Ketamine Attenuates the ACTH Response to Hypoxia in Late-Gestation Ovine Fetus

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Key Words

Endocrinology · Fetal Sheep · Fetus · Hypoxia ·
N-Methyl-D-aspartate receptor

Abstract

Background: Ketamine, a noncompetitive N-Methyl-D-aspartate (NMDA) receptor antagonist, is a commonly used dissociative anesthetic in neonatology. We have proposed that ketamine reduces fetal stress responsiveness to stimuli that involve reduced oxygen supply to the fetal brain. Previously, we have shown that ketamine inhibits plasma ACTH levels in late-gestation fetal sheep subjected to brachiocephalic artery occlusion (BCO), an ischemic hypoxia model that might activate some of the same direct and reflex responses as hypoxia. **Objectives:** We performed the current study to test the hypothesis that ketamine pretreatment will reduce fetal ACTH responses to hypoxic hypoxia (HH). **Methods:** Fetal sheep were chronically catheterized at least 5 days prior to study. Ketamine (3 mg/kg) was administered intravenously to the fetus 10 min prior to normoxia or a period of hypoxia induced by administration of nitrogen gas to the maternal trachea for 30 min. **Results:** Hypoxia significantly increased both fetal ACTH and cortisol levels in both the control and ketamine groups ($p < 0.0005$, interaction effect of time · stimulus in two-way ANOVA), and the ACTH response was blunted by ketamine ($p < 0.005$). **Conclusions:** Ketamine reduced fetal ACTH responses to HH, possibly due to antagonism of the NMDA receptors in the fetal brain. Interestingly,

in contrast to the responses to BCO, ACTH responses to HH were only partially inhibited, suggesting that multiple neurotransmitter pathways mediate the ACTH response to HH.

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Introduction

Ketamine is a unique dissociative anesthetic drug with hypnotic, analgesic, and amnesic properties [1]. The pharmacological properties of ketamine include a fast onset and short duration, and it also preserves respiratory and hemodynamic profiles [2]. Currently, ketamine is used frequently in surgical procedures for inducing anesthesia in neonates, children, animals, and soldiers on the battlefield [1]. Ketamine has also been administered to pregnant women during cesarean delivery [3] and for labor analgesia [4]. Ketamine is both lipid and water soluble [5], thus enabling it to cross the blood-brain barrier [6]. In the brain, ketamine mainly blocks the N-Methyl-D-aspartate (NMDA) receptor to produce an analgesic effect at low concentrations and produce an anesthesia effect at higher concentrations [7]. Recently, ketamine has been shown to possess neuroprotective effects both in vivo and in vitro [8, 9].

We propose that ketamine will decrease hypoxia-induced glutamatergic-NMDA receptor activity, and reduce the HPA response. In our previous work, we have shown that ketamine is able to reduce the fetal plasma

ACTH response to brachiocephalic artery occlusion (BCO), a direct ischemic insult to the fetal brain [10]. The mechanism of response to BCO is likely to be different than to the response to hypoxia, raising the question of whether ketamine suppresses the ACTH response to all hypoxic stimuli (i.e. hypoxia vs. ischemia vs. asphyxia). We therefore performed the present study to test the hypothesis that ketamine reduces the HPA response to hypoxia, similar to its actions with regard to BCO.

Materials and Methods

These experiments were approved by the University of Florida Animal Care and Use Committee and were performed in accordance with the Guiding Principles for Use of Animals of the American Physiological Society. We studied 45 chronically catheterized fetal sheep at a mean gestational age of 126 ± 5 (SD) days (term: 145–147 days). The pregnant ewes were of mixed breeds, and were pregnant with either singleton ($n = 9$) or twin ($n = 18$) fetuses.

Fetal Surgery

All surgical procedures were performed between 115 and 130 days of gestation. The chronic catheterization of fetal and maternal femoral arteries and veins procedures were described previously [10]. Ewes were fasted for 24 h, and received 750 mg of ampicillin (Polyflex[®], Boehringer Ingelheim VetMedica Inc.) preceding anesthesia induction and intubation with 0.5–2% isoflurane with oxygen. Using aseptic techniques, we delivered the fetal hindlimbs from the uterus and surgically placed vascular catheters in both femoral arteries and veins of the fetus, and into the amniotic cavity. In the case of twins, both fetuses were catheterized. Before the uterus was sutured closed, we injected 500 mg of ampicillin into the amniotic fluid. The maternal linea alba and skin were sutured closed in separate layers. We then catheterized both maternal femoral arteries and veins. All catheters were routed subcutaneously to the right flank where they exited the ewe and were gathered into a cloth pouch. To induce maternal hypoxic hypoxia (HH), we surgically implanted a tracheostomy tube (3-mm diameter) in the ewe [11]. After recovery from anesthesia, the ewes received a minimum of 5 days of postoperative treatments before experimentation.

In vivo Experimental Procedures

During the in utero experiments, the ewes were conscious and freestanding in their pens, and were allowed free access to food. We subjected each fetus to only one experiment. The fetal arterial pressure, amniotic fluid pressure, and heart rate (HR) were measured and recorded continuously throughout the entire experiment using standard pressure transducers (Transpac IV; Hospira, Lake Forest, Ill., USA), an analog-to-digital converter (National Instruments, Austin, Tex., USA), and custom-written software (LabView, National Instruments) [10]. The fetal HR was calculated from the phasic arterial pressure signal, and the fetal arterial pressures were corrected by subtraction of the amniotic fluid pressure.

Blood pressure (BP) recording was initiated at least 1 min prior to injection of ketamine. Ketamine (3 mg/kg) was administered intravenously or a sham injection was performed 10 min prior to

30 min of HH. The dose of ketamine was chosen to mimic the clinically used dose [12]. HH was commenced by infusing nitrogen gas directly into the maternal tracheostomy tube to decrease maternal partial pressure of oxygen (P_{aO_2}) from 100 mm Hg to ~50 mm Hg, which corresponds to a decrease of fetal P_{aO_2} from 20 mm Hg to ~10 mm Hg. Fetal and maternal arterial blood samples (5 ml) were collected in K₂EDTA vacutainer tubes at six time points (–10, 0, 5, 10, 20, and 30 min) for measurement of plasma ACTH and cortisol concentrations. Additional samples (1 ml) of fetal and maternal arterial blood were drawn anaerobically into heparinized syringes for measurement of blood gases (ABL80 Radiometer, Copenhagen, Denmark). All blood samples were kept on ice until measurement of blood gases or separation of plasma by centrifugation (3,000 g for 20 min at 4°C). After centrifugation, the plasma was aliquoted into polypropylene tubes and stored at –20°C until the hormones were assayed.

Hormone Assays

Fetal plasma ACTH and cortisol concentrations were measured using commercially available assay kits. Plasma ACTH concentrations were measured using a radioimmunoassay kit (DiaSorin, Stillwater, Minn., USA) according to the manufacturer's instructions. This assay has been previously validated for use in fetal plasma. Plasma cortisol concentrations were measured using an enzyme immunoassay kit (Oxford Biomedical, Oxford, Mich., USA) after ethanol deproteinization. Both of these assays as performed in this laboratory have been completely described elsewhere [10].

Calculations and Statistical Analysis

Data are presented as mean values $\pm$ standard error of the mean (SEM) with consideration for statistical significance at $p < 0.05$. Unless stated, HR, BP, blood gas, pH, and plasma hormone data were analyzed by two-way and three-way ANOVA (time, $\pm$ HH; time, $\pm$ ketamine) with Mixed Procedure of SAS/STAT[®] 9.3, corrected for repeated measures in one dimension (time), and if significant, by a Duncan post hoc test.

Results

Blood Gases

Maternal blood gases and pH measurements are reported in figure 1a–c. Hypoxia decreased the maternal P_{aO_2} from 98 ± 2 mm Hg to 56 ± 2 mm Hg, while during normoxia values remained constant throughout the experiment ($p < 0.0001$, stimulus $\cdot$ time). Maternal partial pressure of carbon dioxide (P_{aCO_2}) decreased from 37 ± 0.5 mm Hg to 32 ± 1 mm Hg during hypoxia, but was unchanged during normoxia ($p < 0.0001$, stimulus $\cdot$ time in two-way ANOVA). The maternal plasma pH increased from 7.47 ± 0.01 to 7.52 ± 0.01 during hypoxia, but did not change during normoxia ($p < 0.0001$, stimulus $\cdot$ time in two-way ANOVA).

Fetal blood gases and pH measurements are reported in figure 1d–f. In both the control and ketamine-treated

Fig. 1. Maternal and fetal P_aO_2 (a, d), P_aCO_2 (b, e), and pH (c, f) were measured 10 min before and during 30 min of HH stimulation in the ewe and HH stimulus $\pm$ ketamine in the fetus (NC = normoxia control; NK = normoxia ketamine; HC = hypoxia control; HK = hypoxia ketamine). Results of the Duncan test for pair-

wise comparison are expressed as: * vs. NC ($p < 0.05$), [^] HC vs. NC ($p < 0.05$), ^a vs. baselines (-10 and 0 min; $p < 0.001$), ^b HC vs. HC baseline (-10 min; $p < 0.05$), ^c HC vs. HC baseline (0 min; $p < 0.005$), ^d HK vs. HK baseline (0 min; $p < 0.001$), and ^e HK vs. HK baseline (-10 min; $p < 0.05$). Data are presented as means $\pm$ SEM.

Fig. 2. Fetal femoral arterial BP 10 min before and during 30 min of HH stimulation. Results of the Duncan test for pairwise comparison are expressed as: # HK vs. NK ($p < 0.05$) and * vs. baselines (average of -10 through 0 min; $p < 0.05$). Abbreviations as in figure 1. Fetal BP and HR values are reported as means $\pm$ SEM. NC, $n = 8$; NK, $n = 9$; HC, $n = 11$, and HK, $n = 11$.

groups, hypoxia decreased fetal P_aO_2 (17 ± 0.7 to 10 ± 0.5 mm Hg for both groups, $p < 0.0001$) and decreased P_aCO_2 (54 ± 0.7 to 52 ± 0.4 mm Hg, $p < 0.001$), resulting in a slight alkalosis. The changes in blood gases during hypoxia were statistically significant when analyzed by two-way ANOVA (stimulus $\cdot$ time: P_aO_2 , $p < 0.0001$; P_aCO_2 , $p < 0.001$; pH, $p < 0.0001$). The consequence of maternal hyperventilation due to hypoxia can be observed in the

Fig. 3. Fetal HR 10 min before and during 30 min of HH stimulation. Results of the Duncan test for pairwise comparison are expressed as: † HC vs. NC ($p < 0.05$), ‡ HC vs. HK ($p < 0.05$), # HK vs. NK ($p < 0.05$), and * vs. baselines (average of -10 through 0 min; $p < 0.05$). Abbreviations as in figure 1. Fetal BP and HR values are reported as means $\pm$ SEM. NC, $n = 8$; NK, $n = 9$; HC, $n = 12$, and HK, $n = 12$.

significant increase of fetal pH ($p < 0.05$, stimulus $\cdot$ time in two-way ANOVA). There was no statistically significant effect of ketamine on the fetal blood gas or pH response to hypoxia.

Fetal Cardiovascular Variables

Analysis of fetal BP (fig. 2a–d) and HR (fig. 3a–d) by three-way ANOVA corrected for repeated measures over

Fig. 4. Fetal plasma concentrations of ACTH and cortisol, and maternal plasma concentration of cortisol. Fetal plasma ACTH (**a**) and cortisol (**b**) concentrations 10 min before and during 30 min of HH stimulation $\pm$ ketamine. **c** Maternal plasma concentrations of cortisol 10 min before and during 30 min of HH stimulation. Results of Duncan pairwise comparisons are expressed as: * vs. NC

($p < 0.05$), # vs. NK ($p < 0.05$), and † vs. HC ($p < 0.005$), a HC vs. HC baseline (-10 min; $p < 0.005$), b HC vs. HC baseline (0 min; $p < 0.01$), and c HK vs. HK baseline (-10 and 0 min; $p < 0.05$). Abbreviations are as in figure 1. All plasma ACTH and cortisol values are reported as means $\pm$ SEM.

time revealed a statistically significant effect of hypoxia and ketamine ($p < 0.05$ for three-way interaction of time, ketamine, and hypoxia on BP, and $p < 0.05$ for three-way interaction for fetal HR). When individual groups were analyzed by one-way ANOVA for repeated measures, BP was increased by hypoxia ($p < 0.001$; fig. 2c) and decreased by ketamine ($p < 0.01$; fig. 2d), but unchanged in the control normoxia group (fig. 2a, b). Ketamine blocked the increase in fetal BP during hypoxia. In the fetuses subjected to hypoxia but not to ketamine, hypoxic stimulus reduced fetal HR for a brief period of time ($p < 0.05$, 11 to 15 and 18 min) when compared to the baseline; however, the fetal HR returned to baseline with prolonged hypoxic

stimulus (fig. 3c). Fetal HR was also not significantly changed in response to hypoxia after ketamine treatment (fig. 3d). Administration of ketamine prior to hypoxia increased HR from 172 ± 4 bpm and reached values of 206 ± 10 bpm during the first 10 min of hypoxia ($p < 0.05$), returning to control levels in the last 20 min (fig. 3d). Ketamine also increased fetal HR in the group not subjected to hypoxia (fig. 3b, $p < 0.05$).

Endocrine Variables

Fetal plasma ACTH was increased by hypoxia in both the control and ketamine groups ($p < 0.0001$, stimulus $\cdot$ time; fig. 4a). Ketamine reduced the fetal ACTH re-

sponse to hypoxia ($p < 0.005$, treatment $\cdot$ time), but did not have an effect on fetal ACTH in the normoxic groups.

As shown in figure 4b, fetal plasma cortisol was increased by hypoxia in both the control and ketamine groups ($p < 0.001$, stimulus $\cdot$ time). In contrast to its effect on fetal plasma ACTH, ketamine did not attenuate fetal plasma cortisol levels when compared with the hypoxic controls. There was also a significant increase in maternal plasma cortisol concentrations during hypoxia, but not during normoxia ($p < 0.0001$, fig. 4c).

Discussion

We have previously reported that ketamine inhibits the fetal ACTH response to BCO, a model of transient brain ischemia and reperfusion in the fetal sheep [10]. The stimulus of HH, used in the present study, differs from BCO in that the stimulus results in a decrease in fetal P_aCO_2 – secondary to maternal hyperventilation – whereas BCO is an asphyxic stimulus. The results of the present study suggest that the ACTH response to BCO and hypoxia share a dependency on NMDA-glutamatergic neurotransmission. In addition, we report that ketamine alters the fetal hemodynamic response to HH.

Ketamine did not alter the magnitude of changes in fetal blood gases or pH, supporting the conclusion that the stimulus intensity was equal in both groups. The fetal BP and HR responses to hypoxia were consistent with responses reported by us [13] and others [14, 15]. Ketamine decreased fetal BP and modified increases in BP and decreases in HR during hypoxia. Whether the effect on BP is purely the result of interruption of the chemoreflex secondary to NMDA blockade, or whether there are opposing but independent effects of hypoxia and ketamine, cannot be discerned from the present experiments. However, interruption of chemoreflex pathways would be consistent with the effect of ketamine to reduce fetal BP and HR responses to BCO [10]. It is also consistent with the known effect of ketamine on fetal chemoreflex control of HR [16]. It is also possible that ketamine produced some vasodilation that countered the vasoconstriction that underlies the redistribution of combined ventricular output during fetal hypoxia [17].

Ketamine blunted the fetal plasma ACTH response to hypoxia, although less dramatically than the decrease in ACTH response to BCO [10]. In a general sense, the inhibition of HPA stress responses by ketamine is consistent with results of studies in adult animals. For example, in adult rats, both acute and chronic treatments of keta-

mine (15 mg/kg) reduced ACTH and corticosterone responses to chronic stress [18]. Ketamine was also shown to inhibit adrenocortical activation in rats undergoing laparotomy stress by decreasing noradrenergic signaling in the hypothalamus [19].

The inhibition of the ACTH response to hypoxia by ketamine suggests that ACTH responses to hypoxia are partially NMDA-glutamate dependent. We know from previous results in this laboratory that NMDA stimulates fetal ACTH secretion [20]. However, it is also known that the fetal ACTH response to hypoxia is dependent on the carotid chemoreflex [10, 21, 22], and that at least one report in the literature suggests that ketamine modulates fetal ovine cardiovascular responses to chemoreceptor stimulation [16]. NMDA receptors are located throughout the afferent sympathetic pathway: nucleus tractus solitarius, rostral ventrolateral medulla, intermediolateral nucleus, and paraventricular nucleus [23]. The modification by ketamine of the cardiovascular response to hypoxia in the present experiments supports the conclusion that the chemoreflex is sensitive to ketamine inhibition, and that blockade of at least a part of this pathway might be the mechanism of the inhibition of ACTH secretion.

While ketamine is thought to work by inhibiting the NMDA receptor, it does have other actions, especially at higher concentrations. Aside from its major action – antagonism of NMDA-glutamatergic receptor – ketamine may have an effect on other receptors: nicotinic acetylcholine, serotonin, dopamine, opioid, muscarinic acetylcholine, and weakly influencing the GABA_A and glycine receptors [24]. However, most of these receptors have less affinity for ketamine than the NMDA receptors and require a higher (10- to 20-fold) concentration of ketamine to obtain the same antagonist effect [25]. While we assume that the blunting of the ACTH response to hypoxia is secondary to blockade of NMDA receptors, we cannot rule out an action of the drug at other receptors. Ketamine can also have actions independent of the chemoreflex response to hypoxia, and was reported to stimulate adrenocortical activity in adult rats [19] and humans [26].

The cortisol response to hypoxia was not attenuated by ketamine. We suspect that the cortisol response to hypoxia in both groups represented the maximal fetal adrenal response to ACTH [27]. In other words, we believe that while the fetal ACTH response to hypoxia was blunted by ketamine, it was still high enough to maximally stimulate the fetal adrenal transiently. This is consistent with our previous observation that ketamine did not reduce the fetal cortisol response to BCO [10, 21, 22].

In conclusion, we have demonstrated that ketamine blunts the fetal ACTH response to maternal hypoxia. The use of ketamine in late pregnancy or in neonates of similar developmental maturity could alter neurohormonal responses to transient hypoxia or brain ischemia, and therefore modify potentially beneficial homeostatic responses. However, it is also possible that ketamine reduces glutamate excitotoxicity and that the reduction in ACTH response is an endocrine indicator of this action.

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