β₂-Agonist ritodrine, unlike natural catecholamines, activates thermogenesis prematurely in fetal sheep

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Bassett, John M., and Michael E. Symonds. β₂-Agonist ritodrine, unlike natural catecholamines, activates thermogenesis prematurely in fetal sheep. Am. J. Physiol. 275 (Regulatory Integrative Comp. Physiol. 44): R112–R119, 1998.—Prolonged administration of the β₂-adrenergic agonist ritodrine to fetal sheep increases nonesterified fatty acid mobilization. To investigate whether changes in fetal growth or functional development of brown adipose tissue (BAT) also occur, ritodrine was infused at 5 µg/min iv into eight fetal sheep (6 twins and 2 singletons at 125–128 days of gestation) for 5 days and then at twice this rate for a further 7–11 days. Fetal growth was reduced significantly (P < 0.02) during ritodrine infusion relative to controls (5.8 ± 17.5 vs. 79.7 ± 10.3 g/day), with growth of skeletal muscles ceasing. Ritodrine reduced perirenal BAT weight by 50% from 18.6 ± 1.89 to 9.3 ± 0.60 g (P < 0.01) and its lipid content by >70% from 6.5 ± 0.96 to 1.9 ± 0.45 g (P < 0.01). Mitochondrial protein in BAT was also less (P < 0.002), but GDP binding to uncoupling protein increased (P < 0.05). In similar experiments, epinephrine and norepinephrine increased plasma nonesterified fatty acid initially, but neither altered perirenal BAT composition. The β₂-adrenergic agonist ritodrine appears able to promote lipid mobilization and thermogenesis in utero.

RITODRINE, a β₂-adrenergic agonist, has been widely used in obstetric practice for the prevention of premature labor (3, 18, 31). When administered to the mother to arrest myometrial contractions, placental transfer of the drug (13) leads to passive treatment of the fetus. Our knowledge of the effects of ritodrine on the human fetus, however, remains limited because of the ethical constraints on such research. Use of the chronically cannulated fetal sheep preparation has enabled studies into acute and chronic effects of ritodrine administered to the mother or directly to the fetus. Fetal responses include tachycardia, hyperglycemia, hyperlactacidemia, hyperinsulinemia, increased lipolysis and O₂ consumption, and maturation of lung function (4, 7, 8, 32–35). During the first 24–48 h of prolonged ritodrine infusion to the fetus, hypoxemia and acidemia develop (4, 7, 32, 33). With more prolonged administration, attenuation of responsiveness to the drug is associated with restoration of normoxemia and metabolic, endocrine, and cardiovascular parameters (7, 8). However, the sensitivity of β₂-adrenergic mechanisms to stimulation by catecholamine infusion is markedly attenuated (8).

In the rat, prolonged administration of the β₂-agonist clenbuterol to the mother significantly reduces fetal body weight and skeletal muscle weight, even though it increases the weight of the fetal heart and has an anabolic effect on maternal skeletal muscle (20). It is not known whether prolonged β₂-agonist administration to the fetal sheep influences fetal growth, despite the dramatic effects on fetal metabolism observed when fetal blood ritodrine concentrations are increased to the same range as that measured in human fetuses after delivery subsequent to maternal infusion for tocolysis (13, 32). It also remains to be determined whether repartitioning of nutrients comparable to that observed in postnatal animals (28) occurs in utero. Chronic β₂-adrenergic agonist administration reduces fat deposition and stimulates the thermogenic activity of brown adipose tissue (BAT) in growing rats (28). However, it has been considered widely that lipid mobilization from perirenal BAT in utero is limited, and thermogenesis cannot be switched on by epinephrine (Epi) or norepinephrine (NE) because of inhibitory effects of adenocine and/or prostaglandins produced by the placenta (2, 14, 15). Ritodrine administration to fetal sheep in utero can result in prolonged mobilization of nonesterified fatty acids (NEFA) (8), but concomitant effects on the lipid content and thermogenic activity of BAT remain to be established. The studies on fetal sheep in late pregnancy reported here were designed to examine the hypothesis that chronic fetal exposure to the β₂-adrenergic agonist ritodrine can prematurely activate BAT in utero, even though the natural catecholamines Epi or NE may not do so. To this end, ritodrine was infused into one fetus continuously for a maximum period of 15 days, while its saline-infused twin acted as a control. Effects of the infusion on fetal blood gas status and plasma concentrations of glucose, lactate, insulin, and NEFA were measured. At the end of infusion, perirenal BAT was sampled and analyzed for its lipid, protein, and mitochondrial protein content as well as the thermogenic activity (i.e., GDP binding) of mitochondrial uncoupling protein (UCP). For comparison, we also determined the composition of perirenal BAT obtained from fetuses that had been infused with Epi or NE for a prolonged period during separate investigations (6).

METHODS

Animal preparation. All surgical procedures and experimental protocols were carried out in accordance with a project license approved by the United Kingdom Home Office under the terms of the Animals (Scientific Procedures) Act 1986. To investigate effects of chronic ritodrine infusion on fetal development, 10 Mule × Suffolk crossbred ewes, of known gestational age, mated with Polled Dorset rams and diagnosed as twin-pregnant by ultrasound scanning at 70–90 days gesta-
ritodrine hydrochloride (Yutopar, Duphar Laboratories, South-

tion were used. During surgery, three of these ewes were

20°C. Fetal carcasses were also frozen and stored at

sucrose and 1 mM EDTA before being frozen and stored at

weighed. Perirenal-abdominal adipose tissue depots were

after 12–16 days of infusion. All experiments were termi-

tration of ritodrine in the infusion solution was doubled after

Black syringes and infusion lines covered in black tape were

intravenously with diluent (sterile 0.9% saline containing

oxidation and infused at a rate of 5 µg/min via the femoral

saline containing 0.3% ascorbic acid to prevent ritodrine

singletons. Ritodrine was diluted to 250 µg/ml in sterile 0.9%

one twin of each of the six twin-pregnant ewes and two

aliquot of blood was taken into a syringe for determination of

metabolite and hormone concentrations was separated by

ritodrine infusion was started. Plasma for determination of

adenosine 5′-diphosphate (GDP). We corrected for the amount of [3H]GDP trapped in

symptoms were declining. Observations were insufficient to

agarose, as previously described (4, 6–8). At surgery, 25 mg of

medroxyprogesterone acetate (Depo-provera, Upjohn, Craw-

and back (longissimus dorsi and dorsal cervical muscles) and

hindlimb (biceps femoris, semimembranosus, semitendino-

ultimally characterize the lipid composition of head, back (longissimus dorsi and dorsal cervical muscles) and hindlimb (biceps femoris, semimembranosus, semitendinosus, adductor femoris, gastrocnemius, and lateral extensors) and back (longissimus dorsi and dorsal cervical muscles) and the principal bones of the hindlimb (pelvis, femur, tibia, and metatarsal) were removed, weighed, and measured using procedures described and validated elsewhere (6).

Analyses. Plasma glucose, lactate, and NEFA concentra-

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concentrations in fetal blood were determined by enzymatic spectrophotometric methods, as in earlier studies (6, 8). Insulin was determined by RIA with use of a highly purified preparation of ovine insulin as a standard and charcoal to separate antibody-bound and free insulin (4, 6–8). Perirenal BAT composition was characterized using methods reported earlier (30). Mitochondria were prepared from frozen perirenal adipose tissue, and cytochrome c oxidase activity was measured to assess recovery of mitochondrial protein. Frozen, rather than fresh, tissue was used for this analysis, inasmuch as we have found that identical values are obtained using ovine tissue of either type (K. Firth, L. Clarke, and M. E. Symonds, unpublished observations). The thermogenic activ-

ity of perirenal adipose tissue was assessed from the in vitro activity of the mitochondrial conductance pathway by use of 2 µM GDP, with nonspecific binding measured using 200 µM GDP. We corrected for the amount of [3H]GDP trapped in extramitochondrial spaces by measuring the trapping of

Effects of ritodrine on perirenal BAT composition were

compared with effects of Epi and NE with use of tissue obtained from 10 fetuses infused with Epi, 6 fetuses infused with NE, and 15 control twins during other experiments (6). Perirenal BAT tissue from these fetuses was collected into ice-cold Tris buffer, as described above, and stored at −20°C. Full details of fetal preparation for these experiments and details of Epi and NE infusion protocols have been reported (6). Briefly, Epi was infused at a rate of 1.0 µg/min for 48 or 72 h (0.25–0.35 µg·kg⁻¹·min⁻¹), and NE was infused at 2.0 µg/min for 72 h (0.5–0.7 µg·kg⁻¹·min⁻¹). Then Epi and NE were infused at twice the initial rate until termination after 7–12 days of infusion.

Calculations. An estimate of fetal body weight at surgery was calculated from measurements of the metatarsal length made routinely at this time by use of an equation calculated from observations on 46 fetuses of similar breeding used in other studies in our laboratory (6) with an approach similar to that of Santucci et al. (26). With assumptions that control fetuses grew at a constant rate throughout the period of study and that the growth of all fetuses was similar to that of the one fetus died shortly after recovery from surgery, so six sets

were administered intramuscularly to the ewe. Each fetus

penicillin G procaine and 250 mg of dihydrostreptomycin

vaseline (250 U/ml) throughout the study. Before the

sterile saline (250 U/ml) throughout the study. Before the

surgery. After recovery from anesthesia, ewes were housed

intravenously after cannulation. Similar amounts of antibiot-

ics were administered once daily for a further 3 days after

determined by RIA with use of a highly purified preparation

of ovine insulin as a standard and charcoal to separate antibody-bound and free insulin (4, 6–8). Perirenal BAT composition was characterized using methods reported earlier (30). Mitochondria were prepared from frozen perirenal adipose tissue, and cytochrome c oxidase activity was measured to assess recovery of mitochondrial protein. Frozen, rather than fresh, tissue was used for this analysis, inasmuch as we have found that identical values are obtained using ovine tissue of either type (K. Firth, L. Clarke, and M. E. Symonds, unpublished observations). The thermogenic activity of perirenal adipose tissue was assessed from the in vitro activity of the mitochondrial conductance pathway by use of 2 µM GDP, with nonspecific binding measured using 200 µM GDP. We corrected for the amount of [3H]GDP trapped in extramitochondrial spaces by measuring the trapping of [14C]sucrose.

Effects of ritodrine on perirenal BAT composition were compared with effects of Epi and NE with use of tissue obtained from 10 fetuses infused with Epi, 6 fetuses infused with NE, and 15 control twins during other experiments (6). Perirenal BAT tissue from these fetuses was collected into ice-cold Tris buffer, as described above, and stored at −20°C. Full details of fetal preparation for these experiments and details of Epi and NE infusion protocols have been reported (6). Briefly, Epi was infused at a rate of 1.0 µg/min for 48 or 72 h (0.25–0.35 µg·kg⁻¹·min⁻¹), and NE was infused at 2.0 µg/min for 72 h (0.5–0.7 µg·kg⁻¹·min⁻¹). Then Epi and NE were infused at twice the initial rate until termination after 7–12 days of infusion.

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than fresh, tissue was used for this analysis, inasmuch as we have found that identical values are obtained using ovine tissue of either type (K. Firth, L. Clarke, and M. E. Symonds, unpublished observations). The thermogenic activity of perirenal adipose tissue was assessed from the in vitro activity of the mitochondrial conductance pathway by use of 2 µM GDP, with nonspecific binding measured using 200 µM GDP. We corrected for the amount of [3H]GDP trapped in extramitochondrial spaces by measuring the trapping of [14C]sucrose.

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RESULTS

Effects of the $\beta_2$-agonist ritodrine on fetal growth and development. The infusion of ritodrine into fetal sheep for a period of 12 days during late gestation significantly decreased fetal body weight relative to that of saline-infused controls (Table 1). There was no significant effect on metatarsal length, crown-rump length, or brain weight. Relative to total body weight, ritodrine-infused fetuses were significantly longer than control fetuses (Table 1). The calculated rate of body weight gain of ritodrine-infused fetuses during infusion was significantly less than that of controls; weight gain of ritodrine-infused fetuses virtually ceased during this period (Table 1).

Effects of ritodrine infusion on the weight of most fetal organs were not significant (Table 2). Lung weight was significantly reduced, but adrenal weight was increased, and there were significant increases in heart and pancreas weight relative to fetal weight. There were no significant differences between groups in the weight or length of the hindlimb bones isolated from the carcass. Although the $\sim 20\%$ decrease in weight of the muscles dissected from carcasses of ritodrine-infused fetuses just failed to reach significance ($P = 0.51$), their growth during the period of infusion ($-0.31 \pm 0.71 \text{ g/day}, n = 6$) was significantly less ($P < 0.02$) than that of control fetuses ($2.1 \pm 0.35 \text{ g/day}, n = 6$). Growth of these muscles evidently ceased in ritodrine-infused fetuses. Similar to the crown-rump length measurement, the total length, relative to body weight, of the four hindlimb bones from ritodrine-infused fetuses ($11.0 \pm 0.35 \text{ cm/kg fetus}, n = 6$) was significantly greater ($P < 0.05$) than that of control fetuses ($9.5 \pm 0.43 \text{ cm/kg fetus}, n = 6$).

Table 1. Body weight, body measurements, and growth rates in saline-infused control twins and in ritodrine-infused fetal sheep

<table>
<thead>
<tr>
<th></th>
<th>Control Fetuses</th>
<th>Ritodrine-Infused Fetuses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age at autopsy, days</td>
<td>138 $\pm$ 0.7</td>
<td>137 $\pm$ 0.7</td>
</tr>
<tr>
<td>Time on infusion, days</td>
<td>13 $\pm$ 0.7</td>
<td>12 $\pm$ 0.8</td>
</tr>
<tr>
<td>Fetal wt</td>
<td>2.2 $\pm$ 0.13</td>
<td>2.5 $\pm$ 0.17</td>
</tr>
<tr>
<td>At autopsy, kg</td>
<td>3.7 $\pm$ 0.19</td>
<td>3.1 $\pm$ 0.14*</td>
</tr>
<tr>
<td>Estimated Δ/day during infusion, g</td>
<td>79.7 $\pm$ 10.34</td>
<td>5.8 $\pm$ 17.5†</td>
</tr>
<tr>
<td>Metatarsal length</td>
<td>10.5 $\pm$ 0.20</td>
<td>10.9 $\pm$ 0.25</td>
</tr>
<tr>
<td>At surgery, cm</td>
<td>12.5 $\pm$ 0.16</td>
<td>12.5 $\pm$ 0.32</td>
</tr>
<tr>
<td>Δ/day, mm</td>
<td>1.09 $\pm$ 0.122</td>
<td>0.87 $\pm$ 0.108</td>
</tr>
<tr>
<td>Estimated Δ/day during infusion, mm</td>
<td>1.09 $\pm$ 0.122</td>
<td>0.74 $\pm$ 0.170</td>
</tr>
<tr>
<td>CRL at autopsy</td>
<td>43.7 $\pm$ 0.74</td>
<td>42.6 $\pm$ 0.68</td>
</tr>
<tr>
<td>cm</td>
<td>12.3 $\pm$ 0.57</td>
<td>14.2 $\pm$ 0.55*</td>
</tr>
<tr>
<td>cm/kg</td>
<td>47.7 $\pm$ 1.18</td>
<td>44.9 $\pm$ 0.87</td>
</tr>
<tr>
<td>Brain wt</td>
<td>13.4 $\pm$ 0.54</td>
<td>14.9 $\pm$ 0.61</td>
</tr>
</tbody>
</table>

Values are means $\pm$ SE of 8 animals in each group, except for brain weight and crown-rump length (CRL), where $n = 6$ in each group. Significant differences between control and ritodrine-infused fetuses: $*P < 0.05; †P < 0.01$.

Table 2. Organ and tissue weights and their relation to body weight in saline-infused control twins and in ritodrine-infused fetal sheep

<table>
<thead>
<tr>
<th></th>
<th>Tissue Wt, g</th>
<th>Tissue-to-Body Wt Ratio, g/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Ritodrine</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>Ritodrine</td>
</tr>
<tr>
<td>Heart</td>
<td>25.6 $\pm$ 1.13</td>
<td>25.7 $\pm$ 1.31</td>
</tr>
<tr>
<td>Liver</td>
<td>82.8 $\pm$ 8.4</td>
<td>92 $\pm$ 14.5</td>
</tr>
<tr>
<td>Lungs</td>
<td>110 $\pm$ 10.4</td>
<td>72 $\pm$ 2.7†</td>
</tr>
<tr>
<td>Kidneys</td>
<td>19.2 $\pm$ 1.26</td>
<td>18.8 $\pm$ 1.58</td>
</tr>
<tr>
<td>Spleen</td>
<td>5.5 $\pm$ 0.75</td>
<td>4.4 $\pm$ 0.54</td>
</tr>
<tr>
<td>Pancreas</td>
<td>3.3 $\pm$ 0.25</td>
<td>3.5 $\pm$ 0.13</td>
</tr>
<tr>
<td>Thymus</td>
<td>6.7 $\pm$ 1.09</td>
<td>4.7 $\pm$ 0.81</td>
</tr>
<tr>
<td>Adrenal</td>
<td>0.51 $\pm$ 0.018</td>
<td>0.69 $\pm$ 0.073*</td>
</tr>
<tr>
<td>Thyroid</td>
<td>0.89 $\pm$ 0.112</td>
<td>0.80 $\pm$ 0.094</td>
</tr>
<tr>
<td>Perirenal BAT</td>
<td>18.6 $\pm$ 1.89</td>
<td>9.3 $\pm$ 0.60‡</td>
</tr>
<tr>
<td>Selected skeletal muscles &amp; Selected hindlimb bones</td>
<td>102 $±$ 7.1</td>
<td>80 $\pm$ 7.0</td>
</tr>
</tbody>
</table>

Values are means $\pm$ SE for 8 control and 7 ritodrine-infused fetuses, except for skeletal muscle and hindlimb bones, where dissections were carried out on carcasses from only 6 control and 6 ritodrine-infused fetuses. BAT, brown adipose tissue. Details of muscles and bones dissected from carcass and measured are given in METHODS. Significant differences between control and ritodrine-infused fetuses: $*P < 0.05; †P < 0.01; ‡P < 0.001$.

Effects of ritodrine, Epi, and NE on the composition of perirenal BAT. At autopsy the perirenal fat of ritodrine-infused fetuses differed markedly in appearance from that of control fetuses; it was highly vascularized with little indication of stored fat. The weight of perirenal fat was significantly reduced by ritodrine infusion (Table 3), and the amount of lipid was reduced to $<30\%$ of that in control twins (Fig. 1). By contrast, prolonged infusions of Epi and NE resulted in only small (nonsignificant) decreases in the weight of perirenal fat (Table 2) and its lipid content (Fig. 1). The total amount of protein and mitochondrial protein in perirenal BAT was also significantly decreased by ritodrine infusion by comparison with controls, but GDP binding to UCP in mitochondria increased significantly (Table 3). Epi and NE resulted in some reduction in the total amount of protein in perirenal BAT, but neither significantly altered the amount of mitochondrial protein or GDP binding to UCP in the mitochondria.

Comparison of the effects of ritodrine on fetal metabolism with effects of Epi and NE. Ritodrine infusion increased fetal plasma NEFA and lactate concentrations significantly ($P < 0.001$) for $\geq 72$ h (Fig. 2). Increases in plasma NEFA and lactate observed during similar prolonged infusions of Epi or NE into fetal sheep (6), which are also illustrated in Fig. 2 for comparison with the effects of ritodrine, were far less prolonged, although the maximum increase during Epi infusion was comparable to that during ritodrine infusion (Fig. 2). In Epi- and NE-infused fetuses, NEFA and lactate concentrations had returned to the preinfusion control range within 2–3 days, when values in ritodrine-infused fetuses were still high. Despite the delay of
The major finding of the present study is that chronic ritodrine infusion resulted in a substantial depletion of fetal adipose tissue stores and a concomitant increase in thermogenic activity. This contrasts greatly with the lack of any significant effect of Epi and NE on fetal perirenal BAT. These biochemical changes in perirenal BAT were accompanied by a marked increase in plasma NEFA concentration and a decrease in fetal PaO₂ over the first 2–4 days of treatment commencing at 125 days gestation (full term = 145–147 days), in agreement with our earlier observations (8). Taken together, the observations provide the first evidence that thermogenic activity in perirenal BAT of the fetal sheep can be switched on prematurely in utero without cooling of the fetus or interruption of the umbilical circulation. Amounts of UCP in perirenal BAT of fetal sheep at this stage of gestation remain to be determined, but its activity as assessed by GDP binding remains low until after 140 days of gestation and increases markedly on delivery (10). Functional activation of thermogenesis in utero has been demonstrated previously only during relatively short-term studies, closer to full term, when

Table 3. Perirenal BAT composition in ritodrine- and Epi- or NE-infused fetal sheep compared with that in their saline-infused control twins

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 7)</th>
<th>Ritodrine (n = 8)</th>
<th>Control (n = 10)</th>
<th>Epi (n = 8)</th>
<th>Control (n = 8)</th>
<th>NE (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Perirenal BAT, g</td>
<td>18.3 ± 2.15</td>
<td>9.4 ± 0.69†</td>
<td>24.9 ± 2.47</td>
<td>21.2 ± 1.51</td>
<td>24.6 ± 0.62</td>
<td>19.8 ± 1.73</td>
</tr>
<tr>
<td>Total protein, mg</td>
<td>699 ± 2.15</td>
<td>322 ± 49.3†</td>
<td>1,103 ± 114.8</td>
<td>882 ± 91.0</td>
<td>1,320 ± 67.4</td>
<td>1,009 ± 94.8</td>
</tr>
<tr>
<td>Mitochondrial protein, mg</td>
<td>372 ± 53.5</td>
<td>84.7 ± 12.36†</td>
<td>441 ± 35.7</td>
<td>443 ± 52.0</td>
<td>621 ± 17.6</td>
<td>563 ± 48.8</td>
</tr>
<tr>
<td>GDP binding to UCP, pmoi/mg mitochondrial protein</td>
<td>153 ± 13.6</td>
<td>209 ± 16.8*</td>
<td>115 ± 21.7</td>
<td>124 ± 18.7</td>
<td>143 ± 16.4</td>
<td>144 ± 22.8</td>
</tr>
</tbody>
</table>

Values are means ± SE. UCP, uncoupling protein; Epi, epinephrine; NE, norepinephrine. Significant differences between controls and ritodrine- or Epi- and NE-infused fetuses: *P < 0.05; †P < 0.01; ‡P < 0.002.

~2.0–2.5 days, the calculated rate at which NEFA and lactate concentrations declined in ritodrine-infused fetuses did not differ from that in Epi-infused fetuses (Fig. 2). Plasma NEFA concentrations decreased from the maximum with a t½ of 1.4 ± 0.22 days (n = 8) in ritodrine-infused fetuses and 1.6 ± 0.29 days (n = 10) in Epi-infused fetuses; calculated t½ values for the decrease in plasma lactate were 1.4 ± 0.11 (n = 8) and 1.6 ± 0.14 (n = 11) days in ritodrine- and Epi-infused fetuses, respectively.

Ritodrine also resulted in a significant reduction (P < 0.05) in PaO₂ but no change in PaCO₂ in the femoral artery during the first 72 h of the study (Fig. 3). Fetal plasma glucose was increased significantly (P < 0.005) throughout the first 5 days of ritodrine infusion. During the first 24 h of infusion, this increase was accompanied by a significant increase (P < 0.001) in plasma insulin concentration (Fig. 3).

DISCUSSION

The major finding of the present study is that chronic ritodrine infusion resulted in a substantial depletion of fetal adipose tissue stores and a concomitant increase in thermogenic activity. This contrasts greatly with the lack of any significant effect of Epi and NE on fetal perirenal BAT. These biochemical changes in perirenal BAT were accompanied by a marked increase in plasma NEFA concentration and a decrease in fetal PaO₂ over the first 2–4 days of treatment commencing at 125 days gestation (full term = 145–147 days), in agreement with our earlier observations (8). Taken together, the observations provide the first evidence that thermogenic activity in perirenal BAT of the fetal sheep can be switched on prematurely in utero without cooling of the fetus or interruption of the umbilical circulation. Amounts of UCP in perirenal BAT of fetal sheep at this stage of gestation remain to be determined, but its activity as assessed by GDP binding remains low until after 140 days of gestation and increases markedly on delivery (10). Functional activation of thermogenesis in utero has been demonstrated previously only during relatively short-term studies, closer to full term, when

Fig. 1. Left: lipid in perirenal brown adipose tissue (BAT) of fetal sheep at autopsy at 138 days of gestation after intravenous infusion of ritodrine (R, n = 7) for 12 days starting at 126 days of gestation and their saline-infused control twins (C, n = 7); right: lipid in perirenal BAT of fetal sheep of similar age infused with epinephrine (Epi; n = 8) or norepinephrine (NE; n = 7) and their control twins (C, n = 13). Values are means ± SE. See METHODS for details of infusion rates. ***P < 0.001.

Fig. 2. Plasma nonesterified fatty acid (NEFA) and lactate concentrations in fetal sheep during prolonged intravenous infusion of ritodrine (▲, n = 7) and in other fetal sheep of similar gestational age receiving prolonged intravenous infusions of epinephrine (●, n = 12) or norepinephrine (●, n = 8) and control twin fetuses receiving prolonged intravenous infusion of saline diluent (○, n = 23). Values are means ± SE; some SE values lie within symbol.
the fetus has been ventilated and placental flow interrupted by umbilical cord occlusion (2, 14–16, 23), but the effect of these manipulations on UCP in BAT has not been determined.

The time course of UCP activation in perirenal BAT has not been defined by this study, since characterization of BAT was only carried out at autopsy 12–14 days after the start of ritodrine infusion. A marked reduction in amount of perirenal adipose tissue and the loss of its lipid stores and mitochondrial protein simultaneously with the increase in GDP binding to UCP in mitochondria are more characteristic of perirenal fat from a postnatal lamb that has utilized its lipid stores for maintenance of homeothermy during the first days of postnatal life (1, 11). The depleted appearance of BAT and the close association of high fetal plasma NEFA concentrations, indicative of increased lipolysis, with a period of hypoxemia at the start of ritodrine infusion suggests that these changes resulted from increased expression and activation of UCP in the BAT of ritodrine-infused fetuses during the first 24–48 h of the infusion. We propose, therefore, that an increase in BAT thermogenic activity could be an important contributor to the increased fetal O2 utilization observed during ritodrine infusion by Van der Weyde et al. (32).

The extent to which ritodrine may activate fetal perirenal BAT through activation of β2-adrenergic receptors alone and/or through β3-adrenergic receptors remains to be established. Activity of ritodrine on β3-adrenergic receptors has not been defined, but Norman and Leathard (22) reported that ritodrine and salbutamol appeared to act at atypical β-adrenergic receptors (i.e., β3-receptors) in the rabbit jejunum. In this study, ritodrine, which is normally less potent than salbutamol at typical β2-adrenergic receptors, was eight times more potent than salbutamol (22). Zhao et al. (36) concluded that it is solely the β2-adrenergic receptor that is coupled to thermogenesis in BAT and that it is via this receptor that Epi and NE, as well as more specific β3-adrenergic agonists, induce thermogenesis. Investigations by Rohlf et al. (25) on immortalized BAT cell lines, however, suggested that simultaneous stimulation of all three β-receptor subtypes may produce the greatest increase in UCP gene expression. Rat studies have shown that naturally occurring catecholamines are as potent as selective β2-adrenergic agonists in stimulating BAT function when given by exogenous injection, but it has yet to be determined whether infused NE is less effective at stimulating fetal BAT than NE released from sympathetic nerve endings located close to brown adipocytes or whether ritodrine, NE, or Epi alters the fraction of NEFA directly oxidized within BAT. The extent to which ritodrine may have a greater ability than NE or Epi to overcome inhibitory influences of placental factors such as prostaglandin E2 or adenosine on lipolysis remains unknown. However, this prostaglandin is proposed to inhibit lipolysis acting through its own receptor (16), whereas adenosine acts

Fig. 3. Arterial O2 and CO2 partial pressures (PaO2 and PaCO2) and plasma concentrations of glucose and insulin in femoral arterial blood of fetal sheep during a prolonged intravenous infusion of ritodrine (●, n = 7) and in saline-infused control twins (○, n = 7). Blood samples were collected daily before morning feed and 6 h after start of infusion. Values are means ± SE; some SE values lie within symbol.
through α-adrenergic receptor stimulation (27). This suggests that the mechanism by which ritodrine promotes fetal BAT function is related to its ability to stimulate β2- or β3-adrenergic receptors, rather than by altering the action of placental inhibitory factors.

The quantitative differences in effects of ritodrine and the natural catecholamines on plasma NEFA and lactate concentrations could provide some further insight into differences in their effects on lipolysis in adipocytes and on the changes in glycogenolysis leading to increased plasma lactate concentration. Changes in plasma NEFA probably reflect alterations in the lipolytic rate, since the apparent half-life of [14C]palmitic acid in the plasma of late-gestation fetal sheep is <1 min (24); therefore, it is unlikely that changes in the kinetics of NEFA clearance play an important role in determining plasma NEFA concentrations in the present studies. Changes in plasma lactate concentrations during the initial stages of infusion are probably also a consequence of changes in lactate production due to changes in the rate of glycolysis, rather than a consequence of changes in clearance. The long time course of the exponential decline in fetal plasma NEFA concentration after stimulation of lipolysis in fetuses infused with ritodrine or with Epi and the strikingly similar rates of decline in fetal plasma lactate concentration suggest that substrate depletion could be involved, but the marked temporal differences among the three adrenergic agonists in the time when this exponential decline commenced (Fig. 2) make this explanation unlikely. These rates, therefore, seem likely to reflect functional downregulation of lipolysis and glycogenolysis by common mechanisms, rather than changes in clearance. Desensitization, phosphorylation, and internalization of receptors appear to be proportional to the efficiency of agonist coupling to β-adrenergic receptors (17) and occur far more rapidly than the return of NEFA or lactate concentrations to the normal range. It is possible that differences among Epi, NE, and ritodrine in the time of the maximum increase in NEFA and lactate and onset of the slow declining phase reflect differences in receptor occupancy at the infusion rates used. There is a 50% reduction in the β-adrenergic receptor population of fetal lung tissue within 24 h of starting ritodrine infusion into fetal sheep (34), but effects of longer infusions have not been reported. Whatever the reason, ritodrine resulted in greater and more prolonged stimulation of lipolysis and glycogenolysis than either of the naturally occurring catecholamines. Also, despite its lower infusion rate, Epi was clearly more efficacious than NE in stimulating lipolysis and glycogenolysis. It seems unlikely that differences between ritodrine and Epi or NE in their effects on the local oxidation of NEFA within BAT could play any significant role in bringing about the differences in the morphological and functional changes observed in perirenal BAT, since their relative effects on lipolysis and glycogenolysis were so similar.

It is difficult to determine the extent to which changes in fetal plasma lactate concentration reflect direct actions of the infusions on glycogenolysis within skeletal muscle and other tissues, including the placenta, rather than increased hepatic glycogenolysis. Ritodrine infusion for 24 h increases phosphofructokinase and glycogen phosphorylase activity in the liver and the lung and causes a marked depletion of glycogen in both tissues (34, 35). Changes in fetal plasma glucose, despite the concomitant stimulation of insulin secretion by ritodrine, suggest that the chronology of increased hepatic glucose output was similar to that indicated by changes in plasma lactate concentration. Inhibition of insulin secretion by Epi and NE infusion (6) makes it difficult to compare their effects on plasma glucose with those of ritodrine. Nevertheless, although Epi had a quantitatively greater effect than NE infusion on plasma glucose, the increasing phase of the response was over within the first 6 h of infusion (6). It is relevant that increased activity of hepatic glycogen phosphorylase and glucose-6-phosphatase brought about by hypoxemia is largely inhibited within 4 h of the start of prolonged hypoxemia in fetal sheep (29). This time course is in keeping with the limitation of lactate responses to NE and Epi infusion in the present study and is in marked contrast to the prolonged activation of phosphofructokinase by ritodrine reported by Warburton et al. (35). Ritodrine has been shown to increase delivery of lactate to the fetus from the placenta (32), but this probably results principally from increased delivery of glucose to the placenta by umbilical arterial blood secondary to increased fetal hepatic glycogenolysis. Direct stimulation of glycogenolysis in placental (32), lung (34), and skeletal muscle tissues could contribute to the increase in plasma lactate observed during ritodrine infusion, but when exogenous glucose is infused into fetal sheep, fetal plasma lactate and fructose concentrations increase in proportion to the increase in fetal plasma glucose (9). Labeling patterns of lactate and fructose in fetal plasma during dual-labeling studies indicate that these metabolites are derived from glucose of the fetal pool, rather than from maternal glucose in transit across the placenta (5). Whatever the site(s) of glycogenolysis, the similar relative increases and chronology of changes in plasma lactate to those in NEFA concentration suggest that similar receptors (presumably β2- rather than β3-adrenergic receptors) are involved in the regulation of glycogenolysis and lipolysis in the fetal sheep. However, this does not preclude the possibility that UCP gene expression and the stimulation of thermogenesis within BAT could be regulated by ritodrine at β2- or β3-adrenergic receptors independently of effects on lipolysis.

Inhibitory actions of placental factors such as adenosine or prostaglandin E2 have been considered to explain the inability of Epi or NE to stimulate thermogenesis in utero in normoxic fetal sheep and to protect it from inappropriate stimulation of increased O2 consumption and thermogenesis before parturition (2, 14–16, 23). However, cesarean-delivered lambs are less able than vaginally delivered lambs to use nonshivering thermogenesis to support body temperature in the early neonatal period (10, 12). This difference reflects differences in the amounts of UCP in perirenal BAT...
and its activation, as assessed by GDP binding (12), and is not consistent with the conclusion that placental inhibitory factors have an important role in utero. The activation of UCP and depletion of lipid stores in fetal BAT by ritodrine, without increases in plasma NEFA concentrations, similar to those observed after cord occlusion and simulated delivery (14–16, 23), raise further doubt about the role of placental inhibitory factors in preventing activation of thermogenesis in utero. The rapidity with which core and BAT temperatures change after clamping or release of the umbilical cord (16) must indicate that any increase in fetal temperature observed after cord occlusion is due to alterations in fetal blood flow, since activation of UCP in BAT would be associated with a sustained increase in body temperature (12). Infusions of NE lasting for <4 h, like ritodrine, have been reported to increase fetal O₂ consumption (20), but the significance of this in relation to their contrasting effects on activation of UCP in BAT at the infusion rates studied here is uncertain. The large difference between ritodrine and the natural catecholamines in their ability to promote and sustain lipolysis and glycogenolysis implies that prolonged activation of β-adrenergic receptors may be essential for the premature upregulation of UCP activation. However, the exact reasons for differences between ritodrine and the catecholamines Epi and NE in their efficacy in promoting activation of UCP in utero have not been defined.

Although effects of prolonged ritodrine administration to fetal sheep on plasma hormone and metabolite concentrations reported here are directly comparable to those observed during earlier investigations by us and others (7, 8, 32–35), this study also suggests that prolonged exposure of the fetus to ritodrine leads to a wider retardation of growth, in addition to its effects on perirenal BAT. The mean body weight of ritodrine-infused fetuses was decreased significantly, and it was evident that muscle growth probably ceased during the period of ritodrine infusion, even though the growth of the skeleton and some other organs was largely unaffected. These tissue-specific actions of ritodrine are largely in accord with the effects of chronic Epi or NE infusion on fetal sheep (6) and with effects of the β₂-adrenergic agonist clenbuterol on growth in the fetal rat (20). The pattern of differential retardation in tissue and organ growth observed after chronic β₂-adrenergic stimulation or the prolonged administration of Epi or NE (6) differs markedly from the growth promotion and repartitioning observed during chronic β₂-adrenergic agonist administration during postnatal life (28). Even so, there was evidence for sparing of cardiac muscle growth, an effect not seen in Epi- or NE-infused fetuses (6). Cardiac hypertrophy also occurs in fetal rats infused with clenbuterol (20) and in chronically hypoxemic fetal sheep (21), yet it is minimal in normoxemic fetal sheep infused with Epi or NE (6).

In conclusion, this study provides evidence that prolonged administration of the β₂-adrenergic receptor agonist ritodrine can promote activation of UCP in brown adipocytes and lead to severe depletion of lipid reserves in the fetal sheep in utero, even though the increase in fetal plasma NEFA concentration is not comparable to that observed during the early postnatal period. The consequences of this and of the adverse effects on skeletal muscle development observed in the fetal sheep for postnatal adaptation and later development remain unknown.

Perspectives

The present study confirms the importance of stimulatory factors in promoting lipolysis and thermogenic activation of fetal BAT in a preocclusal species (12). Primary criteria that appear to determine this response are the type and duration of exposure to β-adrenergic stimulation. This could explain why acute infusion of isoproterenol into hypothermically, oxygenated fetuses, for example, has little effect on lipolysis (15). It is possible that chronic stimulation of the ovine fetus could overcome placental inhibitory effects on lipolysis, although the full extent to which placental factors limit the amount and activity of UCP remains to be established. Attenuated responsiveness of BAT to naturally occurring catecholamines during prolonged exposure may be protective or adaptive to prevent its premature activation in utero. Nevertheless, the results extend the range of adverse consequences for fetal development that may result from passive fetal exposure to selective β₂-adrenergic receptor agonists administered to the mother for prevention of threatened premature labor. The possible effects of such profound changes in fetal growth on postnatal development clearly merit further exploration.

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