Metabolic and cardiorespiratory responses to hypoxia in fetal sheep: adenosine receptor blockade 

ANDREW CHAU AND BRIAN J. KOOS 
Department of Obstetrics and Gynecology, Nicholas S. Assali Perinatal Research Laboratory, and School of Medicine, The Brain Research Institute, University of California, Los Angeles, California 90095-1740

Chau, Andrew, and Brian J. Koos. Metabolic and cardiorespiratory responses to hypoxia in fetal sheep: adenosine receptor blockade. Am. J. Physiol. 276 (Regulatory Integrative Comp. Physiol. 45): R1805–R1811, 1999.—8-Phenyltheophylline (PT), a potent and specific inhibitor of adenosine receptors, was infused intra-arterially into unanesthetized fetal sheep to determine the role of adenosine in hypoxic inhibition of fetal breathing. PT in normoxic fetuses increased heart rate and the incidence of low-voltage electrocortical activity, rapid eye movements (REM), and breathing. Mean breath amplitude increased by 44%. Hypoxia (preductal arterial PO2 = 14 Torr) induced a metabolic acidemia, a transient bradycardia, and hypertension while virtually eliminating REM and breathing. PT administration during hypoxia enhanced the metabolic acidemia, blocked the bradycardia and hypertension, increased the incidence of REM and breathing, and elevated mean breath amplitude. The results indicate that 1) adenosine is involved in fetal glycogenic and cardiovascular responses to hypoxia, 2) activation of central adenosine receptors mediates about one-half the inhibitory effects of hypoxia on REM and breathing, and 3) the depression of breathing may critically depend on a hypoxia-induced reduction in phasic REM sleep.

Heart rate; blood pressure; brain; breathing; eye movements; sleep; thalamus

ADENOSINE (ADO) concentrations in fetal plasma are about four times greater than maternal levels (35) because of lower fetal arterial PO2 (P02) (28) and possibly placental release of the purine nucleoside (35). Because ADO is a vasodilator in most tissues, these increased levels of ADO are likely involved in vasoregulation of the brain (21), heart (21), ductus arteriosus (28), and the umbilical circulation (32, 34). Hypoxia (P02 = 13 Torr) increases fetal plasma ADO concentrations by about twofold (21), and these elevated ADO levels mediate several fetal responses to acute O2 deficiency, including bradycardia and hypertension, through effects on the autonomic nervous system (18, 20). ADO also contributes to hypoxia-induced metabolic acidemia (20) and to the release of arginine vasopressin (23) and atrial natriuretic peptide (30).

Fetal administration of ADO (25) and ADO analogs (4, 36, 37) inhibits fetal breathing. Because hypoxia increases fetal brain ADO concentrations (24), ADO may be involved in the central mechanism that triggers hypoxic inhibition of breathing. Theophylline, a comparatively weak ADO receptor antagonist, blunts the inhibitory effects of hypoxia on fetal breathing (3, 25), providing further evidence that ADO contributes to hypoxic inhibition. However, these latter findings must be interpreted with caution, because the respiratory effects of theophylline may have been caused by other actions of the drug, such as inhibition of phosphodiesterase.

We recently reported that 8-(p-sulfonylphenyl)-theophylline (SPT), a highly specific ADO receptor antagonist, failed to reverse the inhibitory effects of hypoxia on fetal breathing (20). This inability of SPT to blunt hypoxic inhibition may have resulted from the lack of involvement of ADO receptors in hypoxic inhibition or the reduced antagonism of central ADO receptors caused by a decreased ability of this polar drug to penetrate the fetal brain. This study was designed to determine whether a potent and specific ADO receptor inhibitor with greater access to the fetal brain would abolish the inhibitory effects of hypoxia on fetal breathing. The results indicate that central ADO receptor activation accounts for about one-half, but not all, of the inhibitory effects of hypoxia on fetal breathing.

METHODS

Eleven pregnant ewes (Rambouillet-Columbia cross) were operated on at ∼120 days gestation (∼0.8 term). Under halothane anesthesia, polyvinyl catheters (1.0 mm-ID) were placed in the right brachiocephalic trunk, external jugular vein, and carotid artery of the fetus, and in the amniotic sac (27). Bipolar stainless steel electrodes (Cooner Wire, Chatsworth, CA) were sutured to the medial and lateral canthus of an eye to record eye movements and on the parietal dura to record electrocortical activity (27). The fetus and ewe were allowed to recover from surgery for ≥4 days before experiments were begun.

Fetal pressures were measured with pressure transducers (Cobe Laboratories, Lakewood, CO), and heart rate was determined by a cardiotachometer triggered by the arterial pressure pulse. Fetal arterial and tracheal pressures (minus amniotic fluid pressure), heart rate, electrocorticogram (EOG), and electrocorticogram (ECOG) were displayed on a chart recorder (Grass Instruments, Quincy, MA). Heart rate and arterial and tracheal pressures were sampled at 100 Hz by microcomputer, and minute averages of fetal heart rate, mean arterial pressure, and breathing measurements (number of breaths, inspiratory time, breath interval, and breath amplitude) were stored on disk (8). Blood gases and pH were measured using blood gas electrodes (model 1304, Instrumentation Laboratories), with values corrected to fetal temperature (39.5°C).

Three types of cell surface receptors mediate the physiological effects of ADO: A1, A2, and A3, with A2 subdivided into A2a (high-affinity) and A2b (low-affinity) subtypes. 8-Phenyltheophylline
yline (PT) has high affinity for the A2b receptor, with intermediate affinity for A1 and A2a receptors; PT does not bind to the A3 receptor (12). PT has greater affinity for the A1 and A2 receptors than SPT (12). PT is ~10 times more potent as an ADO receptor antagonist than theophylline, with virtually no phosphodiesterase activity (12). PT was dissolved in a minimum volume of a polyethylene glycol-0.1 N NaCl (50:50 vol/vol) solution and diluted with saline to 20 ml. PT was infused into the right brachiocephalic artery at 1.7 mg·min⁻¹·kg⁻¹ for 3 min, then at 0.25 mg·min⁻¹·kg⁻¹ for 1 h. The dose of PT was calculated by the amount of theophylline required to block fetal ADO receptors (25), with the ~10-fold greater affinity of PT for ADO receptors taken into account. The following studies were performed in varied order on separate days to minimize carryover effects.

Normoxia. PT was administered to nine chronically catheterized fetuses to determine the effects of ADO receptor blockade on breathing activity in the basal state. In separate experiments the vehicle for PT was infused into the right brachiocephalic artery for 1 h to control for possible vehicle effects on the fetus.

ADO. ADO was infused (14 mg·min⁻¹·kg⁻¹) into the right external jugular vein of nine fetuses for 1 h to demonstrate the inhibitory effects of ADO on fetal breathing (25). In other experiments, PT was infused into the right brachiocephalic artery for 1 h while ADO was administered intravenously. These studies determined whether the PT dose was sufficient to block the inhibitory effects of ADO on fetal breathing. Infusions of the vehicle (saline) for ADO were not performed, because previous work has shown that slow infusions of saline do not significantly affect fetal heart rate, mean arterial pressure, or breathing activity (25).

Hypoxia. Fetal hypoxia was induced in 1 h in nine fetuses by having the ewe breathe a hypoxic gas mixture (9% O₂-3% CO₂-88% N₂) from a plastic bag (27). Hypoxia was initiated within 5 min of the start of a breathing episode; these experiments demonstrated the inhibitory effects of hypoxia on fetal breathing. Fetal hypoxia was also induced while PT was infused to determine whether PT would blunt the inhibitory effects of hypoxia on fetal breathing.

Data analysis. Fetal breathing was easily distinguished from characteristic negative deflections (>1 mmHg) in tracheal pressure. Inspiratory time, breath interval, and mean breath amplitude were determined from the digitized tracheal pressure recordings with use of data acquisition software (8). These breathing variables were calculated from respiratory efforts having a breath interval ≥3 s and thus excluded isolated breaths or gasps (9).

The incidence of breathing was used as a measure of respiratory activity because of the episodic nature of fetal respiratory activity. Incidence was determined as the number of breaths per minute from computer analysis and the number of minutes per hour of breathing from visual inspection of the chart recordings. In the latter analysis, breathing was considered to be present if it occurred during ≥20 s of each 1-min epoch (25).

In near-term (~0.8 term) fetal sheep the ECoG is clearly differentiated into episodes of high- and low-voltage electrocortical states, which are associated with fetal breathing (9). Rapid, irregular respiratory activity and rapid eye movements (REM) coincide with low-voltage electrocortical states (LV ECoG), but these movements do not occur during high-voltage states (HV ECoG). In these experiments the incidence of REM, LV ECoG, and HV ECoG was determined from visual analysis of the chart recordings, as previously described (18). Breathing, REM, LV ECoG, and HV ECoG were determined to be present if the activity occurred during ≥20 s of each 1-min epoch (25).

Statistical analysis. Two-hour control measurements for ECoG, REM, and breathing were averaged for comparison with the respective measurements during experimental manipulations. Statistical significance was determined using repeated-measures ANOVA with post hoc comparison by Tukey’s least significant difference criterion. Single comparisons between control and experimental measurements were performed using Student’s t-test. A logarithmic transformation of the data was carried out before analysis when it normalized a skewed distribution. Differences were significant at P < 0.05. Values are means ± SE.

RESULTS

Normoxia. During the control period, PaO₂ (23.2 ± 0.8 Torr), arterial PCO₂ (PaCO₂, 49.9 ± 0.7 Torr), and pH (7.337 ± 0.008) in nine fetuses were within the normal range; after 30 min of PT infusion the fetal PaO₂ decreased slightly (P < 0.05) to 20.9 ± 0.7 Torr and returned to control values within 60 min. PaCO₂ and pH were not significantly affected.

Fetal mean heart rate, 159 ± 5.7 beats/min during the control period, increased by ~18 beats/min during the last 30 min of PT infusion; mean arterial pressure (44.3 ± 2.7 mmHg) was not significantly changed.

PT increased the incidence of LV ECoG by ~33% (Fig. 1) and reduced the incidence of HV ECoG from 21.4 ± 1.0 to 14.9 ± 8.0 min/h. PT administration was also associated with an ~26% increase in the incidence of REM (Fig. 1).

PT stimulated breathing as indicated by a 75% increase in breathing incidence, a nearly twofold rise in the number of breaths per hour (Fig. 1), and a 44% increase in mean breath amplitude (control, 2.5 ± 0.2 mmHg; PT, 3.6 ± 0.4 mmHg; P < 0.05). Inspiratory time (0.54 ± 0.02 s) and breath interval (1.43 ± 0.12 s) were not significantly affected.

In control experiments the vehicle for PT was infused over 1 h in nine fetuses. Fetal arterial blood gases (PaO₂ = 22.2 ± 1.0 Torr, PaCO₂ = 50.7 ± 1.2 Torr) and pH (7.316 ± 0.010) were not significantly affected, nor was heart rate or mean arterial pressure. The incidence of LV ECoG, HV ECoG, REM, and breathing activity was not affected; other respiratory measurements (mean breath amplitude, inspiratory time, and breath interval) also were not significantly affected.

ADO. ADO administration to nine fetuses was associated with a significant reduction in arterial pH for measurements 30 (7.305 ± 0.007) and 60 (7.297 ± 0.004) min after the onset of infusion compared with the control of 7.336 ± 0.005. Fetal PaO₂ (23.9 ± 1.5 Torr) and PaCO₂ (49.5 ± 0.8 Torr) were not significantly affected by ADO. Fetal mean heart rate increased by ~30 beats/min during the last 30 min of ADO infusion, but mean arterial pressure was not significantly affected.

ADO significantly reduced the incidence of LV ECoG (Fig. 2) while increasing the incidence of HV ECoG (control, 20 ± 4.0 min/h; ADO, 32 ± 2.4 min/h; P < 0.05) and virtually abolishing REM and breathing activity.
Breath amplitude (2.5 ± 0.1 mmHg), inspiratory time (0.51 ± 0.04 s), and breath interval (1.45 ± 0.12 s) were not significantly changed by ADO. PT was infused intra-arterially in nine fetuses receiving simultaneous administration of ADO. Compared with control (7.332 ± 0.006), the average arterial pH fell significantly to 7.310 ± 0.008 and 7.304 ± 0.006 at 30 and 60 min, respectively, after the onset of infusions. Fetal PaO₂ (22.3 ± 1.4 Torr) and PaCO₂ (50.3 ± 1.3 Torr) were not significantly altered. Fetal heart rate increased by ~25 beats/min during the last 30 min of infusion, while mean arterial pressure was not significantly changed.

The incidence of LV ECoG, REM, and breathing increased significantly during simultaneous administra-
tion of ADO and PT (Fig. 2). The incidence of HV ECoG decreased significantly from 21 ± 1.3 (control) to 12 ± 2.0 min/h (ADO + PT). These infusions were associated with a 44% increase in breathing amplitude (control, 2.5 ± 0.1 mmHg; infusions, 3.6 ± 0.3 mmHg), but inspiratory time (control, 0.51 ± 0.03 s; infusions, 0.44 ± 0.05 s) and breath interval (control, 1.39 ± 0.10 s; infusions, 1.19 ± 0.12 s) were unaffected.

The overall effects of combined ADO and PT administration on ECoG, EOG, and breathing (Fig. 2) were similar to those observed for PT infusion alone (Fig. 1). These experiments showed that PT (0.25 mg·min⁻¹·kg⁻¹) completely blocked the inhibitory effects of ADO on fetal breathing, indicating that the appropriate dose was used for blocking fetal ADO receptors related to breathing.

Hypoxia. In eight fetuses, preductal PaO₂ fell by ~10 Torr during 1 h of hypoxia (Fig. 3). This acute O₂ deprivation was associated with a minimal decrease in PaCO₂ and a progressive acidemia. The average fetal heart rate fell by ~15% during the first 15 min of hypoxia and increased toward control values during the last 30 min of O₂ deficiency (Fig. 4); mean arterial pressure increased significantly by the end of hypoxia, but the incidence of LV ECoG (Fig. 5) or HV ECoG (control, 18 ± 2.1 min/h; hypoxia, 21 ± 1.0 min/h) was not significantly affected. Hypoxia reduced the incidence of REM ~75% and virtually eliminated breathing (Fig. 5). Breath amplitude, inspiratory time, and breath interval were not significantly affected.

PT was infused into eight fetal sheep in which preductal PaO₂ was reduced by ~10 Torr (Fig. 3). Hypoxia was associated with a slight fall in PaCO₂ and a decrease in pH after 60 min of O₂ deficiency. The fall in pH with PT was significantly greater for measurements at the end of hypoxia and during the first 10 min of the recovery period during hypoxia alone. Hypoxia did not significantly change fetal heart rate, but the average heart rate increased significantly after termination of PT infusion and restoration of normoxia (Fig. 4). Acute O₂ deficiency did not significantly alter mean arterial pressure.

Hypoxia with PT administration was not associated with significant changes in HV ECoG (Fig. 5) or LV ECoG (control, 18 ± 2.2 min/h; hypoxia, 19 ± 2.1 min/h) in the five fetuses in which the ECoG was recorded. Although REM declined during hypoxia, the incidence (15 ± 3.4 min/h) was about twice that observed during hypoxia alone (Fig. 5).

PT also blunted the inhibitory effects of hypoxia on fetal breathing (Fig. 5). Breathing incidence during hypoxia with PT was about five times greater than that during hypoxia alone, an effect that was also reflected in the number of breaths per hour. This incomplete
arrest of hypoxic inhibition was not altered by doubling the dose of PT, which indicated that the central ADO receptors related to breathing were completely blocked. During hypoxia, PT increased mean breath amplitude (control, 2.3 ± 0.1 mmHg; hypoxia, 3.3 ± 0.4 mmHg; P < 0.05) without significantly altering inspiratory time or breath interval. The effects of PT on these respiratory variables during hypoxia were similar to those during normoxia.

**DISCUSSION**

ADO receptor blockade with PT prevented ~50% of the hypoxia-induced decline in the incidence of fetal breathing. The blunting of hypoxic inhibition by PT cannot be accounted for by the greater decline in arterial pH in these fetuses, because acidemia at this level of hypoxemia has virtually no effect on breathing incidence (5). These results with a highly specific and potent ADO receptor antagonist indicate that ADO is critically involved in hypoxic inhibition of fetal breathing. Because hypoxic inhibition is blunted by nonpolar PT but not by polar SPT, ADO A1 or A2 receptors in the fetal brain appear to mediate the respiratory depression associated with a hypoxia-induced rise in brain ADO concentrations (22, 24).

Fetal breathing in sheep (>0.8 term) is associated with REM and LV ECoG states but is inhibited during HV ECoG activity (6, 9). Episodes of rapid, irregular breathing during LV ECoG apparently depend on the excitatory drive of phasic REM sleep (14, 15, 31) rather than changes in arterial blood gases or pH (9). The parallel inhibitory effects of hypoxia on fetal breathing and eye movements (27) suggest that hypoxic inhibition of fetal breathing is related to reduced REM activity, although it is unknown how sleep state and acidemia modulate the hypothesized respiratory pacemaker neurons of the pre-Bötzinger complex (33).

The depressant effects of ADO on fetal breathing also appear to be associated with alterations in behavioral state, and the evidence for this is threefold: 1) ADO suppresses REM and breathing movements (25), 2) ADO receptor antagonism by PT increases the incidence of REM and breathing, and 3) ADO receptor blockade by PT blunts hypoxic depression of REM and breathing activity. Hypoxia (PaO2, ~ 14 Torr) increases fetal brain ADO concentrations through extracellular degradation of adenine nucleotides (22); thus a hypoxia-induced rise in brain ADO may be critical to the decrease in phased REM sleep and breathing activity.

The increased breath amplitude associated with PT administration may be mediated by blockade of ADO receptors associated with bulbospinal synaptic transmission of inspiratory drive to phrenic motoneurons (11). However, the ADO receptors that inhibit fetal breathing are not likely associated with brain stem respiratory neurons, because fetal breathing is not depressed by hypoxia or ADO in fetuses with disruptions of the rostral midbrain (17) or thalamus (19).

We recently showed that the parafascicular nuclear complex (Pf) of the posterior thalamus is crucially involved in hypoxic inhibition of fetal breathing (19). Pf, part of the rostral pole of the “reticular activating system,” participates in cortical activation associated with increased discharge rates in wakefulness and REM sleep and integrates sensorimotor function (2). Thus Pf, or sectors associated with Pf, may contain ADO receptors crucial for inhibition of breathing and REM.

PT failed to abolish hypoxic inhibition, indicating that other modulators/neurotransmitters contribute to
the depression of REM and breathing activity during acute O2 deprivation. The relative importance of ADO and other factors involved in hypoxic inhibition probably depends on the gestational age and the severity of O2 deficiency. Additional mechanisms may include activation of central \(\alpha_{2}\)-adrenergic receptors, which inhibit firing of noradrenergic nerves (1), and a reduction in the synthesis and/or release of excitatory amino acids, which contribute to neuronal hyperpolarization (29). Opioids (38), prostaglandins (16), \(\gamma\)-aminobutyric acid (38), and lactate (13) apparently have little or no role in hypoxic inhibition of fetal breathing.

Incomplete blockade of central ADO receptors cannot be totally excluded as a cause of the inability of PT to prevent completely hypoxic inhibition. However, this possibility seems unlikely, because peripheral administration of lipid-soluble PT has been shown to block central ADO receptors in adult rats (7) and because doubling the dose of PT in our fetal studies had no additional effect in attenuating the hypoxic arrest of breathing or REM.

Intravascularly administered ADO stimulates breathing (via the carotid bodies) in adult rabbits, but it depresses ventilation in newborn rabbits through a central mechanism (39). Increased permeability of the blood-brain barrier in the neonate to ADO may underlie this developmental difference, although the exact mechanism remains to be elucidated (39). Thus, as in the neonate, the inhibitory effects of intravascularly infused ADO in the fetus appear to be mediated through a central action of the purine nucleoside. Such a mechanism is supported by observations that SPT, a peripherally acting ADO receptor antagonist, has minimal effects on the hypoxic arrest of breathing, even though circulating levels of ADO during hypoxemia increase to levels similar to those for intravascular infusions of ADO that depress respiratory activity (21).

Although hypoxic inhibition appears to originate within the brain (10, 26), a peripheral contribution may also be involved in initiating the response. For example, the mean lag time from the onset of hypoxia to the cessation of REM and breathing is about two to three times greater than normal in fetuses with bilateral carotid denervation and vagal section (26). These results suggest that peripheral chemoreceptors trigger a more rapid inhibitory response to acute hypoxemia and may represent a component of the hypoxic depression that is not mediated by ADO.

Intravenous administration of ADO decreased fetal arterial pH without significantly altering arterial blood gases, suggesting that activation of ADO receptors modulates lactic acid production. Surprisingly, this ADO-induced fall in pH occurred in the presence of PT, indicating that the principal ADO receptor involved was not blocked by this antagonist. The metabolic effects of ADO, which may be triggered by adrenergic and/or direct effects on glycolysis (20), may be critical to the development of lactic acidemia during fetal O2 deficiency. This latter possibility is supported by our previous studies in which SPT blunted the hypoxia-induced fall in fetal arterial pH (20). In contrast, the present study showed that PT enhanced the fall in fetal arterial pH during hypoxia. This new finding is probably accounted for by a different distribution of these antagonists within fetal tissues. For example, SPT, a polar blocker, would be expected to have limited access to the fetal brain when administered intravascularly, while the nonpolar antagonist PT would be expected to diffuse freely into brain tissue and block central ADO receptors. The distribution of these ADO receptor antagonists may also differ in peripheral tissues where the polar nature of SPT may limit its access to some receptor types.

In our previous studies (20), SPT was shown to eliminate the bradycardia and rise in mean arterial pressure normally elicited by hypoxia in fetal sheep (>0.8 term). These effects of ADO receptor blockade are confirmed by the present study in which PT blocked hypoxia-induced bradycardia and hypertension. Thus a rise in fetal ADO concentrations (21) apparently triggers these fetal cardiovascular responses to acute O2 deficiency. The similar cardiovascular effects of PT and SPT suggest that the ADO receptors that modulate autonomic control of the fetal cardiovascular system reside outside the blood-brain barrier in association with the peripheral arterial chemoreceptors, circumventricular organs, or autonomic nerves (18, 20).

In summary, PT significantly enhanced fetal acidaemia produced by acute O2 deficiency, suggesting that ADO promotes fetal glycolytic responses to hypoxia. The opposing effects of PT and SPT on hypoxia-induced metabolic acidemia may be explained by the heterogeneity of ADO receptors and their accessibility to these ADO receptor antagonists. About 50% of the inhibitory effects of hypoxia on REM and fetal breathing were abolished by ADO receptor blockade, indicating that ADO has a key role in hypoxic inhibition. The parallel effects of PT in blunting hypoxic depression of REM and breathing are consistent with the hypothesis that hypoxic inhibition of breathing depends critically on a reduction in the phasic REM drive to respiration.

We thank Leland Patron for technical assistance.

This study was supported in part by National Institute of Child Health and Human Development Grant HD-18478.

Address for reprint requests and other correspondence: B. J. Koos, 10833 Le Conte Ave., 22-177 CHS, UCLA School of Medicine, Los Angeles, CA 90095-1740 (E-mail: bkoo@jobyn.medsch.ucla.edu).

Received 27 May 1998; accepted in final form 10 February 1999.

REFERENCES