Opiate Withdrawal in the Fetal Rat: A Behavioral Profile

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Received 23 December 1998; Revised 20 October 1999; Accepted 17 November 1999

JONES, K. L. AND G. A. BARR. Opiate withdrawal in the fetal rat: a behavioral profile. PHARMACOL BIOCHEM BEHAV 66(2) 419–424, 2000.—Offspring of women exposed to opiate drugs such as heroin and methadone during pregnancy have a high incidence of morbidity and mortality. Infants also show opiate withdrawal. In this study, we examined the behavioral effects of precipitated withdrawal in morphine-dependent fetal rats at gestational day (GD) 20. The dam was implanted on GD 14 with a pellet containing 75.0 mg of morphine. On GD 20, the dam underwent chemoyelotomy at L1/L2. The uterine horns were externalized and four subject fetuses were selected for behavioral observation, two from each uterine horn. The fetus was then injected subcutaneously with either saline or naltrexone (1.0 mg/kg) and the behaviors of the fetus recorded every 15 sec for 20 min. The results show that naltrexone injected fetuses that had been chronically exposed to morphine demonstrated increased limb and mouth movements, face wiping, and body curls, and spent less time quiet as compared with control fetuses. These results indicate that a morphine withdrawal-like syndrome occurs in the fetal rat. © 2000 Elsevier Science Inc.
ejaculation, increased sensitivity to touch, hostility on handling, teeth chattering, increased exploring, jumping, and wet dog shakes. Although the withdrawal syndrome in the adult animal is well defined, the available data describing the withdrawal syndrome in the developing animal are limited. In the rat, prenatal opiate exposure has effects parallel to those in the human, such as increased fetal and neonatal death and lower birth weights (9,15,19). Although early studies examining the neonatal withdrawal syndrome in the rat report that adult-like withdrawal does not occur until well after weaning in the rat (7), a neonatal withdrawal syndrome characterized by increased levels of ultrasonic vocalizations and specific behaviors appropriate to the age of the animal does occur (2,11,24–26). Morphine-treated rat pups, as young as 7 days of age, tested with naltrexone show increased rolling, stretching, and head and paw movements. The existence of a fetal withdrawal syndrome in the rat has been suggested based on the effects of morphine and naltrexone on the spontaneous activity in rat fetuses. Kirby (12,13) found that morphine caused a depression of activity in fetuses from the 15th through 21st day of gestation that was reversed by naloxone. However, a detailed behavioral profile of opiate withdrawal in the fetal animal has not been described. In an effort to describe behaviors responsive to stimulation in the area of the hindquarters. This produces an irreversible spinal anesthesia posterior to the site of injection rendering the female completely unresponsive to stimulation in the area of the hindquarters. Chemo myelotomy was chosen over spinal transection for its comparability to recent studies of fetal behavior, and its lack of effect on the behavior of the fetus (20). Following the chemo myelotomy, the female was gently restrained in a holding apparatus, the uterus was exteriorized through a midline laparotomy, and her uterus and hindquarters were immersed in a temperature-controlled (37.5°C) bath containing isotonic saline. The number of fetuses in each uterine horn was counted. The mother and fetuses quietly recovered from the anesthesia and were acclimated to the water bath for 20 min before behavioral observations began.

Precipitation of Withdrawal (GD 20)

Following acclimation to the water bath, four subject fetuses were then selected for behavioral observation, two from each uterine horn. The first of the four subject fetuses was prepared for direct observation by the delivery of the subject fetus through a 10- to 15-mm incision in the uterine wall, maintaining the placental-uterine attachment intact, with removal of the amniotic membranes from around the fetus (24). The average weights of the fetuses were determined in preliminary studies. Two subject fetuses received an injection of naltrexone (approximately 1 mg/kg) and two received an injection of saline. The observer was blind to the treatment condition. The behavior of the fetus was then observed. At the end of each observation period the fetus was anesthetized with an intraperitoneal (IP) injection of sodium pentobarbital (10 mg/kg) to prevent interference with the next observed fetus. Although it is possible that the drug injections could spread to other fetuses via placental transport, dilution factors would lower drug concentrations. Furthermore, we observed no noticeable change in the behaviors of fetuses of either uterine horn following each observation. This suggests that there is negligible exchange of injected drugs among fetuses within each uterine horn during the observational period.

Behavioral Observations

Following the injection of naltrexone or saline, fetal behavior was scan sampled every 15 sec for a total of 10 min using a behavioral checklist (Table 1). This checklist was developed by integrating in utero behaviors outlined in previous descriptions of spontaneous behaviors that occur in the fetal rat with withdrawal behaviors of the infant and adult (21). This procedure required the cooperation of two people. One person, who was blind to the treatment condition verbally called out the behavior that the fetus was engaging in at each observation.

### TABLE 1

<table>
<thead>
<tr>
<th>Behavior</th>
<th>Definition</th>
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<tbody>
<tr>
<td>Body curls</td>
<td>Ventrally or lateral flexion of the trunk</td>
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<tr>
<td>Body twitch</td>
<td>Brief spasms along the flank of the fetus</td>
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<tr>
<td>Face wiping</td>
<td>Wiping of both forelimbs across the face</td>
</tr>
<tr>
<td>Foreleg movements</td>
<td>Flexion, extension or rotation of one or both hindlegs</td>
</tr>
<tr>
<td>Head movements</td>
<td>Ventrally, dorsally, or lateral rotary motion of the head</td>
</tr>
<tr>
<td>Hindleg movements</td>
<td>Flexion or extension of one or both hindlegs</td>
</tr>
<tr>
<td>Mouth movements</td>
<td>Opening and closing the mouth</td>
</tr>
<tr>
<td>Quiet</td>
<td>Sedated appearance with no movement</td>
</tr>
<tr>
<td>Stretch</td>
<td>Extension or dorsal flexion of the trunk resulting in an apparent lengthening of the body</td>
</tr>
</tbody>
</table>
FIG. 1. Fetal withdrawal behaviors. These data are the mean number of occurrences ± one SEM of fetal behaviors. Fetuses were exposed to no treatment or chronic morphine exposure. At GD20 they were injected with saline or naltrexone and observed every 15 sec for 10 min. A significant interaction between acute treatment and chronic treatment defined withdrawal. As can be seen there were significant increases in body curls, face wiping, limb movements, and mouth movements. Quiet behavior, which covaries with the other behaviors, was decreased in the withdrawal condition.
Statistics

A factorial analysis of variance (ANOVA) was conducted for each behavior. The 10-min observation period was divided into 2 different time periods, each consisting of 5 min. We did this to assess if there were any time dependent changes in the withdrawal behaviors. There were none, and data are presented for the entire 10-min session. Behaviors were averaged for pups in each litter receiving the same treatment. Therefore the litter was the unit of measure. Averaging the data per litter reduces variability and increases the power of the ANOVA. Naltrexone or saline doses were injected within a single litter, and the two treatments treated as a within-subjects variable.

RESULTS

The results demonstrate increased fetal activity precipitated by naltrexone in morphine-exposed animals. No significant time effects were observed. Face wiping occurred in all morphine-exposed animals treated with naltrexone but never occurred in any other treatment group. Therefore, no analysis was performed for this behavior. The following other behaviors showed significant interaction effects between acute naltrexone treatment and chronic treatment (Fig. 1). The interaction is of interest because withdrawal is defined as occurring only in fetuses given chronic morphine and acute naltrexone: Body curls, \( F(1, 7) = 23.51, p < 0.001 \); forelimb movements, \( F(1, 7) = 25.10, p < 0.001 \); hindlimb movements, \( F(1, 7) = 11.57, p < 0.01 \); mouth movements, \( F(1, 7) = 13.94, p < 0.01 \); and quiet, \( F(1, 7) = 30.08, p < 0.001 \). Behaviors demonstrating no significant effects were body twitch, head movements, and stretch (Fig. 2).

DISCUSSION

The presence of a withdrawal syndrome in the fetal animal has been previously hypothesized (13,15,16), and motor activity increases in naloxone-treated fetuses of dams exposed chronically to morphine (12). The present study indicates that naltrexone-injected GD 20 fetuses that had been passively exposed to morphine (through the placental transport of the drug) show a specific alteration in behavior patterns and provides a detailed behavioral profile that defines the prenatal opiate abstinence syndrome. We were able to identify the same basic patterns of fetal movement as previously reported by Smotherman and Robinson (21) in their study of spontaneous behavior in the fetal rat. The results of the present study show that behaviors that spontaneously occur in the fetus, such as body curls and mouth and limb movements, were greatly increased in naltrexone injected fetuses chronically exposed to morphine as compared with control animals. Other spontaneous behaviors such as body twitch, head movements, and stretching did not increase significantly in

FIG. 2. Fetal behaviors that showed no significant interaction effect. The details are as in Fig. 1. These data are the mean number of occurrences ± one SEM of fetal behaviors. Lateral head moves, stretches, and body twitch were not increased in withdrawal.
experimental animals. Face wiping was unique in that it only occurred in morphine-exposed animals treated with naltrexone. This behavior involved a synchronous movement of the two forelegs along the side of the head and closely resembled grooming patterns observed in adolescent and adult rats during withdrawal (11). Smotherman and Robinson (22) characterize face wiping as a pattern of motor behavior rarely seen in the absence of aversive stimulation such as a tactile probe or chemosensory stimulation. Smotherman and Robinson’s (22) findings taken together with the present results suggest the face wiping may be a motor response evoked by aversive stimuli.

Several animal models have been used to examine the effects of opiate withdrawal in utero. Lichtblau and Sparber (15) found that litters from dams put through withdrawal daily with naloxone injections from day 14 of gestation through term showed an increased rate of stillbirths, decreased pup weight and size, and weight loss 24 hours after birth as compared with morphine-dependent saline treated rats. When morphine-dependent fetal chicks were injected with naloxone, the motility of the opiate-exposed fetuses was significantly increased and the hatchability significantly reduced as compared with control fetuses (14). Following an injection of naloxone into fetuses from morphine-exposed ewes, fetal arterial blood pressure and defecation increased, and fetal heart rate decreased (6). Pups exposed to methadone prenatally, and thus exposed prenatally to the opiate effects of opiate withdrawal in utero, experience withdrawal from opiates in utero suggesting the possibility that abstinence may be in part responsible for some of the adverse effects of opiates observed in animals as well as humans. Furthermore, the description of behaviors associated with opiate withdrawal in the fetus provides some insight into how the effects of opiates and opiate withdrawal on fetal behavior may contribute to the adverse effects observed in offspring of drug-dependent women. For example, past research has emphasized the placental transfer of opiates as the primary mechanism involved in the deleterious effects associated with maternal opiate dependence. Although this mechanism accounts for the direct effects of placental transport (18), it ignores the complexity of the fetal environment, which is constantly interacting with the fetus. Motor activity in the fetus is necessary to promote normal development as well as morphological development (23). It has been previously suggested that these behaviors are not just trivial aspects of the existence of the fetus but rather they are ontogenetic adaptations designed to promote the survival and growth of the fetus (17,23). Therefore anything that influences the behavior of the fetus may be a potential threat to normal development. For example, periodic episodes of heroin withdrawal during pregnancy restrict fetal growth by reducing uterine or placental bloodflow (16). Episodes of maternal withdrawal will increase the activity of the fetus, thereby increasing the metabolic rate and oxygen consumption of the fetus. As a result, the fetus may experience hypoxia or even death (8). Thus the effects caused by opiate withdrawal may be a consequence of both direct and indirect effects of abstinence.

The results of this study indicate that the rat fetus does experience withdrawal from opiates in utero suggesting the possibility that abstinence may be in part responsible for some of the adverse effects of opiates observed in animals as well as humans. Furthermore, the description of behaviors associated with opiate withdrawal in the fetus provides a developmental model to study further the anatomical and physiologic substrates that may mediate this syndrome.

ACKNOWLEDGEMENTS

We thank Dr. Duckhyun Kim for her help with the behavioral observations.

REFERENCES


