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Chronic *in utero* buprenorphine exposure causes prolonged respiratory effects in the guinea pig neonate

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ABSTRACT

Our laboratory studies the effects of *in utero* opioid exposure on the neonate. In this work we test the effects of chronic *in utero* exposure to buprenorphine on the neonate. Buprenorphine is a promising candidate for treatment of opioid addiction during pregnancy and it has been suggested to decrease the neonatal abstinence syndrome in human infants. In our guinea pig model, we focused not only on the respiratory effects of *in utero* exposure on the neonate, but also studied withdrawal signs in the neonate, a major concern of all opioid treatment during pregnancy. Pregnant guinea pigs were treated with daily subcutaneous injections of 0.1 mg/kg buprenorphine during the second half of gestation. We measured weight, locomotor activity and respiratory function in pups of ages 3 to 14 days. Respiratory response was recorded using a two-chamber plethysmograph, while pups were breathing either room air or 5% CO_2. Our results show that chronic *in utero* exposure to buprenorphine induces respiratory effects up to day 14 after birth, while earlier studies have shown that effects of either *in utero* methadone or morphine only persist in the first week after birth in the guinea pig model. These data provide important information for clinical trials of buprenorphine treatment suggesting that duration and severity of respiratory effects of *in utero* buprenorphine exposure should be monitored.

1. Introduction

Currently, the only approved drug treatment in the United States for opioid dependence in pregnant women is methadone maintenance [21,43]. Even though this therapy has proven beneficial for both maternal and fetal outcome, newborns often suffer from neonatal abstinence syndrome (NAS) as a result [27,39,44]. The NAS symptoms caused by *in utero* methadone exposure include dysfunctions of the respiratory system, seizures [15], and an increased risk of sudden infant death syndrome [22,23]. Therefore alternate treatment options are sought that could improve maternal and neonatal outcome.

We have recently shown that in an animal model of chronic *in utero* methadone or morphine exposure, the respiratory effects of methadone on the neonate, though less severe than morphine, are still significant and potentially detrimental [33]. In addition, effects on neonatal weight and excitability, indicators for NAS, are more severe than effects of morphine. In humans these effects are offset somewhat by the socioeconomic factors associated with the treatment program, such as better medical care and nutrition [36].

Buprenorphine is one potential alternative treatment option for pregnant women. It is a long-lasting partial agonist at the mu opioid receptor that has recently been successfully introduced as an alternative treatment for opioid addiction in non-pregnant opioid addicts [4,24,37]. Preliminary studies indicate that pregnant female addicts can safely be transitioned to buprenorphine treatment and that NAS caused by *in utero* buprenorphine exposure may be less severe than that of methadone [11,12,17,20]. However, all these studies are hampered by confounding factors like multi-substance abuse, continued illicit drug use, insufficient medical care, or maternal neglect during and after the pregnancy. Studies using animal models are needed, as they are free of these confounding factors.

This study used the guinea pig model to evaluate the effects of chronic *in utero* buprenorphine exposure on the neonate. It was designed to test the hypothesis that the neonatal abstinence syndrome and respiratory depression effects of buprenorphine are less severe when compared with previously reported data on morphine and methadone *in utero* exposure [16,33].

Pregnant guinea pigs were treated with a once daily dose of buprenorphine during the second half of gestation. Dose selection was based on the survival of the maternal–fetal unit. Respiratory parameters of their litters have been studied in a longitudinal study during the first two weeks after birth using a non-invasive plethysmograph. We show that the respiratory effects of buprenorphine were equally as severe as those of methadone or morphine. Considering respiratory criteria alone, these data suggest that buprenorphine treatment of drug addiction during pregnancy is not more beneficial

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for the newborn child than methadone or morphine, as the respiratory effects persisted for two weeks after birth.

2. Materials and methods

2.1. Animals and treatment groups

All experiments were approved by the Oregon Health & Science University Institutional Animal Care and Use Committee and were conducted in accordance with the National Institutes of Health "Guide for the Care and Use of Laboratory Animals" guidelines. Female Dunkin-Hartley guinea pigs (6 weeks old, 300 to 400 g) from Charles River Laboratories (Wilmington, MA) were bred at Oregon Health & Science University. Pregnant guinea pigs were randomly assigned to receive a daily injection of either 1 ml/kg of 0.9% saline solution (vehicle control) or 0.1 mg/kg buprenorphine in saline. Buprenorphine injection volumes were standardized to 1 ml/kg. Subcutaneous injections in the shaved scruff of the neck started at day 35 of pregnancy prior to critical periods of *in utero* nervous system development in the guinea pig [10,35] and were given once daily in the morning until the day of parturition [33]. Six pups (2 litters, 3 males and 3 females) from two saline treated dams, and eight pups from three buprenorphine treated dams (3 litters, 6 males and 2 females) were included in the study. All pups from a litter were studied, but in order to avoid litter effects, data analysis was performed using the litter as the basic unit.

After parturition, dams and pups were housed together with 12 h controlled light/dark cycles and *ad libitum* food and water. The first day pups were observed in the tub was defined as day 1. A longitudinal study of all pups of a litter was carried out on days 3, 7, 10, and 14.

2.2. Locomotor activity

Locomotor activity of pups was measured on study days immediately prior to respiratory measurements. A pup was separated from its mother, weighed, and placed immediately in an isolated Opto-Varimax mini activity monitor (Columbus Instruments, Columbus, OH) for a period of 15 min. The activity monitor counted breaks of the light beams and separately recorded total movements including grooming, digging, and head movements (every beam break is recorded) and ambulatory movements (two consecutive beam breaks are recorded).

2.3. Ventilatory and metabolic measurements

Ventilatory and metabolic data were collected using the same protocol as in previous studies in our laboratory [16,33]. Pups were placed in a non-invasive, head-out, two-chamber plethysmograph [35] (Buxco Electronics, Sharon, CT) for a 10 min acclimation period. Interchangeable head chambers allowed separate inspiration of the two study gases; "Room Air", (RA; 21% O_2, balance N_2) and "5% CO_2" (5% CO_2, 30% O_2, balance N_2). Therefore both room air and hypercapnic respiratory response could be measured in unanesthesized, unintubated and only slightly restrained pups [3]. First, room air data were collected with a steady flow of RA (1 l min^{-1}) for 10 min, after which the head chamber was quickly changed and the 5% CO_2 gas mixture (1.7 l min^{-1}) was administered for 5 min to measure the hypercapnic response. Metabolic parameters were only measured during RA flow by sampling the head chamber over 30 sec periods. Both oxygen consumption (VO_2; ml min^{-1} 100 g^{-1}) and CO_2 production (VCO_2; ml min^{-1} 100 g^{-1}) were measured and V_I/VO_2 and V_I/VCO_2 were calculated (Oxymax software version 2.4.2, Columbus Instruments, Columbus, OH). From the separate body chamber animal breathing movements were recorded and averaged over 1 min periods. Directly measured parameters were breathing frequency (f_R; breaths min^{-1}), inspiratory time (T_I; s), expiratory time (T_E; s), and respiratory air flow (ml s^{-1}), which were used to calculate tidal volume (V_T; ml 100 g^{-1}), inspiratory minute ventilation (V_I; ml min^{-1} 100 g^{-1}), and inspiratory effort (V_T/T_I; ml s^{-1} 100 g^{-1}) using BioSystem XA software version 2.7.9 (Buxco Electronics, Sharon, CT).

2.4. Data analysis and statistics

Gestational length was defined as the number of days from the first day of vaginal opening to the day pups were observed in the tub. Maternal weight gain was monitored daily and weight recorded on the day before pups were born was reported as maternal weight at parturition. All pups of a litter were studied and averaged to avoid litter effects. Previous studies in the guinea pig in our group as well as clinical observations have shown that respiratory effects of opioids are not sex dependent [5,30,32], therefore both male and female pups were studied and data was collapsed for gender.

Respiratory data of all pups were collected on days 3, 7, 10, and 14 after birth as previously described [33]. In brief, during the 10 min RA breathing period, metabolic parameters were measured for the first 6 min and breathing movements for the remaining 4 min. During the 5 min CO_2 challenge, only breathing movements were measured. In order to ensure a steady-state had been reached, the data reported and analyzed are the averages of the last 2 min of each measurement period. All values are reported as the mean ± standard error.

Significant statistical differences for gestational length, maternal weight, and maternal weight gain were determined using the Student's *t*-test with treatment as factor (SigmaStat version 3.11, SysStat Software Inc., Richmond, CA). All neonate data were averaged for each litter and were analyzed by two-way repeated-measures analysis of variance (ANOVA) with factors of age and treatment. An overall *p*-value of less than 0.05 was considered significant and all post-hoc comparisons were performed using the Holm–Sidak method. Weight data for all litters was analyzed using litter size as a covariant (SPSS, SPSS Inc, Chicago, IL).

3. Results

3.1. Effects of chronic in utero buprenorphine treatment on the dam and pup weight

The buprenorphine dose of 0.1 mg/kg used in these experiments was determined in pilot studies as the maximum tolerable dose for the maternal–fetal unit with no fatal outcome seen for either dams or pups. In the pilot study, four dams had been tested with buprenorphine doses of 0.2 (2 dams), 0.5, and 1.0 mg/kg. The treatment with the two highest doses resulted in unacceptable weight loss of the dams and had to be terminated. The 0.2 mg/ml dose resulted in premature still birth on day 49 and 57, respectively. Table 1 shows the maternal weight data recorded during gestation. Treatment groups were not different in the first half of gestation. Buprenorphine treatment during the second half of gestation caused significantly lower weight gain and resulted in lower maternal weight at parturition. All three dams treated with 0.1 mg/kg of buprenorphine gave birth to live, full term litters.

Pup weight was recorded on the day pups were first observed in the tub (day 1) and on all days respiratory measurements were taken. Weight of *in utero* buprenorphine exposed pups was significantly lower on all days than the weight of the saline exposed control group. The relative weight gain of pups compared to their birth weight was not different between treatment groups (Table 1).

3.2. Effects of chronic in utero buprenorphine treatment on pup activity

Immediately before respiratory measurements were recorded, pups were removed from their moms and their activity was monitored