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Prenatal Administration of Buprenorphine in the Rat: Effects on the Rest-Activity Cycle at 22 and 30 Days of Age

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HUTCHINGS, D. E., A. S. HAMOWY, E. M. WILLIAMS AND A. C. ZMITROVICH. *Prenatal Administration of Buprenorphine in the Rat: Effects on the Rest-Activity Cycle at 22 and 30 Days of Age.* PHARMACOL BIOCHEM BEHAV **55**(4) 607–613, 1996.—Three doses of buprenorphine (BUP) were administered by osmotic minipump from day 8 of gestation through parturition. In addition to 0.3, 1.0, and 3.0 mg/kg/day of BUP, a vehicle control group received sterile water via minipump and a nontreated control group was left undisturbed during pregnancy. All treated and control litters were fostered at birth to untreated dams. BUP produced a dose response reduction in maternal water intake and reduced maternal weight gain among the two high dose groups; resorptions and birthweight were unaffected. BUP increased perinatal mortality in the two high dose groups compared with the vehicle controls and produced inconsistent effects on postnatal growth. To examine the effects of BUP on the rest-activity cycle of the offspring, groups of 3 littermates from each of the treated and control groups were tested for an 8 h observation period on electronic activity monitors at 22 and 30 days of age. Unlike previous effects described for prenatally administered methadone, a disruption in the rest-activity cycle was not observed for any of the BUP treated groups.

Buprenorphine Rat Prenatal Osmotic minipump Rest-activity cycle Developmental toxicity

BUPRENORPHINE is an opioid with mixed agonist-antagonist properties, a characterization derived from its pharmacological activity as a partial agonist and a potent antagonist at k-receptors (2,16,20). Because of its low abuse potential, less severe abstinence syndrome, long duration of action, and enhanced safety profile (12,24), buprenorphine has recently gained new attention as an effective pharmacotherapy for opioid (13–15) and possibly cocaine abuse (14,22). Both adult animal as well as human studies of buprenorphine show either a bell-shaped or an asymptotic dose response curve; in the low dose range, increases in efficacy are dose related whereas higher doses produce either a flattened or decreased function (2,4,17,24). Because the intrinsic activity of buprenorphine is not well understood in the adult there is no way to predict its pharmacological activity in immature animals; it is not at all clear that pharmacological and toxicological effects on the fetus and neonate will yield bell-shaped, linear, or asymptotic dose response effects. Moreover, except for the apparent lack of teratogenicity of buprenorphine in rodents (18) and the observation that prenatal administration transiently downregulates μ-opioid receptors in neonatal and maternal brains of rats (1), little is known of its reproductive or developmental toxicity.

Buprenorphine could eventually prove to be a safe and effective treatment for pregnant clients and, because of its long duration of action, conceivably be associated with less severe abstinence symptoms in the neonate, thus conferring a decided advantage over methadone, the only other therapeutic compound approved for use in opiate dependent individuals during pregnancy. On the other hand, if buprenorphine produces toxic effects (e.g., neonatal depression) that persist and/or cannot be treated or antagonized, it may confer more risk than methadone.

These important safety issues need to be expeditiously

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resolved; our laboratory recently initiated and reported the results of a developmental toxicity study in which doses of 0.0, 0.3, 1.0 or 3.0 mg/kg/day of buprenorphine were administered to pregnant rats using the osmotic minipump (11). The use of the minipump was deemed appropriate for several reasons: First, the half-life of buprenorphine in the rat is approximately 2.5 h (21) and unlike daily single-dose administration, delivering a fixed flow-rate of the compound is more likely to produce physical dependence. Second, Mori et al (19) administered buprenorphine once per day during midgestation and found a dose response decrease in maternal food and water consumption, reduced maternal weight gain, prolonged gestation, increased offspring mortality and a delay rate of postnatal growth. The toxic effects reported for buprenorphine by Mori et al (19) parallel similar effects found for methadone under conditions of once per day administration. Methadone administered by constant infusion (10) compared to once per day administration (7,8) was found to be far less toxic to both the dams and offspring. The enhance toxicity produced by daily, single dose administration probably results from the series of narrow, high drug concentration peaks produced by this technique (5) and we thus chose to administer buprenorphine using constant infusion. Finally, administering a fixed flow rate of the compound more closely approximates pharmacological parameters in human drug maintenance programs in which fluctuations in plasma concentrations tend to be less pronounced and maintained within some therapeutic range.

Because of the opioid properties of buprenorphine, we thought it initially important to examine whether abstinence-like effects would appear in the offspring following prenatal administration. Parenthetically, one of the difficulties of studying buprenorphine is that opioid antagonists neither block nor reverse its effect so that precipitated abstinence as a measure of physical dependence cannot be assessed in either the dams or offspring. For methadone, a synthetic opioid, infant rats, unlike humans, do not appear to undergo passive (i.e., non-precipitated) abstinence at birth but may show a generalized behavioral activation at 7 days of age (e.g., increased ultrasound and locomotion) when administered an opioid antagonist (unpublished observations). However, prenatal methadone appears to produce a prolonged abstinence in the young evidenced by signs of CNS hyperexcitability (or arousal) and delayed growth. These effects are characterized by a disrupted rest-activity cycle at 22 days of age, a slower rate of growth (10), and increased acoustic startle response (9). Paralleling similar observations in human infants, all of these effects are transitory and resolve by 30 days of age (6,25). Compared with methadone, buprenorphine has a longer duration of action and because CNS hyperexcitability might have a delayed onset, effects on the rest-activity cycle may not appear until a later age. In the present study, we examined dose response effects of prenatally administered buprenorphine on rest-activity measured in the offspring both at 22 and 30 days of age.

METHOD

Animals and Timing of Pregnancy

Individual nulliparous Wistar CVF females weighing 200–224 g (Hilltop Lab Animals, Inc., Scottsdale, PA) were paired with males of the same strain in hanging wire cages. The pans beneath the cages were examined by 12:00 pm for the presence of sperm plugs. The day a plug was found was designated Day 1 of gestation, or G1. Gravid dams were then randomly assigned to one of three dose-levels of buprenorphine, a vehicle minipump control, or a nontreated (NT) control group. All animals were housed in standard polycarbonate cages on wood chips. All dams had continuous access to Purina Lab Chow and water. Lights automatically came on at 6:00 am and went off at 6:00 pm.

Drug Administration and Control Groups

We had previously found maternal water intake to be a rapid and sensitive dose response measure of opioid activity (10) and used this end-point as a measure of pharmacological activity for buprenorphine (11). In a range finding study, we determined that a dose of 0.3 mg/kg/day of buprenorphine was the lowest concentration that showed a small but non-significant inhibition of maternal water intake. The highest dose used was determined by the low solubility of the compound in water; concentrations above 8 mg/ml cannot be achieved without the use of a solvent (e.g., methanol) and because of potential drug interactions as well as toxic effects that can occur with solvents, we chose to avoid their use in these initial studies.

Buprenorphine hydrochloride (BUP) was obtained from the NIDA Drug Supply System. Fourteen-day osmotic minipumps (Alza, Palo Alto, CA) were filled with either sterile water or three concentrations of BUP dissolved in sterile water to deliver 0.3, 1.0, and 3.0 mg/kg/day. To deliver these doses, a stock concentration of 2.5 mg/ml was diluted to achieve an initial delivery dose of either 0.3 mg/kg/day (BUP.3) or 1.0 mg/kg/day (BUP1). To achieve an initial delivery dose of 3.0 mg/kg/day (BUP3), a stock concentration of 8.0 mg/ml was used. Vehicle control dams (BUP0) were implanted with pumps filled with sterile water. All drug solutions were mixed and stored in nalgene vials, and kept in the refrigerator. Drug solution was transferred from a syringe to the minipump through a sterile disposable syringe filter (Acrodisc, Gelman Sciences) until overflow. Pumps were weighed before and after filling to verify fill volume. The pumps release the drug at a fixed flow rate so that as the dams gain weight during pregnancy, a proportionately reduced concentration is delivered. By term (G22) the initial doses of 0.3, 1.0, and 3.0 mg/kg/day were reduced to approximately 0.2 , 0.7, and 2.0 mg/kg/day, respectively.

Minipumps were implanted on G8, during early organogenesis; drug treatment thus spanned all of the major prenatal developmental processes. Dams were anesthetized with ketamine-xylazine cocktail (70 mg ketamine/ml, 6 mg xylazine/ml) at a dose of 0.78 ml/kg administered by the intraperitoneal route. The dorsal area behind the neck was shaved and swabbed with alcohol, a 3–4 cm lateral incision was made through the dermal layer and connective tissue, and the pump was inserted subcutaneously along the dorsal mid-line with the minipump cap oriented caudally. The incision was closed using wound clips and the animals were observed during recovery.

Food and water intake were measured daily for each dam from G7 through G22. Food intake was recorded by placing food pellets in stainless-steel hanging food dispensers and weighing the food at 10:00–11:00 am each day to determine the amount consumed during the previous 24 h. Water was presented to the dams in standard water bottles and the amount consumed over 24 h calculated by weight.

Fostering and Weaning

After birth, all treated and control offspring were sexed and weighed. Litters were culled when necessary to 10 pups

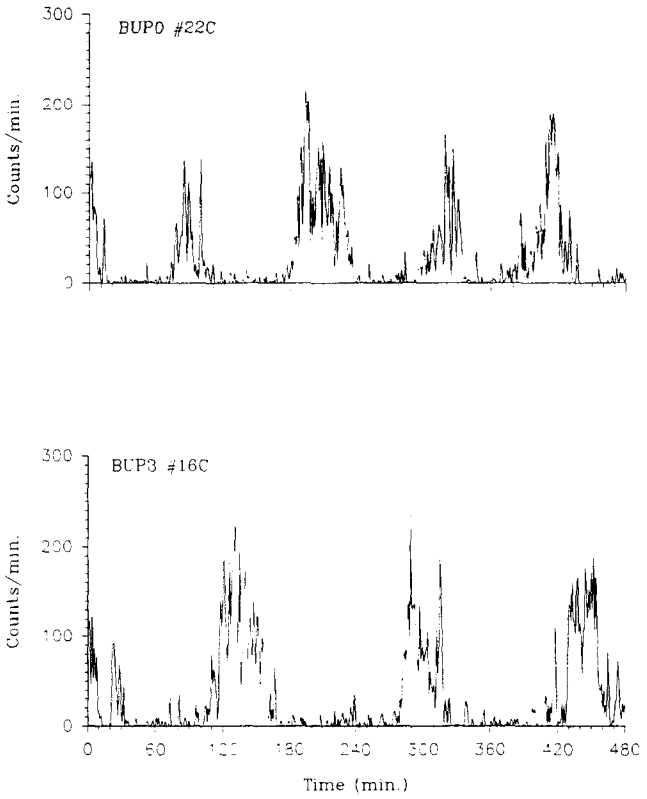

FIG. 1. Activity counts/min for BUP0 and BUP3 offspring. Data represent counts for individual triads.

and those containing less than 8 were also sexed and weighed but excluded from further testing. All experimental and control dams were sacrificed to determine the number of implantation sites and the offspring fostered to normal dams of the same strain that delivered either on the same day or no more than 48 h earlier. Offspring tested on postnatal day (PND) 22 were reared with their foster dams until tested. Offspring tested on PND30 were weaned at PND22. At the time of weaning, all groups were housed by sex with littermates.

Rest-Activity Testing

Previous work showed that groups of young rats comprised of 3–4 normal (untreated) littermates and isolated from their dam on electronic activity monitors for a period of 8 hours show a behavioral ultradian rhythm or "rest-activity cycle" (7). This behavioral cycle is characterized by approximately 60–90 min bouts of locomotor activity that regularly alternate with 60-90 min periods during which the animals huddle and appear to sleep (see Fig. 1). This ultradian behavior is not observed when testing 1 or 2 animals; 3 is the minimum test group size for the effect to be observed. Moreover, the cycle occurs in same-sex or mixed-sex groups and for animals on a standard 12:12 dark-light cycle, it occurs in an identical pattern whether testing is carried out during the dark or light phase. This behavior makes its first appearance around the time of eye-opening, becomes clearly differentiated by 22 days of age and disappears sometime around 30-35 days of age.

Rest-activity testing was carried out between 0900 and 1700 h in a shielded room continuously illuminated with diffuse fluorescent ceiling fixtures and maintained at a temperature of 22–24°C. A 6-channel electronic activity monitor with 6 remote sensors, each measuring 25.4 × 48.3 × 10.2 cm (Stoelting Co., Chicago, IL), was used for testing. The operation of the sensors has been described elsewhere (7). The sensors are stacked 40.6 cm apart in a vertical metal rack with the metal shelving acting as an insulator between the respective sensor fields. The threshold reset time was placed in the normal mode and the activity level control set at 15. This setting was selected so that neither breathing movements nor the myoclonic twitching and fine distal movements that accompany REM sleep produced counts. Activity counts were collected at 1 min intervals using a computer.

Half of the litters were tested at PND22 and the other half at PND30; individual litters were tested only once. However, the Ns for the behavioral tests on PNDs 22 and 30 reported in Tables 2 and 3 exceed those reported in Table 1 for maternal and offspring toxicity. This resulted because 44 treated and control litters came from a previous study that reported effects on maternal and offspring toxicity but not behavior (11). Each litter was separated into three triads consisting of either 2 males and 1 female, or 2 females and 1 male. Each triad was placed in a standard 48.3 × 26.7 × 20.3 polycarbonate cage on approximately 1.5 cm of fresh wood chips. The cage was then placed on one of the 6 monitors and recording began for a test period of 8 h (480 min). Neither food nor water was provided during testing.

Statistical Analysis

Data analyses were carried out using SYSTAT. Analyses of variance (ANOVAs) were performed on normally distributed data including maternal weight gain, implantation sites, litter size, and birth weights. Post hoc univariate F tests were used when appropriate. A repeated measures ANOVA was used for analysis of food and water intake of dams during gestation. The Kruskal-Wallis was the nonparametric test used to analyze maternal and offspring effects expressed as proportions, with Mann-Whitney U tests for post hoc comparisons. For all analyses, the litter was used as the unit of analysis.

RESULTS

Maternal and Offspring Effects

The top panel of Fig. 2 shows that food intake of the drug-treated dams was reduced following pump implantation and drug delivery (G8-G15; repeated measures ANOVA of treatment main effect, $F = 10.83(4,39)$, $p < 0.001$, and one-way ANOVAs on specified gestational days, $p < 0.006$, with post-hoc univariate F tests, $p < 0.05$). On G16-G22, there were no significant differences between the groups, except on G19. The NT and BUP0 consumed food at similar rates throughout gestation, except for a decreased rate of consumption by the BUP0 dams on the day of implantation (G8: one-way anova, $p < 0.001$, post hoc univariate F test, $p < 0.033$), and an increased rate of food consumption by the BUP0 dams on G11, 14, and 19 (one-way ANOVAs on specified gestational days, $p < 0.007$, with post-hoc univariate F tests on G11, 14, 19, $p < 0.04$). As pregnancy progressed the BUP1 and BUP3 dams consumed less food than the other groups on G8,9,10, and 14 (one-way ANOVAs on specified gestational days, $p < 0.005$, with post-hoc univariate F tests, $p < 0.04$).

Following pump implantation, all drug exposed dams showed a decrement in water intake. Unlike food intake, however, this effect persisted throughout late gestation. The bottom panel of Fig. 2 shows that on G11, 12, 14, 15, 16, 17, 18,

TABLE 1
MATERNAL AND OFFSPRING EFFECTS

	NT	BUP0	BUP.3	BUP1	BUP3
Total pregnant	14	11	12	12	12
Maternal weight gain (g)	205 ± 9	205 ± 8	190 ± 9	173 ± 4*	172 ± 11*
Implantation sites	16.5 ± 0.9	16.1 ± 0.8	15.5 ± 0.9	15.2 ± 0.9	14.8 ± 1.3
% Resorption	2.5 ± 1.4	3.7 ± 1.6	8.8 ± 5.3	2.4 ± 1.1	6.6 ± 2.6
% Perinatal mortality	1.1 ± 0.7	0	2.1 ± 1.4	5.6 ± 2.1†	7.8 ± 3.9†
% Total offspring mortality	3.6 ± 1.4	3.7 ± 1.6	5.7 ± 2.0	8.2 ± 2.2	15.0 ± 6.5
% Born					
male	49.8 ± 4.1	47.9 ± 3.0	47.8 ± 5.6	60.2 ± 3.9	47.4 ± 4.6
female	50.2 ± 4.1	52.1 ± 3.0	52.2 ± 5.6	39.8 ± 3.9	52.6 ± 4.6
Litter size	16.0 ± 0.7	15.4 ± 0.7	14.2 ± 1.2	14.1 ± 0.8	13.7 ± 1.3
Birth weight(g)					
male	7.0 ± 0.1	7.3 ± 0.1	7.4 ± 0.2	7.2 ± 0.2	7.5 ± 0.2
female	6.5 ± 0.1	6.7 ± 0.1	6.7 ± 0.1	6.9 ± 0.2	7.0 ± 0.2

*Significantly different from NT and BUP0, $p < 0.02$.
†Significantly different from BUP0, $p < 0.015$.

20, and 21 drug exposed dams consumed significantly less water than both the NT and BUP0 dams (repeated measures ANOVA of treatment main effect, $F = 3.64$ (4,47), $p < 0.013$; one-way ANOVAs on specific gestational days, $p < 0.030$, with significant post-hoc univariate F tests, $p < 0.05$). Throughout gestation, the NT and BUP0 control dams consumed water at similar rates and there were no consistent dose related differences between the BUP.3, BUP1 and BUP3 dams.

Table 1 specifies the Ns for each of the treated and control groups. There were no deaths among any of the treated or control dams. On G1, treated and control dams did not differ with respect to body weight. On the day following pump implantation, there was a modest decrement in rate of maternal weight gain for some of the groups; thereafter, all dams gained weight at similar rates. However, the mean maternal weight gain of the BUP1 and BUP3 dams was significantly less than the controls (one-way ANOVA, $F = 3.44$ (4,54) $p < 0.01$, with significant post-hoc univariate F tests, $p < 0.02$).

Table 1 also includes the analysis of mean implantation sites and percent resorptions which did not reveal significant effects of treatment. Percent perinatal mortality (i.e., pups either stillborn or found dead between PNDs 0-5) was significantly greater in the BUP1 and BUP3 groups compared with the BUP0 ($K = 10.33$, df = 4, $p < 0.036$, post hoc Mann-Whitney-U tests, $p < 0.015$) but the measure of total offspring mortality (% resorptions and % perinatal mortality) was not different between groups. There were no differences in percent male and female offspring born across all four groups nor were there differences in litter size. As shown in Table 1 there were no differences in birthweight of male or female pups across all groups. Among the NT, BUP0, and BUP.3, males weighed significantly more than the females at birth (NT, $F = 10.19$ (1,26) $p < 0.005$; BUP0, $F = 7.982$ (1,20) $p < 0.011$; BUP.3, $F = 9.511$ (1,22) $p < 0.006$). There were no sex differences in birthweight for the BUP1 and BUP3 groups.

Postnatal Growth

There were no significant differences found between groups over time for male or female weights taken on PND0, 5, 10, 22, 30, 40, 50, and 60 as shown by a repeated measures ANOVA. However, analysis of individual days revealed that on PND5, male BUP0 pups weighed significantly more than pups of the other groups and female BUP0 pups tended to (Male: one-way ANOVA on PND5, $F = 2.77$ (4,55) $p < 0.037$, with significant post-hoc univariate F tests, $p < 0.05$; Female: one-way ANOVA on PND5, $F = 2.77$ (4,55) $p < 0.066$, with significant post-hoc univariate F tests, $p < 0.03$). These differences were not seen again through PND60.

Rest-Activity Measure

The activity data of individual triads of BUP0 and BUP3 groups on PND22 shown in Fig. 2 were selected because they are representative of the group means. Counts of 0-5 represent periods when the triad was huddled and either sleeping or at

TABLE 2
DAY 22 REST-ACTIVITY X ± SEM

	NT	BUP0	BUP.3	BUP1	BUP3
Total litters tested (n)	11	11	10	10	10
Mean count	25.8 ± 1.9	26.4 ± 2.5	31.0 ± 3.2	28.7 ± 2.0	28.3 ± 1.8
Max peak activity	213.6 ± 8.6	204.4 ± 7.6	211.4 ± 7.9	204.4 ± 7.6	202.1 ± 5.3
Total rest periods	262.2 ± 15.0	259.8 ± 14.3	229.6 ± 19.6	242.0 ± 14.6	241.3 ± 13.2
Max consecutive rest periods	42.5 ± 6.7	42.2 ± 8.3	38.7 ± 5.3	38.5 ± 4.9	31.9 ± 4.6
Activity change score	46.6 ± 3.7	47.0 ± 2.6	52.2 ± 3.8	49.9 ± 3.6	50.4 ± 4.0

No significant differences found.

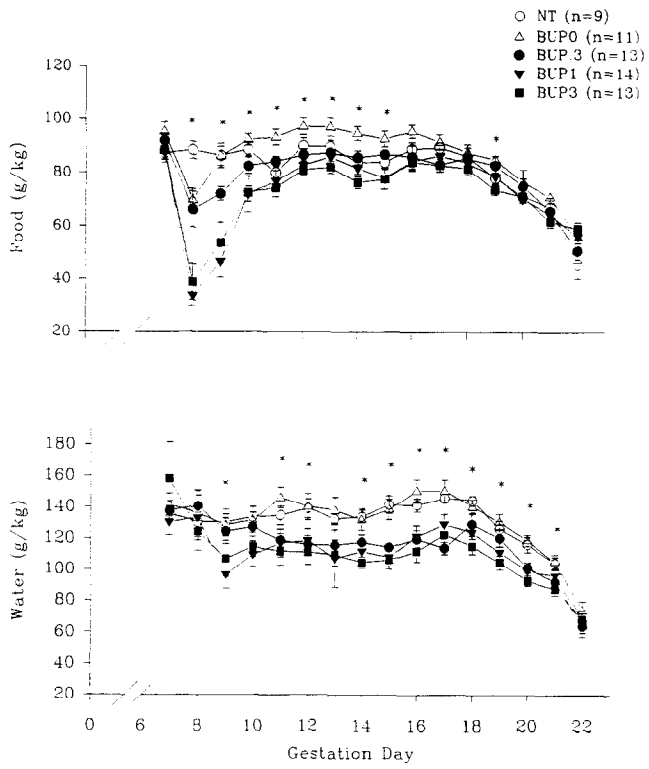

FIG. 2. Mean (± SEM) food (g/kg) and water (ml/kg) intake for drug treated and BUP0 and Nontreated (NT) control dams.

rest and are referred to as "sleep-rest" periods. The brief spikes that range from 6-50 are typically produced by periods of waking of one or more animals and re-positioning within the group; active exploration by the group produces counts of 50 and above.

The BUP0 and BUP3 data shown in the upper and lower panels, respectively, of Fig. 1 are similar in that they show a distinct rhythmicity; periods of active exploration lasting about 45–60 min are separated by sleep-rest periods of about 60–110 min. Mean activity counts/min for the entire test period are shown in Table 2. The mean activity counts were similar across for the drug treated and control groups. Also, the "maximum peak activity" defined as the highest activity count over the entire 480 min test period, also failed to reveal group differences.

Similarly, analysis of mean total rest periods (i.e., number of mins with counts 0-5) failed to reveal a significant effect of treatment nor was an effect found for maximum number of consecutive rest periods (i.e., maximum number of consecutive mins with counts 0-5). Another measure used to detect possible differences is the number of times the activity level crosses the 50 unit line during the 480 min test period, a measure that was significantly altered by prenatal methadone exposure (7, 10). Although the selection of 50 as a "threshold" was arbitrary as representing a point between high and low activity it did yield an "activity change score." The change scores, calculated for each of the test triads and shown in Table 2, are not significantly different across groups.

Although no differences were found on PND22, as shown in Table 2, there were significant differences between these groups for some of these same measures at PND30 (Table 3). The mean activity count for the BUP0 group was significantly higher than the NT and BUP1 groups ($F = 3.10$ (4,48) $p < 0.025$, with significant post-hoc univariate F tests, $p < 0.05$). In addition, the total number of rest periods during the 480 min test period was significantly lower in the BUP0 group compared to the NT and BUP1 groups ($F = 2.63$ (4,48) $p < 0.047$, with significant post-hoc univariate F tests, $p < 0.02$).

DISCUSSION

Dams treated with BUP during gestation consumed less food and water; compared with both control groups and the BUP.3 treated group, the BUP3 dams consumed less food than the other groups on several days during gestation. Water intake was reduced for both the BUP1 and BUP3 dams during mid-gestation and, on several of these days, the decrements were dose related, findings comparable to our previous study. The inhibitory effect of buprenorphine on the dams' water intake parallels similar dose response effects reported for methadone (9,10) suggesting that a common agonistic effect of both compounds is a dose related reduction of water intake in the pregnant dam.

The findings of reduced maternal weight gain and increased perinatal mortality for the two highest doses of buprenorphine do not replicate our previous report of no effects on these same measures (11). Although the reduced maternal weight gain seen in the present study may have resulted from the decrease in maternal food and water intake, it is not clear why buprenorphine increased perinatal mortality. Similarly, the finding of a transitory increase in body weight of BUP0 pups on PND5 was also not previously observed. Because of the inconsistency of these observations, further study will be required to determine the reliability of these effects.

We have found only one other study that administered BUP to pregnant rats using osmotic minipumps. Evans et al

TABLE 3

DAY 30 REST-ACTIVITY X ± SEM

	NT	BUP0	BUP.3	BUP1	BUP3
Total litters tested (n)	12	9	11	10	11
Mean count	31.3 ± 1.7	43.9 ± 4.5*	38.1 ± 2.0	35.0 ± 2.7	40.7 ± 3.0
Max peak activity	224.0 ± 7.8	231.3 ± 10.9	228.4 ± 3.5	228.3 ± 5.1	228.3 ± 5.0
Total rest periods	253.4 ± 7.3	201.6 ± 17.8†	224.5 ± 10.1	244.9 ± 15.6	222.9 ± 11.5
Max consecutive rest periods	30.0 ± 3.4	23.7 ± 2.3	27.5 ± 2.6	29.8 ± 3.3	30.9 ± 3.7
Activity change score	39.8 ± 1.7	48.3 ± 3.9	42.9 ± 2.8	39.1 ± 3.7	43.0 ± 3.3

*BUP0 significantly different from NT and BUP1 ($p < 0.025$).
†BUP0 significantly different from NT and BUP1 ($p < 0.047$).

(3) administered buprenorphine in a methanol vehicle at doses of 2.8, 8.4, and 28.0 μg/h on the last three days of gestation in the rat. The weights of the dams were not reported but if they were in the range of the animals used here, the doses that their animals received on a mg/kg/day basis would be considerably lower than the doses administered here. In contrast to our findings these authors reported prolonged gestation, increased offspring mortality and a decrease in birthweight. However, these toxic effects may have resulted from drug administration being initiated only three days prior to parturition and the incomplete development of tolerance in both the dams and offspring.

Following prenatal administration of methadone, we reported that the offspring exhibit a state of CNS hyperexcitability characterized by a disrupted rest-activity cycle and heightened acoustic startle (9,10). These effects are maximal at 22 days after birth but are no longer evident by 30 days of age. During this period of hyperexcitability, the offspring also show delayed growth but as rest-activity and startle return to normal, rate of growth recovers and weight differences are no longer evident. We have noted that both the disturbed rest-activity cycle and heightened startle bear a striking resemblance to opioid-exposed human infants undergoing prolonged opioid abstinence (5). However, in the present study, neither the mean data nor any of the individual records from animals exposed prenatally to buprenorphine showed any of the disrupted rest-activity or growth effects so readily apparent after prenatal methadone exposure.

One finding difficult to explain is that the BUP0 offspring were more active and showed less rest periods at PND30 compared with the NT and BUP1 offspring. Although the significance of this observation is not clear, it does emphasize the importance of including a nontreated control group in this sort of study. The BUP0 group, in addition to serving as a vehicle control, was also administered anesthesia, and further, underwent a surgical procedure for pump implantation. Although only two effects were seen out of a large number of toxic and behavioral endpoints, without the inclusion of both control groups, it is possible that potential effects produced by some aspect of the control procedure could go undetected.

The observations from this and our previous study (11) indicate that within the range of doses studied here, BUP shows little maternal and offspring toxicity and the offspring do not show behavioral or growth effects suggestive of abstinence or CNS hyperexcitability. However, before any conclusions can be drawn with respect to the potential safety or hazard of BUP, future studies will first need to examine a higher range of doses and resolve some of the pharmacological problems related to this compound. One issue is that BUP has limited solubility in water and the dose of 3.0 mg/kg/day is the highest concentration that can be achieved with a 14-day minipump without the use of a solvent. To address this in a pilot study (unpublished findings), we implanted two pumps per pregnant dam ($N = 14$), each delivering 3.0 mg/kg/day of buprenorphine. Except for two maternal deaths in this 6.0 mg/kg/day group, measures of maternal and offspring toxicity did not reveal any differences compared with a double-pump vehicle control group ($N = 12$). However, BUP is a μ partial agonist (2), dose response effects are bell-shaped or asymptotic and increasingly higher doses are not necessarily associated with increased toxicity (2,23). Thus, based on these limited number toxic end-points and without other pharmacological measures it is not clear where the doses we have studied fall on a dose response curve. One solution for future studies would be to specify the functional significance of the dose range by utilizing a measure of opioid-specific dose response effects in the dam (e.g., analgesia). We suggest that for the development of a clinically relevant animal model of BUP, in addition to studying a full range of doses that include a functional measure of dose response activity, it will also be important that measures of placental transfer and distribution in offspring tissues be included as well as a comprehensive test of standard neurobehavioral outcomes.

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