Purinergic contribution to circulatory, metabolic, and adrenergic responses to acute hypoxemia in fetal sheep

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Giussani, Dino A., David S. Gardner, David T. Cox, and Andrew J. W. Fletcher. Purinergic contribution to circulatory, metabolic, and adrenergic responses to acute hypoxemia in fetal sheep. Am J Physiol Regulatory Integrative Comp Physiol 280: R678–R685, 2001.—This study investigated the effects on femoral vascular resistance, blood glucose and lactate levels, and plasma catecholamine concentrations of fetal treatment with an adenosine receptor antagonist during acute hypoxemia in fetal sheep during late gestation. Under anesthesia, seven fetal sheep were instrumented between 117 and 118 days gestation (term is ~145 days) with vascular and amniotic catheters and an ultrasonic probe around a femoral artery. Six days after surgery, all fetuses were randomly subjected to a 3-h experiment consisting of 1 h of normoxia, 1 h of hypoxemia, and 1 h of recovery. This was done during either intravenous infusion of vehicle or the adenosine receptor antagonist [8-(p-sulfophenyl)-theophylline; 8-SPT] dissolved in vehicle. During vehicle infusion, all fetuses responded to hypoxemia with bradycardia, an increase in arterial blood pressure, and femoral vasoconstriction. Increases in blood glucose and lactate concentrations and in plasma epinephrine and norepinephrine concentrations also occurred in all fetuses during hypoxemia. Fetal treatment with 8-SPT markedly attenuated the bradycardic, hypertensive, vasoconstrictor, glycemic, and adrenergic responses to hypoxemia, but it did not affect the increase in blood lactate concentrations during hypoxemia. These data show that adenosine is involved in the mechanisms mediating fetal cardiovascular, metabolic, and adrenergic responses to hypoxemia in fetal sheep. Fetal treatment with 8-SPT mimics the effects of carotid sinus nerve section on fetal cardiovascular function during hypoxemia, suggesting a role for adenosine in mediating fetal cardiovascular chemoreflexes.

In the sheep fetus during late gestation, acute hypoxemia evokes an integrated cardiovascular, metabolic, and endocrine response that facilitates fetal survival during the period of reduced oxygen availability (13). The fetal cardiovascular responses to acute hypoxemia have been well characterized and include transient bradycardia, an increase in arterial blood pressure, and an increase in femoral vascular resistance (14). The increase in femoral vascular resistance contributes to the peripheral vasoconstriction that redistributes the fetal combined ventricular output in favor of the adrenal, myocardial, and cerebral circulations, which undergo vasodilatation during hypoxemia (7, 13, 14). The fetal metabolic response to acute hypoxemia results in increased blood glucose and lactate concentrations (19).

Control of the fetal cardiovascular responses to acute hypoxemia involves neural, endocrine, and local components. The bradycardia and the initial femoral vasoconstriction during hypoxemia are triggered by the same carotid chemoreflex, as both can be abolished by selective carotid, but not aortic, chemodenervation (13, 14). During hypoxemia, fetal bradycardia is mediated by vagal efferents and femoral vasoconstriction by α-adrenergic efferents (14). Once initiated, fetal peripheral vasoconstriction is maintained during hypoxemia by slower release of vasoactive hormones such as catecholamines (20) and arginine vasopressin (1) into the fetal circulation.

More recently, the purine nucleoside adenosine has been implicated in mediating fetal chemoreflex responses to acute hypoxemia since Koos and colleagues (25) reported that treatment of fetal sheep with the nonselective adenosine receptor antagonist 8-(p-sulphophenyl)-theophylline (8-SPT) prevented fetal bradycardia during acute hypoxemia in a manner similar to bilateral section of the carotid sinus nerves (13, 14). However, the contribution of adenosine to chemoreflex (neural) or chemoreflex-independent (nonneural) mechanisms mediating the redistribution of cardiac output during acute hypoxemia in the sheep fetus remains unknown. In that same study (25), the falls in fetal arterial pH and base excess that accompanied acute hypoxemia were attenuated by fetal treatment with 8-SPT. Koos et al. (25) extrapolated these findings to conclude that adenosine may, in addition, modulate fetal glycolytic responses to acute oxygen deficiency. However, whether adenosine is involved in mediating the actual increase in blood glucose and lactate concentrations during acute hypoxemia in the sheep fetus was not investigated.

Therefore, the present study tested the hypothesis that adenosine mediates fetal chemoreflex and meta-
bolic responses to acute hypoxemia by investigating the effects of fetal treatment with 8-SPT on the femoral vasoconstrictor and the glycemic and lactacidemic responses to acute hypoxemia in late-gestation fetal sheep. The femoral vasoconstrictor response served as an index of fetal cardiac output redistribution and of fetal carotid chemoreflex function. In addition, because increased fetal plasma catecholamines contribute to both vasoconstrictor (13, 14) and metabolic (2) responses to hypoxemia, this study also investigated the effects of 8-SPT on the increase in fetal plasma epinephrine and norepinephrine during acute hypoxemia.

METHODS

Surgical procedures. Food, but not water, was withheld from the pregnant ewes for 24 h before surgery. Seven fetuses were instrumented between 117 and 118 days of gestation (term is 145 days) as described previously (14). In brief, while the mothers and fetuses were under general anesthesia (20 mg/kg iv thiopentone sodium for induction; Intraval Sodium, Dublin, Ireland; 1.5% halothane in 50:50 O2/N2O for maintenance), PVC catheters (Critchly Electrical Products, New South Wales, Australia) were inserted into a fetal femoral artery and vein, with the tips of the catheters in the descending aorta and caudal vena cava, respectively. An ultrasonic flow transducer (2RS with silicone flange; Transonic, Ithaca, NY) was implanted around the other fetal femoral artery (14). Another catheter was placed in the amniotic cavity, the uterine incisions were sewn together in layers, and the abdomen and skin were closed. All catheters were filled with heparinized saline (80 IU heparin/ml in 0.9% NaCl), plugged with brass pins, and exteriorized with the flow probe lead through an incision in the maternal flank.

Postoperative care. Ewes were housed in individual pens, had free access to hay and water, and were fed concentrates twice daily (100 g; Sheep Nuts #6; H & C Bear, Kings Lynn, UK). Antibiotics were administered postoperatively and daily for 3 days to the ewe (9–12 mg im Depocillin; Mycofarm, Cambridge, UK), to the fetus (300 mg iv ampicillin; Penbritin, Smith Kline Beecham Animal Health, Surrey, UK), and into the amniotic cavity (300 mg Penbritin).

Experimental procedure. At least 6 days after surgery, all fetuses were subjected to a 3-h protocol divided into three periods of 60 min: 1 h normoxia, 1 h hypoxemia, and 1 h recovery during a slow fetal intravenous infusion of vehicle (80 IU heparin/ml in 0.9% NaCl). A transparent polyethylene bag was placed over the ewe’s head into which known concentrations of O2, N2, and CO2 were passed at a rate of ~40 l/min. After 1 h of breathing air through the bag (normoxia), hypoxia was induced in the mother for 1 h by switching the gas mixture to 9% O2 in N2 with small amounts of CO2 (18 l/min air: 22 l/min N2; 1–2 l/min CO2). This reduced the fetal descending aortic PaO2 to ~10–11 mmHg without alterations in fetal PaCO2 from baseline. After the hour of fetal hypoxemia, the ewe was returned to breathing air for a further 60 min (recovery).

On a separate day (1–2 days later), isocapnic hypoxemia was also induced during fetal treatment with the nonselective adenosine receptor antagonist 8-SPT (14.5 mg/kg iv bolus followed by intravenous infusion of 1.8 mg·kg−1·min−1; Research Biochemicals) dissolved in vehicle (80 IU heparin/ml in 0.9% NaCl; pH corrected to 7.3 with the use of sodium hydroxide solution) in all fetuses. The dose of 8-SPT employed in this study was based on previous experiments by Koos et al. (25), who reported that fetal treatment with a lower dose of the adenosine receptor antagonist 8-SPT totally abolished the bradycardic response to acute hypoxemia. Fetal treatment with the antagonist started 4 min before the onset of hypoxemia and ran continuously until the end of the hypoxic challenge (25). Experiments involving fetal exposure to acute isocapnic hypoxia with and without 8-SPT were randomized.

During any 3-h experimental protocol, descending aortic blood samples (4 ml) were taken from the fetus at 15 and 45 min of each experimental period to determine blood gases, acid/base status, metabolite and plasma catecholamine concentrations. An additional fetal arterial sample (0.3 ml) was taken 5 min after the onset of hypoxia to confirm that the fetal PaO2 had fallen to the desired level.

In three of the fetuses, ~1 wk later, 8-SPT was also administered (14.5 mg/kg iv bolus and intravenous infusion of 1.8 mg·kg−1·min−1, dissolved in vehicle, 80 IU heparin/ml in 0.9% NaCl; pH corrected to 7.3 with the use of sodium hydroxide solution) during a 1-h period of normoxia, preceded by a 1-h period of baseline and followed by a 1-h period of recovery during vehicle infusion, to determine the effects of the adenosine receptor antagonist alone on circulatory, metabolic, and endocrine variables during normoxia.

At the end of all experiments, the ewes and fetuses were killed with the use of a lethal dose of pentobarbital sodium (200 mg/kg Pentoject; Animalcare, York, UK). The positions of the catheters were confirmed, the flow probe was removed, and the fetuses were weighed.

Measurements, calculations, and biochemical analyses. Fetal arterial pressure was corrected for amniotic pressure. Fetal femoral vascular resistance was calculated by dividing fetal arterial pressure by fetal femoral blood flow. Cardiovascular data collected at 1-s intervals were averaged over every minute. Fetal descending aortic blood gas and acid/base values were determined with the use of an ABL5 blood gas analyzer (Radiometer, Copenhagen, Denmark; measurements corrected to 39.5°C). Blood glucose and lactate concentrations were measured with the use of an automated analyzer (Yellow Springs 2300 Stat Plus Glucose/Lactate Analyzer; YSI, Farnborough, UK). Plasma catecholamine concentrations were determined by high-pressure liquid chromatography with the use of electrochemical detection (10). The limits of sensitivity of the method were 70 pg/ml for epinephrine and 50 pg/ml for norepinephrine. The interassay coefficient of variation for epinephrine and norepinephrine was 7.3 and 6.2%, respectively.

Statistical analyses. Data are expressed as the means ± SE. Cardiovascular variables during the acute hypoxemia protocol are expressed as absolute values averaged every minute. For these absolute data, changes from mean baseline were analyzed by the summary of measures method, previously described in detail (12, 28), to focus the number of comparisons. In brief, the 1-h period of hypoxemia was divided into predetermined intervals known to pick up the salient features of the fetal cardiovascular responses to this challenge (Refs. 11–14): first 15 min (early hypoxemia) and remaining 45 min (late hypoxemia). To provide equivalent time periods of normoxia with which to compare the time periods during hypoxemia, the 1-h episode of normoxia was also divided into an early (first 45 min) and a late (last 15 min) component. In this way, cardiovascular data for the first 15 min of hypoxemia could be compared with the 15 min of normoxic baseline immediately preceding the onset of the hypoxic challenge. In a similar manner, and for similar reasons, the recovery period was also divided into an early (first 15 min) and a late (last 45 min) component. Hence, data for fetal arterial blood pressure, heart rate, femoral blood
flow, and calculated femoral vascular resistance were divided into the following time periods: early (0–45 min) and late (46–60 min) normoxia; early (61–75 min) and late (76–120 min) hypoxemia; and early (121–135 min) and late (136–180 min) recovery.

For each cardiovascular variable, the mean value and the area bounded by the curve during each time period were calculated with the use of a programmed MS-Excel spreadsheet template. Areas were computed by the integration of the areas of trapezia drawn between each consecutive data point and the time axis. Each area was then expressed per unit time (min), by dividing by the time interval, to normalize areas for differences in time interval (12, 28).

Data for changes in plasma epinephrine and norepinephrine concentrations are expressed as the percentage change from baseline during acute hypoxemia to account for differences in basal plasma catecholamine concentrations in individual fetuses.

Statistical significance of any changes in any measured variable within and between treatment groups was assessed with the use of a two-way ANOVA with one repeated measure and the post hoc Tukey’s test. For all statistical comparisons, differences were considered significant when \( P < 0.05 \).

RESULTS

Effects of 8-SPT during normoxia. In normoxia, values for fetal descending aortic blood gases, acid/base status, cardiovascular variables, metabolite and catecholamine concentrations were unchanged by treatment with the adenosine receptor antagonist (Table 1).

Effects of 8-SPT during hypoxemia. In hypoxemia, 5 min after the onset of the challenge, fetal descending aortic PaO₂ fell rapidly to similar values during vehicle infusion (from 20.0 ± 0.8 to 9.3 ± 1.1 mmHg, \( P < 0.05 \)) or treatment with 8-SPT (from 19.0 ± 1.0 to 10.6 ± 0.6 mmHg, \( P < 0.05 \)). Over the whole period of hypoxemia, fetal PaO₂ values were reduced to similar levels during vehicle or 8-SPT infusion (Table 2). Fetal hypoxemia during vehicle or 8-SPT infusion occurred without significant alterations in fetal PaCO₂ from baseline, but it was accompanied by progressive metabolic acidemia. Fetal arterial pH (pHₐ) and base excess fell, and blood lactate concentrations rose, during hypoxemia and recovery after fetal infusion with either vehicle or 8-SPT (Table 2 and Fig. 1). The magnitude of the changes in fetal pHₐ, base excess, and lactate concentrations during hypoxemia and recovery was similar in the two groups. In contrast, although absolute glucose concentrations were not significantly different between the two groups (Table 2), the increment in blood glucose concentrations from mean baseline during acute hypoxemia was significantly greater during vehicle infusion than during treatment with 8-SPT (Fig. 1).

During fetal infusion with vehicle, acute hypoxemia rapidly elicited fetal bradycardia that was transient as heart rate returned toward baseline 10–15 min after the onset of the challenge (Figs. 2 and 3). In addition, acute hypoxemia resulted in a pronounced, sustained increase in fetal arterial blood pressure and an abrupt fall in fetal femoral blood flow that remained depressed until the end of the hypoxic insult (Figs. 2 and 3). This fall in femoral blood flow and the increase in arterial blood pressure resulted in a marked increase in calculated fetal femoral vascular resistance that remained elevated until the end of hypoxemia. Although fetal arterial blood pressure, femoral blood flow, and femoral vascular resistance returned toward baseline values after the end of hypoxemia, a rebound tachycardia occurred during the recovery period (Figs. 2 and 3). In contrast, fetal treatment with 8-SPT markedly attenuated the fetal bradycardia and the increase in fetal arterial blood pressure during acute hypoxemia (Figs. 2 and 3). The fall in femoral blood flow during fetal infusion with 8-SPT was substantially delayed, reaching the low levels measured during vehicle infusion only by the end of the hypoxic challenge (Figs. 2 and 3). During fetal 8-SPT treatment, attenuation of

Table 1. Effects of 8-SPT during normoxia

<table>
<thead>
<tr>
<th></th>
<th>Vehicle Infusion</th>
<th>8-SPT in Vehicle</th>
<th>Vehicle Infusion</th>
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<tr>
<td></td>
<td>N15  N45</td>
<td>N75  N105</td>
<td>N135  N165</td>
</tr>
<tr>
<td>pHₐ</td>
<td>7.34 ± 0.02</td>
<td>7.32 ± 0.01</td>
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<td>PaCO₂, mmHg</td>
<td>56.0 ± 1.2</td>
<td>56.0 ± 1.0</td>
<td>56.0 ± 0.9</td>
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<tr>
<td>PaO₂, mmHg</td>
<td>22.0 ± 1.2</td>
<td>22.0 ± 1.5</td>
<td>21.0 ± 1.5</td>
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<tr>
<td>ABE, meq/l</td>
<td>3.0 ± 0.9</td>
<td>2.0 ± 1.2</td>
<td>2.0 ± 0.9</td>
</tr>
<tr>
<td>Sat Hb, %</td>
<td>57.1 ± 7.8</td>
<td>56.3 ± 8.8</td>
<td>53.1 ± 9.0</td>
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<tr>
<td>[Glucose], mM</td>
<td>0.89 ± 0.08</td>
<td>0.83 ± 0.04</td>
<td>0.87 ± 0.08</td>
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<tr>
<td>[Lactate], mM</td>
<td>1.10 ± 0.03</td>
<td>1.12 ± 0.07</td>
<td>1.15 ± 0.10</td>
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<tr>
<td>Arterial blood pressure, mmHg</td>
<td>51.3 ± 4.5</td>
<td>51.7 ± 3.6</td>
<td>53.8 ± 0.8</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>159 ± 13</td>
<td>167 ± 10</td>
<td>157 ± 2</td>
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<tr>
<td>Femoral blood flow, ml/min</td>
<td>42.6 ± 10.9</td>
<td>39.7 ± 10.3</td>
<td>44.5 ± 12.5</td>
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<tr>
<td>Femoral vascular resistance, mmHg·ml⁻¹·min</td>
<td>1.79 ± 0.61</td>
<td>1.63 ± 0.38</td>
<td>1.46 ± 0.34</td>
</tr>
<tr>
<td>Plasma epinephrine, pg/ml</td>
<td>223 ± 49</td>
<td>193 ± 62</td>
<td>242 ± 22</td>
</tr>
<tr>
<td>Plasma norepinephrine, pg/ml</td>
<td>529 ± 159</td>
<td>713 ± 148</td>
<td>522 ± 83</td>
</tr>
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Values are means ± SE taken at 15 (N15), 45 (N45), 75 (N75), 105 (N105), 135 (N135), and 165 (N165) min of normoxia. Fetuses were infused with vehicle for the first hour, 8-SPT dissolved in vehicle for the second hour, and vehicle again for the third hour (n = 3). pHₐ, arterial pH; PaCO₂, arterial CO₂ partial pressure; PaO₂, arterial O₂ partial pressure; ABE, acid/base excess; Sat Hb, percentage hemoglobin saturation; [Glucose], blood glucose concentration; [Lactate], blood lactate concentration; 8-SPT, 8-(p-sulfophenyl)-theophylline. No plasma epinephrine or norepinephrine concentrations are shown for N135 because blood samples were not taken for hormone analysis at this time point (see METHODS).
and ran continuously until the end of the hypoxemic challenge (bar). Vehicle or antagonist infusion was started 4 min before hypoxemia (H15) and 45 (H45) min of hypoxemia, and at 15 (R15) and 45 (R45) repeated-measures ANOVA hoc analysis indicating a significant main effect of treatment (2-way, repeated-measures ANOVA).

Values are means ± SE taken at 15 (N15) and 45 (N45) min of normoxia, 15 (H15) and 45 (H45) min of hypoxemia, and 15 (R15) and 45 (R45) min of recovery for fetuses during vehicle infusion (n = 6) and during treatment with 8-SPT (n = 7). Significant differences are *P < 0.05, differences by analysis indicating a significant main effect of time (2-way, repeated-measures ANOVA + Tukey’s test).

The hypertensive response and delay of the fall in femoral blood flow resulted in a marked attenuation of the increase in femoral vascular resistance in response to acute hypoxemia (Figs. 2 and 3). However, fetal treatment with 8-SPT during acute hypoxemia did not affect the increase in fetal heart rate or elevations in fetal femoral vascular resistance during the recovery period (Figs. 2 and 3).

Fetal plasma catecholamine concentrations were similar during the baseline period before infusion of either vehicle (epinephrine 103.3 ± 27.3 pg/ml, norepinephrine 747.8 ± 171.5 pg/ml) or 8-SPT (epinephrine 114.1 ± 11.3 pg/ml, norepinephrine 792.0 ± 221.2 pg/ml). During fetal infusion with vehicle, progressive increases in plasma epinephrine and norepinephrine concentrations occurred during acute hypoxemia (epinephrine 524.8 ± 57.3 pg/ml, norepinephrine 3,294.3 ± 274.6 pg/ml; Fig. 4). In marked contrast, fetal treatment with 8-SPT abolished the increase in epinephrine (146.5 ± 29.3 pg/ml) and reduced the norepinephrine (2,137.4 ± 323.3 pg/ml) concentrations during acute hypoxemia (Fig. 4). During recovery, plasma catecholamine concentrations were similar to baseline in both vehicle-infused and 8-SPT-treated groups of fetuses.

**DISCUSSION**

The purine nucleoside adenosine is an intermediate metabolite of cellular ATP that is released from cells under conditions of inadequate tissue oxygenation (36). There is growing evidence that adenosine may regulate carotid body function during hypoxia in adult animals. First, it is known that the carotid body expresses the adenosine A2a receptor gene (23). Second, adenosine has been reported to increase carotid body chemoreceptor fiber activity in the cat (29). Third, increased carotid body afferent discharge as a result of hypoxia can be attenuated by adenosine receptor antagonists (29). Finally, the stimulatory effects of adenosine on ventilation in the adult rat (31) and in humans (35) appear to be mediated within the carotid body.

Previous work by Koos and colleagues (25) introduced the idea that adenosine may also participate in the chemoreflex responses to acute hypoxemia during fetal life. In the fetus, acute hypoxemia abolishes fetal
breathing movements, and fetal survival during the period of oxygen deprivation is dependent on cardiovascular, metabolic, and endocrine responses that redistribute umbilical nutrients and oxygen delivery away from the peripheral vasculature toward the adrenal, myocardial, and cerebral circulations (13). Koos et al. (24) reported that plasma adenosine concentrations increase in the sheep fetus during acute hypoxemia and that treatment of fetal sheep with the nonselective adenosine receptor antagonist 8-SPT prevented the fall in heart rate and the increase in arterial blood pressure during hypoxemia (25). Because 8-SPT does not cross the blood-brain barrier (3, 9), a peripheral action of the antagonist was proposed (25). More importantly, because fetal bradycardia during acute hypoxemia is known to be triggered specifically by the carotid chemoreceptors (13, 14), Koos and colleagues (25) suggested that the site of action of the adenosine antagonist may be at the level of the carotid body in the sheep fetus.

The present study confirms that fetal treatment with the same adenosine receptor antagonist 8-SPT attenuates fetal bradycardia and the increase in fetal arterial blood pressure during acute hypoxemia, and it reports that adenosine receptor antagonism also markedly attenuates the fetal femoral vasoconstrictor response to acute hypoxemia. Because fetal bradycardia and the initial increase in femoral vascular resistance during acute hypoxemia are known to be part of the same carotid chemoreflex (13, 14), these findings provide further strong support for the hypothesis that adenosine mediates cardiovascular responses triggered by the carotid body in the fetus. However, in the present study, fetal treatment with 8-SPT not only markedly attenuated the initial increase in femoral vascular resistance after the onset of acute hypoxemia, but it substantially reduced femoral vasoconstriction throughout the hypoxemic challenge.

The findings of this study therefore suggest that adenosine may, in addition, contribute to nonchemoreflex (endocrine and local) mechanisms mediating the redistribution of the combined ventricular output during acute hypoxemia in the late-gestation sheep fetus.

To further address the mechanisms of action of the adenosine receptor antagonist on the fetal vasocon-
strictor response to acute hypoxemia, the present study also investigated the effects of fetal treatment with 8-SPT on changes in fetal plasma epinephrine and norepinephrine, as increased plasma catecholamines have been suggested to contribute to peripheral vasoconstriction during acute hypoxemia in the late-gestation sheep fetus (13, 20). During hypoxemia, the high concentrations of catecholamines measured in the plasma are of adrenal origin (6, 22). The evidence for this is provided by the study of Jones and colleagues (22), who reported a complete loss of the plasma epinephrine response and a 90% reduction in the plasma norepinephrine response to acute hypoxemia in adrenomedullated fetal sheep during late gestation. The remaining 10% increase in fetal plasma norepinephrine in adrenomedullated fetuses during acute hypoxemia is attributed to spillover from sympathetic nerve terminals (6, 22). Control of the activation of the adrenal medulla to release catecholamines during acute hypoxemia in the sheep fetus involves a neural component that may also be triggered by the carotid body (18) and is mediated by the splanchnic nerves (6, 8). In addition, before 130 days of gestation (as in the present study), the adrenal medulla is able to release catecholamines during hypoxemia by the direct action of hypoxia on the adrenal gland (6, 8, 32). The present study reports that fetal treatment with 8-SPT prevented the increase in fetal plasma epinephrine and markedly reduced the increase in fetal plasma norepinephrine throughout the acute hypoxic challenge. Combined, past and present data therefore suggest that fetal treatment with 8-SPT abolishes the initial femoral vasoconstriction by preventing the actions of carotid body reflexes and may attenuate sustained vasoconstriction during acute hypoxemia by reducing the effects of vasoconstrictors that are released into the fetal circulations by chemoreflex-independent mechanisms, such as the release of adrenal medullary cat-

Fig. 3. Statistical summary of fetal cardiovascular responses to acute hypoxemia. Histograms are the means ± SE of the area bounded by the curve, expressed per minute, for FHR, FBP, FBF, and FVR during the following time periods: early (0–45 min) and late (46–60 min) normoxia, early (61–75 min) and late (76–120 min) hypoxemia, and early (121–135 min) and late (136–180 min) recovery. Fetal cardiovascular responses during vehicle and antagonist infusions are represented by white and black histograms, respectively. Box represents the episode of acute hypoxemia. Saline or antagonist infusion was started 4 min before hypoxemia and ran continuously until the end of the hypoxic challenge (bar). Significant differences are *P < 0.05. †Differences by post hoc analysis indicating a significant main effect of time; ††differences by post hoc analysis indicating a significant main effect of treatment (2-way, repeated-measures ANOVA + Tukey’s test).

Fig. 4. Effect of 8-SPT on fetal plasma catecholamines during acute hypoxemia. Values are the means ± SE of the percentage change from mean baseline at 15 (N15) and 45 (N45) min of normoxia, 15 (H5) and 45 (H45) min of hypoxemia, and 45 (R45) min of recovery. Box represents the episode of acute hypoxemia. Saline or antagonist infusion was started 4 min before hypoxemia and ran continuously until the end of the hypoxic challenge (bar). Significant differences are *P < 0.05. †Differences by post hoc analysis indicating a significant main effect of time; ††differences by post hoc analysis indicating a significant main effect of treatment (2-way, repeated-measures ANOVA + Tukey’s test).
echolamines by the direct actions of hypoxia. The suppression of adrenal catecholamine release in response to direct effects of hypoxia by 8-SPT may be due to blockade of adenosine receptors in the adrenal gland (5). In addition, treatment of fetal sheep with 8-SPT has been reported to attenuate the increase in fetal plasma vasopressin concentrations during acute hypoxemia (26), a response that has been shown to be mediated by carotid chemoreflex-independent mechanisms (11). Remaining increases in femoral vascular resistance late in hypoxemia in fetuses treated with 8-SPT in the present study may therefore be due to influences of other vasoactive agents, counteracting the suppression of chemoreflex and chemoreflex-independent vasoconstrictor mechanisms by 8-SPT.

Previous work by Koos et al. (25) also reported that fetal treatment with 8-SPT attenuated the progressive reductions in fetal brachiocephalic or carotid pHₐ and base excess that accompany acute hypoxemia. The present study reports that fetal treatment with 8-SPT did not affect the fall in fetal descending aortic pH and base excess or the increase in fetal blood lactate concentrations, but it prevented the fetal glycemic response to acute hypoxemia. The reason for the difference in the effect of 8-SPT on the fall in fetal pHₐ and base excess in the present study and that reported by Koos et al. (25) may be due to the sites of blood sampling in the two studies. It is possible that the composition of arterial blood, particularly with regard to H⁺ concentration, in the carotid and brachiocephalic circulations (pseuductus arteriosus) is different to that in the descending aortic circulation (postductus arteriosus) during acute hypoxemia under conditions of adenosine blockade.

The increase in blood glucose concentrations during acute hypoxemia in control fetuses may be due to depression of insulin-dependent glucose uptake by the fetal tissues (20) and/or activation of endogenous glucose production and/or stimulated glycolysis (2, 16, 20, 21). Because fetal treatment with the α-adrenergic antagonist phentolamine prevented the glycemic response but enhanced insulin secretion during hypoxemia (21), both the reduction in insulin-dependent glucose uptake and the increase in glucose production by the fetal tissues may be mediated via sympathetic α-adrenergic pathways under these circumstances. Abolition of the glycemic response to acute hypoxemia after fetal treatment with 8-SPT in the present study may therefore represent an effect of the adenosine receptor antagonist on insulin release and/or on the glucogenic pathways mediated either neurally or via circulating catecholamines or other hormones. It is also possible that 8-SPT may have interfered with the actions of adenosine on ATP-sensitive potassium channels on pancreatic cells that regulate insulin release (33) and on skeletal muscle fibers that regulate glucose uptake (30) during acute hypoxemia. However, the evidence provided in the present study suggests that fetal treatment with 8-SPT may have reduced the glycemic response to acute hypoxemia, at least in part, by interrupting adrenal catecholamine release. Concurrent prevention of the glycemic and adrenergic responses to acute hypoxemia by fetal treatment with 8-SPT supports an important role of increased plasma catecholamines in mediating the increase in blood glucose concentrations during acute hypoxemia in the late-gestation sheep fetus (2).

The increase in circulating lactate concentrations during acute hypoxemia in the fetus may result from an increase in blood and/or tissue glucose availability (27) and increased lactate production by the placenta (34) and by the fetal tissues (15), in particular by those whose O₂ supply is selectively reduced such as the fetal hindlimbs (4). In this regard, it is interesting that fetal treatment with 8-SPT did not significantly affect the lactacidemic response but markedly attenuated the femoral vasoconstriction and hyperglycemia during acute hypoxemia. These findings suggest that organs, in addition to the hindlimbs, contribute to circulating lactate in the fetus during acute hypoxemia. Furthermore, they suggest that the glycemic and lactacidemic responses to acute hypoxemia can be dissociated under certain circumstances. Under normoxic conditions, the placenta releases lactate into the fetal blood at a rate that is ~30% of the turnover rate of fetal blood lactate (34). There are little data on placental metabolism during acute maternofetal hypoxemia, but the available literature suggests that the flux of lactate between the placental and the fetal circulations is, in fact, either reduced or reversed by 4 h of steady-state placentofetal hypoxemia produced by partial compression of the maternal common internal iliac artery (4, 17). However, it is unclear whether an increase in placental lactate production in both groups of animals in the present study contributes to the fetal lactacidemic response to 1 h of acute hypoxemia.

In conclusion, treatment of fetal sheep with the non-selective adenosine receptor antagonist 8-SPT markedly attenuated the bradycardic, hypertensive, vasoconstrictor, glycemic, and plasma adrenergic responses to acute hypoxemia. These results provide strong support to the hypothesis that adenosine mediates fetal carotid chemoreflex and metabolic responses to acute hypoxemia in the fetus and show that the effects of the antagonist on these fetal cardiovascular and metabolic responses may be mediated, at least in part, via suppression of the increase in fetal plasma catecholamines.

**Perspectives**

Previous studies have provided overwhelming evidence for the involvement of adenosine in mediating cardiorespiratory responses to hypoxia in adult animals. The work of Koos and colleagues (25) has extended these observations to suggest that adenosine is also involved in mediating cardiovascular responses to acute hypoxemia in the fetus. The present study demonstrated that treatment of fetal sheep with 8-SPT closely resembles the effects of bilateral section of the carotid sinus nerves on fetal cardiovascular function during hypoxemia (14). This finding strongly supports a role for adenosine in mediating fetal cardiovascular
responses that are specifically triggered by carotid chemoreflexes. Because fetal treatment with 8-SPT also affected the increase in blood glucose and in plasma catecholamine concentrations during acute hypoxemia, a role for adenosine in mediating metabolic responses to acute hypoxemia in the sheep fetus is also implicated. The extent to which metabolic responses to acute hypoxemia are triggered by a carotid chemoreflex remains to be elucidated.

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