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Neonatal Adaptation: Naloxone Increases the Catecholamine Surge at Birth

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ABSTRACT. A marked increase in plasma catecholamines at birth has been described in animals and man. Because the factors that regulate catecholamine secretion are incompletely understood and because it has recently been suggested that endogenous opiates are important in the regulation of catecholamine secretion, we designed studies to determine the influence of opiate receptor blockade prior to delivery on the increase in plasma catecholamines at birth. Term fetal sheep were delivered by cesarean section and randomly assigned to receive naloxone or vehicle. Naloxone was given just prior to umbilical cord cutting as a 2 mg/kg bolus followed by 2 mg/kg/h. Naloxone administration resulted in significantly greater peak levels of plasma norepinephrine (peak levels of 1.5 ± 0.4 *versus* 0.9 ± 0.1 ng/ml) and epinephrine (peak levels of 1.4 ± 0.7 *versus* 0.9 ± 0.3 ng/ml) and higher norepinephrine values throughout the study period. Naloxone administration was associated with significantly elevated heart rate (peak 184 ± 12 *versus* 207 ± 13 beats per min) and blood pressure (peak 95 ± 6 *versus* 88 ± 2 mm Hg). These studies demonstrate that opiate receptor blockade from birth markedly augments the neonatal sympathoadrenal response in the term newborn lamb. (*Pediatr Res* 21:590–593, 1987)

Abbreviations

NE, norepinephrine
E, epinephrine
βEND, β-endorphin
ENK, enkephalin
EGTA, (ethyleneglycol-bis-[β-aminoethyl ether]-N,N,N′,N′-tetracetic acid)
ANOVA, analysis of variance

Fetal and newborn animals secrete catecholamines in response to a variety of stimuli including hypoxia (1), hemorrhage (2), hypothermia (3), labor (4), and delivery (5). The marked increase in catecholamine secretion at birth is particularly important because of the wide range of physiological changes which occur at birth and the importance of the sympathoadrenal system in modulating these changes. In the chronically catheterized fetal sheep, plasma catecholamines begin to rise in the last 3 h of spontaneous labor prior to delivery (4). There is then a further augmentation in plasma levels of both NE and E following delivery and cord cutting (6). We were interested to extend our observations to study the factors that regulate catecholamine release at birth.

Two levels of control of catecholamine release have been recognized, including α 2 receptor-mediated presynaptic inhibition of NE release and inhibition by endogenous opiate peptides. βEND is secreted in response to stress (7) and degrees of hypoxia in fetal sheep known to produce marked catecholamine secretion result in markedly increased levels of circulating βEND (8). Catecholamines and ENK are costored and secreted by the adrenal medulla in response to cholinergic or splanchnic nerve stimulation (9–12). Opiate peptides inhibit catecholamine release by inhibition of neurotransmission in sympathetic ganglia (13, 14) and inhibition of adrenal medullary catecholamine secretion (15). The physiological significance of these observations *in vivo* is unclear. In the present study, in order to investigate the role of endogenous opiates *in vivo* as modulators of sympathoadrenal activity at birth, we compared plasma catecholamine levels with and without continuous opiate receptor blockade following umbilical cord cutting in the term fetal sheep.

MATERIALS AND METHODS

Animals. To control the effects of labor, manipulation, and delivery on sympathoadrenal activity, we used the acutely exteriorized fetal sheep model as described previously (6). Time-dated fetuses were delivered using maternal spinal/epidural anesthesia. The fetal hindlimb was delivered through a small uterine incision, infiltrated with local anesthetic and the dorsal hindlimb artery was catheterized. The hindlimb was returned to the uterus and the incision closed by a purse-string suture. Through a second hysterotomy incision, the fetal head was delivered and fetal breathing was prevented by placing a warm saline filled glove over the head. Following local anesthetic infiltration, a tracheostomy was performed through a midline longitudinal incision with secure placement of an appropriately sized uncuffed endotracheal tube. Breathing was prevented by clamping the endotracheal tube. Breathing efforts were rarely observed prior to umbilical cord cutting. The animals were then delivered onto the maternal abdomen and allowed to stabilize for 20 to 30 min. Care was taken to avoid traction or trauma to the umbilical cord. Heart rate and blood pressure were measured continuously with a pressure transducer connected to the arterial catheter. Body temperature was monitored with an indwelling rectal probe.

Immediately following umbilical cord cutting the animals were ventilated by hand with 100% oxygen and then placed on time-cycled, pressure-limited Sechrist infant ventilators. To prevent movement, the animals were given 0.1 mg/kg pancuronium bromide. The ventilator was adjusted to maintain an arterial pO_2 of 100–150 torr and arterial pH and pCO_2 within normal values.

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The lambs were dried superficially and supported under an infant radiant warmer with supplemental heat lamps as necessary to maintain body temperature at 39 ± 1.0° C. A continuous infusion of normal saline at a rate of 4 ml/kg/h was administered.

Opiate receptor blockade. For the opiate receptor blockade, fetuses from eight time-dated pregnant ewes with twin gestations at term (145 ± 1 day) were randomly assigned to receive either naloxone or control infusion following cord cutting so that if the first fetus of a twin pair received naloxone the second served as a control. In each case the second fetus was delivered within 1 h of the first twin and had comparable blood gas values and catecholamine levels. Naloxone was administered as a 2 ml injection just prior to cord cutting at a dose of 2 mg/kg estimated fetal body weight followed by 2 mg/kg/h via the hindlimb catheter. This regimen has been shown to reverse the circulatory collapse in hemorrhagic and endotoxic shock in dogs and rats (16). Control fetuses received an equivalent amount of normal saline. Blood samples for catecholamines and arterial blood gas measurements were drawn immediately after catheterization, serially after delivery until the time of cord cutting, and sequentially thereafter as described previously (6). All blood samples, heart rate, and blood pressure measurements after cord cutting were done using the umbilical arterial catheter. A total of 3 ml whole blood was removed at each sampling time and the blood was replaced immediately with heparinized residual placental blood drawn following delivery.

In a subset of the control animals (n = 6) a similar bolus followed by a continuous infusion of naloxone was given beginning at 4 h of age. Samples were obtained for arterial blood gases and catecholamine measurements prior to the bolus and at 2, 5, 15, 30, and 60 min during the continuous infusion.

Analytical techniques. Blood samples for catecholamine measurements were placed immediately into chilled tubes containing EGTA and reduced glutathione. Plasma was separated, rapidly frozen, and stored at −70° C for assay within 2 wk. Plasma catecholamine concentrations were determined by radioenzymatic assay sensitive to 10–20 pg/ml of NE and E (17). Blood gases were measured on a Radiometer blood gas instrument maintained at 39° C.

Statistical analysis. All data are presented as mean ± SEM. Comparisons between sequential plasma catecholamine concentrations or biophysical parameters (factor A) in control or naloxone-infused animals (factor B) were made with two-way ANOVA with replication (18). Individual comparisons between or within groups at individual time points were conducted using unpaired Student's t test.

RESULTS

The sequential plasma NE and E values for naloxone treated and control animals are shown in Figure 1. There is a brief, modest elevation in plasma catecholamine levels following catheterization and delivery, similar to previous observations (6). NE rose to a peak of 515 ± 121 pg/ml and E to 70 ± 37 pg/ml during the exteriorization. These values return to baseline during the stabilization period. This allows separation of the neonatal catecholamine surge from the effects of manipulation and delivery. Plasma NE values just prior to cord cutting were 382 ± 76 pg/ml in control animals and 418 ± 77 pg/ml in naloxone-treated animals. Plasma E values were 40 ± 17 and 44 ± 7 pg/ml, respectively. Both the baseline NE and the baseline E values were statistically similar by ANOVA in treated and control animals. In all twin studies the second twin was in stable physiological condition prior to cord cutting as evidenced by normal blood gases and baseline catecholamine values which were not statistically different from the first twin. Fetal and newborn body temperatures remained within a normal range (39 ± 1° C) throughout the experiments.

Following cord cutting there were marked increases in both plasma NE and E in naloxone and control animals. The naloxone-infused animals had higher peak NE and E than the control animals and higher concentrations throughout the period of the study ($p < 0.01$ by ANOVA). The peak plasma NE level in the naloxone-infused animals was 1473 ± 362 pg/ml at 15 min *versus* 909 ± 127 pg/ml in the controls. The peak E concentrations also occurred at 15 min in both groups (1398 ± 698 *versus* 864 ± 278 pg/ml, respectively). These differences were both significant ($p < 0.01$). There was more variability in the plasma E responses following naloxone than in the NE values. However, all animals "responded"; *i.e.* the naloxone-treated twin had higher NE and E values and the results would be similar if expressed as percentage change from baseline prior to cord cutting.

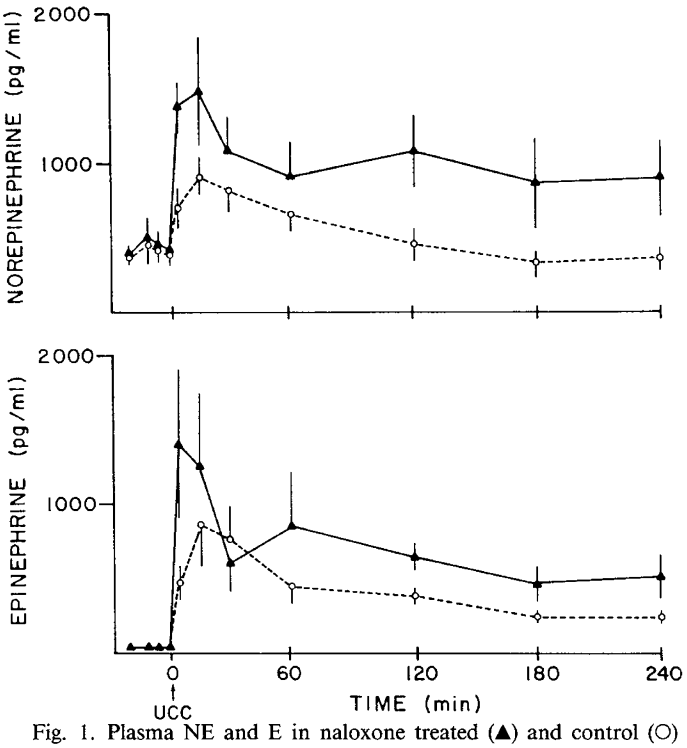

Fig. 1. Plasma NE and E in naloxone treated (▲) and control (○) newborn sheep following delivery. *UCC*, umbilical cord cutting. Naloxone treatment regimen as described in "Materials and methods."

The results of the serial arterial blood gas, heart rate, and blood pressure from naloxone treated and the control animals are shown in Figure 2. The initial increase in pO_2 after cord cutting and ventilation was followed by adjustments in fractional inspired oxygen concentration to maintain a pO_2 of 100–150 mm Hg. Arterial pCO_2 and pH were within physiological limits. The similar arterial blood gas and pH values demonstrated the comparable physiological state of the two groups. Heart rate and systolic blood pressure were both significantly elevated in naloxone-treated animals ($p < 0.01$ by ANOVA). Peak heart rate in the naloxone animals was 207 ± 13 beats per min at 60 min *versus* 184 ± 12 beats per min at 60 min in the controls. Peak systolic pressures were 95 ± 6 mm Hg at 60 min *versus* 88 ± 2 mm Hg at 60 min, respectively.

Serial NE and E levels following the injection and continuous infusion of naloxone at 4 h of age are shown for the control animals in Figure 3. By 4 h of age plasma NE levels had returned to 342 ± 78 pg/ml, a value similar to the baseline level prior to cord cutting (382 ± 76 pg/ml). The plasma E concentration at 4 h of age was 271 ± 24 pg/ml, a level significantly greater than the baseline value of 60 ± 24 pg/ml just prior to cord cutting. Following naloxone administration, there was a small increase in plasma NE concentration to a maximum of 494 pg/ml at 5 min. There was a greater increase in plasma E concentration, which rose to a peak of 509 pg/ml at 2 min. Neither of these

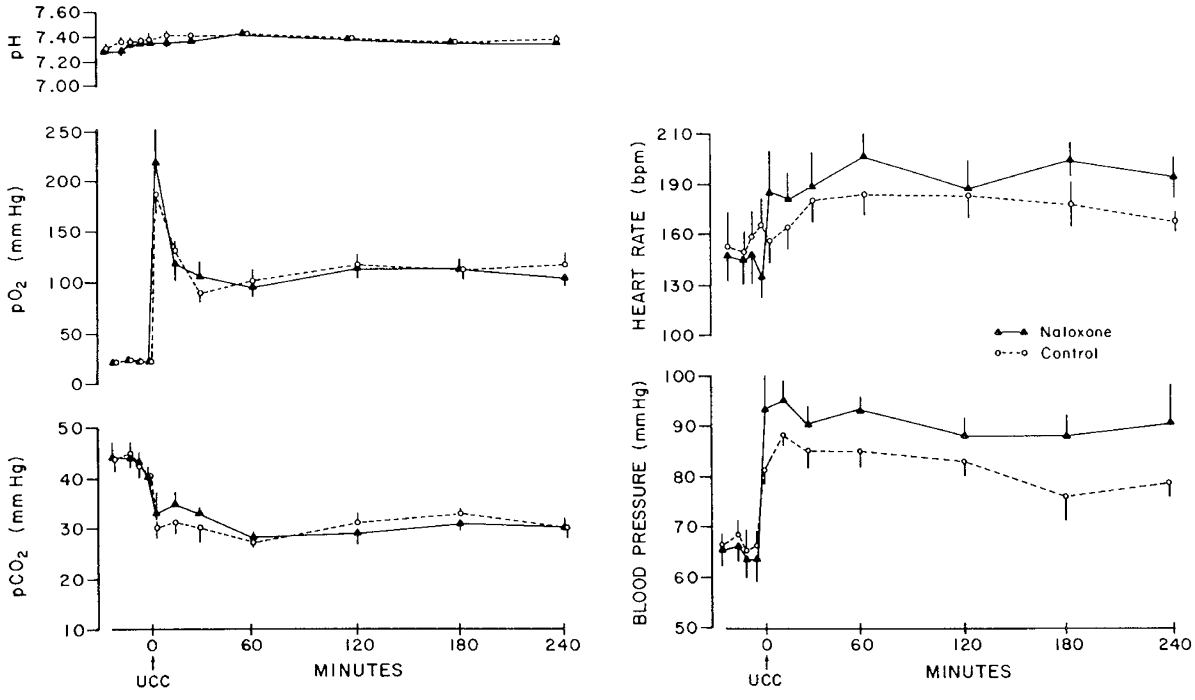

Fig. 2. Serial blood gases, heart rate, and systolic blood pressure in naloxone-treated (▲) and control (O) newborn sheep following delivery. *UCC*, umbilical cord cutting. Naloxone treatment as described in "Materials and methods."

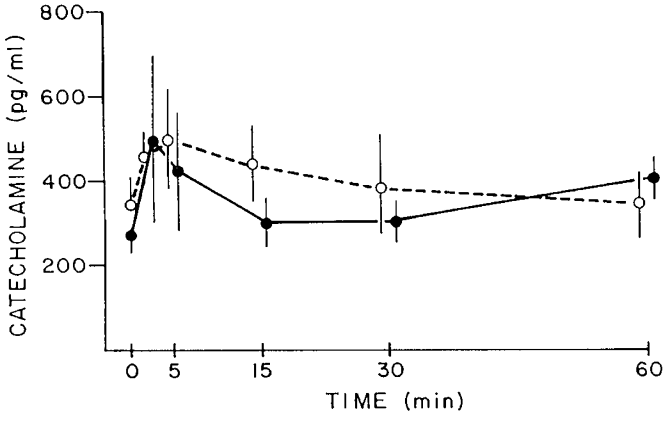

Fig. 3. Plasma NE (O) and E (●) in term sheep treated with naloxone at 4 h of age.

values nor the overall responses were significantly different from the pretreatment values. There were no significant blood gas, heart rate, or blood pressure changes following naloxone administration to control animals at 4 h of age.

DISCUSSION

The present results demonstrate that opiate receptor blockade increases the catecholamine surge at birth. We observed higher concentrations of circulating NE and E in sheep which received a continuous infusion of naloxone from birth when compared to control animals. Not only were peak plasma levels of NE and E greater in the naloxone-infused animals but the higher NE concentrations were sustained throughout the period of observation. The reliability of these differences in plasma catecholamines is supported by the similarity in plasma catecholamine responses of the control animals in this study and the term animals of comparable gestational ages reported in previous studies using this model (6, 19). The physiological importance of these concentration differences is suggested by the elevations in heart rate and blood pressure and seen in the naloxone-infused animals.

It is unclear whether the naloxone was antagonizing opiate peptide inhibition of sympathoadrenal activity centrally or peripherally and whether the higher circulating NE values were derived from sympathetic nerves or adrenal medullary secretion. The distribution of endogenous opiates and their receptors throughout the nervous system suggests their importance in the regulation of autonomic function. Opiate peptides have been localized in sympathetic ganglia (20), preganglionic nerve terminals (20–22), the adrenal medulla (20, 22), and the anterior pituitary (7) and in specific brainstem and hypothalamic nuclei (16). The effectiveness of intracisternal naloxone in shock models at doses which have no effect when administered peripherally suggests that one of their primary actions is to antagonize endogenous opiate inhibition of sympathoadrenal outflow at central autonomic sites. The relatively high dose of naloxone used in this study has been shown to be effective at these central nervous system sites (16, 23). It is also unclear which opiate receptor subtypes were antagonized by this large dose of naloxone. Mu receptors are antagonized at lower doses of naloxone than δ or κ receptors (24), but the dosage used in this study does not permit discrimination of action at opiate receptor subtypes. It is also possible that other neurotransmitter/modulator systems were affected.

A potential role for adrenal enkephalins in the regulation of adrenal medullary catecholamine release in the developing rat has also been suggested. Neonatal rats secrete adrenal catecholamines directly in response to hypoxia or reserpine prior to the development of splanchnic nerve innervation, and thus potential central control (25, 26). Pretreatment with naloxone potentiates whereas methadone inhibits this reserpine-induced adrenal catecholamine depletion in neonatal rats but not adults following the development of an intact splanchnic innervation. In the fetal sheep a similar developmental transition in direct *versus* neural control of adrenal medullary catecholamine secretion takes place between approximately 130 days gestation and term (145–150 days) (27). In the present study we saw an increase in adrenal E

secretion in the naloxone-treated group. However, because these were term sheep it is unclear whether this was due to altered central or peripheral regulation of adrenal E secretion. Further *in vivo* physiological studies are needed to clarify this mechanism.

Plasma catecholamines have been measured before and after induced hemorrhage in adult rabbits and following naloxone administration. Naloxone caused a nearly 100% increase in circulating NE and return of blood pressure to normal with a much smaller increase in plasma E (28). In adult humans with septic shock, naloxone administration evoked a 10-fold increase in plasma E levels associated with improvements in circulatory function (29). Similar improvements in blood pressure and perfusion following naloxone have been observed in septic newborn humans (30) and neonatal piglets (31). In contrast, fentanyl, a potent opiate agonist, causes a dose-dependent inhibition of plasma NE and E in humans (32) and adult dogs (33) as well as decreases in heart rate, blood pressure, and cardiac output (33). The effect on NE is greater than E. These effects are all reversed by naloxone administration.

After 4 h, circulating plasma NE and E concentrations in control animals had returned nearly to baseline values. Naloxone administration at this time had no significant effect on circulating plasma catecholamine levels. These results, considered with the early naloxone infusion results, suggest that endogenous opiate peptides are important inhibitors of sympathoadrenal activity *in vivo* but that this regulation is most important during periods of increased sympathoadrenal activity (34).

The potential role of endogenous opiates in regulating the enhanced sympathoadrenal activity seen at birth is substantial. Elevated levels of circulating endorphins and related peptides are seen in laboring women and newborn infants (35). Significant functional alterations in a variety of organ systems are required for successful neonatal adaptation to extrauterine life. These include changes in the cardiovascular system, pulmonary adaptation, thermogenesis and mobilization of energy substrates, and onset of respiration and establishment of respiratory control. Ample evidence exists to suggest that increased sympathoadrenal activity is vital to these adaptive changes. Central or peripheral regulation of sympathoadrenal involvement in these aspects of neonatal adaptation should provide fruitful areas of investigation of the physiological role of endogenous opiates.

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