Enhanced insulin secretion responsiveness and islet adrenergic desensitization after chronic norepinephrine suppression is discontinued in fetal sheep

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Enhanced insulin secretion responsiveness and islet adrenergic desensitization after chronic norepinephrine suppression is discontinued in fetal sheep. Am J Physiol Endocrinol Metab 306: E58–E64, 2014. First published November 19, 2013; doi:10.1152/ajpendo.00517.2013. — Intrauterine growth-restricted (IUGR) fetuses experience prolonged hypoxemia, hypoglycemia, and elevated norepinephrine (NE) concentrations, resulting in hypoinsulinemia and β-cell dysfunction. Previously, we showed that acute adrenergic blockade revealed enhanced insulin secretion responsiveness in the IUGR fetus. To determine whether chronic exposure to NE alone enhances β-cell responsiveness afterward, we continuously infused NE into fetal sheep for 7 days and, after terminating the infusion, evaluated glucose-stimulated insulin secretion (GSIS) and glucose-potentiated arginine-induced insulin secretion (GPAIS). During treatment, NE-infused fetuses had greater (P < 0.05) plasma NE concentrations and exhibited hyperglycemia (P < 0.01) and hypoinsulinemia (P < 0.01) compared with controls. GSIS during the NE infusion was also reduced (P < 0.05) compared with pretreatment values. GSIS and GPAIS were approximately fourfold greater (P < 0.01) in NE fetuses 3 h after the 7 days that NE infusion was discontinued compared with age-matched controls or pretreatment GSIS and GPAIS values of NE fetuses. In isolated pancreatic islets from NE fetuses, mRNA concentrations of adrenergic receptor isoforms (α1D, α2A, α2C, and β1), G protein subunit-α7, and uncoupling protein 2 were lower (P < 0.05) compared with controls, but β-cell regulatory genes were not different. Our findings indicate that chronic exposure to elevated NE persistently suppresses insulin secretion. After removal, NE fetuses demonstrated a compensatory enhancement in insulin secretion that was associated with adrenergic desensitization and greater stimulus-secretion coupling in pancreatic islets.

adrenergic receptor; β-cell; intrauterine growth restriction; uncoupling protein 2; catecholamines

SMALL-FOR-GESTATIONAL AGE or intrauterine growth-restricted (IUGR) infants are at greater risk for developing metabolic diseases such as type 2 diabetes mellitus (29, 40, 54). Impaired insulin secretion is associated with a diabetic phenotype indicating that in utero complications can permanently compromise β-cell development and function (27, 28). A fetal sheep model with placental insufficiency-induced intrauterine growth restriction shares many similarities with human IUGR fetuses, such as asymmetric growth, hypoxemia, hypoglycemia, hypoinsulinemia, and hypercatecholaminemia [epinephrine and norepinephrine (NE)] (4, 16, 18, 22, 23, 32, 41, 47). Furthermore, glucose-stimulated insulin secretion (GSIS) and β-cell mass are lower in IUGR sheep fetuses, which also replicate features in human IUGR fetuses (34–36, 41, 53). Several characteristics of the fetal IUGR environment, including hypoglycemia, hypoxemia, and hypercatecholaminemia, are proposed to cause hypoinsulinemia and pancreatic β-cell dysfunction (22).

NE inhibits insulin secretion via the α2-adrenergic receptors (ARs) in fetal sheep and other species (26, 48, 51, 56). Chronic exposure to high catecholamine concentrations often causes ARs to become desensitized by downregulation of their transcription (5, 12, 14). However, in IUGR fetal sheep islets, α2A-AR mRNA concentrations were elevated (32), and genetic variants that increase α2A-AR are associated with a higher incidence of type 2 diabetes in man (6, 17, 49). In our previous studies in IUGR sheep fetuses, we showed that elevated plasma NE concentrations chronically suppress insulin concentrations (32, 37). Strikingly, AR antagonists administered to acutely block the action of chronically elevated NE concentrations revealed an enhancement in glucose-stimulated insulin concentrations in IUGR fetuses that was equivalent to control fetuses, although β-cell mass was less in IUGR fetuses (32, 35). There was also an enhancement in fractional insulin release from isolated islets in IUGR fetuses that indicates β-cell-specific compensatory adaptations (12, 36). Therefore, chronic exposure to high NE appears to produce a β-cell compensatory response distal to the ARs and enhanced insulin stimulus-secretion coupling.

In sheep fetuses, previous experiments have demonstrated that a 7-day infusion of NE slows growth and attenuates cardiovascular and metabolite effects (3). The objective of this study was to determine whether sustained exposure to elevated NE, independent of other intrauterine growth restriction-related deficiencies, acutely enhances secretagogue-stimulated insulin secretion after the exogenous administration of NE has been terminated for ≥3 h. In addition, we associated the compensatory enhancement in insulin secretion responsiveness in NE-infused fetuses with lower expression of AR and uncoupling protein 2 (UCP2) in pancreatic islets compared with vehicle-infused controls while also demonstrating that regulatory genes for insulin secretion or β-cell function were unaffected (2, 11, 19, 42).

MATERIALS AND METHODS

Ethical approval. Twelve Columbia-Rambouillet crossbred ewes carrying singleton fetuses were purchased from Nebecker Ranch (Lancaster, CA) and managed in compliance with the Institutional Animal Care and Use Committee at the University of Arizona, which approved our study and is accredited by the American Association for Accreditation of Laboratory Animal Care. Ewes were fed Standard-Bread Alfalfa Pellets (Sacate Pellet Mills) and provided water ad libitum.
Animal preparations. Animals were randomly assigned to control (n = 6) or NE (n = 6) treatments. At 126 ± 1 days of gestational age (dGA), indwelling polyvinyl catheters were surgically placed in the fetus and ewe, as described previously (33). Fetal catheters for blood sampling were placed in the abdominal aorta via hindlimb pedal arteries, and infusion catheters were placed in the femoral veins via the saphenous veins. Maternal catheters were placed in the femoral artery and vein for arterial sampling and venous infusions. All catheters were tunneled subcutaneously to the ewe’s flank, exteriorized through a skin incision, and kept in a plastic mesh pouch sutured to the ewe’s skin. Ewes were allowed to recover for 5 days before the first GSIS and glucose-potentiated arginine-induced insulin secretion (GPAIS) studies were conducted.

Experimental design and treatment protocol. Fetal GSIS and GPAIS studies were performed at 132 ± 1 dGA to establish pretreatment insulin secretion responsiveness. Chronic infusions of NE and vehicle (described below) were initiated following the studies (treatment day 0). On day 7, chronic infusions were terminated for ≥3 h prior to acute insulin secretion responsiveness being evaluated. GSIS studies were also completed on each NE-infused fetus on day 3 (mid-NE treatment) to evaluate the effectiveness of NE suppression during treatment.

In the chronic NE treatment group, NE bitartrate (Bedford Laboratories) diluted with 0.3% ascorbic acid and saline (0.9% NaCl) was infused continuously in the fetal vein. During the 7-day infusion period, the dose of NE concentration was started at 1 μg/min for the first 24 h, increased to 2 μg/min for days 2–4, and finally increased to 4 μg/min for days 5–7, as described previously (3). Control fetuses received an infusion of saline with 0.3% ascorbic acid. After the final GSIS study at 139 ± 1 dGA, both ewes and fetuses were euthanized with an overdose of pentobarbital sodium (86 mg/kg, Euthasol; Virbac Animal Health, Fort Worth, TX). Perirenal adipose, liver, heart, lung, and kidneys were dissected and weighed.

GSIS and GPAIS studies. Square-wave hyperglycemic clamps were performed as described previously (21). Three baseline blood samples were collected at 5-min intervals before the hyperglycemic clamp was initiated. Following the last baseline sample, at time designated 0 min, a glucose bolus (110 mg/kg estimated fetal weight) was given to the fetuses and followed immediately by a continuous infusion of 33% dextrose solution (Abbott Laboratories, Abbott Park, IL) that was varied to maintain fetal glucose concentration at ~2.4 mM. This is the recommended value to achieve maximum glucose-stimulated insulin concentrations in singleton sheep fetuses (21). During the hyperglycemic clamp, three fetal arterial plasma samples were collected every 5 min starting from 35 min. Following the hyperglycemic clamp, an arginine bolus (261 mg of arginine in 0.4 M sodium acetate) was infused continuously in the fetal vein. During the 7-day infusion period of NE, square-wave hyperglycemic clamps were performed on each NE-infused fetus on days 2–4 (mid-NE treatment) to evaluate the effectiveness of NE suppression during treatment.

Blood collection and analysis. Fetal blood was collected in syringes lined with EDTA (Sigma-Aldrich, St. Louis, MO) and centrifuged (16,000 g) for 2 min at 4°C to separate plasma. Plasma glucose concentrations were measured immediately with an YSI model 2700 SELECT Biochemistry Analyzer (Yellow Springs Instruments, Yellow Springs, OH). Plasma insulin and NE concentrations were measured with an ovine insulin ELISA (Alpco Diagnostics, Windham, NH) and Noradrenaline ELISA (Labor Diagnostica Nord), respectively, as described previously (20). Additional fetal blood samples were collected in heparin-lined syringes (Elkins-Sinn, Cherry Hill, NJ) for blood gas and oxygen saturation measurements with an ABL 725 (Radiometer, Copenhagen, Denmark).

Pancreatic islet isolation. Islets were isolated from the fetal pancreas after a Liberase BlendZyme III (0.175 mg/ml; Roche, Indianapolis, IN) digestion and purified with a Histopaque density gradient (Sigma-Aldrich), as described previously (36, 50). After isolation, islets were washed in Krebs-Ringer buffer containing 0.5% BSA and then cultured overnight at 37°C in 95% O2-5% CO2 in RPMI 1640 media supplemented with 2.8 mM glucose (Sigma-Aldrich), 2% fetal bovine serum, and penicillin-streptomycin-neomycin (50 U, 50 μg, and 100 μg, respectively, Sigma-Aldrich). Islets were hand-picked into a 1.5-ml tube and centrifuged at 800 g for 5 min. After removing the supernatant, the pellets were stored at −80°C until RNA was extracted.

RNA extraction. RNA was extracted from isolated pancreatic islets with the AllPrep DNA/RNA-Protein Mini Kit (Qiagen, Valencia, CA) according to the manufacturer’s instructions. Quality and concentration of the RNA were determined by measuring absorbance at 260 and 280 nm with the NanoDrop ND-1000 Spectrophotometer (NanoDrop, Wilmington, DE). RNA integrity was evaluated with an Experion Automated Electrophoresis System (Bio-Rad Laboratories, Hercules, CA). PCR amplification was performed on RNA (~1 μg) that was reverse transcribed into cDNA with Superscript III reverse transcriptase (Invitrogen Life Technologies, Carlsbad, CA).

Primer design and cDNA cloning. Synthetic oligonucleotide primers were designed against sequences for ovine α1A, B, D1-ARs, D2A, B, C1-ARs, β1, 2, 3-ARs (12), insulin (GenBank accession no. U006599, pancreatic duodenal homebox 1 (PDX-1; JF728302), glucose transporter 2 (Glut2; AJ1318925), Glut3 (JX012067), Gαs (JX012068), Gαi3 (JX012069), Gαo (JX012070), Gαq (JX012071), Gαo (forward, 5′-GAC CAG CAC GTG CTT CCG TGC-3′; reverse, 5′-AGC CTC CAG CAC GTG GGT G3′), Gβ3 (forward, 5′-CGG ACC AGC AGA CAA CCA CCA TGT GAT GTG-3′; reverse, 5′-TCT TCC GCG AGT TGC TGG TGC-3′), Gβ1 (forward, 5′-CCG GCC GGC AGC AGA CAA CCA CCA TGT GAT GTG-3′; reverse, 5′-CTG CCC GCA CAT CCC TCG TGC-3′), Gβ2 (JX012072), Gβ3 (JX012073), UCP2 (forward, 5′-TCT CCT GTC GAG GTG GTG TTC A-3′; reverse, 5′-TGC AGC TGC AGC CAA TGC TGA TCA G3′), Kir6.2 (forward, 5′-ACA GCA GTG CTT CAC CAC GCT-3′; reverse, 5′-CGA AAA GGA GTG GTG CCT CAC AAC G3′), and L-type voltage-dependent calcium channel α1D subunit (CACNA1D; forward, 5′-GCC GAG AAC ACC CGG AGA TAC A-3′; reverse, 5′-AGG CCG CTC AAA GGA TCA GCT G3′). All DNA products were cloned and sequenced as described previously (12, 13).

Quantitative real-time PCR. The relative expression levels of mRNA transcripts were determined by quantitative PCR (qPCR) using SYBR Green (Qiagen) in an iQ5 Real-Time PCR Detection System (Bio-Rad). After an initial denaturation for 15 min, all reactions went through 40 cycles at 96°C (30 s), annealing temperature (30 s), and 72°C (10 s), at which point the fluorescent measurement was collected. Optimal annealing temperatures for different primer sets were determined using a temperature gradient (54–62°C) and examined on an agarose gel for specificity. Melt-curve analysis, starting at 60°C with an increase of 0.2°C every 6 s to 96°C, was performed at the end of the amplification to confirm product homogeneity.

PCR efficiency was determined with gene-specific plasmid DNA, for which threshold cycles (Ct) were linear over six orders of magnitude. Samples were run in triplicate. The results were normalized to the reference gene ribosomal protein S15, the average ΔCt was analyzed by the comparative ΔCt method (Ct reference gene), and fold change was determined by the 2−ΔΔCt method (46).

Statistical analysis. Data for body and organ weights and qPCR (ΔCt) were analyzed by one-way ANOVA, using the general linear model procedure of SAS (SAS Institute, Cary, NC). Insulin concentrations during basal and hyperglycemic steady-state periods were compared between treatments and studies. GSIS responsiveness was taken to be the difference in mean insulin concentration between the hyperglycemic and baseline states and was compared between treatments and studies. GPAIS was analyzed as the area under the curve relative to basal insulin concentrations for each sheep fetus (P value ≤0.05). GraphPad Software, La Jolla, CA). Biochemical, hematometabolic, and hormone measurements for daily, GSIS, and GPAIS studies were analyzed by ANOVA, using the MIXED procedure with
fetus as a random effect (SAS 9.3). The midtreatment GSIS in NE fetuses was compared with pretreatment values with a nonparametric Kruskal-Wallis test (proc npar1way; SAS 9.3). All values are expressed as means ± SE. P values ≤ 0.05 were considered significant.

RESULTS

Body weights and organ weights. Body weights were not different between control ewes (56.0 ± 4.5 kg) and ewes with NE-infused fetuses (49.8 ± 3.3 kg) or between control (3.8 ± 0.4 kg) and NE-infused fetuses (3.3 ± 0.2 kg). No treatment effects were found for fetal organ weights (Table 1).

Daily fetal plasma biochemical values. Fetal plasma glucose, insulin, and NE concentrations were not different between groups prior to treatment (Fig. 1). During the 7-day infusion, average NE concentrations were ninefold higher in NE-infused fetuses than control fetuses (9.343 ± 1.047 vs. 1.059 ± 142 pg/ml, P < 0.01; Fig. 1A). Insulin concentrations were 61% lower in NE fetuses compared with control fetuses (0.12 ± 0.01 vs. 0.31 ± 0.03 µg/l, P < 0.01; Fig. 1C). NE-infused fetuses had greater plasma glucose concentrations (1.54 ± 0.04 vs. 0.99 ± 0.03 mM, P < 0.01; Fig. 1B) and partial pressure of oxygen (Po2; 31.29 ± 0.65 vs. 22.98 ± 0.56 mmHg, P < 0.01; Fig. 1D) compared with controls. Hematocrit and blood pH were not different between treatments throughout the 7-day infusion period.

GSIS studies. Among the pretreatment GSIS studies, fetal plasma glucose concentrations of NE fetuses were not different from controls at baseline but were lower (P < 0.05) during the hyperglycemic clamp (Table 2). In the posttreatment GSIS studies, plasma glucose concentrations at baseline were 19% greater in NE fetuses than controls. However, posttreatment GSIS hyperglycemic glucose concentrations of NE-infused fetuses were not different from controls or from the pretreatment hyperglycemic values. During all GSIS studies, the mean glucose infusion rates needed to maintain the hyperglycemic steady state were similar between treatment groups within the study period.

In the pretreatment GSIS studies, fetal plasma insulin concentrations at baseline and hyperglycemic steady-state periods were not different between control fetuses and NE fetuses (Fig. 2). Insulin concentrations during the NE infusion were decreased at both the baseline and hyperglycemic periods (0.09 ± 0.01 µg/l, baseline; 0.40 ± 0.16 µg/l, hyperglycemic; P < 0.01) compared with NE pretreatment values (0.44 ± 0.05 µg/l, baseline; 0.99 ± 0.10 µg/l, hyperglycemic). Three hours after the 7-day NE infusion was terminated, NE fetuses had fivefold greater (P < 0.01) insulin concentrations at baseline and fourfold greater (P < 0.01) insulin concentrations during the hyperglycemic clamp compared with their own pretreatment GSIS studies. Posttreatment baseline and hyperglycemic insulin concentrations in NE fetuses were greater than control insulin concentrations. Among controls, insulin concentrations during the GSIS studies (both at baseline and during hyperglycemic steady state) were not different between pre- and posttreatment periods (Fig. 2).

Hematocrit, Po2, and pH at baseline and hyperglycemic steady states were similar between NE and control fetuses prior to the chronic infusion (Table 2). During the posttreatment GSIS, the hematocrit was 12% lower (P < 0.01) at baseline and hyperglycemic states in NE fetuses compared with controls. Po2 was greater (P < 0.01) during the baseline and hyperglycemic clamp in NE fetuses compared with controls.

During the pretreatment GSIS studies, fetal plasma NE concentrations were similar between treatment groups at baseline, but control fetuses had higher (P < 0.05) plasma NE concentrations at hyperglycemic state compared with NE fetuses. During the posttreatment GSIS studies, plasma NE concentrations of NE fetuses were 3.5-fold greater (P < 0.01) than control fetuses at baseline and hyperglycemic steady states (Table 2).

Table 1. Organ and tissue weights in control and NE-infused fetal sheep

<table>
<thead>
<tr>
<th>Organ and tissue weight</th>
<th>Control (n = 6)</th>
<th>NE (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
<td>52.5 ± 1.7</td>
<td>50.9 ± 1.5</td>
</tr>
<tr>
<td>Heart</td>
<td>25.0 ± 2.2</td>
<td>24.4 ± 1.1</td>
</tr>
<tr>
<td>Kidneys</td>
<td>19.9 ± 1.5</td>
<td>18.4 ± 1.2</td>
</tr>
<tr>
<td>Liver</td>
<td>98.8 ± 11.6</td>
<td>88.3 ± 5.1</td>
</tr>
<tr>
<td>Lungs</td>
<td>123.8 ± 8.9</td>
<td>109.8 ± 5.9</td>
</tr>
<tr>
<td>Perirenal adipose</td>
<td>13.8 ± 1.5</td>
<td>15.2 ± 0.8</td>
</tr>
</tbody>
</table>

Values are means ± SE. NE, norepinephrine.

Fig. 1. Daily biochemical values during treatment conditions. Plasma norepinephrine (NE) concentrations (A), plasma glucose concentrations (B), plasma insulin concentrations (C), and arterial blood partial pressure of oxygen (Po2; D) are presented for control (n = 6) and NE fetuses (n = 6) relative to days of treatment. The samples collected on day 0 were prior to the chronic NE or vehicle infusions being initiated. Subsequent sampling was performed during the chronic infusions initiated on day 0 following the pretreatment studies and continued through day 7. Significant differences between control and NE-infused fetuses are indicated in RESULTS.
POST-NE infusion

GSIS, glucose-stimulated insulin secretion; NS, not significant. Differences were determined between treatment groups for pre- and posttreatment GSIS studies.

fetuses. **Significance,

concentrations during baseline and hyperglycemic steady-state periods for pre-

Fig. 2. Insulin concentrations during pre- (pre) and posttreatment (post) GPAIS studies. In the GPAIS studies, area under the curve calculations for plasma insulin concentration are presented for control and NE-infused fetuses during the pretreatment and posttreatment studies. **Significance, P < 0.01.

DISCUSSION

In the present study, we show that chronic infusion of NE suppressed fetal insulin concentrations throughout the 7-day treatment and was accompanied by hyperglycemia. Circulating NE concentrations resembled those observed in placental insufficiency-induced IUGR sheep fetuses, which have 69% lower insulin concentrations (36). After the NE infusion was terminated, we observed a compensatory augmentation of GSIS and GPAIS in NE fetuses, although plasma NE concentrations remained elevated compared with controls. The α2A-

Table 2. Hematological values and glucose infusion rate during GSIS studies

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Condition</th>
<th>Control</th>
<th>NE</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-NE infusion</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose, mmol/h</td>
<td>Infusion rate</td>
<td>10.75 ± 1.44</td>
<td>10.69 ± 1.12</td>
<td>NS</td>
</tr>
<tr>
<td>Glucose, mM</td>
<td>Baseline</td>
<td>1.19 ± 0.07</td>
<td>1.14 ± 0.03</td>
<td>NS</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>Hyperglycemia</td>
<td>2.48 ± 0.03</td>
<td>2.24 ± 0.03</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>P/O2, mmHg</td>
<td>Baseline</td>
<td>34.78 ± 0.82</td>
<td>34.00 ± 1.00</td>
<td>NS</td>
</tr>
<tr>
<td>P/O2, mmHg</td>
<td>Hyperglycemia</td>
<td>34.73 ± 0.80</td>
<td>34.22 ± 0.96</td>
<td>NS</td>
</tr>
<tr>
<td>NE, pg/ml</td>
<td>Baseline</td>
<td>24.48 ± 0.96</td>
<td>24.39 ± 0.83</td>
<td>NS</td>
</tr>
<tr>
<td>NE, pg/ml</td>
<td>Hyperglycemia</td>
<td>23.66 ± 0.99</td>
<td>23.75 ± 0.70</td>
<td>NS</td>
</tr>
<tr>
<td>Post-NE infusion</td>
<td>Infusion rate</td>
<td>1,262.33 ± 178.76</td>
<td>588.89 ± 65.86</td>
<td>NS</td>
</tr>
<tr>
<td>Post-NE infusion</td>
<td>Baseline</td>
<td>1,434.80 ± 98.11</td>
<td>693.23 ± 95.80&lt;0.05</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE and represent plasma glucose and NE concentrations, hematocrit, and partial pressure of blood oxygen (P/O2) for treatment conditions. GPAIS, glucose-stimulated insulin secretion; NS, not significant. Differences were determined between treatment groups for pre- and posttreatment GSIS studies.

GPAIS studies. Fetal plasma insulin concentrations following the arginine bolus reached maximum concentrations after 5 min in all fetuses. The pretreatment GPAIS area under the curve measurement was not different between controls and NE fetuses. During the posttreatment GPAIS studies, insulin concentrations of NE-infused fetuses were greater (P < 0.05), and the area under the curve was 3.6-fold greater (P < 0.01) compared with NE pretreatment and control values (Fig. 3).

Islet gene expression. To interpret compensatory enhancement of GSIS in NE-infused fetuses, we evaluated factors regulating aspects of insulin synthesis and secretion. Pancreatic islets from NE fetuses had lower (P < 0.05) mRNA concentrations for α1D-AR, α2A-AR, α2C-AR, β1-AR, G protein subunit αs-2, and UCP2 (Fig. 4). Insulin, PDX-1, Glut2, Kir6.2, CACNA1D, other ARs, and Goα1, Goα3, Goαq, Goαs, Goαz, and GBi,3 mRNA concentrations were not different in pancreatic islets (data not shown).

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AR, α_{2C}-AR, and G_{01,2} mRNA concentrations were lower in pancreatic islets from NE-infused fetuses compared with controls, indicating AR desensitization in fetal islets (45, 52). No differences were noted for measured β-cell regulatory factors; however, the reduction of UCP2 mRNA indicates improved insulin stimulus-secretion coupling in islets from NE fetuses. These findings show that chronic exposure to elevated NE induces a compensatory enhancement in β-cell insulin secretion responsiveness.

As expected, there was no difference in GSIS responsiveness between the two groups before the treatment (Fig. 2). In control fetuses, GSIS was also not different following the 7-day vehicle infusion (Fig. 2). Comparison between the two GSIS studies in control fetuses demonstrates that there is no significant developmental enhancement or maturation in β-cell function at this gestational stage (33), in contrast to that found for younger fetuses (1). During the NE treatment, insulin concentrations at basal and hyperglycemic steady states were inhibited. After removing the chronic NE infusion, basal, hyperglycemic, and GPAIS insulin concentrations were approximately fourfold higher than pretreatment measurements in NE fetuses. Posttreatment measurements in control fetuses (Fig. 2). Therefore, these findings support the hypothesis that NE induces a compensatory hyperinsulin secretion state in IUGR fetuses similar to that observed after adrenergic antagonists administered (32).

Enhanced insulin secretion responsiveness was observed despite higher than normal plasma NE concentrations 3 h after the chronic NE infusion was terminated. According to previous studies, the whole body clearance rate of NE is 178 ± 28 ml·kg^{-1}·min^{-1} in near-term sheep fetuses and is not dependent on plasma catecholamine concentration (43). Since the average blood volume in fetal sheep is 120 ml/kg at 131 dGA (7), theoretically, the half-life of circulating plasma NE is <2 min. However, longer than expected clearance rates were shown in a previous long-term NE infusion study (38), indicating that chronic NE exposure decreased NE clearance, as found previously (9). In the current study, our primary objective was to look at the immediate insulin secretion responsiveness following a chronic NE infusion. Despite NE’s inhibitory effect on insulin secretion, NE fetuses with relatively greater plasma NE concentrations still had enhanced insulin secretion responsiveness compared with control fetuses.

Hyperglycemia was observed throughout the NE treatment and represents a confounding factor that might also negatively influence β-cell function. The 55% increase in fetal plasma glucose concentrations is most likely due to reduced glucose oxidation in peripheral tissues of the NE fetus, which is further supported by the chronic hyperoxemia (3, 39). Previous studies in fetal sheep have found that chronic hyperglycemia for 7–10 days results in impaired β-cell function and decreased insulin secretion (10). Additionally, 2 wk of pulsatile hyperglycemia also lowers GSIS in fetal sheep and increases the rate of accumulation for reactive oxygen species in isolated pancreatic islets at 11.1 mM glucose compared with controls (20). Together, these findings indicate that fetal sheep islets exposed to hyperglycemia exhibit glucotoxicity and β-cell dysfunction (25). Interestingly, the islets from NE fetuses, also exposed to chronic hyperglycemia, were not affected in a similar fashion, as insulin secretion was enhanced after the 7 days of exposure. This shows that NE may protect islets from the ensuing hyperglycemia and subsequent glucotoxicity, possibly by inducing metabolic quiescence (30, 31).

Chronic exposure of high catecholamine concentrations has been shown to persistently reduce both mRNA and protein expression of ARs (5, 12, 14) and related G proteins (52), which is also supported in the current study (Fig. 4). NE inhibits insulin secretion principally through α2-ARs, but not α1-ARs or β-ARs (31). Thus, this lower expression of α2C-AR and α2C-AR might lead to decreased adrenergic inhibition of insulin secretion (51, 55). The decreased expression of G_{01,2} is also postulated to lower NE inhibitory effects on insulin secretion (52). Therefore, desensitization of both α2-ARs and G_{01,2} contributes to a higher insulin concentration as well as higher insulin responsiveness after the 7-day NE infusion is removed, whereas plasma NE concentrations remain greater than controls.

As the exclusive proton leak regulator in the mitochondria of the β-cell, UCP2 negatively affects β-cell responsiveness by decreasing ATP production (15, 57). Chronic hyperglycemia stimulates UCP2 expression in rat and human islet cultures (8, 44). In glucokinase knockout mice, whether incubated with or without chronic hyperglycemia, decreased rates of glucose utilization lower UCP2 expression and activity in pancreatic islets (15). Thus, expression and function of UCP2 are associated with glucose metabolism in islets, which is suppressed by α2-AR stimulation (31).

In conclusion, a 7-day infusion of NE in fetal sheep chronically suppresses plasma insulin concentrations and lowers α2-ARs and G_{01,2} mRNA in islets. After NE infusion is terminated, NE-infused fetuses have enhanced insulin secretion responsiveness to both glucose and arginine stimulation, although NE concentrations are greater than controls. These findings demonstrate that 7-day NE suppression during late gestation contributes AR desensitization and a compensatory enhancement of β-cell function. In addition, lower expression of UCP2 in pancreatic islets may facilitate enhanced insulin stimulus-secretion coupling. Therefore, these data begin to explain how endocrine factors such as catecholamines may facilitate prenatal adaptations for thriftiness in utero (24) but...
contribute to postnatal catchup growth, early onset obesity, and other metabolic diseases.

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DISCLOSURES

The authors have no conflicts of interest, financial or otherwise, to disclose.

AUTHOR CONTRIBUTIONS

X.C. and S.W.L. contributed to the conception and design of the research; X.C., A.S.G., A.R.M., D.T.Y., A.C.K., and S.W.L. edited and revised experiments; X.C. and S.W.L. prepared the figures; X.C. drafted the manuscript; X.C., A.S.G., A.R.M., D.T.Y., A.C.K., and S.W.L. analyzed the data; X.C., A.S.G., A.R.M., D.T.Y., A.C.K., and S.W.L. interpreted the results of the experiments; X.C. and S.W.L. prepared the figures; X.C. drafted the manuscript; X.C., A.S.G., A.R.M., D.T.Y., A.C.K., and S.W.L. edited and revised the manuscript; X.C., A.S.G., A.R.M., D.T.Y., A.C.K., and S.W.L. approved the final version of the manuscript.

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