

TRANSLATIONAL PHYSIOLOGY |

Sustained sympathetic nervous system support of arterial blood pressure during repeated brief umbilical cord occlusions in near-term fetal sheep

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Galinsky R, Jensen EC, Bennet L, Mitchell CJ, Gunn ER, Wassink G, Fraser M, Westgate JA, Gunn AJ. Sustained sympathetic nervous system support of arterial blood pressure during repeated brief umbilical cord occlusions in near-term fetal sheep. *Am J Physiol Regul Integr Comp Physiol* 306: R787–R795, 2014. First published March 19, 2014; doi:10.1152/ajpregu.00001.2014.—Sympathetic nervous system (SNS)-mediated peripheral vasoconstriction plays a key role in initial maintenance of blood pressure during rapid-onset asphyxia in the mammalian fetus, but it is attenuated after the first few minutes. It is unclear whether the SNS response is sustained during the brief, but frequently repeated, episodes of asphyxia characteristic of labor. In the present study, 14 fetal sheep at 0.85 of gestation received either chemical sympathectomy with 6-hydroxydopamine (6-OHDA; $n = 7$) or sham injection (control; $n = 7$), followed 4–5 days later by repeated 2-min episodes of complete umbilical cord occlusion every 5 min for up to 4 h or until mean arterial blood pressure (MAP) fell to <20 mmHg for two successive occlusions. In controls, umbilical cord occlusions were associated with a rapid initial fall in fetal heart rate (FHR) and femoral blood flow (FBF), with initial hypertension, followed by progressive development of hypotension during ongoing occlusions. Sympathectomy was associated with attenuation of the initial rise in MAP during umbilical cord occlusion, and after the onset of hypotension, a markedly more rapid fall of MAP to the nadir, with a correspondingly slower fall in FBF ($P < 0.05$). In contrast, MAP and FHR between successive occlusions were higher after sympathectomy ($P < 0.05$). There was no significant difference in the number of occlusions before terminal hypotension (6-OHDA; 16.1 ± 2.2 vs. control; 18.7 ± 2.3). These data show that SNS activity provides ongoing support for fetal MAP during prolonged exposure to brief repeated asphyxia.

fetal sheep; repeated asphyxia; sympathetic nervous system; sympathetic nervous system

DURING PROFOUND ASPHYXIA, the mammalian fetus adapts by a rapid, vagally mediated bradycardia that reduces myocardial work (4, 7, 28) with peripheral vasoconstriction, mediated by sympathetic nervous system (SNS) activity, which redistributes combined ventricular output away from peripheral organs (e.g., skin, gut, and kidneys) to maintain perfusion of vital organs, such as the heart, brain, and adrenals (18, 27). For example, in fetal sheep, chemical sympathectomy blunted the peripheral and central vascular responses to 2 min of profound

asphyxia (29) and 40 min of moderate hypoxia (25), supporting a key role for SNS activity in the initial fetal hemodynamic adaptation to asphyxia. These neural responses are coordinated by chemoreflex activity (4). There is evidence that other reflexes, such as the baroreflex and mechanoreceptor reflexes, do not contribute significantly (7, 9, 19). The baroreflex, for example, is incomplete in the near-term fetus and triggers vasodilation rather than vasoconstriction (9), and during umbilical cord occlusion, blood pressure increases after the initial rapid fall in fetal heart rate (7).

During sustained severe asphyxia, after the first few minutes, bradycardia is sustained by nonreflex-mediated myocardial hypoxia (2), while the early SNS-mediated vasoconstriction is progressively attenuated (2, 10, 40, 47). It is important to appreciate, however, that asphyxia during established labor is often characterized by brief, but repeated, episodes of acute asphyxia associated with uterine contractions (48). Mild fetal stress induced by repeated mild asphyxia has been associated with attenuation of the chemoreflex (19, 24). In contrast, severe fetal compromise during repetitive labor-like episodes of asphyxia was actually associated with an increase in the slope and magnitude of the initial FHR deceleration consistent with enhanced chemoreflex responses (7). It is unclear whether SNS support of fetal adaptation to such labor-like insults is also maintained over time.

Therefore, in the present study, we administered 6-hydroxydopamine (6-OHDA), a neurotoxin that destroys sympathetic nerve terminals but leaves the adrenal medulla structurally intact, to near-term fetal sheep to dissect the effect of sympathetic nerve activity on the hemodynamic responses to repeated brief episodes of asphyxia induced by umbilical cord occlusion.

MATERIALS AND METHODS

Surgical procedures. Fourteen Romney/Suffolk sheep (116–122 days of gestation) were operated on using sterile techniques (31). Food, but not water, was withdrawn 18 h before surgery. All procedures were approved by the Animal Ethics Committee of the University of Auckland. Ewes were given 5 ml of Streptopen [procaine penicillin (250,000 IU/ml) and dihydrostreptomycin (250 mg/ml), Pitman-Moore, Wellington, New Zealand] intramuscularly for prophylaxis 30 min prior to the start of surgery. Anesthesia was induced by intravenous injection of Saffan (Alphaxalone and Alphadolone, 3 mg/kg, Schering-Plough Animal Health, Wellington, New Zealand), and general anesthesia was maintained using 2–3% isoflurane in O₂. A 20-gauge intravenous catheter was placed in the maternal cephalic vein, and the ewes were placed on a constant infusion saline drip to

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Table 1. *Fetal arterial blood gases and glucose and lactate concentrations*

	pH		PaO ₂ , mmHg		PaCO ₂ , mmHg		Glucose, mmol/l		Lactate, mmol/l	
	Control	6-OHDA	Control	6-OHDA	Control	6-OHDA	Control	6-OHDA	Control	6-OHDA
Baseline	7.41 ± 0.01	7.41 ± 0.01	22.0 ± 0.96	24.3 ± 1.1	46.3 ± 2.0	44.2 ± 1.5	0.72 ± 0.08	0.85 ± 0.11	0.70 ± 0.06	0.74 ± 0.07
First	7.34 ± 0.01§	7.36 ± 0.01§	19.7 ± 0.61	22.3 ± 1.3	52.2 ± 2.1§	49.3 ± 1.7§	0.95 ± 0.11§	1.25 ± 0.12§	1.57 ± 0.14§	1.54 ± 0.20§
Middle	7.24 ± 0.02§	7.18 ± 0.04§	19.2 ± 0.18	20.2 ± 2.4	61.0 ± 1.8§	64.3 ± 6.0§	1.61 ± 0.11§	1.85 ± 0.13§	3.47 ± 0.25§	3.95 ± 0.51§
Last	6.99 ± 0.03§	6.95 ± 0.03§	21.4 ± 1.43	24.4 ± 0.61	61.4 ± 2.0§	69.3 ± 4.8§	1.30 ± 0.29§	2.39 ± 0.42§	12.5 ± 0.75§	12.3 ± 0.78§

All values are means ± SE for controls ($n = 7$) and 6-OHDA ($n = 7$) fetal sheep. First, the first five occlusions; middle, the middle five occlusions; and last, the final five occlusions. § $P < 0.05$ vs. baseline within groups. 6-OHDA; 6-hydroxydopamine.

maintain maternal fluid balance. Staff trained in administering anesthesia monitored the depth of anesthesia and maternal respiration.

Catheters were placed in a fetal femoral artery and vein, an axillary artery, and the amniotic sac. An inflatable silicone occluder was placed around the umbilical cord of all fetuses (In Vivo Metric, Healdsburg, CA). Ultrasound blood flow probes (size 3S; Transonic Systems, Ithaca, NY) were placed around a femoral artery and carotid artery for the measurement of femoral blood flow (FBF) and carotid blood flow (CaBF). ECG electrodes were sewn across the chest to record fetal heart rate (FHR). All fetal leads were exteriorized through the maternal flank, and a maternal long saphenous vein was catheterized to provide access for postoperative care and euthanasia. Antibiotics (80 mg gentamicin; Rousell, Auckland, New Zealand) were administered into the amniotic sac prior to closure of the uterus. A period of 4–6 days postoperative recovery was allowed before the experiments, during which time, antibiotics were administered daily for 4 days to the ewe [600 mg iv benzylpenicillin sodium (Crystapen) and 80 mg iv gentamicin]. Fetal catheters were maintained patent by continuous infusion of heparinized isotonic saline (20 U/ml at 0.2 ml/h), and the maternal catheter was maintained by daily flushing.

Experimental protocol. Fetuses were randomly assigned to either saline (control; $n = 7$) or sympathectomy ($n = 7$). Experiments were conducted after 3 to 4 days of postoperative recovery at 120–126 days of gestation, when neural development approximates that of term human infants (3, 37). Umbilical cord occlusions were performed by inflating the occluder with a known volume of saline and deflating it after 2 min. This procedure was repeated 3 min later, and the process continued for up to 4 h, or until mean arterial pressure (MAP) fell below 20 mmHg during two successive occlusions or failed to recover to baseline when the next occlusion was due. The ewes and fetuses were killed by an overdose of pentobarbital sodium (9 g iv to the ewe; Pentobarb 300, Chemstock International, Christchurch, New Zealand).

Sympathectomy. Fetal sympathectomy was performed 1 day after surgery using an intravenous infusion of 6-OHDA (20 mg/ml) at a rate of 2.5 ml/h over 3 h (29). To confirm that the sympathectomy was effective, a tyramine bolus (2 µg iv) was administered after 48 h. Tyramine raises blood pressure by stimulating the release of noradrenaline from peripheral nerve endings (31). During the tyramine chal-

lenge the mean increase in MAP was 13.5 ± 1.6 mmHg in controls ($P < 0.05$) vs. 2.3 ± 1.4 mmHg in the 6-OHDA group, confirming effective sympathectomy.

Fetal recordings. Fetal MAP corrected for maternal movement by subtraction of amniotic fluid pressure (Novatrans II, MX860; Medex, Hilliard, OH) (34), FBF and CaBF (T208 Ultrasonic Flowmeter; Transonic Systems), and ECG were recorded continuously. The blood pressure signal was collected at 64 Hz and low pass filtered at 30 Hz. The raw ECG was analog filtered between 0.05 and 80 Hz and digitized at 512 Hz (51). CaBF was measured as an index of changes in global cerebral blood flow (6, 8, 21, 23). Data were collected by computer and stored to disk for off-line analysis.

Fetal arterial blood gas analysis (Ciba-Corning Diagnostics 845 blood gas analyzer and co-oximeter; Ciba-Corning, Cambridge, MA), and measurements of glucose and lactate concentrations (YSI model 2300; Yellow Springs Instruments, Yellow Springs, OH) were performed immediately before the first occlusion, and before every fifth occlusion until the end of occlusions.

Hormone assays. Fetal plasma was collected before the first occlusion, then immediately after release of occlusion at 2, 12, and 42 min during the occlusion period, and 0, 4, and 24 h after the last occlusion for assessment of cortisol and catecholamine concentrations, as previously described (30, 31). Cortisol was eluted using 1 ml of ethyl acetate (Merck, Darmstadt, Germany) and measured using mass spectrometry. The internal standard was cortisol-d₂. After removal of the organic supernatant, samples were dried, resuspended in 100 µl of mobile phase [80% methanol (Merck) and 20% water], and transferred to HPLC injector vials. Twenty-five microliters were injected onto an HPLC mass spectrometer system consisting of a Waters Alliance 2690 Separations Module (Waters, Milford, MA) followed by an Ion Max APCI source on a Finnigan TSQ Quantum Ultra AM triple quadrupole mass spectrometer, all controlled by Finnigan Xcaliber software (Thermo Electron, San Jose, CA). The mobile phase was isocratic, flowing at 600 µl/min through a Luna 3µ C18(2) 100A 250 × 4.6 mm column at 40°C (Phenomenex, Auckland, New Zealand). Retention time was 6.1 min. Ionization was in positive mode and Q2 had 1.2 mTorr of argon. The mass transitions followed were cortisol-d₂ 365.3·121.2 at 28 V, cortisol 363.3·122.2 at 28 V. Mean interassay and intra-assay coefficient of variation values were 11.2

Table 2. *Epinephrine and norepinephrine concentrations*

	Epinephrine, pmol/l		Norepinephrine, pmol/l	
	Control	6-OHDA	Control	6-OHDA
Baseline	118 ± 20	176 ± 47	1,401 ± 236	939 ± 219
2 min	44,495 ± 9,590§	44,338 ± 9,557§	88,937 ± 17,374§	79,238 ± 25,443§
12 min	79,843 ± 11,771§	132,438 ± 41,507§	16,8571 ± 8,278§	159,888 ± 29,796§
42 min	74,283 ± 14,509§	60,550 ± 10,718§	115,417 ± 12,502§	85,567 ± 20,188§
End of occlusions	68,057 ± 22,470§	66,840 ± 22,660§	83,214 ± 11,768§	100,480 ± 28,214§
4 h postocclusions	1,263 ± 287§	1,116 ± 269§	8,513 ± 2,337§	3,317 ± 860§
24 h postocclusions	325 ± 123§	808 ± 216§	3,897 ± 976§	2,215 ± 271§

Data are expressed as means ± SE. Epinephrine and norepinephrine concentrations in control ($n = 7$) and 6-OHDA fetal sheep ($n = 7$). Two, 12, and 42 min indicate the time during the occlusion period. § $P < 0.05$ vs. baseline within groups. 6-OHDA, 6-hydroxydopamine.

Table 3. Cortisol concentrations

	Cortisol, ng/ml	
	Control	6-OHDA
Baseline	4.39 ± 1.31	5.83 ± 3.29
2 min	3.76 ± 2.08	7.54 ± 2.14
12 min	14.74 ± 3.54§	21.33 ± 4.13§
42 min	18.07 ± 3.95§	24.48 ± 3.98§
End of occlusions	21.74 ± 7.00§	18.57 ± 1.24§
4 h postocclusions	10.97 ± 5.16	13.78 ± 3.94
24 h postocclusions	4.19 ± 1.73	3.78 ± 1.16

Cortisol concentrations in control (*n* = 7) and 6-OHDA fetal sheep (*n* = 7). Two, 12 and 42 min indicate the time during the occlusion period. Data are mean ± SE. §*P* < 0.05 vs. baseline within groups.

and 7.1%, respectively. Cortisol was measured using LC/MS/MS single reaction monitoring during an 11-min isocratic run following the addition of deuterated cortisol as an internal standard and extraction with ethyl acetate.

Epinephrine and norepinephrine were eluted with acetic acid. The extracted catecholamines were separated and measured with

reverse-phase HPLC with electrochemical detection, as previously described (20).

Data analysis and statistics. Off-line physiological data analysis was performed using LabVIEW-based customized programs (LabVIEW for Windows, National Instruments, Austin, TX). Femoral vascular conductance (FVC) was calculated as mean blood flow/MAP. Conductance was calculated instead of the reciprocal, vascular resistance, because during umbilical cord occlusion, the denominator of resistance, blood flow, approaches zero, leading to highly nonlinear, nonparametric changes. In contrast, conductance changes more linearly, allowing parametric statistics to be used (26). To enable accurate assessment of the initial peripheral hemodynamic response to umbilical cord occlusions, 10-s averages of MAP and FBF were derived for each fetus. The baseline period was taken as the mean of 1 h before occlusions. Because of the variation in the number of occlusions between fetuses, data were grouped into the first, middle, and last set of occlusions. Each third included either three occlusions per animal for the 10-s average data, or 5 occlusions for all other data. The first third begins with the first occlusion and the final third ends with the final occlusion for each fetus. The middle third was defined as the median of the interval. This approach means that the number of fetuses in each group is the same for all time points presented.

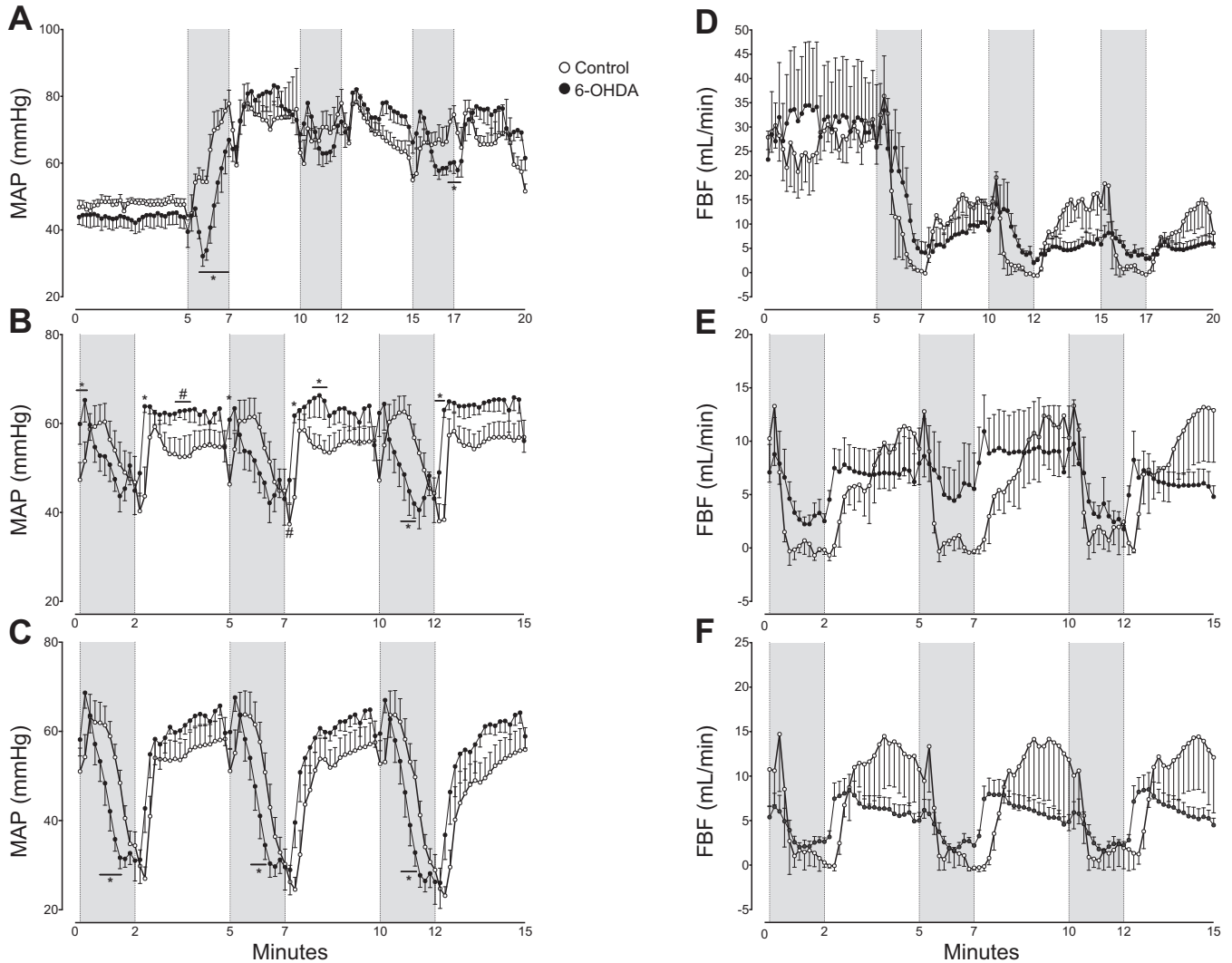


Fig. 1. Mean arterial pressure (MAP; A–C) and femoral blood flow (FBF; D–F) during the first (A and D), middle (B and E) and last three occlusions (C and F) in control (○) and sympathetomized fetal sheep (●). The shaded region denotes the period of asphyxia. Data are means ± SE. **P* < 0.05 control vs. 6-OHDA, #*P* < 0.07 control vs. 6-OHDA.

The rate of change in MAP, FBF, and FVC during the first 60 s of occlusion and during the first 90 s of recovery was derived by calculating the slope (y) for each of the variables outlined where y is the difference in pressure, flow, or conductance/time. Use of data during the first 60 s of occlusion and 90 s of recovery ensured data collection early enough to reflect the immediate hemodynamic response during and after asphyxia.

Statistical analyses were undertaken using SPSS (SPSS, Chicago, IL) and SigmaPlot software (v12.0; Systat Software, Washington, IL). Between- and within-group comparisons of fetal blood gases, glucose, lactate, cortisol, and catecholamine concentrations, MAP, FHR, CaBF, FBF, and FVC for the three time periods were performed by two-way repeated-measures ANOVA. Physiological data for the first, middle, and last set of occlusions and recovery were analyzed individually. When statistical significance was found between groups or between group and time, a Holm-Sidak post hoc test was used to determine the time points at which differences occurred. Statistical significance was accepted as $P < 0.05$. All data are expressed as means \pm SE.

RESULTS

Baseline pH, blood gases, glucose, lactate, cortisol, catecholamines, MAP, FBF, FHR, and CaBF were not different between groups. The total number of occlusions was not significantly affected by sympathectomy (16.1 ± 2.2 vs. control; 18.7 ± 2.3 occlusions).

Fetal blood gases, glucose, and lactate concentrations during occlusions. In both groups, fetal PaCO_2 increased, pH decreased, and glucose and lactate were increased during occlusions ($P < 0.05$; Table 1), with no significant effect of 6-OHDA (Table 1).

Fetal plasma cortisol and catecholamine concentrations during occlusions. Fetal plasma cortisol and catecholamine concentrations increased during the occlusion series ($P < 0.01$; Tables 2 and 3), with no significant effect of 6-OHDA.

Fetal hemodynamics during occlusions. In the control group, MAP rose during each occlusion for the first three occlusions. Thereafter, a biphasic response developed with initial hypertension followed by a fall to a nadir shortly after the release of the occluder. In the 6-OHDA group, the first umbilical cord occlusion was associated with an initial fall in MAP that recovered over the remainder of the occlusion period (Fig. 1A). Thereafter, sympathectomy was associated with an initial rapid increase in MAP during occlusion, which was followed by a more rapid fall in MAP during the first 60 s of occlusion compared with controls ($P < 0.05$; Figs. 1, A–C, and 3A).

In both groups, umbilical cord occlusions were associated with a transient brief rise in FBF, followed by a rapid fall to a nadir around the time the occluder was released (Fig. 1, D–F). Minimum FHR, CaBF, and minimum CaBF did not differ between groups during occlusions (Fig. 2, A–C). The 6-OHDA group showed an attenuated rate of fall in FBF and FVC at the beginning of the first and last three occlusions compared with controls ($P < 0.05$; Fig. 3, B and C).

Interocclusion period. Both control and sympathectomy animals showed a rapid and similar fall in FHR during occlusions, followed by overshoot acceleration after release of occlusion. There was a significant effect of time vs. group for mean interocclusion FHR, such that, overall, FHR between occlusions was higher in the 6-OHDA group than in controls ($P < 0.05$). Post hoc testing suggested that sympathectomized fetuses showed greater FHR between occlusions after the 4th

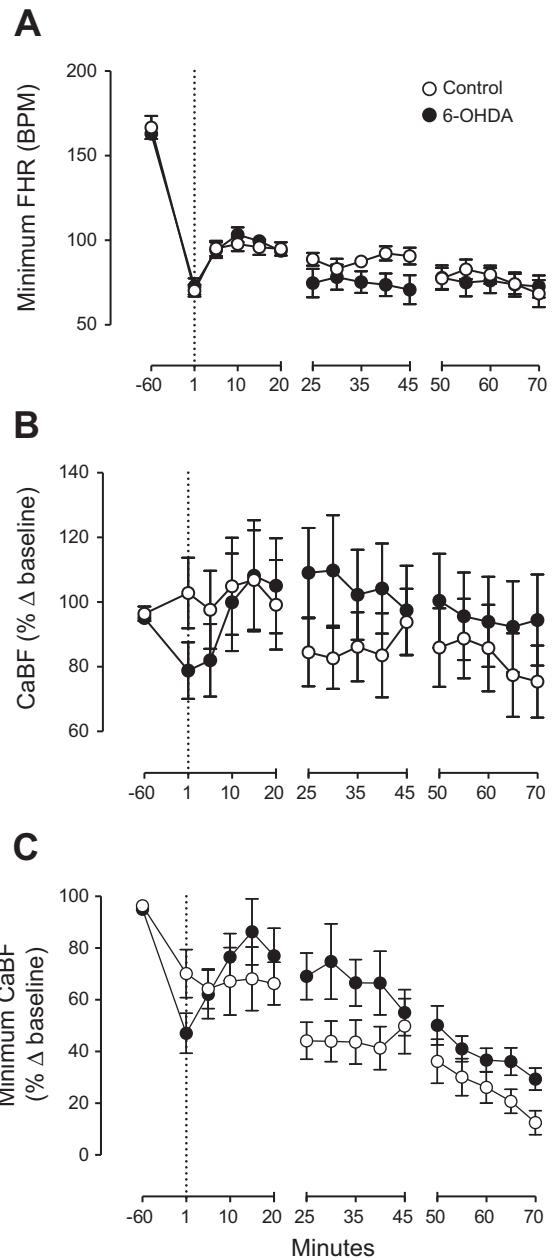


Fig. 2. Minimum fetal heart rate (FHR; A), mean and minimum carotid blood flow (CaBF; B and C, respectively) 60 min before and during the first, middle and last five umbilical cord occlusions in control (○) and sympathectomized fetal sheep (●). The dashed line indicates the beginning of the occlusion period. Data are expressed as means \pm SE.

and 5th occlusions than controls (Fig. 4A). A similar time vs. group interaction was seen for maximum interocclusion FHR ($P < 0.05$), such that the 6-OHDA group achieved a greater transient increase in FHR after release of occlusions ($P < 0.05$; Fig. 4B).

Interocclusion MAP was greater in sympathectomized fetuses than controls throughout the occlusion series ($P < 0.05$; Figs. 1B and 4C), whereas there was no difference in mean CaBF (Fig. 4D). In sympathectomized fetuses, the rate of recovery of FBF and FVC was significantly slower after the first three occlusions ($P < 0.05$; Fig. 5, A and B, respectively)

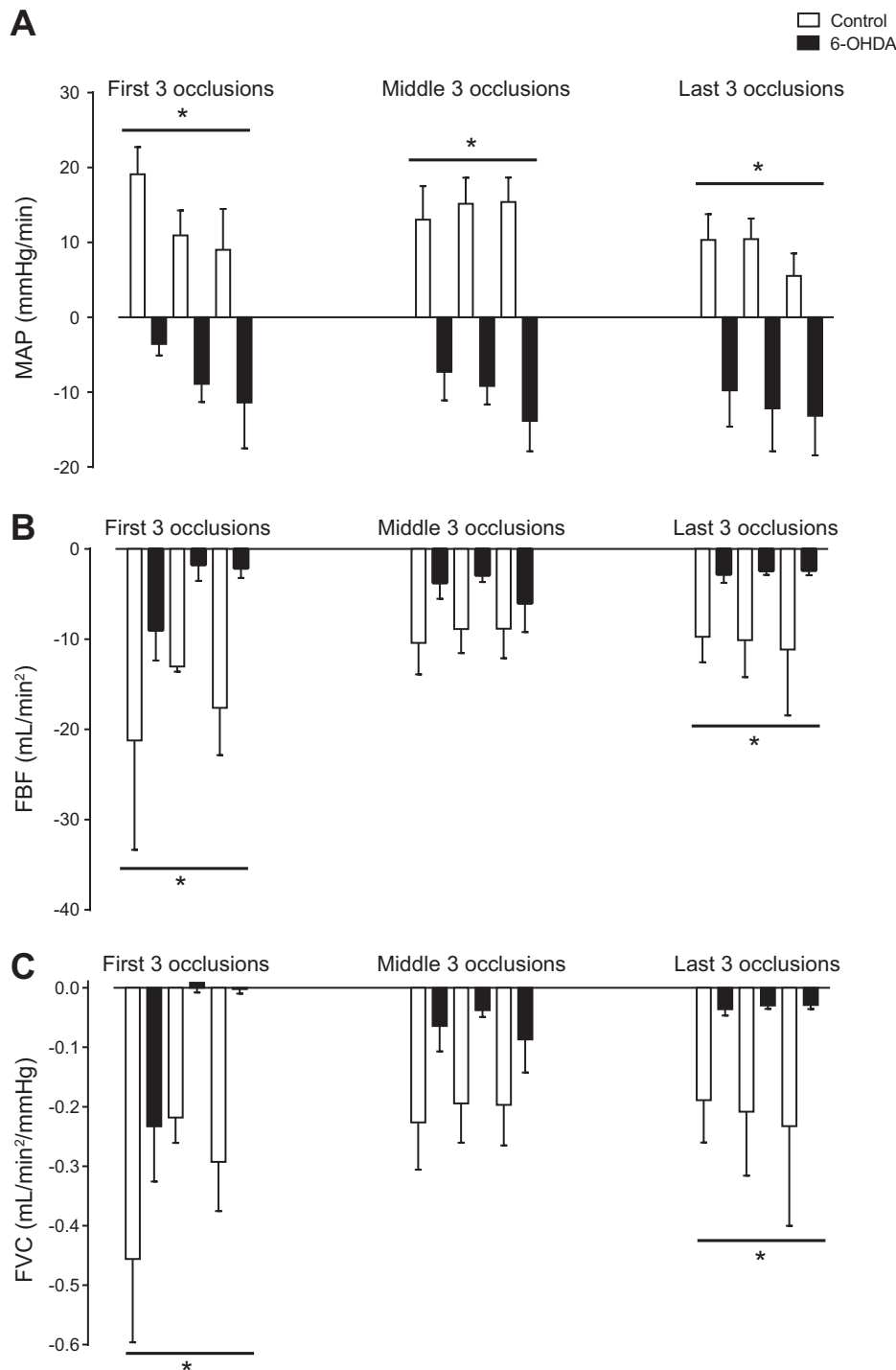


Fig. 3. Rate of change (slope) of mean arterial pressure (MAP; A), femoral blood flow (FBF; B), and femoral vascular conductance (FVC; C) during the first minute of the first, middle, and last three umbilical cord occlusions in control (open bars) and sympathectomized fetal sheep (solid bars). Data are expressed as means \pm SE. * $P < 0.05$ control vs. 6-OHDA.

but not statistically less during the last three occlusions ($P < 0.07$; Fig. 5B).

DISCUSSION

Acute but brief repeated asphyxia is common during labor and delivery (53). In the present study, repetitive brief episodes of asphyxia were associated with a sustained chemoreflex response, characterized by bradycardia with initial hypertension and peripheral vasoconstriction, as shown by reduced

FVC. Fetal chemical sympathectomy markedly delayed the rate of peripheral vasoconstriction and impaired maintenance of MAP during repeated occlusions, despite similar increases in circulating catecholamines and cortisol between groups. These data demonstrate a central role of the SNS in initiating and maintaining peripheral vasoconstriction and arterial blood pressure during prolonged exposure to brief repeated asphyxia with severe metabolic acidosis. However, fetal survival was not significantly impaired.

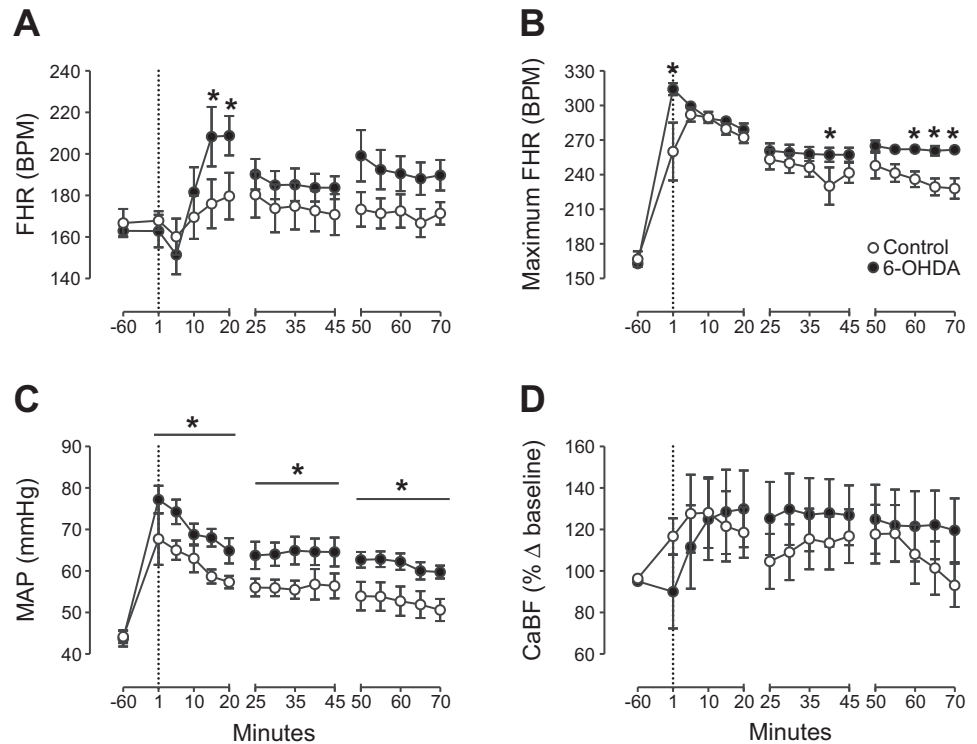


Fig. 4. Mean and maximum fetal heart rate (FHR; *A* and *B*, respectively), mean arterial pressure (MAP; *C*) and carotid blood flow (CaBF; *D*) 60 min before occlusions and during the interocclusion period between the first, middle and last 5 umbilical cord occlusions in control (○) and sympathectomized fetal sheep (●). The dashed line indicates the beginning of the occlusion period. Data are expressed as means \pm SE. * P < 0.05 control vs. 6-OHDA.

Cardiovascular adaptation of the fetus to acute episodes of hypoxia can be considered in two broad phases (5). The first phase involves an initial rapid, chemoreflex-mediated adaptation that is primarily mediated by the carotid chemoreceptors (2, 4, 18, 19). The second phase is characterized by progressive hypoxic decompensation, when the cardiovascular defenses fail, leading to hypotension and organ hypoperfusion (7, 49–52). In controls, during the first three occlusions, we observed profound bradycardia with a rapid increase in MAP that was mediated by rapid and sustained peripheral vasoconstriction. These data demonstrate that the near-term fetus is capable of rapidly establishing and maintaining centralization of blood flow throughout repeated brief episodes of asphyxia. Previous studies have shown that the increase in peripheral vascular tone during asphyxia is sympathetically mediated by α -adrenergic stimulation (18) and that the bradycardia is mediated by vagal efferents (18). During the middle and last three occlusions, progressive hypoxic decompensation was marked by a rapid fall in MAP during each occlusion.

In sympathectomized fetuses, we observed markedly delayed peripheral vasoconstriction during the first umbilical cord occlusion that was associated with a fall in MAP, consistent with previous acute observations (25, 29). In contrast, subsequent episodes of asphyxia were associated with an initial increase in MAP despite sympathectomy but impaired ability to sustain MAP during occlusions compared with controls. This was likely mediated by slower peripheral vasoconstriction during asphyxia in sympathectomized fetal sheep, with a slower rate of reduction in FBF and FVC than controls. This strongly indicates that rapid and complete peripheral vasoconstriction cannot be achieved without SNS-mediated activation of α -adrenergic efferents. It is possible that an additional factor may have been reduced cardiac contractility mediated by

impaired activation of β -adrenergic efferents after sympathectomy.

The nadir of MAP during ongoing occlusions (\sim 20–30 s before the end of occlusion) and of peripheral vascular conductance was similar in sympathectomized fetuses and controls. Further, during the interocclusion period, maximum and mean MAPs were higher in sympathectomized fetuses than controls. This was coupled with slower peripheral reperfusion in sympathectomized fetuses, as shown by a reduced rate of increase in FBF and FVC immediately after occlusions. This combination of findings strongly suggests that MAP was largely supported by vasoactive humoral factors after sympathectomy. In addition, given that stroke volume is relatively constrained in the fetus (22), it is likely that the increase in FHR, particularly in the first and last thirds of the occlusion series, also contributed, in part, to increased interocclusion MAP. Presumptively, this greater overshoot increase in FHR reflected greater release of parasympathetic activity (50).

It is likely that the substantial increases in both adrenal catecholamines and cortisol during repeated umbilical cord occlusions helped to increase MAP in both groups. Increased release of circulating catecholamines from the adrenal gland is known to contribute to the maintenance of peripheral vasoconstriction during prolonged asphyxia (18). Similarly, in fetal sheep, elevated plasma cortisol is strongly associated with elevated blood pressure (41), by augmenting cardiac contractility (39) and peripheral vasoconstriction (13, 16, 43). However, we observed no significant difference in plasma adrenal catecholamines or cortisol levels after sympathectomy, consistent with previous data that chemical sympathectomy did not alter catecholamine release during moderate hypoxia in late-gestation fetal sheep or impair

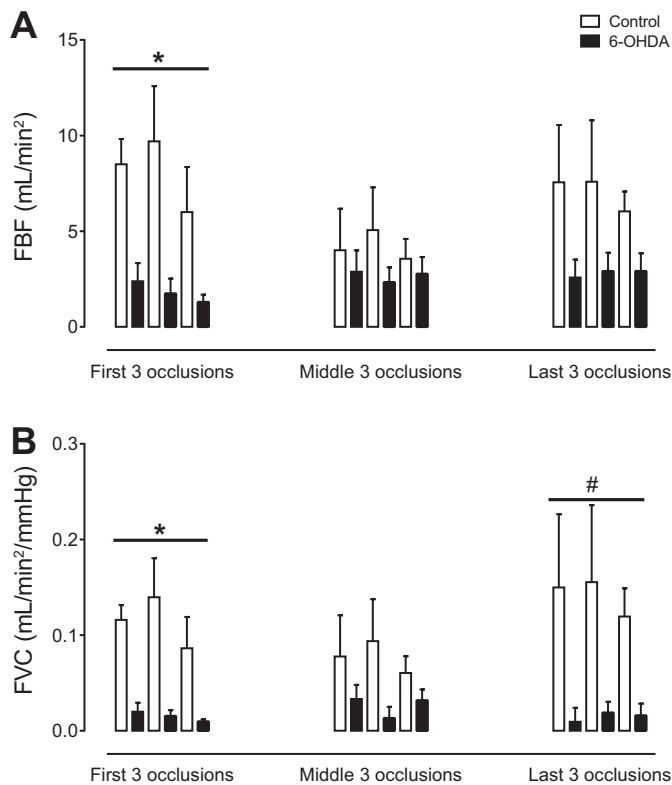


Fig. 5. Rate of change (slope) of femoral blood flow (FBF; A) and femoral vascular conductance (FVC; B) during the first 90 s of recovery after the first, middle and last three umbilical cord occlusions in control (white bars) and sympathectomized fetal sheep (black bars). Data are expressed as means \pm SE. * $P < 0.05$ control vs. 6-OHDA, # $P < 0.07$ control vs. 6-OHDA.

cortisol release (33). In contrast to previous studies (35, 36), we did not observe an increase in baseline catecholamine levels in sympathectomized fetal sheep and, supporting this, baseline arterial blood pressures were similar between groups. We speculate that this difference may be related to differences in 6-OHDA dosage and timing of administration. Although this study does not have the power to exclude small differences, there was considerable overlap in plasma levels between groups.

A number of factors likely underlie the lack of effect of chemical sympathectomy on the endocrine responses to repeated asphyxia. Cortisol release from the adrenal gland is primarily mediated by ACTH release from the anterior pituitary gland (44), but can be augmented by SNS activity during stress, as shown by the lack of effect of bilateral splanchnic nerve section on baseline cortisol levels in near-term to term fetal sheep, but an attenuated rise in plasma cortisol during acute hypotension despite no change in ACTH release (38). In contrast, chemical sympathectomy was associated with exaggeration of the cortisol response during moderate hypoxia in fetal sheep (33). There was no effect on ACTH release and thus, presumably, this greater cortisol release was mediated by indirect mechanisms. Speculatively, the lack of effect of sympathectomy on cortisol levels in the present study may reflect the much greater severity of asphyxia and systemic compromise compared with previous studies, leading to near-maximal stimulation of glucocorticoid release.

The lack of effect of sympathectomy on catecholamine levels is consistent with evidence that adrenal catecholamine release during hypoxia in fetal life can be mediated by direct, nonneurally mediated mechanisms (11), including detection of reduced levels of reactive oxygen species (46) and activation of voltage-sensitive Ca^{2+} channels in the chromaffin cell membrane (1). There is evidence that SNS input becomes more important for catecholamine release during moderate hypoxia in late-gestation fetal sheep, although the response to profound asphyxia has not been studied (11, 12).

It is important to note that the present study was conducted in near-term fetal sheep, when neural development corresponds most closely to that of the full-term human infant (3, 37). Given the maturation of sympathetic neural input to the adrenal medulla noted above and that baseline fetal cortisol levels increase from 126 to 141 days of gestation in fetal sheep (38), it is possible that full-term fetuses may be more dependent on SNS input or be able to mount a greater endocrine response to repeated asphyxia. Nevertheless, previous studies have shown that during asphyxia, fetal sheep at 0.7 of gestation have a similar relative increase in plasma cortisol to late-gestation fetal sheep (0.9 of gestation) (41). Given the similar elevation in plasma cortisol levels between groups in the present study, it is likely that the hemodynamic responses would have been similar in more mature fetuses. However, this question is worthy of further investigation. Finally, in this study, fetal arterial blood samples were taken immediately after release of occlusions. Given that in late-gestation fetal sheep, the half-life of cortisol is ~ 20 min (38), and the half-life of catecholamines is ~ 1 min (32), it is relatively unlikely that the timing of samples substantially affected the magnitude of the endocrine measurements.

In part, the increase in interocclusion MAP after sympathectomy could potentially be mediated by increased receptor sensitivity to catecholamines, as previously reported in fetal sheep (45). Alternatively, there is evidence in near-term fetal sheep that cortisol and angiotensin act synergistically to increase MAP, so that even relatively small changes might be physiologically significant (17). Studies in near-term fetal sheep have shown that unilateral renal denervation did not affect renin angiotensin system activity during renal hypoperfusion, confirming that angiotensin production can occur independently of renal sympathetic nerve activity (42). Thus, it is possible that there may have been a compensatory increase in angiotensin levels. Finally, vasopressin release also contributes to fetal vasoconstriction during prolonged hypoxia (15). However, previous studies suggest that the plasma vasopressin response to hypoxia was reduced after chemical sympathectomy in near-term fetal sheep (25), and thus, it is less likely that vasopressin played a major role in supporting arterial blood pressure in the sympathectomized fetus. Unfortunately, insufficient plasma was available to measure angiotensin and vasopressin levels.

Carotid blood flow did not differ between groups during repeated asphyxic episodes. These data are consistent with previous studies that showed during a single prolonged episode of moderate hypoxia, sympathectomized fetal sheep were capable of increasing myocardial, adrenal, and cerebral perfusion while reducing perfusion of peripheral vascular beds (25). However, as shown here, redistribution of blood flow away from the periphery during occlusions and subsequent reperfu-

sion after release of each occlusion occurred more slowly after sympathectomy. These observations suggest that in the absence of functional α -adrenergic efferents, other local and/or humoral mechanisms are important for redistributing and maintaining combined ventricular output toward vital organs, such as the brain during short repeated asphyxic episodes.

Perspectives and Significance

The SNS is a key factor in fetal adaptation to repeated asphyxia, as may be seen during intense established labor. During sustained asphyxia, for example, during clinical sentinel events, such as cord prolapse or placental abruption, fetuses show progressive attenuation of peripheral vasoconstriction after the first few minutes, ultimately leading to severe hypotension and relative reperfusion of peripheral tissues (14, 47). In contrast, strikingly, the current study shows that in near-term fetal sheep, SNS activity was essential to maintain peripheral vasoconstriction and MAP during repeated episodes of umbilical cord occlusion despite the development of profound metabolic acidosis and hypotension. While this response is primarily mediated by activation of α -adrenergic efferents, further studies are required to better understand the local and humoral factors that partially compensate for loss of SNS activity and whether these responses change in late gestation.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

Author contributions: R.G., E.C.J., C.J.M., E.R.G., G.W., M.F., J.A.W., and A.J.G. performed experiments; R.G., E.C.J., C.J.M., E.R.G., G.W., M.F., J.A.W., and A.J.G. analyzed data; R.G., E.C.J., L.B., C.J.M., E.R.G., G.W., M.F., J.A.W., and A.J.G. interpreted results of experiments; R.G., E.C.J., and C.J.M. prepared figures; R.G. and E.C.J. drafted manuscript; R.G., L.B., E.R.G., G.W., M.F., J.A.W., and A.J.G. edited and revised manuscript; R.G., E.C.J., L.B., C.J.M., E.R.G., G.W., M.F., J.A.W., and A.J.G. approved final version of manuscript; L.B., J.A.W., and A.J.G. conception and design of research.

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