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Chronic umbilical cord compression results in accelerated maturation of lung and brown adipose tissue in the sheep fetus during late gestation

M. G. Gnanalingham,[1] D. A. Giussani,[2] P. Sivathondan,[2] A. J. Forhead,[2] T. Stephenson,[1] M. E. Symonds,[1] and D. S. Gardner[1]

[1]*Centre for Reproduction and Early Life, Institute of Clinical Research, University of Nottingham; and* [2]*The Physiological Laboratory, University of Cambridge, Cambridge, United Kingdom*

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Gnanalingham, M. G., D. A. Giussani, P. Sivathondan, A. J. Forhead, T. Stephenson, M. E. Symonds, and D. S. Gardner. Chronic umbilical cord compression results in accelerated maturation of lung and brown adipose tissue in the sheep fetus during late gestation. *Am J Physiol Endocrinol Metab* 289: E456–E465, 2005. First published April 26, 2005; doi:10.1152/ajpendo.00053.2005.—Umbilical cord compression (UCC) sufficient to reduce umbilical blood flow by 30% for 3 days, results in increased fetal plasma cortisol and catecholamines that are likely to promote maturation of the fetal lung and brown adipose tissue (BAT). We determined the effect of UCC on the abundance of uncoupling protein (UCP)1 (BAT only) and -2, glucocorticoid receptor (GR), and 11β-hydroxysteroid dehydrogenase (11β-HSD)1 and -2 mRNA, and mitochondrial protein voltage-dependent anion channel (VDAC) and cytochrome *c* in these tissues. At 118 ± 2 days of gestation (dGA; term ~145 days), 14 fetuses were chronically instrumented. Eight fetuses were then subjected to 3 days of UCC from 125 dGA, and the remaining fetuses were sham operated. All fetuses were then exposed to two 1-h episodes of hypoxemia at 130 ± 1 and 134 ± 1 dGA before tissue sampling at 137 ± 2 dGA. In both tissues, UCC upregulated UCP2 and GR mRNA, plus VDAC and cytochrome *c* mitochondrial proteins. In lung, UCC increased 11β-HSD1 mRNA but decreased 11β-HSD2 mRNA abundance, a pattern reversed for BAT. UCC increased UCP1 mRNA and its translated protein in BAT. UCP2, GR, 11β-HSD1 and -2 mRNA, plus VDAC and cytochrome *c* protein abundance were all significantly correlated with fetal plasma cortisol and catecholamine levels, but not thyroid hormone concentrations, in the lung and BAT of UCC fetuses. In conclusion, chronic UCC results in precocious maturation of the fetal lung and BAT mitochondria, an adaptation largely mediated by the surge in fetal plasma cortisol and catecholamines that accompanies UCC.

cortisol; catecholamines; mitochondria; proteins

A COMMON FORM OF REVERSIBLE adverse intrauterine condition in human pregnancies is umbilical cord compression (UCC), which has been reported to occur at an incidence of up to 40% (10, 11, 52). UCC can result from nuchal cord (11) torsion of the umbilicus during gestation (52), oligohydramnios (39), or compression of the cord during the actual processes of labor and delivery (66). It increases the susceptibility of the fetus to perinatal complications and, potentially, neurodevelopmental handicap (10, 43). In fetal sheep, partial compression of the umbilical cord to reduce umbilical blood flow by 30% from baseline for a period of 3 days produces reversible, mild fetal asphyxia, a transient increase in fetal plasma adrenocorticotropic hormone (ACTH) concentration, and a progressive and sustained increase in fetal plasma cortisol (26, 28). This chronic elevation in fetal plasma cortisol before birth may result in accelerated maturation of fetal organ systems, since in sheep the ontogenic increase in fetal plasma cortisol toward term, in conjunction with fetal plasma triiodothyronine (T_3), is necessary for the appropriate maturation of both the fetal lung and brown adipose tissue (BAT) (20, 44). In sheep, the prepartum cortisol surge coincides with an increase in hepatic 5′-monodeiodinase activity (12). As a consequence of increased deiodination of thyroxine (T_4) to T_3, the circulating T_3 concentration in the fetus also increases toward term (24). At the same time, there is a peak in glucocorticoid receptor (GR) and 11β-hydroxysteroid dehydrogenase (11β-HSD) type 1 mRNA abundance near to term in the sheep lung (32), which is likely to determine glucocorticoid sensitivity in fetal BAT. 11β-HSD1 behaves predominantly as an 11-oxoreductase, catalyzing the conversion of cortisone to bioactive cortisol, and as an intracellular amplifier of glucocorticoid access to the GR (3, 58). Conversely, 11β-HSD2 behaves as an 11-dehydrogenase, catalyzing the inactivation of cortisol to inert cortisone (58).

The abundance of the BAT-specific uncoupling protein (UCP)1 in the fetus is dependent on an intact adrenal gland, the associated prepartum increase in plasma cortisol and T_3 (13, 44), sympathetic innervation (30), β-adrenergic receptor density (8), and plasma catecholamine concentration (17), with fetal plasma norepinephrine constituting 40–60% of total catecholamines in the fetal sheep adrenal gland (14). UCP1 has a defined role in nonshivering thermogenesis at birth by avoiding ATP synthase and allowing proton reentry into the mitochondrial matrix, thus creating the proton electrochemical gradient to be dissipated as heat (29). The peak in UCP1 at birth coincides with maximum expression of other mitochondrial membrane proteins within adipose tissue, including voltage-dependent anion channel (VDAC), located on the outer mitochondrial membrane, and cytochrome *c*, present within the intermembrane space (45). The role of VDAC in adipose tissue has not been established, although it is a component of the mitochondrial permeability pore, which regulates the supply of mitochondrial adenosine diphosphate and adenosine triphosphate, and is proposed to have a role in apoptosis (15, 33). Cytochrome *c* is an essential component of the mitochondrial respiratory chain and is a mobile electron transporter, involved in the electron transfer from complex III to complex IV (37, 41). The regulation of VDAC and cytochrome *c* proteins by fetal plasma cortisol, T_3, and catecholamines has not been

Address for reprint requests and other correspondence: M. E. Symonds, Academic Division of Child Health, School of Human Development, Queen's Medical Centre, Univ. Hospital, Nottingham NG7 2UH, UK (e-mail: michael.symonds@nottingham.ac.uk).

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established. The potential thermogenic capacity of fetal BAT, measured as guanosine diphosphate (GDP) binding, also peaks at birth (13), although this does not appear to be affected by the manipulation of fetal plasma cortisol or T_3 (44). In the sheep lung, GDP binding activity is minimal (32), although it remains to be ascertained whether its abundance is affected by fetal plasma cortisol, T_3, and catecholamine manipulation.

In the sheep lung, although the abundance of cytochrome *c* protein remained unchanged with age, VDAC peaked at 7 days of postnatal age, coinciding with the maximal abundance of UCP2 protein, which, although undetectable in the fetal lung, follows the peak in UCP2 mRNA at 6 h of age before declining rapidly in postnatal life (32, 45). UCP2, a recently discovered member of the UCP subfamily of inner mitochondrial membrane carriers, is highly abundant in the lung (50) and has postulated roles in energy regulation (4, 6), reactive oxygen species production (38, 48), and apoptosis, potentially in conjunction with VDAC and cytochrome *c* proteins (63). Although the glucocorticoid (20), thyroid (60), and catecholamine (42, 56) dependence of fetal lung development is well established, it remains to be established whether these hormones impact on the abundance of mitochondrial proteins within the lung.

The impact of adverse intrauterine conditions produced by controlled, partial compression of the umbilical cord on fetal lung and BAT development has not been previously examined. We hypothesized that prolonged, reversible compression of the umbilical cord would result in the premature maturation of these fetal organs at the level of the mitochondria by upregulating the abundance of mitochondrial proteins and components of glucocorticoid sensitivity. Thus the aim of this study was to use the chronically catheterized ovine fetus preparation to determine the impact of prolonged reversible UCC for a 3-day period on the mRNA abundance of UCP1 (in BAT only), UCP2, GR, 11β-HSD1 and -2, and protein abundance of UCP1 (in BAT only), VDAC and cytochrome *c*, in the fetal lung and BAT during late gestation.

MATERIALS AND METHODS

Procedures

Animal experimentation. Full details of the surgical preparation, postoperative care, experimental procedure, measurements, and hormone analysis have been previously published (26, 28). Briefly, under 1–2% halothane anesthesia, 14 Welsh Mountain sheep fetuses were chronically instrumented at 118 ± 2 days of gestation (dGA; term ~145 dGA) with an inflatable occluder cuff around the umbilical cord, amniotic and femoral vascular catheters, and a transit-time flow probe around an umbilical artery inside the fetal abdomen. At 125 dGA, umbilical blood flow was reduced by 30% from a predetermined 24-h baseline for 3 days by automated servo-controlled inflation of the occluder cuff [umbilical cord compressed (UCC), $n = 8$]. The occluder was then deflated, allowing return of umbilical blood flow to baseline. The remaining six fetuses were used as sham-operated control animals in which the implanted occluder was not inflated throughout. For the purpose of another study, all fetuses were then subsequently subjected to two periods of acute hypoxemia, elicited by reducing maternal $F_{I_{O_2}}$, at 2 ± 1 (130 ± 1 dGA) and at 5 ± 2 (134 ± 1 dGA) days after the end of cord or sham compression. The protocol for acute fetal hypoxemia involved a 3-h experiment consisting of 1 h of normoxia, 1 h of hypoxemia, and 1 h of recovery, as previously described in detail (31). None of the fetuses examined in the present study was then subjected to the ACTH challenge following the second hypoxemia protocol, as described previously (28).

At 137 ± 2 dGA, the ewes and fetuses were humanely killed using a lethal dose of pentobarbital sodium (200 mg/kg Pentoject; Animal Ltd, York, UK), and tissues were rapidly dissected (sham: $n = 6$ for lung and for BAT; UCC: $n = 8$ for lung and $n = 6$ for BAT), weighed, and then placed in liquid nitrogen and stored at −80°C until analysis. All procedures were performed under the UK Animals (Scientific Procedures) Act, 1986.

Measurements and Hormone Analyses

Maternal and fetal arterial blood samples (4 ml) were taken simultaneously before umbilical cord compression at −1 day and −1 h, during umbilical cord compression at +1 h, +8 h, +1 day, +2 days, and +3 days, and subsequently at 1 day following deflation of the occluder cuff for measurement of blood gases, acid/base status, and hormone concentrations. Plasma catecholamines, epinephrine and norepinephrine, were analyzed by high-performance liquid chromatography using electrochemical detection (22). Plasma cortisol, triiodothyronine (T_3) and thyroxine (T_4) were determined by a radioimmunoassay validated for use in ovine plasma (21, 23). These results, with the exception of fetal plasma thyroid hormones, have been previously published elsewhere (26, 28).

Laboratory Analyses

Protein detection. Mitochondria were prepared from 1 g of frozen lung or BAT (specifically, perirenal adipose tissue, which constitutes ~80% of adipose tissue in a newborn sheep) (59), and protein content of each preparation was determined (40). Mitochondrial protein (10 μg) was loaded onto each gel for every sample, and Ponceau red staining of all membranes confirmed that equal amounts of protein were transferred from each gel to membrane before immunodetection (45). Abundance of cytochrome *c* protein was determined using an antibody (Santa Cruz Biotechnology, Santa Cruz, CA) at a dilution of 1:1,000. VDAC protein abundance was determined using an antibody raised in rabbits to ovine VDAC, purified from the kidney of a newborn lamb, as described by Mostyn et al. (45) and used at a dilution of 1:2,000. UCP1 content was measured as described by Mostyn et al. (45). Densitometric analysis was performed on each gel, and all values were expressed in densitometric units. Specificity of detection was confirmed using nonimmune rabbit serum. A range of molecular weight markers was included on all gels. Densitometric analysis was performed on each membrane following image detection, using a Fujifilm LAS-1000 cooled charge-coupled device (CCD) camera (Fuji Photo Film, Tokyo, Japan). All gels were run in duplicate, and a reference sample (an appropriate ovine mitochondrial sample) was included on each to allow comparison between gels.

GDP binding. The thermogenic activity of adipose and lung tissue ($n = 6$ per group) was assessed from the in vitro activity of the mitochondrial conductance pathway using GDP at a concentration of 2 mM, with nonspecific binding measured using a 200 mM concentration of GDP (59). Mitochondrial protein prepared from perirenal adipose tissue from a 1-day-old lamb acted as the positive control on this assay, and all measurements were made in triplicate.

Messenger RNA detection. Total RNA was isolated from lung and adipose tissue using Tri Reagent (Sigma, Poole, UK) and the expression of UCP2, GR, and 11β-HSD types 1 and 2 mRNA determined by reverse transcriptase-polymerase chain reaction (RT-PCR), as previously described by Gnanalingham et al. (32). The analysis used oligonucleotide cDNA primers to UCP1, UCP2, GR (type 2), 11β-HSD types 1 and 2 genes, generating specific intron-spanning products (Table 1). Briefly, the PCR program consisted of an initial denaturation (95°C, 15 min), amplification (stage I, 94°C, 30 s; stage II, annealing temperature, 30 s; stage III, 72°C, 60 s) and final extension (72°C, 7 min; 8°C, "hold"). The annealing temperature and cycle number of all primers were optimized so as to be in the linear range for the relevant tissue (Table 1). Agarose gel electrophoresis

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Table 1. *Primer sequences and optimal PCR conditions used in BAT and lung in late-gestation ovine fetus*

Primer Set	Product Size, BP	Primer Sequence	Annealing Temp, °C		Cycle No.	
			Lung	BAT	Lung	BAT
UCP2	513	F 5′-GGG ACT CTG GAA AGG GAC AT-3′ R 5′-AAG AGA GGG ATG GGG AGA GA-3′	59.2	59.0	27	25
GR (type 2)	150	F 5′-ACT GCC CCA AGT GAA AAC AGA-3′ R 5′-ATG AAC AGA AAT GGC AGA CAT T-3′	58.9	58.7	30	32
11β-HSD1	160	F 5′-GTG CCA GAT CCC TGT CTG GAT-3′ R 5′-AGC GGG ATA CCA CCT TCT TT-3′	58.2	58.5	31	32
11β-HSD2	260	F 5′-CGC ATT GTG ACC GTA AGC-3′ R 5′-CAG GCA GGC AGG ATG ATG-3′	58.5	58.7	31	38
UCP1	301	F 5′-AAA GTC CGG CTA CAG ATCCA-3′ R 5′-TGA CCT TGA CCA CCT CTG TG-3′		60.0		24
18S	324	Ambion Classic II 18S Internal Standards Catalog no. 1717				

BAT, brown adipose tissue; UCP, uncoupling protein; GR, glucocorticoid receptor; 11β-HSD, 11β-hydroxysteroid dehydrogenase; F, forward; R, reverse.

(2.0–2.5%) and ethidium bromide staining confirmed the presence of both the product and 18S at the expected sizes. Densitometric analysis was performed on each gel by image detection using a Fujifilm LAS-1000 cooled CCD camera and UCP1, UCP2, GR (type 2), 11β-HSD types 1 and 2 and 18S mRNA abundance determined. Consistency of lane loading for each sample was verified, and all results were expressed as a ratio of a reference sample to r18S abundance. All analyses and gels were conducted in duplicate, with appropriate positive and negative controls, and a range of molecular weight markers. The resultant PCR product was extracted (QIAquick gel extraction kit, Qiagen, catalog no. 28704) and sequenced, and results were cross-referenced against the GenBank website to determine specificity of the target gene.

Statistical Analyses

All data are presented as means ± SE. Significant differences ($P < 0.05$) between values obtained from sham and UCC groups were determined by Mann-Whitney *U*-test. Significant correlations ($P < 0.05$) between fetal plasma hormone concentrations and physiological and molecular parameters were undertaken independently by two-tailed Spearman's rank order test (SPSS v11.0) in sham and UCC groups on *day 1* post-UCC in the fetal lung and BAT, since this was the last time point when all measured parameters were recorded from all of the fetuses. When multiple correlations were present with a molecular parameter, additional partial correlation analyses were undertaken to determine which factor had the greatest impact.

RESULTS

Effect of UCC on Fetal Blood Gases and Plasma Hormone Concentrations

Chronic UCC for a 3-day period produced reversible mild fetal asphyxia for the duration of the challenge, with significant falls in fetal arterial pH (pH_a), arterial oxygen partial pressure (Pa_{O_2}) and percent saturation of oxygen in hemoglobin, together with a significant increase in arterial carbon dioxide partial pressure (Pa_{CO_2}) (26). UCC produced a transient increase in fetal plasma ACTH and norepinephrine concentrations, with all values for blood gases and hormones returning toward baseline concentrations 1 day after UCC. In contrast, UCC produced a progressive increase in fetal plasma cortisol concentration during the period of compression, with values remaining significantly higher than baseline 3 days after the onset of UCC (*day 1* post-UCC: sham 26.53 ± 9.70, UCC 50.43 ± 9.29 ng/ml, $P < 0.05$) (28).

The two periods of acute hypoxemia following UCC resulted in similar slopes for the correlation between fetal plasma ACTH and cortisol concentrations in UCC- and sham-compressed groups (second hypoxemia: sham, $y = 0.052x + 35$, $r^2 = 0.11$; UCC, $y = 0.118x + 55$, $r^2 = 0.19$), with fetal plasma cortisol and norepinephrine concentrations being the only humoral factors that remained at significantly different levels relative to sham fetuses before, during, and after the period of acute oxygen deprivation (28).

In contrast to the data above (26, 28), the following results on fetal thyroid hormone concentrations have not been previously published. UCC did not have any effect on fetal plasma T_3 or T_4 concentrations during or 1 day after compression (1 day post-UCC, T_3: sham 0.26 ± 0.06, UCC 0.24 ± 0.04 ng/ml, $P > 0.05$; T_4: sham 63.21 ± 15.55, UCC 62.46 ± 7.32 ng/ml, $P > 0.05$). In addition, there was no overall correlation between fetal plasma cortisol and T_3 concentrations ($r^2 = 0.11$, $P > 0.05$).

Effect of UCC on Abundance of mRNA and Mitochondrial Proteins and GDP Binding in Fetal Lung and BAT

For lung and BAT, UCC significantly ($P < 0.01$) upregulated UCP2 and GR mRNA (Fig. 1, *A* and *B*), and VDAC and cytochrome *c* protein abundance, compared with shams (Fig. 2, *A* and *B*). The abundance of 11β-HSD1 mRNA was increased ($P < 0.01$) and the abundance of 11β-HSD2 mRNA decreased ($P < 0.01$) for the lungs of the UCC group compared with shams, a pattern that was reversed for BAT (Fig. 1, *C* and *D*). In BAT, UCC increased ($P < 0.01$) UCP1 mRNA, its translated protein, and GDP binding compared with shams (Fig. 3). In contrast, GDP binding was decreased by UCC in the lung (shams 11.82 ± 0.94, UCC 5.29 ± 0.43 pmol/mg mitochondrial protein, $P < 0.01$), compared with shams. The relative abundance of GR and 11β-HSD1 mRNA was higher in the fetal lung than in BAT, whereas the reverse was true for the relative abundance of 11β-HSD2 mRNA and VDAC and cytochrome *c* protein abundance. UCC did not affect body weight, total lung weight, lung weight relative to body weight, total protein, mitochondrial protein (total, per gram of tissue or per total tissue weight) or RNA (total, per gram of tissue or per total tissue weight) concentration between sham and UCC fetuses (data not shown).

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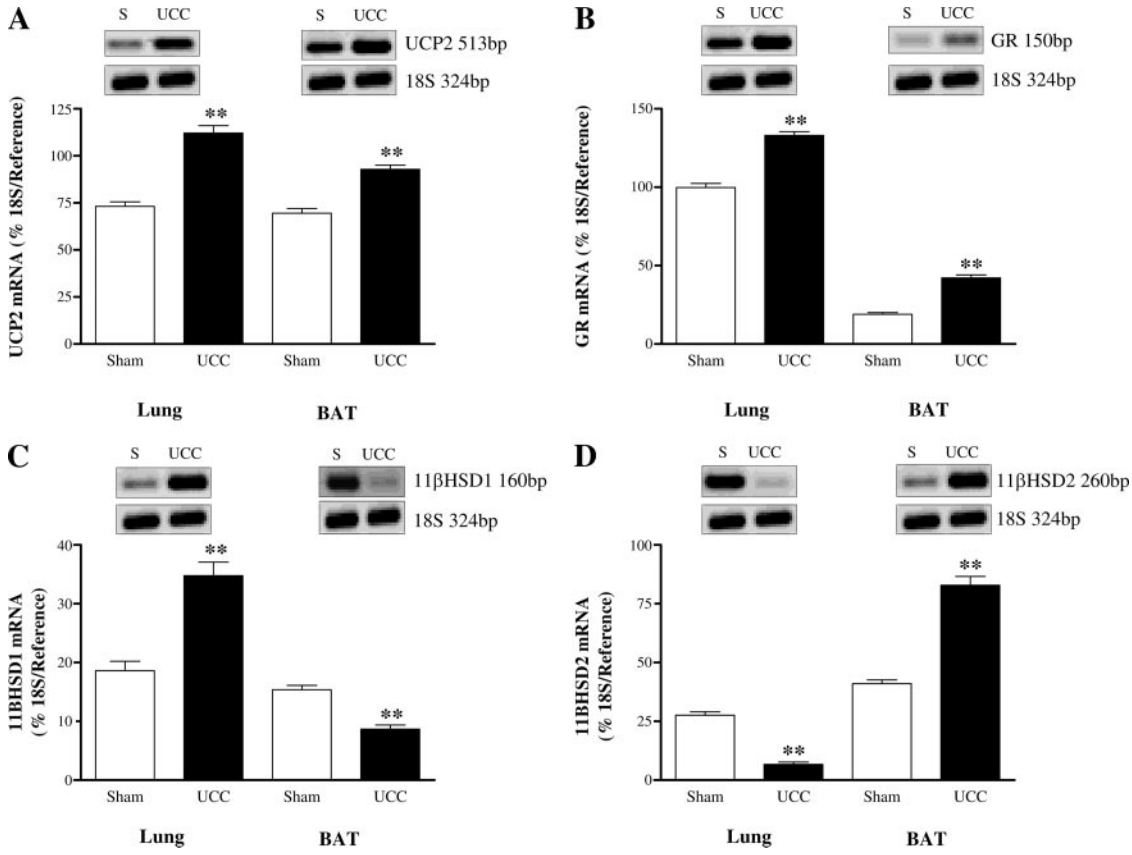

Fig. 1. Effect of umbilical cord compression (UCC, 30% reduction of umbilical blood flow from baseline) or sham (S) compression on the abundance of uncoupling protein-2 (UCP2) mRNA (*A*), glucocorticoid receptor (GR) mRNA (*B*), 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) mRNA (*C*), and 11β-HSD2 mRNA (*D*) in lung and brown adipose tissue (BAT) of sheep fetuses at 137 ± 2 days gestation (term ~145 days). Examples of gene mRNA expression are given. Values are means ± SE (n = 6–8 per group). $^{**}P < 0.01$, UCC mean value significantly different from sham group.

Relationships Between Fetal Plasma Hormone Concentrations and Abundance of mRNA and Mitochondrial Proteins and GDP Binding in Fetal BAT

A number of significant relationships were observed between fetal plasma ACTH, cortisol, and catecholamines and physiological and molecular variables measured on *day 1* post-UCC in fetal BAT of sham and UCC groups, as outlined in Table 2. However, no such correlations were observed with either fetal plasma T_3 or T_4 concentrations and measured molecular parameters.

In UCC fetuses, UCP2 was positively correlated with fetal plasma cortisol, norepinephrine, and epinephrine (r^2 = 0.49, P = 0.008) compared with a negative correlation with fetal plasma cortisol concentration in shams. Partial correlation analyses revealed that only fetal plasma cortisol ($P < 0.05$), independently of fetal plasma norepinephrine and epinephrine

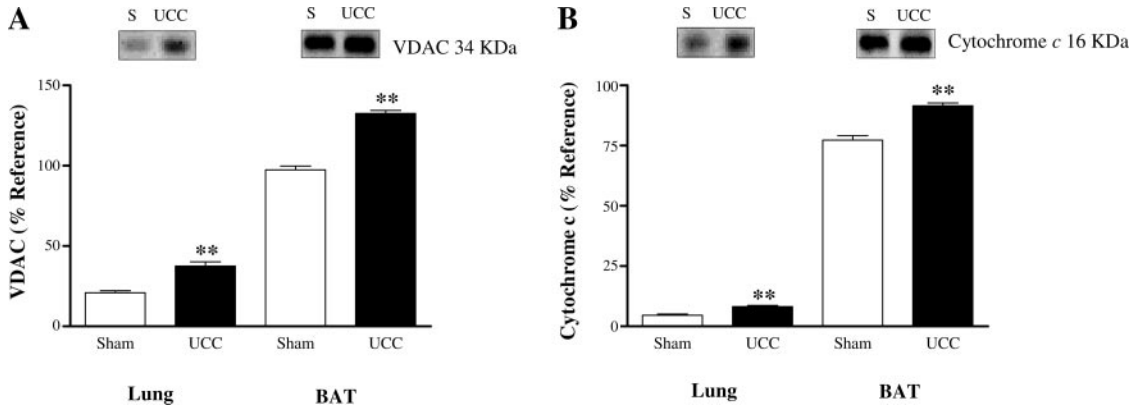

Fig. 2. Effect of UCC (30% reduction of umbilical blood flow from baseline) or sham (S) compression on abundance of voltage-dependent anion channel (VDAC; *A*) and cytochrome *c* mitochondrial proteins (*B*) in lung and BAT of ovine fetuses at 137 ± 2 days gestation (term ~145 days). Examples of protein expression are given. Values are means ± SE (n = 6–8 per group). $^{**}P < 0.01$, UCC mean value significantly different from sham group.

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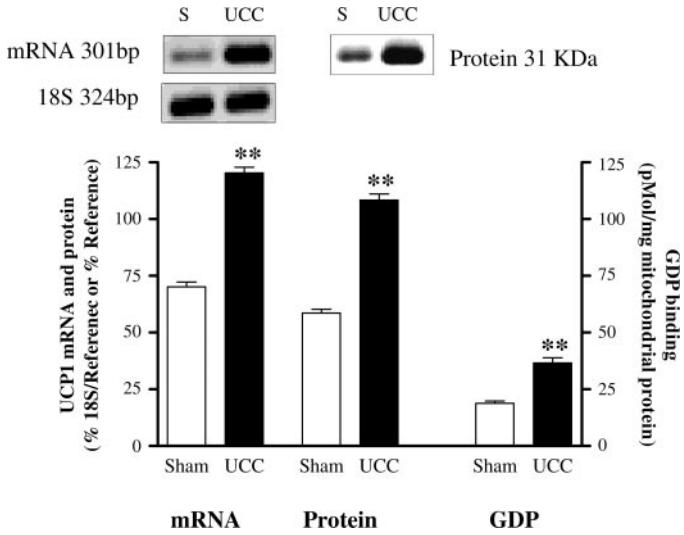

Fig. 3. Effect of UCC (30% reduction of umbilical blood flow from baseline) or sham (S) compression on abundance of UCP1 mRNA and its translated protein and on GDP (potential thermogenic capacity) binding in BAT of ovine fetuses at 137 ± 2 days gestation (term ~145 days). Examples of mRNA and protein expression are given. Values are means ± SE (n = 6–8 per group). **$P < 0.01$, UCC mean value significantly different from sham group.

concentrations, regulated UCP2 expression in BAT of UCC fetuses. UCP2 was also positively correlated with cytochrome *c* in these fetuses. In sham fetuses, GR was negatively correlated with fetal plasma norepinephrine concentration. In UCC fetuses, 11β-HSD1 was positively correlated with fetal plasma epinephrine concentration ($r^2 = 0.71$, $P = 0.019$). In UCC fetuses, 11β-HSD2 was positively correlated with fetal plasma norepinephrine and negatively correlated with fetal plasma cortisol concentration. Partial correlation analyses revealed that neither fetal plasma cortisol nor plasma norepinephrine concentration independently regulated 11β-HSD2 expression in BAT of UCC fetuses. 11β-HSD2 was also positively correlated with fetal plasma ACTH in shams.

VDAC was positively correlated with fetal plasma norepinephrine concentration in all fetuses. In UCC fetuses, cytochrome *c* was positively correlated with fetal plasma cortisol, norepinephrine, and epinephrine concentrations ($r^2 = 0.31$, $P = 0.039$). Partial correlation revealed that only fetal plasma norepinephrine ($P < 0.05$), independently of fetal plasma cortisol and epinephrine concentration, regulated cytochrome *c* expression in BAT of UCC fetuses. In shams, UCP1 mRNA was positively correlated with its translated protein, which was positively correlated with GDP binding in UCC fetuses. GDP binding was also negatively correlated with fetal plasma cortisol concentration in shams.

Relationships Between Fetal Arterial Blood Gases, Fetal Plasma Hormone Concentrations, and Abundance of mRNA and Mitochondrial Proteins and GDP Binding in Fetal Lung

A number of significant relationships were observed between fetal arterial blood gases, fetal plasma ACTH, cortisol, and catecholamines, and physiological and molecular variables measured on *day 1* post-UCC in the fetal lung of sham and UCC groups, as outlined in Table 3. Again, no such correlations were observed with either fetal plasma T_3 or T_4 concentrations and the measured parameters.

In UCC fetuses, UCP2 was positively correlated with fetal plasma cortisol and norepinephrine concentrations. Partial correlation analyses revealed that only fetal plasma cortisol ($P < 0.05$), independently of fetal plasma norepinephrine concentration, regulated UCP2 expression in the lung of UCC fetuses. UCP2 was also positively correlated with Pa_{O_2} in shams. In all fetuses, GR was positively correlated with fetal plasma ACTH and negatively correlated to fetal plasma norepinephrine concentration in shams. Partial correlation analyses revealed that neither fetal plasma ACTH nor plasma norepinephrine concentration independently regulated GR expression in the lung of UCC fetuses. In UCC fetuses, 11β-HSD1 was positively correlated with VDAC and with fetal plasma cortisol and norepinephrine concentrations. Partial correlation analyses revealed that only fetal plasma cortisol ($P < 0.05$), independently of fetal plasma norepinephrine concentration, regulated 11β-HSD1 expression in the lung of UCC fetuses. In shams, 11β-HSD1 was negatively correlated with fetal pH_a and Pa_{O_2}, whereas 11β-HSD2 was positively correlated with fetal Pa_{O_2} and negatively correlated with Pa_{CO_2} in shams. 11β-HSD2 was also positively correlated with fetal plasma cortisol concentration and negatively correlated with cytochrome *c* in UCC

Table 2. *Significant relationships in BAT between fetal plasma hormone concentrations and molecular parameters measured on day 1 post-UCC or sham-operated in late-gestation sheep fetus*

Group	Independent Variable	Dependent Variable	r^2	n	P Value	Relationship
UCC	Cortisol	UCP2 mRNA	0.52	6	0.008	+
UCC	Norepinephrine	UCP2 mRNA	0.72	6	0.036	+
Sham	Cortisol	UCP2 mRNA	0.87	6	0.037	−
UCC	UCP2 mRNA	Cytochrome *c*	0.81	6	< 0.0001	+
Sham	Norepinephrine	GR mRNA	0.91	6	0.037	−
UCC	Cortisol	11βHSD2 mRNA	0.43	6	0.019	−
UCC	Norepinephrine	11β-HSD2 mRNA	0.61	6	0.042	+
Sham	ACTH	11β-HSD2 mRNA	0.43	6	0.037	+
UCC	Norepinephrine	VDAC	0.80	6	0.042	+
Sham	Norepinephrine	VDAC	0.51	6	0.037	+
UCC	Cortisol	Cytochrome *c*	0.44	6	0.019	+
UCC	Norepinephrine	Cytochrome *c*	0.93	6	0.019	+
Sham	UCP1 mRNA	UCP1 protein	0.75	6	0.037	+
UCC	UCP1 protein	GDP binding	0.64	6	0.037	+
Sham	Cortisol	GDP binding	0.67	6	0.037	−

UCC, umbilical cord compression; VDAC, voltage-dependent anion channel; GDP, guanosine diphosphate.

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Table 3. *Significant relationships in lung between fetal plasma hormone concentrations, physiological and molecular parameters measured on day 1 post-UCC or sham-operated in late-gestation sheep fetus*

Group	Independent Variable	Dependent Variable	r^2	n	P Value	Relationship
UCC	Cortisol	UCP2 mRNA	0.55	8	0.016	+
UCC	Norepinephrine	UCP2 mRNA	0.37	8	0.042	+
Sham	Arterial Pa_{O_2}	UCP2 mRNA	0.98	6	<0.0001	+
UCC	ACTH	GR mRNA	0.30	8	0.037	+
Sham	ACTH	GR mRNA	0.66	6	0.019	+
Sham	Norepinephrine	GR mRNA	0.86	6	<0.0001	−
UCC	11β-HSD1 mRNA	VDAC	0.78	8	0.004	+
UCC	Cortisol	11β-HSD1 mRNA	0.34	8	0.046	+
UCC	Norepinephrine	11β-HSD1 mRNA	0.77	8	0.019	+
Sham	Arterial pH	11β-HSD1 mRNA	0.67	6	0.005	−
Sham	Arterial Pa_{O_2}	11β-HSD1 mRNA	0.62	6	0.019	−
Sham	Arterial Pa_{O_2}	11β-HSD2 mRNA	0.40	6	0.050	+
Sham	Arterial Pa_{O_2}	11β-HSD2 mRNA	0.71	6	0.008	−
UCC	Plasma cortisol	11β-HSD2 mRNA	0.62	8	0.004	+
UCC	11β-HSD2 mRNA	Cytochrome *c*	0.79	8	0.031	−
Sham	Cortisol	VDAC	0.87	6	0.037	+
UCC	Norepinephrine	Cytochrome *c*	0.37	8	0.041	−
Sham	Norepinephrine	Cytochrome *c*	0.69	6	0.042	−
Sham	Arterial Pa_{O_2}	GDP binding	0.84	6	0.008	−

fetuses. In shams, VDAC was positively correlated with fetal plasma cortisol concentration. Cytochrome *c* was negatively correlated with fetal plasma norepinephrine concentration in all fetuses. In shams, GDP binding was negatively correlated with fetal Pa_{O_2}.

DISCUSSION

We have shown for the first time that chronic stress produced by partial compression of the umbilical cord for 3 days results in the premature maturation of mitochondria in the lung and BAT of the late-gestation ovine fetus 7 days after the initial insult. Critically, these adaptations are correlated with the surge in fetal plasma cortisol and catecholamines that accompanies UCC. Our results therefore emphasize for the first time the primary contribution of fetal plasma cortisol and catecholamines in determining the abundance of mitochondrial mRNA and protein in the fetal lung and BAT, independent of fetal plasma thyroid hormones. Indeed, experimental models examining adverse intrauterine conditions have largely concentrated on the fetal endocrine response characterized by a sustained elevation in basal fetal plasma cortisol despite unaltered fetal ACTH levels (9, 25, 26, 28, 53). Others have focused on the impact on insulin-like growth factors and their binding proteins, which play an important role in fetal growth (36) following intermittent UCC (34).

It is unlikely that the two periods of hypoxemia following UCC were directly responsible for the changes in mitochondrial protein abundance, because both UCC-compressed and sham-compressed groups were exposed to the same experimental protocol; moreover, the slopes for the correlation between fetal plasma ACTH and cortisol between groups were similar during the periods of hypoxemia following UCC (28). Moreover, it is conceivable that the observed effects on the abundance of mitochondrial mRNA and protein in the fetal lung and BAT are due to the combined effects of the transient hypoxia and hypercarbia present during the challenge, in conjunction with the sustained elevation in fetal plasma cortisol and catecholamines. However, although the inflicted fetal asphyxia was transient during the period of UCC, the response in fetal plasma cortisol and catecholamines was sustained and, hence, more likely to be influencing the observed effects on the abundance of mitochondrial mRNA and protein in the fetal lung and BAT. Clearly, to fully differentiate between the potential confounding variables of raised fetal plasma cortisol and catecholamine concentrations, hypoxia and hypercarbia on the observed effects on lung and BAT mitochondria require further experimentation to differentiate not only between these factors during UCC but also with the subsequent hypoxic episodes imposed within this study.

Interestingly, the current study employed a model of reversible fetal asphyxia that produced marked effects on fetal plasma hormone and physiological and molecular parameters independently of changes in fetal plasma thyroid hormone concentrations. However, because we did not have cord plasma samples at post mortem, we cannot exclude the possibility that the developmental increase in fetal plasma cortisol and T_3 during late gestation may be different between sham and UCC groups. In addition, the lack of overall correlation between fetal plasma cortisol and T_3 during late gestation in this present study is in contrast to the established link between cortisol and T_3 (19, 44). Cortisol induces hepatic activity of the 5′-monodeiodinase responsible for deiodinating T_4 to T_3 and, hence, leads to a concomitant rise in fetal plasma T_3 (12). However, the low fetal pH_a and Pa_{O_2}, and increased fetal Pa_{CO_2} evident during UCC, may affect and/or oppose the ability of cortisol to stimulate thyroid hormone bioavailability (61, 68). The changes in mitochondrial mRNA and protein abundance following UCC appear to be independent of changes in fetal plasma thyroid hormone concentrations, in contrast to fetal plasma cortisol and catecholamines, which appear to have a more defined role.

Effect of Chronic UCC on Mitochondria of Fetal BAT

In fetal BAT, chronic UCC results in upregulation of UCP1, VDAC, and cytochrome *c* proteins, all of which have defined roles in energy production and, potentially, in newborn thermogenesis (13, 33, 41, 45). Indeed, the increased energy requirements needed for thermoregulation by UCP1 may be

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reflected in the higher relative abundance of VDAC and cytochrome *c* proteins in fetal BAT compared with that in the lung. Our results also demonstrate that the abundance of VDAC and cytochrome *c* proteins is positively associated with fetal plasma cortisol and/or catecholamines in the UCC groups. Whereas the appearance and activation of the BAT-specific UCP1 is dependent on fetal plasma cortisol and catecholamines (17, 44), this has not been previously demonstrated for other mitochondrial proteins. We were, however, not able to demonstrate such a relationship between UCP1 and either fetal plasma cortisol and/or norepinephrine in this study. Potential reasons for the lack of any such association may include the potential confounding effects of acidosis, hypoxia, and hypercarbia directly or indirectly impacting on fetal plasma cortisol and norepinephrine and their regulation of UCP1. The absence of any major change in fetal plasma thyroid hormones with UCC may, directly or indirectly, impact on the regulation of UCP1 in fetal BAT, since both fetal plasma cortisol and T_3 have been shown to be equally important in its regulation (44).

In contrast to the defined role of UCP1 in fetal BAT, the function and regulation of UCP2 remains a subject of intense debate (47). Our results suggest that both fetal plasma cortisol and catecholamines are potentially important in the regulation of UCP2 mRNA in fetal BAT. Previous in vitro and in vivo studies in rodents examining the relationship between norepinephrine and UCP2 mRNA in BAT have given conflicting findings. Treatment of mouse brown adipocytes in primary culture with norepinephrine triggered a dose-dependent increase in UCP2 mRNA. This was coupled with a downregulation of β_3-adrenoreceptor mRNA, the main β-adrenoreceptor through which norepinephrine mediates its effects, suggesting a role for UCP2 in rodent thermogenesis (54). However, mice lacking the dopamine β-hydroxylase gene and, hence, incapable of synthesizing norepinephrine or epinephrine, have increased UCP2 mRNA in BAT, which did not explain the increased basal metabolic rate or cold intolerance, which was attributable to impaired peripheral vasoconstriction and minimal UCP1 mRNA abundance (62).

The observed positive association between UCP2 mRNA and cytochrome *c* protein in the UCC group could indicate their combined roles in promoting apoptosis (37, 63), although it remains to be established what impact this would have on fetal BAT growth or function. One potential role for such a process may be in the transition from brown to white adipose tissue following birth in the neonatal period, which involves the proliferation and differentiation of preadipocytes and cell loss via apoptosis of adipocytes and possibly by other processes such as adipocyte dedifferentiation (51). The upregulation of UCP2, VDAC, and cytochrome *c* in fetal BAT may have roles in this orchestrated transition in late gestation, when fetal adipose tissue formation is maximal (12). However, further studies are warranted to determine the exact role and regulation of UCP2 in fetal tissues during late gestation.

The decreased glucocorticoid sensitivity within fetal BAT following chronic UCC as indicated by upregulation of 11β-HSD2 mRNA and downregulation of 11β-HSD1 mRNA were also differentially associated with fetal plasma cortisol and catecholamines. Although the 11β-HSD types 1 and 2 were positively associated with fetal plasma catecholamines, the latter enzyme was also negatively associated with fetal plasma cortisol in the UCC groups. These data taken together potentially suggest that 11β-HSD2 may be the primary determinant of glucocorticoid sensitivity within fetal BAT, with fetal plasma cortisol and catecholamines having opposing effects on this enzyme. However, we cannot exclude the potential direct or indirect effects of acidosis, hypoxia, and/or hypercarbia impacting on these and other associations observed with measured molecular parameters. Although 11β-HSD2 has an established role in the development of hypertension (64), this is the first study to determine the abundance of 11β-HSD2 in fetal BAT and is in contrast to Whorwood et al. (67), who detected 11β-HSD2 expression only in the neonatal kidney and adrenal gland in the sheep. However, their employed Northern blotting technique is less sensitive than the RT-PCR method employed in the current study. In addition, these 11β-HSD isoform mRNA changes are likely to be accompanied by parallel changes in enzyme activity (67). Interestingly, chronic hypoxemia selectively downregulates 11β-HSD2 expression in the fetal sheep kidney, leading to increased glucocorticoid sensitivity and possibly fetal hypertension (46).

In our study, although UCP1 mRNA was translated to its protein, we could not confirm this for UCP2, because the antibody raised against UCP2 cross-reacts with UCP1 (50), so it is impossible to determine the abundance of UCP2 in mitochondria that possessed UCP1. Overall, these adaptations within the mitochondria of fetal BAT suggest an improved ability of the newborn to thermoregulate following periods of chronic stress by optimizing energy production and local glucocorticoid sensitivity, although additional studies are required to clarify these initial observations.

Effect of Chronic UCC on Mitochondria of Fetal Lung

Chronic UCC resulted in upregulation of UCP2 and glucocorticoid sensitivity in the fetal lung. In the sheep lung, the peak abundance in GR and 11β-HSD1 mRNA occurs close to term, whereas UCP2 mRNA peaks just after birth, to being barely detectable after 1 mo of age (32). In contrast, UCP2 protein is undetectable in the fetal lung (45). UCP2 mRNA in the fetal lung is associated primarily with fetal plasma cortisol in the UCC group, as was the case in BAT. In addition, UCP2 mRNA abundance was positively influenced by a decrease in Pa_{O_2} during the period of UCC, suggesting that the combination of transient hypoxia during the challenge, in conjunction with the sustained elevation in fetal plasma cortisol and catecholamines, may be particularly important in regulating UCP2 mRNA expression in the fetal lung. Peccquer et al. (50) suggested that the increase in lung UCP2 with lipopolysaccharide injection was caused by macrophage receptors by lipopolysaccharide stimulating the production of proinflammatory cytokines, such as tumor necrosis factor-α (55), which activates the nuclear factor-κB pathway, thereby increasing levels of intracellular reactive oxygen species. It has also been shown that lipopolysaccharide-stimulated signals suppress UCP2 expression by interrupting the function of the intronic enhancer, leading to upregulation of intracellular reactive oxygen species, which activate the signal transduction cascade of nitric oxide synthase 2 expression (38). Chronic UCC results in increased nitric oxide activity in the fetus (27), and this may predispose to a burst of oxidative activity during periods of hypoxia-reperfusion associated with UCC and other adverse intrauterine conditions (1). A similar explanation may account

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for the decrease in GDP binding activity in the fetal lung with UCC and for the negative association between GDP binding and Pa_{O_2} in shams. The levels of GDP binding found in the fetal lung do not indicate a direct thermogenic role, as they are substantially lower than that found in BAT. Moreover, the lack of any significant positive association between GDP binding activity and fetal plasma cortisol, thyroid hormones, and catecholamines in either the fetal lung or BAT UCC groups suggests that these hormones may not directly influence GDP binding activity.

The direct impact of hypoxia on UCP2 in the lung has not been studied to date. In vivo and in vitro models of hypoxia in other tissues have found conflicting results with respect to the effect on UCP2. For example, exposure of an human adipocyte cell line to 6% oxygen for 2 days markedly decreased UCP2 mRNA (35), whereas in the rat heart a 7-day exposure to 11% oxygen did not change UCP2 mRNA (18). However, Ookawara et al. (49) found higher UCP2 mRNA in the skeletal muscle of untrained male students following 3 mo of endurance training, associated with an increase in their maximal oxygen uptake level during training, which is comparable to our findings of increased UCP2 mRNA abundance in the fetal lung and BAT following chronic UCC. In the current study, the effects on mitochondrial mRNA and protein abundance in the fetal lung and BAT by the imposed transient fetal asphyxia are likely to have been confounded by the sustained elevation in fetal plasma cortisol and catecholamines during UCC and potentially by the two subsequent hypoxic episodes.

The increased abundance of UCP2 mRNA, VDAC, and cytochrome *c* proteins with chronic UCC may promote apoptosis within the fetal lung (48, 63). Apoptotic activity has been observed during all six (embryonic, pseudoglandular, canalicular, saccular, alveolar, and microvascular) stages of lung development, suggesting its important role during this highly orchestrated process (16). After birth, apoptosis also emerges as an important process after extensive proliferation and subsequent transformation of primary saccules into functional alveoli (5, 57). Hence, the increased abundance of apoptotic mitochondrial proteins UCP2, VDAC, and cytochrome *c* with chronic UCC in the lung may promote fetal lung maturation during late gestation and after birth. The upregulation of these three mitochondrial proteins by UCC may also be reflecting increased energy production within the mitochondrial respiratory chain in the fetal lung during late gestation, in preparation for birth and extrauterine adaptation (4, 33, 41). The upregulation of VDAC protein with chronic UCC could promote solute exchange within the fetal lung, since it has recently been located in the plasma membrane (2) and appears to have a role in fluid secretion (7).

Enhanced glucocorticoid sensitivity in the fetal lung following upregulation of GR and 11β-HSD1 mRNA and downregulation of 11β-HSD2 mRNA contrasts with fetal BAT. In addition, fetal plasma ACTH, cortisol, and norepinephrine were positively associated with increased glucocorticoid sensitivity in the UCC group, indicating the potential importance of fetal plasma cortisol and catecholamines in the development of the fetal lung (20, 65). Increased glucocorticoid sensitivity has also been associated with an increase in arterial contractile sensitivity to norepinephrine and vascular resistance in ovine uterine arteries (69). Interestingly, although 11β-HSD2 appeared to be the key determinant of glucocorticoid sensitivity within fetal BAT, 11β-HSD1 appears to determine glucocorticoid sensitivity within the fetal lung. The relative abundance of 11β-HSD1 mRNA in the fetal lung was much higher than that in BAT, and this enzyme was associated not only with fetal plasma cortisol and catecholamines but also with arterial fetal pH, Pa_{O_2} and Pa_{CO_2}, which were all affected by chronic UCC (26). Overall, during chronic intrauterine stress, adaptations within mitochondria of the fetal lung may enable optimal pulmonary maturation and for the fetus to establish effective ventilation following birth. However, additional measurements of physiological lung function, including morphometry and/or surfactant analysis, are warranted to determine actual maturational effects on the lung architecture, in addition to the observed maturational effects on the lung mitochondria.

In conclusion, we have shown for the first time that chronic stress induced by partial compression of the umbilical cord for a 3-day period results in precocious maturation of mitochondria within the fetal lung and BAT by upregulating mitochondrial proteins and glucocorticoid sensitivity. Furthermore, these adaptations are mediated, in part, by the surge in fetal plasma cortisol and catecholamines that accompanies UCC. These parallel changes in the mitochondria within the fetal lung and BAT may better prepare the compromised fetus for preterm birth and extrauterine adaptation by establishing and maintaining effective ventilation and thermoregulation hand in hand.

GRANTS

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