Total body catecholamine kinetics before and after birth in spontaneously hypoxemic fetal lambs

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Smolich, Joseph J., and Murray D. Esler. Total body catecholamine kinetics before and after birth in spontaneously hypoxemic fetal lambs. Am. J. Physiol. 277 (Regulatory Integrative Comp. Physiol. 46): R1313–R1320, 1999.—To study the effect of fetal hypoxemia on perinatal norepinephrine and epinephrine total body kinetics, 13 near-term fetal lambs were instrumented with vascular catheters under general anesthesia. One week later, norepinephrine and epinephrine kinetics were measured in normoxemic (n = 7) or spontaneously hypoxemic fetuses (n = 6) with isotope dilution methodology. Hypoxemic fetuses had lower body (P < 0.02) and placental (P = 0.01) weights and a threefold elevation in plasma norepinephrine (P < 0.005) and epinephrine (P < 0.025) associated with correspondingly higher total body norepinephrine (P < 0.005) and epinephrine (P < 0.05) spillovers. After birth, total body norepinephrine and epinephrine spillover increased 45% and 3.2-fold, respectively, in normoxemic animals (both P < 0.001). However, in the hypoxic group, norepinephrine total body spillover was unchanged between fetal and 1-h lambs and then fell in 4-h lambs (P < 0.005). In addition, total body epinephrine release rose postnatally (P < 0.05) but less than in the normoxic group (P < 0.02). No differences in norepinephrine or epinephrine total body clearance occurred between normoxic and hypoxic groups in either fetal or newborn lambs. These findings indicate that in hypoxemic and growth-restricted fetuses 1) elevated circulating norepinephrine and epinephrine levels are related to increased sympathoadrenal activity and 2) birth is associated with an initial maintenance and subsequent decline in global sympathetic activity but a blunting of adrenal medullary activation.

ALTHOUGH SUSTAINED FETAL hypoxemia may be accompanied by an elevation in the circulating levels of norepinephrine (7, 11, 21), epinephrine (17), or both of these catecholamines (14, 29), the basis of these alterations is not well understood. One possibility is that the release of catecholamines into the circulation (i.e., spillover) is increased by chronic fetal hypoxemia. Because norepinephrine is the principal neurotransmitter within sympathetic nerves (2, 5) and epinephrine is mainly derived from the adrenal medulla (5, 16, 26), this explanation would suggest that sustained fetal hypoxemia augments sympathoadrenal activity. However, because the circulating level of catecholamines is dependent on the balance between their entry into and exit from the circulation (5), such elevations could also be related to an impairment of catecholamine removal processes (i.e., clearance). The nature of changes in catecholamine kinetics accompanying in utero hypoxemia is of further importance because the transition from fetus to newborn is normally accompanied by surges in circulating norepinephrine and epinephrine levels (2, 26, 30), which are related not only to increased catecholamine spillover but also reduced catecholamine clearance (30). Alterations in catecholamine spillover or clearance accompanying sustained fetal hypoxemia are therefore likely to have secondary postnatal consequences, not only for changes in norepinephrine and epinephrine kinetics but also circulating levels.

This study therefore had two main aims. The first was to determine the relative contribution of alterations in catecholamine spillover and clearance to increases in circulating norepinephrine and epinephrine levels in sustained fetal hypoxemia. The second was to define the manner in which preexisting fetal hypoxemia influenced birth-related changes in catecholamine kinetics. Fetal and newborn total body norepinephrine and epinephrine kinetics were determined with isotope dilution methodology using a combined tracer infusion of 3H-labeled norepinephrine and epinephrine. Studies were performed in chronically instrumented near-term spontaneously hypoxic fetal lambs, before and after cesarean section delivery, and results were compared with data obtained from a group of normoxic animals prepared within a similar experimental period. Data from some of the normoxic animals have been included in a previous report from this laboratory examining perinatal changes in total body norepinephrine and epinephrine kinetics (30).

MATERIALS AND METHODS

All studies were approved by the Monash University Animal Experimentation Committee and were in accord with guidelines established by the National Health and Medical Research Council of Australia.

Animal preparation. Thirteen fetal lambs with known breeding dates were chronically instrumented under aseptic conditions at 133–134 days of gestation (term 147 days) as described previously (30–32). In brief, fasted Border-Leicester cross ewes were anesthetized with propofol (5 mg/kg iv), intubated, and then mechanically ventilated with 1–3% halothane and a 2:1 nitrous oxide-oxygen mixture. The uterus was exposed through a midline laparotomy and incised over the fetal hindlimbs. Polyvinyl catheters (ID 1 mm, OD 1.5 mm) were inserted into a posterior tibial artery and lateral saphenous vein and advanced into the abdominal aorta and inferior vena cava, respectively. After delivery of the fetal
head, left forelimb, and upper thorax through a second hysterotomy, a thoracotomy was performed in the third left interspace and the pericardium was incised over the pulmonary trunk and left atrium. A Teflon cannula connected to a polyvinyl catheter was inserted into the distal part of the pulmonary trunk, and a polyvinyl catheter was introduced into the left atrial cavity through a purse-string suture. A Silastic catheter was also passed into the origin of the coronary sinus via the left hemiazygous vein. The pericardium was then loosely closed, the ribs were reapproxosed, and the overlying muscle layers were repaired. After incision of the neck ventrally in the midline, a Teflon cannula attached to a polyvinyl catheter was inserted nonocclusively into the left carotid artery and a polyvinyl catheter was passed into the superior vena cava via the left external jugular vein. Both catheters were tunneled subcutaneously to the chest incision. In all fetuses, a Silastic catheter (1D 0.8 mm, OD 1.7 mm) was introduced into the upper part of the trachea via an intercartilaginous space and exteriorized through the cephalic end of the neck incision for later withdrawal of lung liquid. Lastly, a wide-bore catheter was sutured to the anterior chest wall for measurement of amniotic fluid pressure. The fetus was then returned to the uterus, all incisions were closed, and antibiotics (500 mg streptomycin and 5 × 10⁵ units penicillin) were instilled into the amniotic cavity. The vascular catheters were filled with sodium heparin solution (1,000 IU/ml) and exteriorized on the right flank of the ewe. After surgery, antibiotics were administered daily, either as an intramuscular injection to the ewe or directly into the amniotic cavity, while vascular catheters were flushed on the first postoperative day and every second day thereafter.

Experimental protocol. Experiments were performed 7 days after surgery, at a gestation of 140–141 days. To exclude potential effects of the surgical procedure itself, sustained hypoxemia was defined as the presence of an ascending aortic hemoglobin O₂ saturation of ≤40% for at least 1 day prior to the experiment and was evident in six fetuses. The remaining seven normoxic fetuses all had an ascending aortic hemoglobin O₂ saturation of ≥45%. The ensuing experimental protocol was identical in both animals groups. To measure fetal catecholamine total body spillover and clearance rates, [³H]norepinephrine and [³H]epinephrine were simultaneously infused for 30 min into the fetal hindlimb venous catheter in all the normoxic and four hypoxic fetuses, and into the left atrial catheter in the remaining two hypoxic fetuses. After recording of hemodynamics and removal of twice the catheter dead space volume, 2.5 ml of blood were simultaneously withdrawn from the carotid artery, pulmonary trunk, and abdominal aorta for catecholamine analysis, and hemato-crit determination. Fetal ventricular outputs and major regional blood flows were measured with radioactive microspheres using the reference sample method (10), and blood samples were collected aeronically from the carotid artery for hemoglobin and blood gas analysis. The tritiated catecholamine infusion was then stopped, and low spinal anesthesia was induced in the ewe with an intrathecal injection of 3–5 ml of 0.5% bupivacaine. After withdrawal of 30–40 ml of lung liquid via the tracheal catheter to facilitate the rapid establishment of pulmonary gas exchange after birth (4), fetuses were quickly delivered by cesarean section, the tracheal catheter was removed, and the umbilical cord was clamped and cut. The ewe was killed immediately after cesarean section delivery with an intravenous overdose of pentobarbital sodium. All lambs breathed spontaneously and rapidly established a rhythmic breathing pattern. Newborn studies were performed 1 and 4 h after cord clamping. As in the fetus, an infusion of [³H]norepinephrine and [³H]epinephrine was begun 30 min before each study. With the [³H]catecholamine infusion continuing, hemodynamics were then recorded, blood samples were taken for hematocrit, blood gas and catecholamine analysis, and left ventricular (LV) output was measured with radioactive microspheres.

Physiological measurements. Mean abdominal aortic blood pressure was referenced to amniotic fluid pressure in fetuses and to atmospheric pressure at the mid-chest position in newborn lambs. Both aortic blood pressure and amniotic fluid pressure were monitored with strain-gauge pressure transducers (model 1280B, Hewlett Packard, Waltham, MA), which were calibrated against a water manometer before each experiment. Heart rate was measured with a tachometer triggered by the arterial pulse. Signals were displayed on an eight-channel paper recorder (model 8002; Nemedix Systems, Sydney, New South Wales, Australia).

Blood pH, P O₂, and P CO₂ were measured with a blood analyzer (model 168, Corning Medical, Halstead, Essex, UK), at 39°C in fetuses and the measured rectal temperature in newborn lambs. Blood hemoglobin concentration and hemoglobin O₂ saturation were measured in duplicate with a hemometer (model OS52, Radiometer, Copenhagen, Denmark).

Radiotracer infusions. Stock solutions of radiolabeled norepinephrine (levo-[2, 5, 6]¹H)norepinephrine) and epinephrine (levo-[3,4,5,6]¹H)epinephrine; New England Nuclear, Boston, MA), dissolved in 0.2 M acetic acid and containing 1 mg/ml ascorbate, were stored at −80°C. Before the study, an aliquot of each radiotracer was thawed and added to 40 ml of 0.9% sodium chloride, which was then infused with a syringe pump at a rate of 0.18 ml/min. The infusion rate of [³H]norepinephrine was 43.7 ± 3.3 nCi·kg⁻¹·min⁻¹ in the normoxic and 66.6 ± 8.1 nCi·kg⁻¹·min⁻¹ in the hypoxic group. The infusion rate for [³H]epinephrine was 54.3 ± 4.5 nCi·kg⁻¹·min⁻¹ in the normoxic and 67.5 ± 8.9 nCi·kg⁻¹·min⁻¹ in the hypoxic group. A sample of the infusate was stored at −80°C for subsequent assay of norepinephrine and epinephrine content.

Blood samples withdrawn during infusion of [³H]-labeled tracers were transferred to tubes containing EDTA and immediately centrifuged. The plasma fraction was pipetted into Eppendorf tubes, which were initially placed on dry ice and then stored at −80°C until analysis.

Assay of catecholamines. Endogenous and tritiated catecholamines were extracted from plasma samples using alumina adsorption and separated with high-performance liquid chromatography as previously described (30). Concentrations of total catecholamines in 1 ml plasma and 10 µl infusate samples were quantified by electrochemical detection, while timed collection of the eluant leaving the electrochemical cell allowed fractionation of [³H]-labeled catecholamines into scintillation vials for counting by liquid scintillation spectroscopy. The within-assay coefficient of variation was 2.0% for norepinephrine and 2.3% for epinephrine. Endogenous levels of norepinephrine and epinephrine were not corrected for the contribution of exogenous [³H]-labeled catecholamines, because, on average, the infused [³H]norepinephrine contributed <1% to endogenous norepinephrine levels, whereas the contribution of [³H]epinephrine to endogenous epinephrine was <2%.

Radioactive microsphere technique. Radioactive microspheres, 15 µm in diameter and labeled with one of five gamma-emitting isotopes ([¹⁴C]Ce, [¹¹³Sn, [⁸⁵Sr, [⁵⁹Nb, or [⁴⁶Sc; New England Nuclear) were ultrasonicated for 10–15 min before injection and then injected over 30–45 s with 10 ml isotonic saline. In fetuses, two different microsphere labels were injected simultaneously, one into the left atrium to measure LV output and the other into either the superior
vena cava or coronary sinus to measure right ventricular (RV) output (32). Approximately $1 \times 10^6$ microspheres were injected per microsphere label, while reference samples were drawn simultaneously from the carotid artery, pulmonary trunk, and abdominal aorta. In newborn lambs, about $0.5 \times 10^6$ microspheres were injected into the left atrium to measure LV output, while reference samples were obtained from the carotid artery and the abdominal aorta. All reference samples were drawn at a rate of 4.1 ml/min with a mechanical pump (model 901A; Harvard Apparatus, South Natick, MA). Reference sample collection was commenced 5–10 s before injection and continued for an additional 75 s after the end of injection. Blood withdrawn in the reference samples was simultaneously replaced with fetal blood mixed with a plasma substitute (Haemaccel, Behring, Marburg, Germany).

After completion of the experimental protocol, lambs were killed with an intravenous overdose of pentobarbital sodium and the position of all catheters was carefully checked. The lungs and placenta were placed in Formalin fixative for 7–10 days and then carbonized at a temperature of 280°C in a vented box furnace. The carbonized tissue was ground into a coarse powder and packed into plastic counting vials to a height of $\leq 2$ cm. The radioactivity of the blood reference samples and the tissue vials was counted in a gamma counter (model 1282 CompuGamma; LKB-Wallac, Turku, Finland) at the appropriate window settings and the photopeaks of the individual isotopes were separated by an online computer program.

Calculation of blood flows. Radioactive microsphere measurements of ventricular output and tissue blood flow were calculated using the general relation $Q = (Q_{\text{Ref}} \cdot R)/R_{\text{Ref}}$, where $Q$ is flow (ml/min) and $R$ is radioactivity (counts/min). With use of this relation, LV output ($Q_{\text{LV}}$) in fetal and newborn lambs was equal to $(Q_{\text{Ref}} \cdot R_{\text{LA}})/R_{\text{LA-Ca}}$, where $R_{\text{LA}}$ is the radioactivity of the label injected into the left atrial cavity and $R_{\text{LA-Ca}}$ is the radioactivity of the same label collected in the carotid arterial reference sample. Fetal RV output ($Q_{\text{RV}}$) was equivalent to $(Q_{\text{Ref}} \cdot R_{\text{RV}})/R_{\text{VP}}$, where $R_{\text{RV}}$ is the radioactivity of the venous label passing into the right ventricle, calculated as the injected radioactivity of this label minus that portion crossing the foramen ovale to appear in the LV output, and $R_{\text{VP}}$ is the radioactivity of the venous label in the pulmonary reference sample (32).

Fetal lung blood flow ($Q_{\text{L}}$) was calculated as $(Q_{\text{Ref}} \cdot R_{\text{L}})/R_{\text{VP}}$, where $R_{\text{L}}$ is the radioactivity of the venous label passing to the lungs. Placental blood flow ($Q_{\text{P}}$) was calculated as $(Q_{\text{Ref}} \cdot R_{\text{P}})/R_{\text{AA}}$, where $R_{\text{P}}$ is the radioactivity of the placenta and $R_{\text{AA}}$ is the radioactivity of the venous microsphere label in the umbilical aortic reference sample. With use of previously derived equations (32), fetal upper body flow ($Q_{\text{UB}}$) was computed as $Q_{\text{LV}} - [(Q_{\text{Ref}} - Q_{\text{L}})(R_{\text{LA-CA}} - R_{\text{LA-AA}})]$, and the combined fetal lower body and placental flow ($Q_{\text{LBP}}$) as $Q_{\text{RV}} - (Q_{\text{VP}})$, where $R_{\text{LA-CA}}$ is the radioactivity of the same label collected in the carotid arterial reference sample. Fetal lung blood flow was calculated as $Q_{\text{L}} = Q_{\text{UB}} + Q_{\text{L}} + Q_{\text{VP}}$, where $Q_{\text{UB}}$, $Q_{\text{L}}$, and $Q_{\text{VP}}$ are defined in the previous text.

Total body plasma catecholamine clearance and spillover rate was obtained with previously described formulas (5, 30). The total body plasma clearance of catecholamines (TBCl) was calculated as $IR/(\text{[3H-Cat}_a) \cdot BW$, where $IR$ is the rate at which $\text{[3H-Cat}_a$ is infused into the circulation, $\text{[3H-Cat}_a$ is the steady-state mean systemic arterial plasma concentration of $\text{[3H-Cat}_a$, and BW is the body weight. To provide an accurate measure of TBCl in newborn lambs receiving intravenous infusion of $\text{[3H-Cat}_a$ tracers, IR was reduced by a correction factor that corresponded to the pulmonary clearance of catecholamines (9). The magnitude of this correction factor was 17.1 ± 5.6% for $\text{[1^H-Inorepinephrine and 2.2 ± 1.0% for [1^H-Epinephrine. Division of TBCl by the systemic plasma flow yielded the total body fractional extraction, i.e., the proportion of catecholamine extracted on a single pass through the circulation. Systemic plasma flow was computed as $CO - (1 - Hct)$, where $CO$ is the combined LV and RV output in the fetus and the LV output in the newborn and Hct is the hematocrit.

The total body spillover rate of catecholamines into plasma was computed as $TBCl \cdot Cat_a$, where $Cat_a$ is the mean arterial plasma concentration of norepinephrine or epinephrine.

Statistics. Changes in physiological variables, catecholamine clearance, and spillovers between fetal and newborn lambs were analyzed with repeated measures one-way ANOVA (33). In the case of the epinephrine results, this was preceded when necessary by logarithmic transformation of nonnormally distributed data. The sum of squares from the ANOVA was orthogonally partitioned into individual degrees of freedom, and the significance of changes between the fetal and newborn periods was evaluated using the Bonferroni procedure as appropriate for multiple tests (34). Differences between the normoxic and hypoxic groups were compared with one-way ANOVA if normally distributed or a Mann-Whitney U test if not normally distributed. Results are reported as means ± SE, and $P < 0.05$ was considered significant.

RESULTS

Weights, hemodynamics, and blood gas variables. The presence of fetal hypoxemia was associated with a reduced fetal body weight (3.90 ± 0.10 vs. 3.30 ± 0.18 kg, $P < 0.02$) and placental weight (0.53 ± 0.03 vs. 0.36 ± 0.03 kg, $P = 0.01$). Hemoglobin concentration, mean arterial blood pressure, heart rate, and systemic plasma flow were similar in normoxic and hypoxic fetuses, but the latter had a lower pH ($P < 0.025$) and $P_O_2$ ($P = 0.005$), as well as a higher $P_CO_2$ ($P < 0.05$). The pattern of change in blood gas and hemodynamic variables after delivery was similar in normoxic and hypoxic groups, and apart from a lower arterial hemoglobin $O_2$ saturation in the hypoxic group at 1 h ($P < 0.05$), postnatal variables were not significantly different between the two groups (Table 1).

Endogenous catecholamine plasma concentrations. The average arterial norepinephrine concentration in hypoxic fetuses, 3.027 ± 648 pg/ml, was 3.2-fold that of normoxic fetuses, 941 ± 35 pg/ml ($P < 0.005$; Fig. 1A). In the normoxic group, the arterial norepinephrine level rose by 120% after birth ($P < 0.001$). By contrast, in the hypoxic group, the arterial norepinephrine level rose by only 39% in 1-h lambs ($P < 0.05$), an increment that was less than in the normoxic group ($P < 0.05$), and then tended to fall in 4-h lambs. Thus, whereas the arterial norepinephrine in the hypoxic
Table 1. Hemodynamics and ascending aortic blood gas variables before and after birth

<table>
<thead>
<tr>
<th>Group</th>
<th>Fetuses</th>
<th>1-H NB</th>
<th>4-H NB</th>
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<tr>
<td>Hb, g/dl</td>
<td>N</td>
<td>10.2±0.3</td>
<td></td>
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<tr>
<td></td>
<td>H</td>
<td>11.0±0.8</td>
<td></td>
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<tr>
<td>pH</td>
<td>N</td>
<td>7.335±0.020</td>
<td></td>
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<tr>
<td></td>
<td>H</td>
<td>7.264±0.015</td>
<td></td>
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<tr>
<td>HbO₂ satu-</td>
<td>N</td>
<td>48.7±1.1</td>
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</tr>
<tr>
<td>ration, %</td>
<td>H</td>
<td>32.8±2.8</td>
<td></td>
</tr>
<tr>
<td>PO₂, mmHg</td>
<td>N</td>
<td>21.5±1.0</td>
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<td></td>
<td>H</td>
<td>16.0±1.2</td>
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<tr>
<td>Pco₂, mmHg</td>
<td>N</td>
<td>50.9±0.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>H</td>
<td>54.1±1.3</td>
<td></td>
</tr>
<tr>
<td>MABP, mmHg</td>
<td>N</td>
<td>52±1.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>H</td>
<td>51±1.1</td>
<td></td>
</tr>
<tr>
<td>Heart rate,</td>
<td>N</td>
<td>155±5.1</td>
<td></td>
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<tr>
<td>beats/min</td>
<td>H</td>
<td>153±4.1</td>
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<tr>
<td>Systemic</td>
<td>N</td>
<td>324±27.7</td>
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</tr>
<tr>
<td>plasma flow</td>
<td>H</td>
<td>321±15.9</td>
<td></td>
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<tr>
<td>ml·min⁻¹·kg⁻¹</td>
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Values are means ± SE; n = 7 normoxemia and 6 hypoxemia lambs. N, normoxemic group; H, hypoxic group; 1-H NB, 1-h newborn lambs; 4-H NB, 4-h newborn lambs; Hb, hemoglobin; MABP, mean systemic arterial blood pressure. *P < 0.05, **P < 0.025, ***P < 0.005 normoxemic vs. hypoxemic fetuses. 1-P < 0.05, 2-P < 0.01, 3-P < 0.005, fetuses vs. 1- and 4-H NB; 4-P < 0.005, 5-P < 0.001, 6-HNB vs. 1-HNB; 7-P < 0.005, H vs. N.

The arterial epinephrine concentration in hypoxemic fetuses, 42±12 pg/ml (P < 0.025; Fig. 1B). In the normoxemic group, the arterial epinephrine increased 5.4-fold with birth (P < 0.001). The circulating epinephrine concentration also increased after birth in the hypoxemic group (P < 0.005), but the 1.9-fold increment was less than in the normoxemic group (P < 0.02). The plasma epinephrine concentration was not different between normoxemic and hypoxemic groups in 1- or 4-h lambs (Fig. 1B).

Catecholamine total body clearance. Norepinephrine total body clearance per unit body weight was not significantly different between normoxemic (145 ± 9 ml·min⁻¹·kg⁻¹) and hypoxemic fetuses (133 ± 14 ml·min⁻¹·kg⁻¹; Fig. 2A). In the normoxemic group, norepinephrine total body clearance decreased by 32% between fetal and newborn lambs (P < 0.001). However, norepinephrine total body clearance in the hypoxemic group decreased by 29% between fetal and 1-h lambs (P < 0.005) and by a further 29% in 4-h lambs (P < 0.02; Fig. 2A).

The epinephrine total body clearance was similar in normoxemic (131 ± 7 ml·min⁻¹·kg⁻¹) and hypoxemic fetuses (118 ± 10 ml·min⁻¹·kg⁻¹; Fig. 2B). In the normoxemic group, epinephrine total body clearance decreased by 35% between fetal and newborn lambs (P < 0.001). By contrast, in the hypoxemic group, epinephrine total body clearance decreased by 32% between fetal and 1-h lambs (P < 0.001) and then fell by a further 18% in 4-h lambs (P < 0.03; Fig. 2B).

Catecholamine total body fractional extraction. The norepinephrine total body fractional extraction was similar in normoxemic (0.46 ± 0.03) and hypoxemic fetal lambs (0.43 ± 0.07), and neither changed significantly after birth (Fig. 3A). Similarly, the epinephrine total body fractional extraction was not statistically different in normoxemic (0.42 ± 0.04) and hypoxemic fetal lambs (0.37 ± 0.05), and neither was altered to any significant extent after birth (Fig. 3B).
lower in 4-h lambs (P < 0.005; Fig. 4A). Thus, whereas norepinephrine total body spillover was higher in the hypoxemic group in 1-h lambs (P < 0.01), no difference was evident between the two groups 4 h after birth.

Epinephrine total body spillover in hypoxic fetuses (16.5 ± 4.9 ng·min⁻¹·kg⁻¹) was 3.2-fold that of normoxic fetuses (5.1 ± 1.2 ng·min⁻¹·kg⁻¹; P < 0.05). However, the 94% increment in epinephrine total body spillover in the hypoxic group between fetal and newborn lambs was less than the 3.6-fold rise evident in the normoxic group (P < 0.02).

DISCUSSION

Four main findings have emerged from this study, which has examined total body catecholamine kinetics in near-term spontaneously hypoxic fetal lambs before and after cesarean section delivery. First, elevations in circulating norepinephrine and epinephrine levels were primarily related to increased release of these catecholamines into the circulation. Second, rises in circulating norepinephrine and epinephrine levels were attenuated after birth. Third, total body norepinephrine spillover was initially unchanged and then reduced after birth. Last, the increase in whole body epinephrine release occurring with birth was less pronounced than normal. Taken together, these observations suggest that while sustained in utero hypoxemia elevates fetal circulating norepinephrine and epinephrine levels via an enhancement of sympathoadrenal activity, it abolishes the perinatal increase in global sympathetic activation and blunts birth-related rises in adrenal medullary activity.

Spontaneous hypoxemia in fetal lambs is a recognized phenomenon that has been studied previously in relation to its effects on systemic blood flow and oxygenation (6, 8, 28), as well as placental morphology (27). Although the precise duration of the hypoxemia in our study was uncertain, two factors suggested that it was long-standing. First, fetal arterial blood pressure and heart rate were not different from the control values, a
finding that not only contrasts with the hypertension and bradycardia seen with acute hypoxemia (15, 17) but is similar to that noted in experimental models of chronic fetal hypoxemia produced by uteroplacental (19) or placental (7, 21) embolization, prolonged maternal hypoxemia (17), or placental restriction secondary to surgical removal of uterine caruncles (29). Furthermore, the hypoxemia in our study was associated with a reduction in both fetal body and placental weights, suggesting that it was most likely an accompaniment of fetal growth restriction occurring secondary to placental insufficiency (24). Interestingly, the pattern of change in hemodynamic and blood gas variables were similar in normoxic and hypoxic fetal groups following delivery, implying that in utero hypoxemia did not markedly interfere with birth-related cardiorespiratory alterations. However, the tendency for blood gas differences between the two groups to persist in at least the initial hour after birth suggested that antecedent fetal hypoxemia influenced the temporal course of early postnatal respiratory adjustments.

The threefold increase in the plasma concentration of both norepinephrine and epinephrine in hypoxic fetuses of the present study closely resembled the changes in circulating catecholamines observed in the curundectomy model of chronic fetal hypoxemia close to term (29). However, this pattern differed from that of prolonged fetal hypoxemia produced by placental embolization of late-gestation fetal sheep (7, 21) or a reduction in uterine blood flow (11), which are associated with an increase in plasma norepinephrine but not epinephrine, or in long-term maternal hypoxemia (17), which is accompanied by an elevation in circulating epinephrine without a significant change in norepinephrine. The mechanisms underlying the emergence of these differing catecholamine responses is not entirely clear at present. However, as this diversity may reflect fundamental differences in the presence and extent of underlying changes in norepinephrine and epinephrine spillover and/or clearance, caution will clearly need to be exercised in the extrapolation of catecholamine kinetic findings obtained in one experimental model of chronic in utero hypoxemia to other models.

Increases in circulating norepinephrine and epinephrine levels in hypoxic fetuses in the present study were accompanied by proportionally similar rises in total body norepinephrine and epinephrine spillover but unchanged total body catecholamine clearance rates and fractional extractions. As circulating norepinephrine in late-gestation fetal lambs is mainly derived from sympathetic nerves (2) while the adrenal medulla is the main source of epinephrine in utero (16, 26), these results are consistent with the notion that the elevated circulating catecholamine levels in hypoxic fetuses were related to increased sympathoadrenal activity. On the other hand, the similarity of total body catecholamine clearance rates and fractional extractions in hypoxic and normoxic fetuses suggests that overall catecholamine uptake mechanisms were relatively resilient to the effects of prolonged in utero hypoxemia. However, given that catecholamine uptake exhibits regional differences and occurs via both neuronal and nonneuronal processes (5), we cannot exclude the possibility that the overall preservation of catecholamine clearance was also associated with opposing changes in catecholamine neuronal and nonneuronal uptake or in catecholamine clearance between the fetal body and placenta.

Under normal circumstances, the circulating level of norepinephrine increases two- to fivefold with birth (2, 26, 30). Moreover, recent findings from this laboratory have suggested that this rise is related to an elevation in total body norepinephrine spillover that is indicative of a global increase in sympathetic activation and a fall in total body norepinephrine clearance occurring secondary to loss of the placenta (30). However, perinatal changes in norepinephrine plasma levels and kinetics in hypoxic fetuses differed in two major respects from normoxic animals. First, the rise in circulating norepinephrine after birth in hypoxic fetuses was not only attenuated in magnitude but also appeared to be more transient in duration. Second, total body
norepinephrine spillover in the hypoxic group was not altered significantly between fetal and 1-h lambs but then fell to a similar level as the normoxic group by 4 h after birth. This suggests that, whereas the globally elevated degree of sympathetic activation present in hypoxic fetuses was initially maintained after birth, it declined toward normal within hours of birth. By contrast, the pattern of change in total body norepinephrine clearance and fractional extraction in the normoxic and hypoxic groups were essentially similar in the perinatal period, apart from a more pronounced postnatal decline in total body norepinephrine clearance between the 1- and 4-h lambs of the hypoxic group.

Given the lack of change in norepinephrine spillover in the hypoxic group between the fetal and 1-h postnatal time points in the present study, it would at first appear not unreasonable to presume that the increase in circulating norepinephrine occurring in this interval was primarily related to the birth-related reduction in norepinephrine clearance arising from loss of the placenta, a major site of catecholamine removal (13). However, an additional consequence of loss of the placenta occurring at birth is a contraction of the systemic vascular compartment, a change that amplifies the effect of perinatal rises in spillover on circulating catecholamine levels and can even increase these levels in the absence of any alteration in spillover (30). Indeed, using a similar approach, as described in our previous study (30), we estimate that with a systemic plasma flow of 321 ml·min⁻¹·kg⁻¹, the spillover rate of 403 ng·min⁻¹·kg⁻¹ in the hypoxic fetuses contributed an average of 1,297 pg/ml to the circulating norepinephrine level of 3,027 pg/ml. However, in 1-h lambs with a systemic plasma flow of 176 ml·min⁻¹·kg⁻¹, the norepinephrine spillover rate of 365 ng·min⁻¹·kg⁻¹ contributed 2,074 pg/ml to the circulating norepinephrine level of 4,203 pg/ml. Thus the statistically unchanged norepinephrine total body spillover accounted for 777 pg/ml (i.e., 2,074–1,297 pg/ml) or ~70% of the increase in circulating norepinephrine of 1,176 pg/ml occurring between fetal and 1-h lambs of the hypoxic group.

The circulating level of epinephrine typically rises 5-10-fold with birth (2, 26, 30), and is underpinned not only by an increase in total body epinephrine release that is suggestive of enhanced adrenal medullary activity but also a reduction in total body epinephrine clearance accompanying loss of the placenta (30). However, whereas perinatal increases in epinephrine circulating levels in hypoxic fetuses were maintained, they were also less pronounced than in normoxic fetuses. Furthermore, these increases in circulating epinephrine levels were accompanied by an attenuated rise in total body epinephrine release, suggesting that the perinatal augmentation of adrenal medullary activity was blunted after sustained in utero hypoxemia. At first glance, this blunting appears somewhat puzzling given that an increase in the weight of the adrenal gland relative to body weight has been often reported in the setting of chronic fetal hypoxemia (1, 7, 12). However, in accord with our observation, recent findings suggest that chronic fetal hypoxemia is associated with a specific reduction of adrenal mRNA levels of the epinephrine-synthesizing enzyme phenylethanolamine N-methyltransferase (PNMT), the magnitude of which not only bears a strong inverse relationship to the fetal arterial Po₂ but is also accompanied by a contraction in the extent of the PNMT-containing region of the medulla (1).

**Perspectives**

Clinically, chronic fetal hypoxemia is commonly associated with intrauterine growth restriction and a low birth weight (23), as well as increased perinatal morbidity and mortality (18, 20). Moreover, epidemiological studies have pointed to a link between low birth weight and an enhanced risk of development of cardiovascular disorders such as hypertension in adult life (3). The present study suggests that sustained spontaneous hypoxemia is associated with an alteration in sympathetic mechanisms not only in the fetus but also in the immediate period after birth. It still remains to be determined, however, to what extent any long-term sequelae of such alterations contribute to the pathophysiology of cardiovascular disease in adulthood.

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