dose of 400 μg/kg LPS (from *E. coli* 055:B5, Difco Labs, Detroit, MI, in a volume of 1 ml/kg). This dose of LPS was determined according to previous experiments with LPS-induced analgesia in our laboratory [e.g., (51)]. Additional HPL measurements were taken 30 min and 2, 4, and 6 h after the injection. To evaluate the effect of LPS on pain sensitivity, an analgesia score was computed, by subtracting baseline HPL from the post-LPS values of HPL.

Experiment 3—LPS Effects on Activity and Body Temperature

Activity and body temperature (BT) were continuously measured using battery-operated biotelemetric transmitters

(model VM-FH, Mini Mitter Co. Inc., Sunriver, OR) implanted in the peritoneal cavity. Output was monitored by a receiver board (model RA-1010) placed under each animal's cage and fed into a peripheral processor (BCM100) connected to a personal computer. Activity was measured by monitoring the number of pulses generated by the transmitter as the animal moved about its cage, which was proportional to the distance the animal moved. The number of pulses generated in 10-min periods was recorded. BT was detected by a sensor embedded in the transmitter and recorded at 10-min intervals.

Because we have noticed in previous studies conducted in our laboratory that LPS-induced fever is more pronounced during the light phase of the circadian cycle, animals of this experiment (n = 11 MR, 10 PF, 6 AL) were maintained under normal light–dark cycle, with lights on between 0730 and 1930, throughout the experiment. Rats were implanted with intraabdominal biotelemetric transmitters under ether anesthesia, and then returned to their home cages for recovery. At least 2 weeks later, animals were individually housed in cages that were placed over the receiver boards, in the biotelemetric measurement room. Data recording began after a 3-day adaptation period and was carried out for three consecutive 24 h periods (from noon to noon of the next day). Activity and BT recorded during the first 24-h period represented baseline data; on the beginning of the second 24-h, all animals were injected with saline 1 ml/kg, IP, and on the beginning of the third 24-h period they were injected with LPS, 50 μg/kg, IP.

Experiment 4—LPS Effects on Open-Field Behavior, Social Exploration, and Ingestive Behavior

The effects of LPS on open-field behavior, social exploration, and food intake was tested in male rats (n = 8 MR, 8 PF, and 8 AL). To measure baseline level of social exploration, each rat was placed in a semicircular observation box (30 cm high, 60 cm in diameter), the front wall of which was made of transparent Plexiglas. The animal was allowed 15 min of habituation, following which a juvenile male rat (26–30 days old) was introduced into the cage for 3 min. The cumulative time spent by the experimental rat in exploration (sniffing and grooming) of the juvenile was recorded by an experimenter blind to the prenatal history of the experimental rat, using a computerized event recorder. At the completion of the social–exploration test, each animal was weighed, housed in an individual cage, and supplied with a weighed amount of food pellets (100 g). Twenty-four hours later, each animal was weighed again, and the amounts of food left were recorded, to give baseline data of daily body weight gain and food consumption. (Preliminary experiments showed that food spillage was negligible.)

On the morning of the test day, rats were weighed and injected with LPS (50 μg/kg, IP), or with saline. Weighed food (100 g) was supplied. Social exploration was assessed 2 and 6 h after the injection. Behavior in the open-field test was run 4 h after the injection. The open-field apparatus consisted of wooden box (95 × 95 × 60 cm) painted black, with a floor divided by white strips into 25 identical squares. The rat was placed in one of the corners of the open field, and the incidence of line crossing (with all paws), rearing and grooming episodes was recorded by an observer blind to the animal's prenatal history, over a period of 3 min. Body weight and food consumption were measured 24 h after the injections.

Statistical Analysis

Data were analyzed using analyses of variance (ANOVA) according to prenatal treatment, with repeated measures wherever applicable. Where no significant difference was detected between the two control groups (AL and PF), their data were pooled together, for comparison of MR rats with as a single control group. A significance level of 0.05 was predetermined for all statistical analyses.

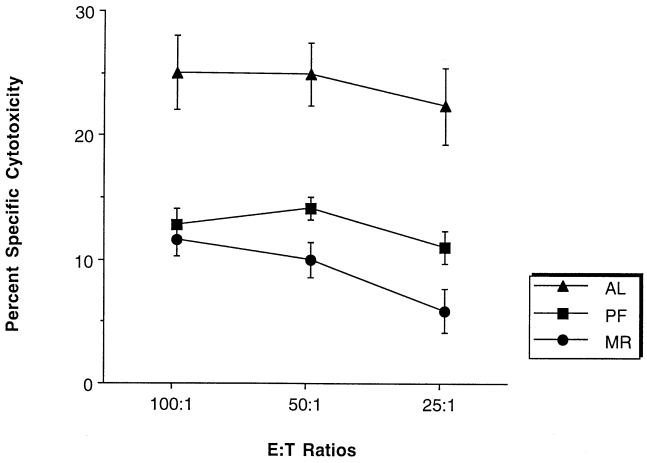


FIG. 1. NK cytotoxicity (mean ± SEM) in male rats of the three prenatal treatment groups (MR = prenatally morphine-exposed rats; PF = pair-fed controls; AL = ad lib controls). Spleen cells were coincubated with ^{51}Cr-labeled YAC-1 cells at E:T ratios of 100:1, 50:1, and 25:1. Cytotoxicity was significantly lower in MR and PF animals than in AL animals, and was lower in MR than in PF animals.

RESULTS

Experiment 1—NK Cell Cytotoxicity

NK cell cytotoxic activity was significantly, $F(2, 21) = 13.2$, suppressed in rats of both MR and PF groups, compared with AL rats (Fig. 1). Suppression was evident at all E:T ratios, indicating impaired NK activity in rats exposed to morphine or to food restriction during their fetal development. NK cell suppression was significantly (Games-Howell post hoc comparison) more pronounced in MR than in PF rats, suggesting that the effect of prenatal morphine was not merely due to malnutrition or stress.

Experiment 2—LPS-Induced Analgesia

In agreement with previous reports [e.g., (51)], LPS induced hyperalgesia at 30 min postinjection, and hypoalgesia at later time points (2–6 h post-LPS; Fig. 2). LPS analgesia scores of MR rats were significantly, $F(1, 50) = 4.31$, different from the pooled analgesia scores of the two control groups: MR rats showed no hyperalgesia at 30 min post-LPS, and showed higher hypoalgesia at all later time points.

Experiment 3—LPS Effects on Body Temperature and Activity

Body temperature was elevated following LPS injection, especially at 5–9 h after the injection (Fig. 3). This febrile response was significantly lower in MR compared with the pooled BT of the two control groups, $F(1, 25) = 4.67$ (Fig. 3).

Motor activity was significantly suppressed by LPS. Mean (±SEM) change of motor activity in the saline day (saline–baseline values) was −5.9 ± 2.23, and in the LPS day (LPS–

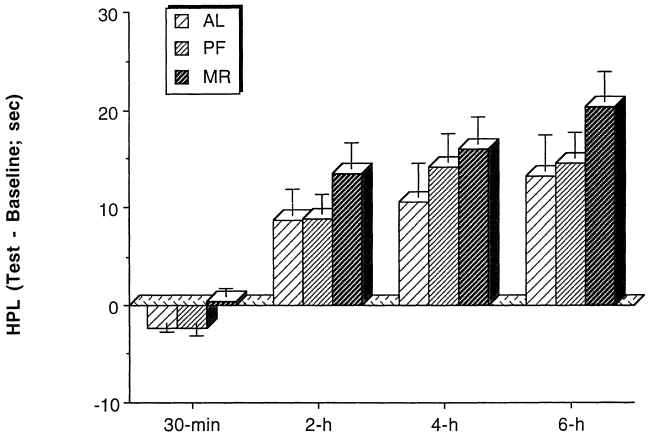


FIG. 2. Changes in hot-plate latency, induced by LPS, 400 μg/kg, in male rats of the three prenatal treatment groups. MR animals exhibited no hyperalgesia at 30 min postinjection and enhanced hypoalgesia at later time points (2–6 h).

baseline values) −18.6 ± 2.25, $F(21, 42) = 8.24$. There were no significant differences among the prenatal treatment groups, although MR rats had a tendency for higher baseline activity compared with AL and PF rats.

Experiment 4—LPS Effects on Open-Field Behavior, Social Exploration, and Ingestive Behavior

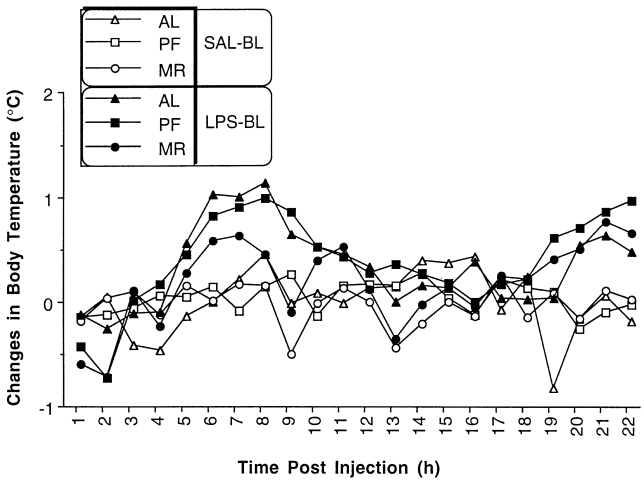
LPS significantly suppressed the incidence of line crossing, $F(1, 42) = 42.12$, rearing, $F(1, 42) = 37.31$, and grooming, $F(1, 42) = 13.56$, in all rats (Fig. 4). A two-way ANOVA revealed a significant interaction of prenatal treatment × experimental injection for line crossing, $F(2, 42) = 7.34$, and for rearing, $F(2, 42) = 4.55$. The effect on grooming was almost significant, $F(2, 42) = 3.1$, $p = 0.055$. This interaction indicates that saline-treated MR rats exhibited more line crossing, rearing, and grooming than AL and PF rats treated with saline; while LPS-treated MR rats exhibited more pronounced suppression of line crossing, rearing, and grooming compared with LPS-treated AL and PF rats (Fig. 4).

LPS significantly suppressed food consumption, $F(1, 42) = 191.68$, and body weight gain, $F(1, 42) = 66.82$, as measured 24 h after the injection (Fig. 5). LPS also suppressed social exploration at 2 and 6 h postinjection (data not shown). Weight gain, food intake, and social exploration were not differentially affected by LPS in the three prenatal treatment groups.

DISCUSSION

Prenatal exposure to morphine suppressed the cytotoxic activity of NK cells, and modulated LPS responsiveness at adulthood. LPS-induced fever and hyperalgesia were attenuated in MR rats, whereas LPS-induced suppression of open-field activity and hypoalgesia were augmented in these animals, compared to AL and PF rats. LPS-induced aphagia, body weight loss, suppression of social exploration, and suppression of home-cage activity were similar in MR, AL, and PF rats.

Prenatal exposure to morphine appears to induce long-term increase in the sensitivity/activity of endogenous opiate systems. For example, MR rats exhibited increased analgesic response to acute morphine administration (13), enhanced preference for sweet solutions (13), and higher preference for a



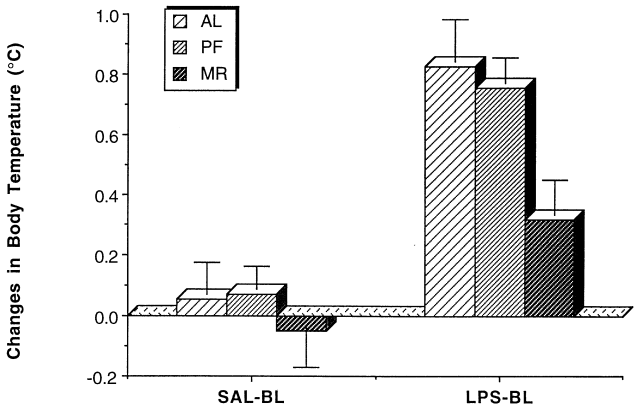


FIG. 3. Changes in body temperature induced by saline and by LPS, 50 μg/kg, in male rats of the three prenatal treatment groups. Body temperature was continuously recorded using biotelemetric transmitters, and expressed as differences between the temperature measured during the test and the respective baseline records. Top: continuous records taken throughout the day following saline injection (open symbols) and the next day following LPS injection (closed symbols). Bottom: temperature changes averaged across the period of 5–9 h after each injection. Febrile response was lowest in MR rats.

place associated with morphine via a classical conditioning paradigm (14). Other reports have shown that prenatal morphine or β-endorphin induced an increase in μ opiate receptors in the striatum and nucleus accumbens (15,21,52), increased proenkephalin mRNA levels, decreased Met-enkephalin levels in the striatum of newborns (43), and enhanced development of Met-enkephalin–containing neurons (10).

Previous research demonstrated that LPS activates endogenous opiate systems. Endogenous opiate levels are higher in the plasma of LPS-treated animals (4). Mononuclear cells, particularly B cells, synthesize large quantities of opiates upon exposure to LPS (1,17). LPS-induced elevation of endogenous opiates may be mediated by the secretion of IL-1α, as ICV administration of IL-1α increases immunocyte concentration of β-endorphin (38). Furthermore, endogenous opiates appear to mediate at least some of the physiological effects of LPS: naloxone attenuates the physiological effects

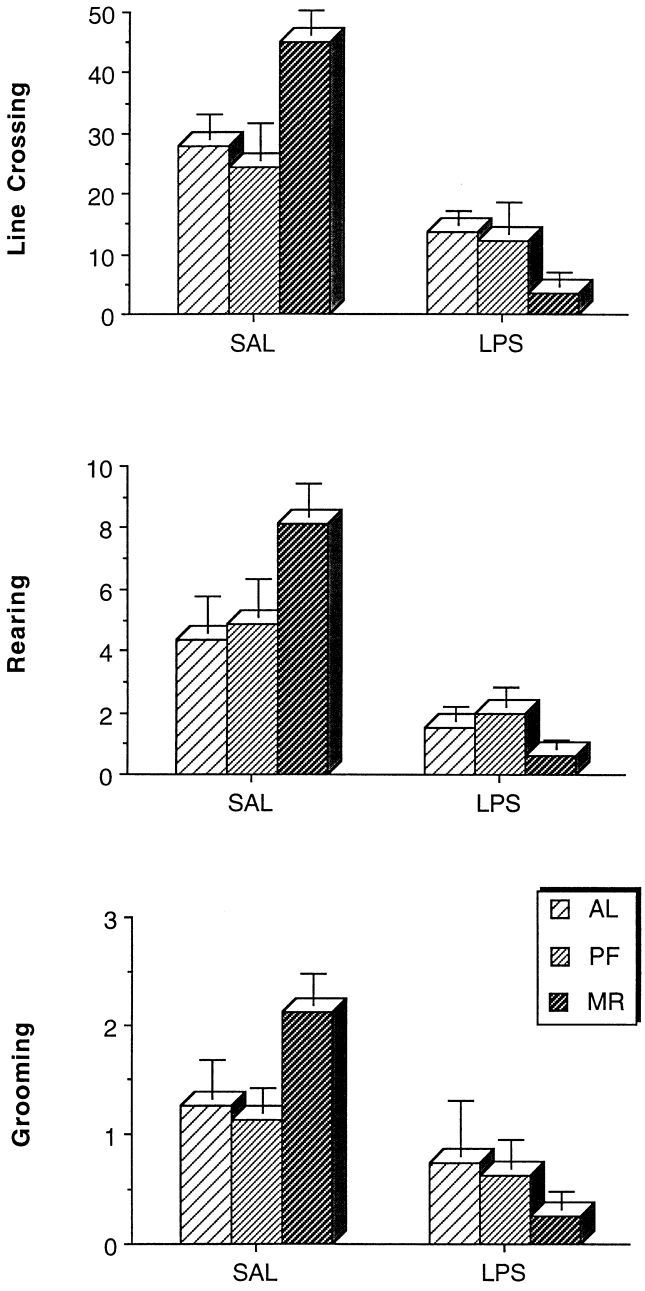


FIG. 4. Open-field behavior following injection of saline or LPS, 50 μg/kg, in male rats of the three prenatal treatment groups. Incidence of line crossing (top), rearing (middle), and grooming (bottom), were recorded during a 3-min test period, 4 h after injection. MR rats exhibited higher incidence of open-field behavior following saline injection, and more pronounced suppression of activity following LPS, compared with control rats.

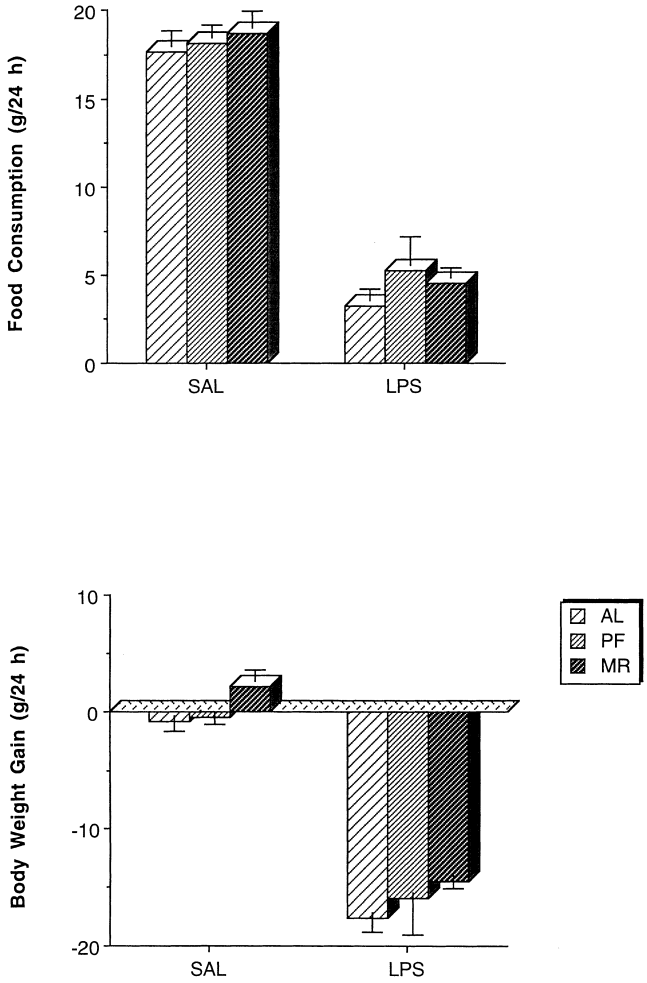


FIG. 5. Daily food consumption (top) and body weight gain (bottom) following injection of saline or LPS, 50 μg/kg, in male rats of the three prenatal treatment groups. LPS suppressed food consumption and body weight. However, no differences were detected among MR, PF, and AL animals.

of LPS and increases the overall survival rate of LPS-treated rats (20). Thus, alterations in physiological and behavioral responsiveness to LPS in adult MR rats may be related to the increased sensitivity/activity of endogenous opiate systems, which follows prenatal exposure to morphine.

LPS produced significant elevation in body temperature, which started 5 h after the injection and returned to baseline in 12 h. This febrile response was significantly attenuated in MR rats. Several mechanisms may underlie this effect, including opiate-mediated alterations in the release of endogenous pyrogens, or changes in the neural substrates of body temperature regulation. The release of endogenous pyrogens, such as IL-1, IL-6, TNF-α, and IFN, has not been tested in MR rats yet. However, in adult animals (not treated prenatally) morphine and endogenous opiates have been reported to modulate LPS-induced production and secretion of these cytokines. For example, LPS-induced release of TNF-α from peripheral blood mononuclear cells (PBMC) in vitro was suppressed both by morphine (5), and Met-enkephalin (28). Although some studies have suggested that TNF-α acts as a cryogen [e.g., (25)], others have indicated a pyrogenic effect of this cytokine (19,26,42). LPS-induced production of TNF-α and nitric oxide was inhibited in mixed glia cultures by ultralow concentrations of dynorphins (23). Administration of morphine in vivo was also shown to inhibit LPS-induced elevation in plasma TNF-α levels (2). Adult rats, chronically exposed to morphine, exhibited attenuated production of hippocampal IL-1β (34). On the other hand, LPS-induced release of IL-1 by either

PBMC or microglia in vitro was potentiated by Met-enkephalin (9,28). The findings that opiates modulate LPS-induced release of TNF-α and IL-1 may be particularly relevant in MR rats, which exhibit increased activity of endogenous opiate systems, and therefore show attenuated LPS-induced fever.

In control animals, LPS induced hyperalgesia at 30 min and hypoalgesia at 2–6 h postadministration. Similar biphasic effects of LPS on pain perception have been reported previously in studies using both the tail-flick and the hot-plate tests (36,51). LPS hyperalgesic response appears shortly after injection and lasts for about 1 h (27,29); according to previous reports, this effect is not attenuated by pretreatment with naltrexone (51). LPS hyperalgesia is probably mediated by cytokine secretion, particularly IL-1β and TNF-α (47); both cytokines were reported to produce hyperalgesia following exogenous administration (48). In addition, pretreatment of rats with the IL-1 receptor antagonist (IL-1ra) has been shown to block the hyperalgesic effect of LPS (47). Unlike LPS hyperalgesia, the hypoalgesic effect of LPS was completely blocked by pretreatment with naltrexone (51), suggesting its mediation by endogenous opiates. MR rats exhibited no hyperalgesia at 30 min, and significantly higher hypoalgesia at later time points. As discussed above, LPS-induced secretion of cytokines is attenuated by endogenous opiates; because opiate effects are more pronounced in MR rats, this may explain the lack of hyperalgesia in these rats. The augmented hypoalgesic response could be related to the enhanced sensitivity/activity of endogenous opiate systems in these rats, corroborating our previous finding of enhanced hypoalgesia in MR rats following acute morphine administration (13).

MR rats exhibited higher activity in the open-field test, reflected by increased incidence of line crossing, rearing, and grooming episodes following acute saline. A similar trend was observed in measurements of locomotor activity within the home cage (in Experiment 3). These findings are consistent with a clinical study that found increased activity levels in 7-year-old children exposed to methadone in utero (37). Prenatal exposure to morphine has been shown to alter the development of noradrenergic and dopaminergic systems in the brain (46). Because these systems are involved in the regulation of motor activity and in clinical hyperactivity syndromes, their modulation by prenatal morphine may be related to the increased activity observed in MR rats this study.

LPS suppressed open-field activity in all animals. This effect was significantly more pronounced in MR than in control rats. LPS-induced suppression of open-field activity could not be attributed to an endogenous opiate mechanism, because we previously demonstrated that it was not blocked by naltrexone (51). Neither is the effect of LPS on open-field behavior mediated by IL-1, because it was recently found that it was not reversed by IL-1ra (unpublished data). Further research is required to elucidate the mechanism underlying LPS-induced suppression of open-field activity, and its enhancement in MR rats.

The lack of differential effect of LPS on social exploration is puzzling. Social exploration is affected by the level of IL-1 (8), and as argued above, IL-1 secretion should be attenuated due to prenatal morphine. Therefore, LPS-induced suppression of social exploration should logically be attenuated in MR rats. This issue also requires further investigation.

NK cytotoxic activity was significantly suppressed in MR and PF compared with AL rats. Although partly explained by nonspecific effects of malnutrition (because dams of these two groups consumed less food than AL dams during pregnancy), it should be noted that NK activity was further suppressed in MR rats, compared with PF rats. The latter observation suggests that prenatal morphine has a specific suppressive effect on NK activity, beyond the nonspecific effect of food reduction. The suppression observed in MR rats may be related to increased activity of endogenous opiate systems: several studies indicated that both exogenous and endogenous opiates suppress NK cytotoxic activity, via central mechanisms (30,39,49). Moreover, based on the finding that anti-β-endorphin antibodies significantly enhanced mitogen-induced splenocytes proliferation, it has been suggested that β-endorphin exerts a tonic inhibitory effect on immune functions (33). It may be speculated that increased activity of the endogenous opiate systems in MR rats causes suppression of immune functions, including NK activity.

In summary, the present findings indicate that prenatal exposure to morphine induces long-term impairment of host–defense mechanisms, including NK activity and responsiveness to endotoxin challenge. NK cells play an important role in immune surveillance against virally infected and tumor cells. The febrile and behavioral responses to immune challenge are also important in host resistance against pathogens. Fever has been shown to facilitate immune reactivity and suppress the growth of some viruses and bacteria (22). In addition, sickness behavior is considered an adaptive response, which facilitates recovery from infections (8,18). Because MR rats exhibited suppressed NK activity, reduced febrile response, and impaired regulation of LPS-induced changes in pain sensitivity and motor activity, it is suggested that exposure to opiates during pregnancy renders the offspring more susceptible to infectious diseases.

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