Effect of Perinatal Buprenorphine Exposure on Development in the Rat

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ABSTRACT
The developmental effects of exposure to various doses of buprenorphine, methadone, or water during the perinatal period were studied in the rat. Rats were exposed to buprenorphine (0.3, 1.0, or 3.0 mg/kg/day), methadone (9 mg/kg/day), and/or water prenatally, postnatally, or both pre- and postnatally, via maternally implanted osmotic minipumps. Fetal and maternal mortality and morbidity were assessed, as well as the acquisition of several developmental milestones, pup weight gain, precipitated withdrawal, and the antinociceptive effect of morphine. Although perinatal exposure to buprenorphine failed to produce severe maternal and fetal or neonatal mortality, it was associated with a significant amount of perinatal mortality and perturbations of pup development. Pups developed physical dependence to both drugs, as evidenced by the ability of naloxone challenge to precipitate withdrawal. Both drugs induced tolerance to the antinociceptive effects of morphine in the tail-flick test. The effects of buprenorphine varied with the dose used, and the highest dose did not always produce the greatest effect. There were some similarities between the effects of perinatal buprenorphine and perinatal methadone; however, differences were also observed between the effects of the two drugs, which may be related to the different affinities and efficacies of the drugs at different opioid receptor subtypes.

The partial μ-opioid agonist buprenorphine is currently being considered for maintenance of pregnant addicts, as it may offer advantages over the use of methadone. Although it is generally accepted that methadone maintenance of pregnant narcotic addicts is preferable to subjecting the fetus to erratic drug levels and repeated intrauterine withdrawal (Jarvis and Schnoll, 1994), prenatal opioid exposure is known to produce severe maternal and fetal or neonatal mortality, it was associated with a significant amount of perinatal mortality and perturbations of pup development. Pups developed physical dependence to both drugs, as evidenced by the ability of naloxone challenge to precipitate withdrawal. Both drugs induced tolerance to the antinociceptive effects of morphine in the tail-flick test. The effects of buprenorphine varied with the dose used, and the highest dose did not always produce the greatest effect. There were some similarities between the effects of perinatal buprenorphine and perinatal methadone; however, differences were also observed between the effects of the two drugs, which may be related to the different affinities and efficacies of the drugs at different opioid receptor subtypes.

The partial μ-opioid agonist buprenorphine is currently being considered for maintenance of pregnant addicts, as it may offer advantages over the use of methadone. Although it is generally accepted that methadone maintenance of pregnant narcotic addicts is preferable to subjecting the fetus to erratic drug levels and repeated intrauterine withdrawal (Jarvis and Schnoll, 1994), prenatal opioid exposure is known to produce severe maternal and fetal or neonatal mortality, it was associated with a significant amount of perinatal mortality and perturbations of pup development. Pups developed physical dependence to both drugs, as evidenced by the ability of naloxone challenge to precipitate withdrawal. Both drugs induced tolerance to the antinociceptive effects of morphine in the tail-flick test. The effects of buprenorphine varied with the dose used, and the highest dose did not always produce the greatest effect. There were some similarities between the effects of perinatal buprenorphine and perinatal methadone; however, differences were also observed between the effects of the two drugs, which may be related to the different affinities and efficacies of the drugs at different opioid receptor subtypes.

Preliminary studies in pregnant addicts, on the other hand, suggest that buprenorphine may not produce a neonatal abstinence syndrome (Fischer et al., 1998). Furthermore, studies in the rat have failed to detect changes in rest-activity cycles in buprenorphine-exposed pups, supporting the contention that buprenorphine does not produce a protracted abstinence syndrome (Hutchings et al., 1996).

Pharmacologically, buprenorphine differs from methadone in several ways; therefore, the effects of buprenorphine on the developing animal may be very different from those of methadone. Unlike methadone, buprenorphine is a partial agonist at the μ-opioid receptor, and its effects at this receptor follow a biphasic curve (Dum and Herz, 1981; Cowan, 1995). Hence, the developmental effects of buprenorphine could also be biphasic in nature. In addition to acting as a μ-opioid partial agonist, buprenorphine also binds to κ- and δ-opioid receptors (Richards and Sadee, 1985; Cowan, 1995). Buprenorphine is currently considered to be a potent κ-opioid antagonist, although its role at δ receptors remains unclear. μ- and κ-opioid receptors are expressed very early in the brain and spinal cord, beginning prenatally, whereas δ-opioid receptors are expressed early in the postnatal period (Attali et al., 1990; De Vries et al., 1990; Xia and Haddad, 1991; Rahman et al., 1998), allowing for the possibility of drug-receptor interactions during the perinatal period. Actions at
these additional opioid receptors may further disrupt development or even counteract changes produced by stimulation of the \( \mu \)-opioid receptor.

Two previous studies reported the effects of buprenorphine on the pregnant rat and offspring (Hutchings et al., 1995, 1996). However, these studies involved only prenatal exposures and failed to assess the effects of buprenorphine exposure on several developmental milestones. It must be emphasized that the rat is born at a stage of development equivalent to the end of the second trimester in humans (Dobbing and Sands, 1979). To obtain a degree of exposure comparable with that of human offspring, buprenorphine exposure must continue into the early postnatal period. Osmotic minipumps have been used for pre- or postnatal delivery of methadone, producing a dependent state without excessive maternal or fetal mortality (Enters et al., 1991; Kunko et al., 1996). Using a similar method of drug delivery, we now compare the developmental effects of buprenorphine to the effects of methadone in the rat.

Materials and Methods

Subjects. This study was conducted in accordance with the National Institutes of Health Guidelines for the Care and Use of Animals in Research and under protocols approved by the Animal Care and Use Committee of Virginia Commonwealth University. Nulliparous female Sprague-Dawley CD rats (Harlan Bioproducts for Science, Indianapolis, IN) were allowed to habituate to a temperature- and humidity-controlled vivarium for at least 1 week before breeding. All animals were maintained on a 12-h light/dark cycle with ad libitum access to food and water. Females were placed individually with male conspecifics during breeding. The detection of a seminial plug was used to indicate successful mating; the day this happened was designated as gestational day (GD) 0. After confirmation of mating, females were housed singly in plastic breeding cages with hardwood chip bedding.

Experimental Procedures. Rats were exposed to buprenorphine or to methadone prenatally, postnatally, or both pre- and postnatally. On day 7 of gestation, dams were implanted subcutaneously, under methoxyflurane anesthesia, with 28-day osmotic minipumps (Alza, Palo Alto, CA) which delivered sterile water, buprenorphine HCl (initial dose rate 0.3, 1, or 3 mg/kg/day), or methadone HCl (initial dose rate, 9 mg/kg/day). In this way, drug exposure began immediately before development of the central nervous system. Buprenorphine has been shown to enter the fetus after maternal intramuscular injection (Walter and Inturrisi, 1995). Previously, it has been demonstrated that methadone enters fetal and pup brains after maternal dosing via osmotic minipumps (Kunko et al., 1996). Because of the greater lipid solubility of buprenorphine, there is little doubt that it will enter fetal or neonatal brains under similar circumstances. The toxicity of the treatment regimen was assessed by weighing the pregnant females and their food and water intake daily. The day of birth was denoted as postnatal day (PD) 0. Within 24 h of parturition, litters were culled to 10, maintaining equal numbers of males and females when possible, and cross-fostered, resulting in the following prenatal/postnatal exposure groups: water/water, methadone/water, water/methadone, methadone/methadone, buprenorphine/water, water/buprenorphine, and buprenorphine/methadone. Litters containing 8 to 10 pups were fostered intact. On PD 1, pups were grouped according to sex and weighed. Thereafter, individual pups were weighed daily. On PD 10, minipumps were replaced with new preactivated 14-day pumps filled with the appropriate solution. On PD 21, the day of weaning, uteri were checked for implantation sites to determine the number of fetuses resorbed and to calculate the mortality index for each litter [mortality index = (number of stillbirths + number of resorptions) / (number of implantation sites)].

Precipitated Withdrawal. On PD 1, at the time of culling, culled pups were tested for naloxone-precipitated withdrawal by an observer blinded to the dam’s exposure group and to the challenge drug. Pups were injected with naloxone (2 mg/kg, s.c., from a 0.5 mg/ml solution) or 0.9% saline (4 ml/kg) and observed over 30 min for several withdrawal-related behaviors: hyperactivity, vocalization, tongue and mouth movements, stretching, and “face-washing”. Each pup was given a “yes” or “no” score based on the manifestation of the behavior, and the number of affirmative scores was summed to provide a total withdrawal score.

Developmental Assessment. The onset of several developmental milestones was assessed, as described previously (Kunko et al., 1996). One male and one female from each litter were assigned to one of five measures of development, to minimize time away from the dams and the possibility of stress as a cause of developmental disruption. Neuromuscular development was assessed by testing for the surface righting reflex (PDs 1–4), negative geotaxis response (PDs 7–20), and performance on a vertical screen task (PDs 7–20). Physical maturation was determined by noting the day on which eyes or ears opened (PDs 7–21). Once a pup acquired a given milestone, testing for that milestone ceased. Additionally, all pups were weighed daily from PD 1 through PD 21.

Assessment of Antinoicception. On PD 4, the response to an opiate challenge (morphine) in the presence of a noxious stimulus was measured using the tail-flick test (Dewey et al., 1970). Baseline reaction times of 2 to 4 s and a cutoff time of 10 s were used. Antinoicception was quantified as percent of maximum possible effect (Harris and Pierson, 1984) using the following formula: percent of maximum possible effect = 100 × [(test – baseline)/(100 – baseline)]. One male and one female pup from each litter were injected s.c. with morphine every 30 min using a cumulative dosing paradigm (Adams et al., 1990), so that final doses of 0.2, 0.4, 0.8, 1.2, and 1.6 mg/kg morphine were given. The tail-flick procedure was performed 25 min after each injection. Pups were returned to their dams during intertesting intervals.

Data Analysis. The unit of measurement was the litter. Only one male and one female from each litter were used to avoid inflation of the litter size or a litter effect. In the case of pup weight, mean weights were obtained for male pups and female pups in each litter. In the case of precipitated withdrawal, one pup from each cull group was injected with saline and one with naloxone, with no attempt to control for sex. The withdrawal data represent a discrete random variable and were analyzed by the Kruskal-Wallis test, followed by post hoc tests, where appropriate, using the method of Siegel and Castellan (1989). After confirmation of parallelism (Tallarida and Murray, 1986), ED\(_{50}\) values in the tail-flick test were calculated by least-squares linear regression analysis, followed by calculation of confidence limits (Bliss, 1967). Potency ratios and their associated confidence limits were calculated according to the method of Colquhoun (1971). The remaining data were analyzed by analysis of variance (ANOVA), followed by Dunnett’s post hoc test, and repeated-measures ANOVA when time (days) was included as a factor. \( p < 0.05 \) was accepted as statistically significant.

Results

Maternal Variables. Data for maternal weight gain and food and water consumption were analyzed by two-way (treatment × day) ANOVA, with day as a repeated measure. Dams exhibited no differences in weight gain, food intake, or water intake for the first 7 days of gestation. After implantation of minipumps on GD 7, there was no significant treatment effect on dam weight gain [treatment effect: \( F(4,192) = 0.82, \ p > 0.05 \); however, a significant treatment × day interaction was observed: \( F(52,2496) = 3.78, \ p < 0.05 \).
ternal weight gain was significantly less on several days in the various buprenorphine exposure groups than in the water-exposed dams (Fig. 1A). On no day was a statistically significant difference in weight gain observed between the water- and methadone-exposure groups. However, across GDs 7 to 20, a significant treatment effect was observed on water consumption \[ F(4,192) = 5.00, p < 0.05; \text{Fig. 1B}. \] When the data were collapsed across day, less water was consumed by all four opioid treatment groups as compared with the water controls \( (p < 0.05, \text{Dunnett’s test}) \). A significant treatment effect \[ F(4,192) = 7.17, p < 0.05 \] and a treatment \( \times \) day interaction \[ F(52,2496) = 1.53, p < 0.05 \] were observed for maternal food consumption (Fig. 1C) on GDs 7 to 20. Buprenorphine-treated dams (1.0 or 3.0 mg/kg/day) consumed significantly less food than did the water controls on GDs 7, 8, and 9 \( (p < 0.05, \text{Dunnett’s test}) \). As was the case with water consumption, overall food consumption was significantly reduced in all four opioid treatment groups compared with the water controls \( (p < 0.05, \text{Dunnett’s test}) \).

**Litter Variables.** None of the treatments significantly affected the number of live pups born \[ F(4,192) = 1.25, p > 0.05 \], although the number of males born to dams exposed to methadone or 3.0 mg/kg/day buprenorphine was significantly reduced \[ F(4,192) = 4.04, p < 0.05, \text{Table 1} \]. However, the ratio of males to females in each litter did not differ significantly from that of the water controls \[ F(4,192) = 1.30, p > 0.05 \]. The mortality index was significantly increased in all three of the buprenorphine exposure groups \[ F(4,192) = 12.60, p < 0.05, \text{Table 1} \]. There was a statistically significant greater occurrence of resorptions \[ F(4,192) = 6.64, p < 0.05 \], and in the case of the 3 mg/kg/day buprenorphine group, a statistically significant increase in the number of stillbirths \[ F(4,192) = 5.87, p < 0.05, \text{Table 1} \]. Furthermore, pups born to opioid-exposed dams tended to weigh less on PD 1, the first day on which the pups were weighed \( \text{[females, } F(4,192) = 2.90, p < 0.05; \text{males, } F(4,192) = 3.17, p < 0.05] \). Females from litters exposed to methadone or 0.3 mg/kg/day buprenorphine and males from litters exposed to 0.3 mg/kg/day buprenorphine weighed less than their counterparts in water-exposed litters (Table 1).

Perinatal opioid exposure affected pup weight as measured out to PD 21 \( \text{[main effect for treatment, } F(12,174) = 3.88, p < 0.05; \text{treatment } \times \text{day interaction, } F(228,3306) = 2.66, p < 0.05] \). There was no interaction of treatment \( \times \) sex or treatment \( \times \) day \( \times \) sex, so data were collapsed across sex. One-way ANOVAs, followed by Dunnett’s test, were conducted to compare treatments on individual days. For clarity of presentation, data are grouped as prenatal exposure only (Fig. 2A), postnatal exposure only (Fig. 2B), and combined pre- and postnatal exposure (Fig. 2C). Pups exposed to methadone (9 mg/kg/day) or buprenorphine (0.3, 1.0, or 3.0 mg/kg/day) only during the prenatal period were not significantly different in weight from the water-exposed controls on any day in the first 3 postnatal weeks (Fig. 2A). However, pups exposed to opioids postnatally exhibited significant reductions in weight, whether the exposure period was postnatal only or both pre- and postnatal (Fig. 2, B and C). The weights of pups exposed to either methadone or the largest dose of buprenorphine (3.0 mg/kg/day) were significantly reduced for a duration of 4 to 10 days, depending upon the treatment. Interestingly, the pattern of the effect varied according to the exposure paradigm. Pups exposed to opioids both pre- and postnatally weighed less mainly during the first 2 postnatal weeks, whereas the pups exposed only postnatally were primarily affected over the 2nd and 3rd postnatal weeks.

**Developmental Milestones.** Significant treatment effects were observed for the acquisition of several developmental milestones (Table 2). A significant treatment effect was observed for development of the righting reflex \[ F(12,192) = 2.73, p < 0.05 \], with acquisition of the righting reflex actually occurring at an earlier age in pups whose dams were administered 0.3 mg/kg/day buprenorphine in the postnatal period (0.3 mg/kg buprenorphine/buprenorphine and water/0.3 mg/kg buprenorphine groups). Acquisition of the vertical screen task was also significantly affected \[ F(12,192) = 6.45, p < 0.05 \] with all three of the 0.3 mg/kg/day buprenorphine exposure groups exhibiting a significant delay in acquisition (Table 2). A significant treatment effect was observed on the day of eye opening \( F(12,192) = 2.18, p <
0.05). Rats exposed prenatally to methadone opened their eyes slightly, but significantly, earlier than the water-exposed controls. On the other hand, the treatment effect [F(12,192) = 2.95, p < 0.05] observed on the day of ear opening represented a significant delay in pups exposed to 1.0 mg/kg/day buprenorphine either prenatally or postnatally, but not in those exposed both prenatally and postnatally (Table 2).

Precipitated Withdrawal. Data regarding precipitated withdrawal are presented in Fig. 3. Pups were challenged with naloxone (2 mg/kg, s.c.) or saline (4 ml/kg). Analysis of the withdrawal scores by the Kruskal-Wallace test indicated a significant treatment effect (H = 36.12, p < 0.05). Post hoc testing according to Siegel and Castellan (1999) indicated that pups exposed to methadone prenatally exhibited significantly higher withdrawal scores in response to naloxone challenge than pups born to water-treated dams, or saline-challenged pups born to either methadone- or water-treated dams. Likewise, pups exposed prenatally to 0.3 mg/kg/day buprenorphine exhibited significantly higher withdrawal scores in response to naloxone challenge than pups born to water-exposed dams or saline-challenged pups born to water-treated or to similarly dosed dams. Finally, pups born to dams exposed to 3.0 mg/kg/day buprenorphine exhibited higher withdrawal scores than saline-challenged pups born to water-treated dams, but variability in the data precluded a statistically significant difference from buprenorphine-exposed pups challenged with saline.

Antinociception. Sensitivity to the antinociceptive effects of morphine was assessed in 4-day-old pups using the tail-flick test (Fig. 4). It was not possible to achieve greater than 50% of the maximum possible effect in this test, because the efficacy of morphine in the tail-flick test is not fully developed in rats of this age (Barr et al., 1986). Therefore, ED<sub>25</sub>, instead of ED<sub>50</sub>, values were calculated (Table 3). The ED<sub>25</sub> values of morphine were increased at each dose of buprenorphine, although this effect was observed in pups exposed postnatally to the two higher doses of buprenorphine. A significant increase in the ED<sub>25</sub> was observed in the methadone/water exposure group; in the methadone/buprenorphine or buprenorphine/water exposure groups relative to those of the water/water group. In the water/buprenorphine exposure groups, a significant increase in ED<sub>25</sub> values was observed only with 3.0 mg/kg/day buprenorphine-exposed pups compared with water-exposed pups; p < 0.05, 3.0 mg/kg/day buprenorphine-exposed pups compared with water-exposed pups. Dunnett’s test was used in each of these comparisons.

Table 1

Effect of prenatal opioid exposure on litter variables

<table>
<thead>
<tr>
<th></th>
<th>Water</th>
<th>Methadone, 9 mg/kg/day</th>
<th>Buprenorphine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>72</td>
<td>66</td>
</tr>
<tr>
<td>Stillbirths</td>
<td>1.0 ± 0.06</td>
<td>2.0 ± 0.1</td>
<td>2.0 ± 0.2</td>
</tr>
<tr>
<td>Resorptions</td>
<td>1.5 ± 0.2</td>
<td>1.8 ± 0.8</td>
<td>2.4 ± 0.5</td>
</tr>
<tr>
<td>Mortality index</td>
<td>0.09 ± 0.01</td>
<td>0.15 ± 0.06</td>
<td>0.18 ± 0.03</td>
</tr>
<tr>
<td>Total live pups</td>
<td>13.3 ± 0.3</td>
<td>12.5 ± 0.5</td>
<td>13.7 ± 0.5</td>
</tr>
<tr>
<td>Females</td>
<td>6.9 ± 0.2</td>
<td>6.3 ± 0.4</td>
<td>6.5 ± 0.4</td>
</tr>
<tr>
<td>Males</td>
<td>7.1 ± 0.2</td>
<td>5.5 ± 0.4</td>
<td>6.8 ± 0.4</td>
</tr>
<tr>
<td>Male/female ratio</td>
<td>1.2 ± 0.08</td>
<td>0.9 ± 0.07</td>
<td>1.3 ± 0.1</td>
</tr>
<tr>
<td>Average pup weight (g)</td>
<td>6.8 ± 0.1</td>
<td>6.3 ± 0.2</td>
<td>6.4 ± 0.1</td>
</tr>
<tr>
<td>Females</td>
<td>6.6 ± 0.1</td>
<td>6.1 ± 0.2</td>
<td>6.2 ± 0.1</td>
</tr>
<tr>
<td>Males</td>
<td>7.0 ± 0.1</td>
<td>6.5 ± 0.2</td>
<td>6.5 ± 0.1</td>
</tr>
</tbody>
</table>

* p < 0.05 compared with water group by Dunnett’s test.

Fig. 2. The effect of prenatal (A), postnatal (B), or pre- and postnatal (C) opioid exposure on pup weight in the early postnatal period. Data are expressed as mean ± S.E.M., n = 10 to 44 for each point. *, p < 0.05, 9 mg/kg/day methadone-exposed pups compared with water-exposed pups; †, p < 0.05, 3.0 mg/kg/day buprenorphine-exposed pups compared with water-exposed pups. Dunnett’s test was used in each of these comparisons.
exposure to methadone reduced sensitivity to morphine only in pups that had the methadone exposure continue through the postnatal period.

**Discussion**

In the present study, we report the effects of perinatal exposure to various doses of buprenorphine on several measures of postnatal development, on neonatal precipitated withdrawal, and on the antinociceptive response to morphine, as well as the effects of methadone on these parameters. Differences in the effect of perinatal exposure to these two drugs might be anticipated, because methadone is a full agonist selective for μ-opioid receptors, whereas buprenorphine is a partial agonist that binds to μ-, κ-, and δ-opioid receptors (Richards and Sadee, 1985; Kristensen et al., 1995).

Although perinatal buprenorphine exposure was not associated with profound mortality or toxicity, it produced more effects on the dam and offspring than did methadone exposure. Methadone produced no significant effect on maternal weight, whereas buprenorphine significantly reduced maternal weight gain during the first few days following implantation of the osmotic minipumps. Although both drugs significantly reduced food and water consumption over gestation, the pattern of disruption differed between methadone and buprenorphine, suggesting that actions at different opioid receptors may be involved. Part of the effect of methadone and buprenorphine on food consumption may result indirectly from decreased water consumption, a well known effect of opioids (Chance and Rosecrans, 1977). However, the two higher doses of buprenorphine suppressed eating over the first few days after minipump implantation to a much greater extent than they suppressed drinking, whereas water consumption was reduced uniformly throughout pregnancy following implantation of minipumps containing either opioid. Because κ-opioid receptors stimulate food consumption (Morley and Levine, 1983), κ-antagonist properties of buprenorphine may be responsible for the sharp reduction in food intake, although it appears that tolerance rapidly develops to this effect. Reduced food consumption may also be attributed to the sedation of the buprenorphine-treated dams evident in the first few days after initiation of dosing, an effect also associated with κ receptors.

Increased fetal mortality was observed in the buprenorphine-exposed litters. All three doses of buprenorphine significantly increased the mortality index, due to increased resorptions, stillbirths, or both. The highest dose of buprenorphine produced the greatest mortality. A study by Hutchings et al. (1996) in a different strain of rats reported similar increases in perinatal mortality in buprenorphine-exposed rats. Although methadone tended to increase the mortality index, the increase was not statistically significant,
as observed previously (Kunko et al., 1996). Both methadone and buprenorphine tended to reduce birth weight, but, in general, gross fetotoxicity was not observed.

In addition to reducing birth weight, perinatal exposure to methadone and the highest dose of buprenorphine produced small, but statistically significant, reductions in pup weight gain. It is unlikely that this effect resulted from decreased maternal care or lactation. Pups exposed to opioids only in the postnatal period exhibited the greatest weight reductions in the 2nd and 3rd postnatal weeks, at which time rodent chow provides an increasing proportion of their nutritional intake. Furthermore, the decreased weight gain in pups exposed to methadone or buprenorphine both pre- and postnatally was most pronounced during the early postnatal period. If maternal behavior were a factor in this effect, the pattern of reduced weight gain would be similar in the two postnatal exposure groups (i.e., water/buprenorphine versus buprenorphine/buprenorphine and water/methadone versus methadone/methadone). However, it is possible that prenatal exposure to opioids alters neurotransmitter systems downstream from the affected opioid receptors so that different responses to opioids are observed when exposure occurs in the postnatal period. Although \( \kappa \) antagonist properties of buprenorphine may explain reduced food consumption and concomitant loss of weight in the buprenorphine-exposed pups, this does not explain reduced weight gain in the methadone-exposed pups.

Few effects were observed on developmental milestones. The most consistent effect was a substantial delay in the acquisition of the vertical screen task in the pups exposed perinatally to the lowest dose of buprenorphine. Although the acquisition of the negative geotaxis task was somewhat delayed in these same rats, development of the righting reflex was actually accelerated. Therefore, exposure to buprenorphine does not appear to produce a generalized delay in neuromuscular development. It is interesting that the lowest dose of buprenorphine produced the greatest effects on developmental milestones, whereas the highest dose had no significant effect.

Hutchings et al. (1996) found no evidence of protracted withdrawal in pups born to buprenorphine-treated dams. However, the present study demonstrates that naloxone precipitates an abstinence syndrome in neonatal buprenorphine-exposed rats, as has been shown in human adults maintained on buprenorphine (Eissenberg et al., 1996). Thus, not only do the buprenorphine-exposed pups develop physical dependence in utero, it is possible to precipitate...
Perinatal Buprenorphine Exposure

TABLE 3
Effect of perinatal opioid exposure on morphine-induced antinociception in the tail-flick test in 4-day-old rats
Morphine was injected subcutaneously 25 min prior to each tail-flick measurement.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>ED_{50}</th>
<th>Potency Ratio (versus Water/Water group)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water/water</td>
<td>0.479(0.414–0.554)</td>
<td></td>
</tr>
<tr>
<td>Water/methadone</td>
<td>0.410(0.340–0.460)</td>
<td>1.14(0.55–1.54)</td>
</tr>
<tr>
<td>Methadone/water</td>
<td>0.655(0.535–0.802)</td>
<td>0.75(0.55–1.01)</td>
</tr>
<tr>
<td>Methadone/methadone</td>
<td>0.882(0.584–1.331)*</td>
<td>0.59(0.39–0.82)*</td>
</tr>
<tr>
<td>Water/buprenorphine</td>
<td>0.486(0.425–0.555)</td>
<td>0.97(0.71–1.32)</td>
</tr>
<tr>
<td>0.3 mg/kg/day</td>
<td>0.696(0.553–0.875)</td>
<td>0.71(0.51–0.97)*</td>
</tr>
<tr>
<td>1.0 mg/kg/day</td>
<td>0.860(0.580–1.275)*</td>
<td>0.53(0.36–0.72)*</td>
</tr>
<tr>
<td>Buprenorphine/water</td>
<td>0.725(0.611–0.859)*</td>
<td>0.69(0.48–0.94)*</td>
</tr>
<tr>
<td>0.3 mg/kg/day</td>
<td>0.908(0.718–1.149)*</td>
<td>0.55(0.38–0.75)*</td>
</tr>
<tr>
<td>1.0 mg/kg/day</td>
<td>1.417(1.216–1.651)*</td>
<td>0.40(0.27–0.53)*</td>
</tr>
<tr>
<td>3.0 mg/kg/day</td>
<td>1.111(0.756–1.633)*</td>
<td>0.53(0.34–0.76)*</td>
</tr>
<tr>
<td>Buprenorphine/buprenorphine</td>
<td>0.791(0.614–1.102)*</td>
<td>0.64(0.45–0.87)*</td>
</tr>
<tr>
<td>1.0 mg/kg/day</td>
<td>1.087(0.878–1.345)*</td>
<td>0.50(0.34–0.67)*</td>
</tr>
<tr>
<td>3.0 mg/kg/day</td>
<td>1.111(0.756–1.633)*</td>
<td>0.53(0.34–0.76)*</td>
</tr>
</tbody>
</table>

*p < 0.05 compared with water group.

Values in parentheses represent the 95% confidence interval.

...and postnatally were more resistant to the antinociceptive actions of morphine. It has been suggested that opioid receptors are most sensitive to agonist exposure in the immediate perinatal period (Windh et al., 1995). Mechanisms responsible for tolerance may not become functional until immediately after birth. Because buprenorphine is very lipid-soluble and dissipates slowly from receptors (Hambrook and Rance, 1976), the drug administered perinatally may remain in the body well into the postnatal period. Hence, perinatally administered buprenorphine may have a greater effect on developing receptors than perinatally administered methadone. Because it remains associated with the receptors longer, buprenorphine may induce tolerance more rapidly than methadone when introduced in the postnatal period. This may explain why morphine ED_{50} values increased in pups exposed postnatally to buprenorphine, but not in pups exposed postnatally to methadone. Alternatively, this discrepancy could be attributed to the actions of buprenorphine at receptors other than the µ receptor. Yet another possibility is that rather than tolerance, the decreased potency of morphine reflects an antagonist effect of buprenorphine at opioid receptors. Large doses of buprenorphine administered perinatally may remain in the body and antagonize the effects of morphine. The fact that the highest doses of buprenorphine produced the greatest tolerance also supports this argument. However, the full agonist methadone, when administered pre- and postnatally, also produced this apparent tolerance, which argues against this mechanism. Furthermore, no differences were observed in the baseline tail-flick latencies of the various treatment groups, suggesting that either insufficient buprenorphine or methadone remained to affect nociception, or that opioid tolerance had indeed developed.

Differences and similarities between the effects of methadone and buprenorphine, as well as the actions of the different doses of buprenorphine, should be addressed. Buprenorphine’s effects at the µ-opioid receptor follow a biphasic curve (Dum and Herz, 1981; Cowan, 1995). Although the doses used in the present study were selected to span that curve, administration of the drug by minipump over an entire day differs from administration of the same dose of the drug as a single daily injection. Therefore, it is currently difficult to state definitively whether the doses of buprenorphine used represent agonist or antagonist doses. In instances where buprenorphine and methadone produce the same effect, one may assume that both drugs are acting via agonist actions at the µ-opioid receptor, or at least not exclusively via κ or δ receptors, for which methadone has very low affinity (Kristensen et al., 1995). The areas in which methadone and buprenorphine produce a different spectrum of effects, including perinatal mortality, dam food intake, antinociception, and developmental milestones, may reflect buprenorphine’s actions at additional opioid receptor subtypes. The fact that methadone and the highest dose of buprenorphine share similar effects on pup weight suggests that even the largest dose of buprenorphine retained µ-opioid agonist activity. On the other hand, some behaviors were affected more by the lower doses of buprenorphine than the highest dose, suggesting that antagonist actions may occur at the higher doses of buprenorphine. This explanation requires that different behaviors affected by buprenorphine possess different sensitivities to buprenorphine, if in some cases one dose...
exerted an antagonist effect and in others the same dose exerted agonist effects.

It is certain that endogenous opioids play a role in central nervous system development. Perinatal perturbations of opioid receptors have been found to produce a multitude of effects, whether the drugs involved are agonists or antagonists (Hans, 1992; Zagon and McLaughlin, 1992). In general, opioid agonists delay development, whereas opioid antagonists may actually accelerate development. Although many of the sequelae of perinatal opioid exposure may be relatively minor, there is evidence for more lethal outcomes, such as an increased incidence of sudden infant death syndrome (Hans, 1992). However, declining to treat pregnant addicts with maintenance drugs would be inadvisable, because sporo-adic exposure to "street" drugs will almost certainly produce adverse outcomes. For these reasons, an optimal maintenance schedule must be developed. Currently, it is not possible to state that either buprenorphine or methadone is clearly superior to the other for use in the pregnant addict.

In summary, although perinatal buprenorphine exposure fails to produce severe maternal and fetal or neonatal mortality and morbidity, it is associated with a finite amount of perinatal mortality and disruption of pup development. While there are similarities between the effects of perinatal buprenorphine and perinatal methadone exposure, there are also several differences between the effects of the two drugs, likely reflecting different affinities and efficacies of the two drugs at different opioid receptors. Furthermore, the effects of buprenorphine vary with the dose used, and the highest dose does not always produce the greatest effect. Because of its partial agonist properties, it may be possible to carefully adjust buprenorphine dosages to avoid adverse outcomes associated with opioid full agonists. However, it should be emphasized that none of the doses of buprenorphine used was without effect on some aspect of pup development. Buprenorphine may eventually represent an improvement over methadone in the management of the pregnant addict. However, great care should be taken in selecting appropriate doses.

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References


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