Prolactin and the expression of suppressor of cytokine signaling-3 in the sheep adrenal gland before birth

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Submitted 12 April 2006; accepted in final form 23 June 2006

Gentili, S., J. S. Schwartz, M. J. Waters, and I. C. McMillen. Prolactin and the expression of suppressor of cytokine signaling-3 (SOCS-3) in the sheep adrenal gland before birth. Am J Physiol Regul Integr Comp Physiol 291: R1399–R1405, 2006.-The fetal pituitary-adrenal axis plays a key role in the fetal response to intrauterine stress and in the timing of parturition. The fetal sheep adrenal gland is relatively refractory to stimulation in midgestation (90–120 days) before the prepartum activation, which occurs around 135 days gestation (term = 147 ± 3 days). The mechanisms underlying the switch from adrenal quiescence to activation are unclear. Therefore, we have investigated the expression of suppressor of cytokine signaling-3 (SOCS-3), a putative inhibitor of tissue growth in the fetal sheep adrenal between 50 and 145 days gestation and in the adrenals of the growth-restricted fetal sheep in late gestation. SOCS-3 is activated by a range of cytokines, including prolactin (PRL), and we have, therefore, determined whether PRL administered in vivo or in vitro stimulates SOCS-3 mRNA expression in the fetal adrenal in late gestation. There was a decrease (P < 0.005) in SOCS-3 expression in the fetal adrenal between 54 and 133 days and between 141 and 144 days gestation. Infusion of the dopaminergic agonist, bromocriptine, which suppressed fetal PRL concentrations but did not decrease adrenal SOCS-3 mRNA expression. PRL administration, however, significantly increased adrenal SOCS-3 mRNA expression (P < 0.05). Similarly, there was an increase (P < 0.05) in SOCS-3 mRNA expression in adrenocortical cells in vitro after exposure to PRL (50 ng/ml). Placental and fetal growth restriction had no effect on SOCS-3 expression in the adrenal during late gestation. In summary, the decrease in the expression of the inhibitor SOCS-3 after 133 days gestation may be permissible for a subsequent increase in fetal adrenal growth before birth. We conclude that factors other than PRL act to maintain adrenal SOCS-3 mRNA expression before 133 days gestation but that acute elevations of PRL can act to upregulate adrenal SOCS-3 expression in the sheep fetus during late gestation.

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gestation, after which the PRL receptors are localized to the adrenal cortex (10). It is unknown, however, whether PRL acts to regulate SOCS-3 expression in the adrenal before birth. To assess whether SOCS-3 expression in the fetal adrenal is tonically regulated by PRL, we have investigated the effect of infusion of the dopaminergic agonist, bromocriptine, which suppresses endogenous PRL secretion, on fetal adrenal SOCS-3 expression during late gestation. We have also investigated whether acute PRL administration results in increased SOCS-3 expression in the fetal adrenal in vivo. Furthermore, we have examined the effect of PRL action in vitro on SOCS-3 expression in fetal adrenocortical cells.

The fetal adrenal plays a major role in the fetal response to intrauterine stress. In ewes in which the growth of the placenta has been experimentally restricted from conception, the fetus is growth restricted, and it has been shown that the growth of the fetal brain and adrenal are relatively spared (27). Furthermore, growth-restricted fetuses are chronically hypoxic, and plasma cortisol concentrations are elevated; however, this occurs in the absence of an increase in circulating ACTH concentrations (36). It is currently unclear how intrauterine growth restriction and exposure to chronic intrauterine hypoxia result in an upregulation of fetal adrenal growth and steroidogenesis. It has previously been demonstrated that plasma PRL concentrations are low in chronically hypoxic, placently restricted fetal sheep, and one possibility is that this results in a relative decrease in adrenal SOCS-3 expression, which, in turn, results in a relative increase in adrenal growth and/or steroidogenesis. We have, therefore, determined the impact of fetal growth restriction, induced by experimental restriction of placentation growth, on fetal adrenal SOCS-3 expression in late gestation.

MATERIALS AND METHODS

Animals. All procedures were approved by the Animal Ethics Committee of The University of Adelaide. Ewes were housed at The University of Adelaide in individual pens in rooms with a 12:12-h light-dark cycle and provided with food and water ad libitum throughout the duration of the experiment.

Ontogeny study. Twenty-one pregnant Merino ewes carrying either singleton or twin fetuses between 54 and 144 days gestation (term = 147 ± 3 days) were used in this study. Ewes were killed by intravenous overdose of pentobarbitonal sodium. Fetuses (54 days, n = 4; 90–110 days, n = 5; 131–133 days, n = 5; 140–144 days, n = 7) were delivered, weighed, and killed by decapitation. Fetal adrenals were collected, weighed, and snap frozen in liquid nitrogen and stored at −80°C for subsequent analysis.

In vivo prolactin study. Surgery was performed on 11 pregnant ewes (5 ewes carrying singleton fetuses, 5 ewes carrying twin fetuses and 1 ewe carrying triplets) between 117 and 124 days gestation under aseptic conditions, as previously described (6). Surgery was performed on each singleton fetus, one fetus in three of the twin pregnancies, both fetuses in the other twin pregnancies, and on one triplet fetus (n = 13 fetuses). General anesthesia was induced by an intravenous injection of sodium thiopentone (1.25 g Pentothal; Rhone Merieux, Pinkenba, QLD, Australia) and maintained with 2.5–4% halothane (fluothane; ICI, Melbourne, VIC, Australia) in O2. Fetal vascular catheters were implanted in a fetal carotid artery and jugular vein. All catheters were filled with heparinized saline, and fetal catheters were exteriorized through an incision made in the ewes’ flank. Ewes and fetuses received an intramuscular injection of antibiotics (procaine penicillin, 250 mg/ml; dihydrostreptomycin, 250 mg/ml; and procaine hydrochloride 20 mg/ml; Penstrep Illium; Troy Laboratories, Smithfield, NSW, Australia) at the time of surgery. Animals were allowed to recover from surgery for at least 4 days before experimentation.

Fetuses were randomly assigned to either a bromocriptine (Bromo; n = 8) or a saline (Sal; n = 5) infusion group. Bromocriptine (0.03 µg/h) or saline was infused intravenously for a 48-h period between 124 and 138 days gestation. After 48 h of continuous infusion, fetuses infused with bromocriptine were administered either a saline (Bromo + Sal; n = 4) or a single bolus of ovine PRL (oPRL) (60 µg/kg of predicted fetal body weight; Bromo + oPRL) [National Hormone and Pituitary Program, NIDDK, Baltimore, MD]; n = 4), and fetuses infused with saline were administered a saline bolus (Sal + Sal; n = 5). The bromocriptine or saline infusion continued postbolus administration. Fetal arterial blood samples (2.5 ml) were collected into heparinized tubes at 24 h and again immediately before the start of the infusion. Additional blood samples were collected at +24 h and +48 h after the start of the bromocriptine infusion and at regular intervals (between +5 to +240 min) after administration of the oPRL or saline bolus. Samples were centrifuged at 1,500 g for 10 min at 4°C, and the plasma was stored at −200°C for subsequent determination of PRL concentrations. In addition, fetal arterial blood (0.5 ml) was collected to assess blood gas and pH status of the fetus (ABL 520 Blood Gas Analyzer, Radiometer, General Microsystms, Copenhagen, Denmark). Post mortem was performed at between 15 and 240 min after administration of the saline or oPRL bolus. Fetal organs were removed and weighed, and samples of the fetal adrenal were snap frozen in liquid nitrogen and stored at −80°C for analysis.

In vitro prolactin study. Adrenal cortical cells were isolated and cultured from adrenals collected from fetuses between 144 and 147 days gestation (n = 7). Adrenals were quickly dissected free from connective tissues and placed in sterile HEPES dissociation buffer (HDB; 137 mM NaCl, 5 mM KCl, 0.7 mM Na2HPO4, 25 mM HEPES, 10 mM glucose) for dissociation and culture of adrenocortical cells, as previously described (42). Briefly, the adrenal was cut longitudinally, and the medulla was teased free from the cortex, which was then separated from the capsule. The cortex was minced, and adrenocortical cells were digested in collagenase type I (0.4% in HDB; Sigma, St. Louis, MO) and deoxyribonuclease (1:1,000 dilution, Sigma) followed by a brief digestion in trypsin (0.5 mg/ml in HDB, Sigma). Cells were washed by centrifugation three times in culture media (Dulbecco’s modified Eagle’s medium and Ham’s F-12 medium mixed 1:1, 10% fetal calf serum; all from Gibco BRL, Victoria, Australia), with the addition of penicillin and streptomycin sulfate in 0.85% saline (100 units/ml and 100 µg/ml, respectively; Gibco BRL). Isolated cortical cells were combined from twin fetuses to increase the final cell yield. Viable cells were determined by trypsin blue exclusion, and cells were plated in 24-well culture plates (Falcon, Franklin Lakes, NJ) in 1 ml culture medium at a density of 2 × 10⁵ cells/well. Culture medium was refreshed every 24 h. Forty-eight hours post-isolation, cells were incubated in the presence of oPRL (National Hormone and Pituitary Program, NIDDK, Baltimore, MD) at either 0, 10, 20, or 50 ng/ml (n = 4 replicate wells per oPRL concentration tested) in serum-free incubation media (FCS replaced by 0.1% FBS; Sal; n = 5). The bromocriptine or saline infusion continued to increase the final cell yield. Viable cells were determined by trypsin blue exclusion, and cells were plated in 24-well culture plates (Falcon, Franklin Lakes, NJ) in 1 ml culture medium at a density of 2 × 10⁵ cells/well. Cells were cultured at 37°C in the presence of 5% CO2, and culture medium was replenished every 24 h. Forty-eight hours post-isolation, cells were incubated in the presence of oPRL (National Hormone and Pituitary Program, NIDDK, Baltimore, MD) at either 0, 10, 20, or 50 ng/ml (n = 4 replicate wells per oPRL concentration tested) in serum-free incubation media (FCS replaced by 0.1% Polypep, Sigma) for 1 h. After incubation, media were collected, cells washed twice in HDB, and culture plates were stored at −80°C for analysis.

Placental restriction study. Sixteen ewes between 140 and 144 days gestation were used in this study. In 9 ewes (placental restriction group, PR), surgery was performed to remove the majority of the endometrial caruncles from the uterus of the nonpregnant ewe before mating, as previously described (7). Following the carunculectomy surgery, the ewes were monitored for 4–7 days and were mated after ~10 wk. Ewes in the control (n = 7) and PR (n = 9) groups were transported to the animal holding facility, and fetal adrenals were collected at post mortem between 140 and 144 days gestation, weighed and snap frozen in liquid nitrogen, and stored for subsequent analysis.

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Total RNA extraction and quantification of SOCS-3 and β-actin mRNA expression by RT-PCR. Total RNA was extracted from adrenal samples collected from fetuses in the Ontogeny study (n = 21), the Placental Restriction study (n = 16) and in the in vivo PRL study after administration of bromocriptine and/or oPRL (n = 13), as previously described (12). Total RNA was also extracted from cultured adrenocortical cells. Cortical cells were lysed on the culture plate, and total RNA was extracted using a commercially available kit (RNaseq Mini Kit, Qiagen, Valencia, CA). The spectrophotometric absorbance at 260 and 280 nm was used to determine RNA quality and concentration. The quality of the RNA was determined by electrophoresis on a denaturing agarose gel with ethidium bromide staining to visualize 28S and 18S ribosomal RNA using a UV transilluminator.

Ovine SOCS-3 and β-actin mRNA were amplified using RT-PCR validated and optimized for use in sheep tissues (12). cDNA was generated by reverse transcription of 5 μg total RNA with random-sequence hexanucleotides (GeneWorks, Adelaide, Australia), using Super-Script III (Invitrogen, Victoria, Australia), as recommended by the manufacturer. A fragment of ovine SOCS-3 and β-actin cDNA were amplified through 31 cycles of 60 s at 96°C, 60 s at 58°C and 90 s at 72°C (Hybaid PCR Express, Teddington, UK) from 3 μl of 1:1 diluted reverse transcription product using native Taq DNA polymerase (Invitrogen, Victoria, Australia), as recommended by the manufacturer with 5'-GCTGGTGGTGAACCGAGTGC-3' and 5'-GGTTCTTGACGCTAGGGTG-3' as SOCS-3 primers and 5'-TGTTTGGGTATATGGGTC-3' and 5'-TAGATGGGCCACA-GTGTGGGTTATATGGGTC-3' as β-actin primers (GeneWorks, South Australia, Australia). These produced a 164 bp fragment of ovine SOCS-3 cDNA and a 349-bp fragment of ovine β-actin cDNA, both previously validated for use in sheep tissues (12, 51). RT-PCR products (15 μl) were electrophoresed through a 2% (wt/vol) agarose gel in TBE buffer. PCR products were visualized using ethidium bromide, and the gel was scanned using the Molecular FX Scanner (Bio-Rad, Hercules, CA). The band intensity of the RT-PCR products was determined using Quantity One software (Bio-Rad). Molecular sizes of PCR products were estimated by comparing their electrophoretic migration with those of fragments of pUC19 ds DNA digested with Hpa II (GeneWorks, Adelaide, Australia). The mobility of the SOCS-3 fragment was intermediate between that of 147 and 190 bp markers and was calculated to have a molecular weight in the in vitro cultures of adrenocortical cells incubated with increasing concentrations of oPRL were compared using a one-way ANOVA. Statistical significance for all analyses was taken as a probability value of less than 0.05 (P < 0.05).

**RESULTS**

**Ontogeny of SOCS-3 expression in the fetal adrenal.** The relative weight of the fetal adrenal was highest at 54 days gestation and decreased (P < 0.001) and then remained constant between 95 and 144 days gestation (Fig. 1A). The relative expression of SOCS-3 in the fetal adrenal was highest between 54 and 133 days gestation and then decreased significantly.

**Statistical analyses.** All data are expressed as the means ± SE. All SOCS-3-β-actin mRNA data were generated from duplicate PCR assays for each animal or in vitro treatment group. Relative adrenal weight in the different gestational age groups and the level of SOCS-3 mRNA expression in adrenals in the different gestational age groups were compared using a one-way ANOVA and the Duncan’s post hoc test to determine significant differences between mean values. Mean fetal weight, relative adrenal weight, and adrenal SOCS-3 expression in the placental restriction study were compared using a Student’s t-test. Mean plasma PRL concentrations were determined during the following time periods: 24 h before the start of the bromocriptine or saline infusion, 48 h during the bromocriptine or saline infusion and after the administration of the oPRL or saline bolus on the day of post mortem. Differences in mean plasma PRL concentrations between saline- and bromocriptine-infused fetuses before and during the infusion period were compared using a two-way ANOVA. There was no difference in adrenal SOCS-3 mRNA expression at between 15 and 240 min after the administration of the oPRL bolus, therefore the SOCS-3 mRNA data in the Bromo + oPRL group were pooled, and the mean values in the Sal + Sal, Bromo + Sal and Bromo + oPRL groups were compared using a one-way ANOVA. Relative adrenal weight, mean fetal arterial PO2, and arterial blood pH in the PRL study were compared using a one-way ANOVA. SOCS-3 expression levels in the in vitro cultures of adrenocortical cells incubated with increasing concentrations of oPRL were compared using a one-way ANOVA. Statistical significance for all analyses was taken as a probability value of less than 0.05 (P < 0.05).
(P < 0.005) by 141–144 days gestation (Fig. 1B). There was no relationship between SOCS-3 mRNA expression in the adrenal and either the absolute or relative weight of the adrenal in late gestation.

**Effect of bromocriptine and exogenous PRL on fetal plasma PRL concentrations.** There was no difference between experimental treatment groups or between singleton and twin fetuses in mean fetal arterial PO2 or arterial blood pH (data not shown). There was no difference in mean plasma PRL concentrations before the start of either the saline or bromocriptine infusion (Table 1). During the 48-h infusion period, plasma PRL concentrations decreased in all the bromocriptine-infused fetuses compared with saline-infused fetuses (Table 1). On the day of post mortem, plasma PRL concentrations were higher (P < 0.05) after the administration of the oPRL bolus (Table 1).

**DISCUSSION**

We have demonstrated for the first time that SOCS-3 is expressed in the fetal sheep adrenal throughout gestation and that suppression of endogenous circulating PRL does not decrease adrenal SOCS-3 expression. Acute administration of oPRL, however, increased the expression of SOCS-3 mRNA in the fetal adrenal both in vivo and in vitro. Adrenal SOCS-3 mRNA expression was not different in growth-restricted and normally grown fetal sheep in late gestation.

SOCS-3 expression has previously been demonstrated in a range of tissues in the adult mouse (46), as well as in the adrenal of the adult rat (47). We have also reported that SOCS-3 is expressed in a range of tissues in the fetal sheep, including the liver and perirenal adipose tissue during late gestation (12). In the present study, we have demonstrated that SOCS-3 is expressed in the fetal adrenal from as early as 54 days gestation and that adrenal SOCS-3 mRNA expression remains relatively high until 133 days gestation, after which there is a ∼24% decrease in SOCS-3 mRNA levels at 141–144 days gestation. This ontogenetic profile of adrenal SOCS-3 expression does not parallel the changes in fetal adrenal weight through mid- to late gestation. Relative adrenal weight was highest at 54 days gestation before decreasing to remain relatively constant between 95 and 144 days gestation. There was, therefore, no relationship between the relative weight of the adrenal and adrenal SOCS-3 mRNA expression. The factor(s) responsible for the relatively high expression of SOCS-3 mRNA within the fetal adrenal between 54 and 133 days gestation remain unknown. The expression of TGF-β1, the

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**Table 1. The effect of either saline or bromocriptine infusion followed by either a saline or oPRL bolus on fetal plasma PRL concentrations, before and during the infusion period and on the day of post mortem**

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Plasma PRL before infusion period, ng/ml</th>
<th>Plasma PRL during 48 h infusion, ng/ml</th>
<th>Plasma PRL on day of post mortem, ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sal + Sal Group</td>
<td>49.6 ± 7.6</td>
<td>64.4 ± 15.9b</td>
<td>62.7 ± 15.3b</td>
</tr>
<tr>
<td>Bromo + Sal Group</td>
<td>46.2 ± 10.5</td>
<td>17.3 ± 6.2**</td>
<td>10.0 ± 5.3**</td>
</tr>
<tr>
<td>Bromo + oPRL Group</td>
<td>41.1 ± 9.4</td>
<td>18.1 ± 6.3*</td>
<td>147.1 ± 17.8**</td>
</tr>
</tbody>
</table>

Values are presented as means ± SE. Different superscripted letters denote mean values that were significantly different from each other between groups (P < 0.05). bSignificant differences within the group compared to baseline measures, i.e., plasma PRL before the infusion period (P < 0.05). PRL, prolactin; Sal, saline; Bromo, bromocriptine.

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Fig. 2. The effect of bromocriptine (Bromo) infusion and oPRL administration on relative SOCS-3 expression in the fetal adrenal from the saline + saline (Sal + Sal; n = 5), Bromo + Sal (n = 4) and Bromo + oPRL (n = 4) groups. oPRL, ovine prolactin. Different superscript letters denote mean values that are significantly different from each other (P < 0.05).
negative regulator of adrenal growth (22, 39, 43–45), is relatively high at around 100 days gestation then decreases during the last ~30 days of gestation (5). Evidence derived from studies in osteoclasts and macrophages suggests that TGF-\(\beta\) can upregulate the expression of SOCS-3 in vitro (8, 23). Although it is possible that intra-adrenal TGF-\(\beta\)1 may act to upregulate and maintain high SOCS-3 expression in the adrenal during late gestation, it is unlikely that it would stimulate adrenal SOCS-3 mRNA expression at around 50 days gestation, as it has been demonstrated that fetal adrenal growth and steroidogenic capacity are high, rather than low, during this period. In the present study, there was a relatively limited increase in fetal adrenal weight at 141–144 days gestation, suggesting that the prepartum increase in adrenal growth was not fully activated at this age range in this series of fetal sheep. Interestingly, there was a decrease in adrenal SOCS-3 expression at 141–144 days gestation, and one possibility is that the decrease in the expression of the inhibitor SOCS-3 after 133 days gestation may be permissive for a subsequent increase in adrenal growth and steroidogenic capacity.

The cytokine PRL is detected in lactotroph cells of the fetal sheep pituitary as early as 58 days gestation, with plasma PRL being detectable by 90 days gestation (14). Signaling in PRL-target cells involves the JAK/STAT cascade and upregulation of SOCS-3 mRNA expression in the adrenal of the adult mouse (47), and PRLR mRNA expression has been detected in the human (11), rat (10), and mouse (2) adrenal throughout gestation, but the role of PRL in the regulation of the fetal adrenal is unclear. The expression profile of the PRLR changes dramatically after birth, such that the PRLR is expressed in fetal cortical cells, as well as the definitive neo-cortex (10), and by adult life, PRLR is detected in all three zones of the cortex (zona glomerulosa, fasciculata, and reticularis). The change in PRLR localization from pre- to postnatal life may suggest that the role of PRL changes during this period. There is evidence that PRL has a direct effect on steroid hormone production (17), as primary human adrenal cultures, consisting of both medulla and cortical cells, have shown to secrete cortisol in culture when treated with PRL (13). Similarly, in vivo PRL treatment of adult pigs and rats has been shown to significantly elevate steroid production (1, 20). Thus it has been proposed that acute PRL administration upregulates steroidogenesis, and there is then stimulation of SOCS-3 expression, which acts in a negative feedback manner to limit further PRL signaling in the adrenal.

The dopaminergic agonist, bromocriptine, acts specifically at the D2 subclass of dopamine receptors present on lactotrophic cells (9) to suppress PRL secretion both in adult life and in the late-gestation sheep fetus (15, 24). In the present study, bromocriptine administration did not, however, change adrenal SOCS-3 expression. Therefore, despite the almost complete absence of circulating PRL, there was no decrease in adrenal SOCS-3 mRNA expression. Thus it appears that adrenal SOCS-3 expression is relatively unresponsive to changes in basal plasma concentrations of PRL within the physiological range, that is, 5–60 ng/ml. We did find, however, that adrenal SOCS-3 expression was responsive to acute stimulation by PRL. The administration of a single bolus of exogenous oPRL resulted in an increase in adrenal SOCS-3 mRNA levels.

![Fig. 3. The effect of PRL on SOCS-3 expression in in vitro adrenocortical cells collected from fetuses at 144–147 days gestation. Adrenocortical cells were treated with 0, 10, or 50 ng/ml oPRL for 1 h. Relative SOCS-3 expression in the in vivo fetal adrenal at 141–144 days gestation is shown on the left-hand side of the histogram. Different superscript letters denote mean values, which were significantly different from each other (\(P < 0.05\)).](image)

![Fig. 4. A: Relative fetal adrenal weight in control (n = 7) and placentally restricted (PR; n = 9) fetuses. B: relative adrenal SOCS-3 mRNA expression in control and PR at 140–144 days gestation. *Mean values, which are significantly different from each other (\(P < 0.05\)).](image)
SOCS-3 mRNA levels increased around 10% in the presence of a 15-fold acute increase in plasma PRL concentrations compared with bromocriptine- or saline-infused animals. These data suggest that PRL can act acutely to upregulate the expression of SOCS-3 in the fetal adrenal. Tam and colleagues (47) have previously reported that there is an increase in adrenal SOCS-3 mRNA expression in the adult rat after administering PRL at relatively high doses, that is, an approximate 2- to 18-fold increase in circulating PRL concentrations. The relationship between PRL stimulation and adrenal SOCS-3 mRNA expression was also examined in isolated adrenocortical cells cultured in the presence or absence of oPRL. We found that in the absence of exogenous serum, SOCS-3 was expressed in adrenocortical cells in vitro. The cells were cultured in a defined medium without exogenous growth factors. After exposure to this medium for an hour, SOCS-3 expression was ~75% of that detected in the in vivo adrenal. One possibility is that adrenal SOCS-3 expression is normally upregulated by either circulating or paracrine factors present within the fetus in vivo, which are absent in the in vitro culture medium. It is known that the cortical cells can synthesize factors such as IGF peptides, basic fibroblast growth factor, epidermal growth factor, and TGF-β (18, 29), which are all known to upregulate the expression SOCS-3 in other tissues. When adrenocortical cells were stimulated with a high concentration of exogenous oPRL (50 ng/ml), there was a small but significant increase in the expression of SOCS-3. This response was similar to that present in the fetal adrenal after PRL stimulation in vivo. Our conclusions from this set of experiments are that firstly, adrenal SOCS-3 expression is not tonically regulated by PRL concentrations present in the normal circulating range in the late-gestation sheep fetus but that acute elevations of PRL can stimulate adrenal SOCS-3 expression in vivo and in vitro. In contrast to the fetal adrenal, SOCS-3 mRNA expression in the fetal sheep liver is regulated by PRL acting within the concentration range normally present in the fetal circulation (12). The lack of a role for endogenous PRL in the regulation of SOCS-3 mRNA within the fetal adrenal is further supported by the observation that adrenal SOCS-3 mRNA expression was not altered in the growth-restricted sheep fetus.

Removal of the majority of endometrial caruncles before conception reduces the development and nutrient transfer capacity of the placenta during pregnancy, thus restricting fetal growth and resulting in the relative sparing of the growth of the fetal brain, heart, and adrenal (27). Interestingly, plasma PRL concentrations in the placenta of restricted fetal sheep are significantly lower than those detected in normally grown fetuses (34) and are similar to the plasma PRL concentrations in the present studies during the 48-h bromocriptine infusion period. Thus, taken together, the studies reported above provide no evidence to support a primary role for either endogenous PRL or adrenal SOCS-3 expression in the tonic regulation of adrenal growth in normally grown or growth-restricted fetal sheep.

In summary, we have found that SOCS-3 expression in the adrenal decreases during late gestation, preceding the preparum increase in adrenal growth and steroidogenesis. We have found no evidence to support a role for endogenous PRL in the maintenance of adrenal SOCS-3 mRNA levels during late gestation in either the normally grown or growth-restricted sheep fetus, but we have demonstrated that acute PRL administration results in an increase in adrenal SOCS-3 expression both in vivo and in vitro. Thus the factor(s) maintaining adrenal SOCS-3 expression at relatively high levels between 50 and 133 days gestation and whether the fall in adrenal SOCS-3 expression is or is not permissive for the subsequent increase in fetal adrenal growth and steroidogenesis remain to be determined.

ACKNOWLEDGMENTS

We wish to acknowledge the Australian Research Council for financial support of this work. We are also grateful to Laura O’Carroll for her assistance with the surgical and experimental procedures.

GRANTS

This work was funded by the Australian Research Council.

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