Effect of prolonged catecholamine infusion on heart rate, blood pressure, breathing, and growth in fetal sheep

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Abstract: Norepinephrine and epinephrine were infused into fetal sheep for 24 h to compare the effects on fetal heart rate, blood pressure, breathing movements, and tissue growth with those of prolonged reductions in uterine blood flow. Norepinephrine concentrations increased ($p < 0.01$) from 871 ± 71 to 6831 ± 1090 pg/mL (2 h) with norepinephrine infusion, and epinephrine concentrations increased from 310 ± 95 to 1424 ± 288 pg/mL (2 h) with epinephrine infusion. Fetal pH decreased ($p < 0.01$) from 7.37 ± 0.01 to 7.29 ± 0.02 at 0.5 h of the norepinephrine infusion and returned to control values by 2 h, whereas fetal lactate concentrations increased ($p < 0.05$) from 1.6 ± 0.2 to 4.6 ± 1.0 mmol/L at 2 h and remained elevated for 12 h. Lactate concentrations also increased with epinephrine infusion. Fetal heart rate increased ($p < 0.05$) from 176 ± 5 to 246 ± 6 and 220 ± 6 beats/min in the 1st h of norepinephrine and epinephrine infusions, respectively, with a subsequent decline. Fetal blood pressure increased ($p < 0.05$) from 43 ± 3 and 40 ± 2 to 53 ± 3 and 47 ± 2 mmHg (1 mmHg = 133.3 Pa) during the 1st h of norepinephrine and epinephrine infusions, respectively, remaining elevated for 24 h. Fetal body weights were not different between the groups of animals, although liver/body weight ratio was less ($p < 0.05$) in epinephrine-infused fetuses (0.030 ± 0.001) compared with vehicle-infused animals (0.036 ± 0.002). There was no change in DNA synthesis rate in any of the fetal organs, despite changes in organ-specific DNA and protein content. Our results indicate that the changes in fetal cardiovascular and behavioural function, as well as tissue growth, that occur with prolonged reductions in uterine blood flow are not mediated solely by elevated circulating catecholamine concentrations.

Key words: fetal physiology, catecholamines, pregnancy.

Résumé : On a perfusé des brebis foetales avec de la norépinéphrine et de l'épinéphrine pendant 24 h pour comparer leurs effets sur la fréquence cardiaque, la pression sanguine, les mouvements respiratoires et la croissance tissulaire avec les effets de réductions prolongées du débit sanguin utérin. Les concentrations de norépinéphrine ont augmenté de 871 ± 71 à 6831 ± 1090 pg/mL (2 h) suite à la perfusion de norépinéphrine, et les concentrations d'épinéphrine ont augmenté de 310 ± 95 à 1424 ± 288 pg/mL (2 h) suite à la perfusion d'épinéphrine. Le pH foetal a diminué ($p < 0,01$) de 7,37 ± 0,01 à 7,29 ± 0,02, 0,5 h après la perfusion de norépinéphrine, et est retourné aux valeurs témoins près de 2 h plus tard, alors que les concentrations de lactate foetal ont augmenté ($p < 0,05$) de 1,6 ± 0,2 à 4,6 ± 1,0 mmol/L, 2 h après la perfusion, et sont demeurées élevées pendant 12 h. Les concentrations de lactate ont aussi augmenté après la perfusion d'épinéphrine. La fréquence cardiaque foetale a augmenté ($p < 0,05$) de 176 ± 5 à 246 ± 6 et 220 ± 6 battes/min durant la première heure de perfusion de norépinéphrine et d'épinéphrine, respectivement, et a diminué par la suite. La pression sanguine foetale a augmenté ($p < 0,05$) de 43 ± 3 et 40 ± 2 à 53 ± 3 et 47 ± 2 mmHg (1 mmHg = 133,3 Pa) durant la première heure de perfusion de norépinéphrine et d'épinéphrine, respectivement, et est demeurée élevée pendant 24 h. Les poids corporels foetaux des deux groupes

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d'animaux n'ont pas différé, bien que le rapport poids hépatique/poids corporel ait été plus faible ($p < 0,05$) chez les foetus perfusés avec de l'épinéphrine (0,030 ± 0,001) comparativement à ceux perfusés avec un véhicule (0,036 ± 0,002). Le taux de synthèse de l'ADN n'a varié dans aucun organe foetal, malgré des variations de la teneur en protéines et en ADN spécifiques à chaque organe. Nos résultats indiquent que, dans le foetus, les variations des réponses comportementales et cardio-vasculaires, ainsi que les variations de croissance tissulaire provoquées par des réductions prolongées du débit sanguin utérin, ne sont pas véhiculées uniquement par des concentrations élevées de catécholamines circulantes.

Mots clés : physiologie foetale, catécholamines, gestation.
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Introduction

It is well established that acutely induced maternal hypoxemia in sheep results in an increase in circulating concentrations of catecholamines (Cohen et al. 1982; Jones and Robinson 1975) within the fetus. The major source of the elevation in norepinephrine and epinephrine concentrations in fetal sheep under these conditions is the adrenal medulla (Cohen et al. 1984). Recently, we have investigated the effects of a prolonged period (24 h) of hypoxemia, secondary to the reduction of uterine blood flow (RUBF) in pregnant sheep on fetal behaviour (Bocking et al. 1988*a*), cardiovascular responses (Bocking et al. 1988*b*), endocrine changes (Challis et al. 1989; Hooper et al. 1990), and tissue growth (Hooper et al. 1991; McLellan et al. 1992). Briefly, prolonged RUBF leads to an initial bradycardia followed by tachycardia and an initial inhibition of fetal breathing movements (FBMs) followed by a return to normal incidence. The major effect of RUBF on tissue growth in fetal sheep is a reduction in DNA synthesis in the fetal lung, thymus gland, and quadriceps muscle. Prolonged RUBF also leads to a significant increase in fetal norepinephrine concentrations, which is sustained for 24 h, and an increase in epinephrine concentrations, which return to near control values by 18 h (Hooper et al. 1990).

In this study our aim was, therefore, to determine whether the cardiovascular, behavioural, and tissue growth changes that occur in the hypoxemic sheep fetus during prolonged RUBF are mediated through the elevations in circulating catecholamine concentrations. To test this hypothesis, we infused norepinephrine or epinephrine separately for 24 h into fetal sheep at approximately 125 days of gestation (term, 147 days) in dosages sufficient to achieve concentrations observed with our previous studies of RUBF (norepinephrine, 4000–6000 pg/mL; epinephrine, 1000–1500 pg/mL) (Hooper et al. 1990) and examined the effect on fetal cardiovascular function, FBMs, and tissue growth.

Methods

Animal preparation

Surgery was performed using sterile techniques on 15 pregnant sheep between 112 and 120 days of gestation, with a mean age of 114 ± 1 (SEM) days. Anesthesia was induced using intravenously administered thiopental sodium (Abbott Laboratories, Montréal, Que.) and maintained with 1.0–1.5% halothane (Halocarbon Laboratories, Hackensack, N.J.) in oxygen at a flow rate of 5–6 L/min. A low midline abdominal incision was made and the uterus opened. Polyvinyl catheters (V4, Bolab, Lake Havasu City, Ariz.) were placed in the fetal carotid artery, jugular vein, and trachea. Catheters (V11, Bolab) were also placed in the amniotic cavity and maternal femoral vein. All catheters were then exteriorized through the flank of the ewe and the uterine and abdominal wall incisions repaired.

Sodium penicillin G (1 000 000 U) was infused into the fetal jugular vein and amniotic cavity at the time of surgery and then daily for the next 3 days. Sodium penicillin G (800 000 U) and streptomycin (250 mg) were injected intramuscularly into the ewe at the same time intervals. The animals were then placed in individual cages with free access to food and water, and were allowed at least 5 days to recover from surgery before experiments were conducted. All animals were treated in compliance with guidelines established by the Canadian Council on Animal Care, and according to protocols approved by the Animal Care Committees of the Lawson Research Institute and The University of Western Ontario.

Experimental protocol

Infusions commenced at 09:00 for all experiments and were administered through the fetal jugular vein using a Harvard constant-infusion pump. In 6 animals, norepinephrine was infused at a rate of 1.0 $\mu g \cdot kg^{-1}$ estimated fetal weight $\cdot$ min^{-1}. In 5 animals, epinephrine was infused at a rate of 0.25 $\mu g \cdot kg^{-1}$ estimated fetal weight $\cdot$ min^{-1}. In 4 animals, 0.9% NaCl (vehicle) was administered at a rate of 0.5 mL/h. Fetal weight was estimated on the basis of previous determinations of fetal weights for pregnant sheep in our laboratory (approximately 3 kg at 125 days of gestation). Continuous recordings of fetal blood pressure (FBP), fetal heart rate (FHR), tracheal pressure, and amniotic pressure were performed throughout the infusion period. Fetal arterial blood samples (3.0 mL) were obtained in chilled syringes before (0) and at 2, 12, and 24 h during infusions for measurement of plasma catecholamine and whole-blood lactate concentrations. Plasma levels of glucose, insulin, glucagon, and insulin-like growth factor binding proteins (IGFBPs) were determined from these samples and have been reported elsewhere (Hooper et al. 1994). Blood used for measurement of lactate concentrations (0.3 mL) was rapidly frozen using dry ice, stored at −20°C, and analyzed within 48 h. Blood used for measurement of catecholamines (1.0 mL) was immediately centrifuged for 10 min at 2500 rpm, and the plasma was stored at −70°C for subsequent analysis. In addition, fetal arterial blood samples (0.6 mL) were obtained before and during (0.5, 1, 2, 4, 12, and 24 h) the infusions for measurement of blood gases, pH, oxygen saturation (Sa_{O_2}), and hemoglobin (Hb).

Tritiated thymidine at a dose of 1 mCi (1 Ci = 37 GBq) per kilogram estimated fetal weight (specific activity 20 Ci/mmol; ICN Biomedicals Inc., Irvine, Calif.) was injected into 14 fetuses at 16 h of infusion time to determine the effect of elevated catecholamine concentrations on DNA synthesis rate over the last 8 h of infusion. One norepinephrine-infused fetus did not receive [^{3}H]thymidine. The animals were killed at the end of the 24-h infusion, using an intravenous injection of pentobarbital sodium and propylene glycol (Euthanyl, MTC Pharmaceuticals, Cambridge, Ont.). The fetus was then removed and weighed, fetal organs were dissected, and wet weight was determined. Representative tissue samples were taken from the brain, right and left myocardial free wall, liver, lung, thymus, adrenal gland, kidney, and placental cotyledons and were rapidly frozen in liquid nitrogen and stored at −70°C for subsequent analysis.

Data analysis

Fetal tracheal, arterial, and amniotic pressures were measured using Statham pressure transducers (Gould, Cleveland, Ohio) and direct current pressure amplifiers (model 7P1F, Grass Instruments, Quincy, Mass.), then displayed continuously on a polygraph (Grass 7D). Mean fetal blood pressure was calculated as diastolic pressure + 0.4(systolic−diastolic pressure) after the subtraction of amniotic pressure. FHR was determined from the pulsatile blood pressure using a tachograph preamplifier (model 7P44B, Grass) and displayed continuously. FBMs were defined as repeated negative deflections in tracheal pressure (corrected for amniotic pressure) of >2 mmHg. An episode of FBM was considered to be present if negative deflections occurred continuously for 30 s (Bocking and Harding 1986).

Arterial Po_2 (Pao_2), Pco_2 ($Paco_2$), pH (pHa), and HCO_3^- were measured using an ABL-3 blood gas analyzer (Radiometer, Copenhagen) at 37°C and then corrected for a fetal temperature of 39.5°C. Sao_2 and Hb were measured in duplicate using an OSM2 Hemoximeter (Radiometer). Plasma catecholamine concentrations were determined using high-pressure liquid chromatography followed by electrochemical detection (Allenmark et al. 1980). Whole-blood lactate concentrations were measured using the lactate oxidase method (model 23A, Yellow Springs Instruments, Yellow Springs, Ohio).

Protein content was measured using the Bio-Rad Protein Assay Kit (Bio-Rad, Hercules, Calif.). DNA content was measured by fluorometric assay using calf thymus DNA standards with bis-benzimide (Hoechst 33258; Sigma Chemical Co., St. Louis, Mo.) as the fluorochrome. Fluorometric readings were performed at 356-nm (excitation) and 480-nm (emission) wavelengths. DNA and protein content were then calculated in order to allow for possible changes in tissue extracellular water and then presented as a proportion of fetal body weight to eliminate the variation due to differences in body size between animals. Results for the right and left ventricle of the heart, thymus gland, and quadriceps muscle are presented as a concentration (mg/g tissue) because of the inherent difficulties in accurately measuring total weights for these organs. DNA synthesis rate was determined by calculating the amount of [^{3}H]thymidine incorporated into DNA and was expressed as disintegrations per minute per microgram of DNA as previously described (Hooper et al. 1991).

All results are presented as mean values ± SEM. Statistical significance was determined using a two-way analysis of variance followed by t tests using the Bonferroni correction both within and between treatment groups.

Results

Fetal plasma catecholamine concentrations

Fetal plasma catecholamine concentrations from these experiments have been reported elsewhere (Hooper et al. 1994). Briefly, fetal norepinephrine concentration was 871 ± 71 pg/mL before the infusion of norepinephrine and increased to 6831 ± 1090 ($p < 0.01$), 8682 ± 1122 ($p < 0.01$), and 9882 ± 1856 ($p < 0.01$) pg/mL at 2, 12, and 24 h of norepinephrine infusion, respectively. There was no change in norepinephrine concentration with either vehicle or epinephrine infusion. Fetal epinephrine concentration was 310 ± 95 pg/mL before the infusion of epinephrine and increased to 1424 ± 288, 1378 ± 395, and 1304 ± 946 pg/mL at 2, 12, and 24 h of epinephrine infusion, respectively. There was no change in epinephrine concentration with either vehicle or norepinephrine infusion.

Fetal blood gas tensions, pH, and O_2 saturation

Fetal pHa decreased significantly from 7.37 ± 0.01 before to 7.29 ± 0.02 at 0.5 h ($p < 0.01$) and 1 h ($p < 0.01$) of norepinephrine infusion but returned to control values by 2 h and remained unchanged for the remainder of the infusion (Fig. 1). Base excess decreased from 2.8 ± 0.6 before to −3.5 ± 0.5 mmol/L ($p < 0.01$) at 1 h of norepinephrine infusion and returned to control values by 4 h, with no further change (Fig. 1). A similar transient but less severe decrease in pH and base excess was seen with the infusion of epinephrine. Fetal $Paco_2$ decreased from 51.0 ± 1.3 before to 47.7 ± 2.0 mmHg ($p < 0.05$) at 1 h of norepinephrine infusion and remained significantly depressed for the duration of the infusion (Fig. 1). Similarly, fetal $Paco_2$ decreased from 46.2 ± 1.7 before to 43.5 ± 1.5 mmHg ($p < 0.001$) at 2 h of epinephrine infusion and remained lower throughout the infusion, although not significantly.

Fetal Pao_2 was 23.9 ± 1.7, 21.9 ± 1.2, and 25.8 ± 1.8 mmHg before infusions of vehicle, norepinephrine, and epinephrine, respectively. Fetal Sao_2 was 68.5 ± 3.9, 61.6 ± 3.4, and 67.3 ± 3.3% before infusions for vehicle-, norepinephrine-, and epinephrine-infused animals, respectively. There was no significant change in Pao_2 or Sao_2 with the infusion of norepinephrine, epinephrine, or vehicle.

Fetal lactate and bicarbonate concentrations

Fetal whole-blood lactate concentration increased from 1.6 ± 0.2 and 1.7 ± 0.2 mmol/L to peak concentrations of 5.5 ± 1.6 and 4.4 ± 0.4 mmol/L at 12 h after commencing the infusion of norepinephrine and epinephrine, respectively (Fig. 2). Although lactate concentrations remained higher than preinfusion values at 24 h with the infusion of norepinephrine, this was not statistically significant. There was no change in fetal lactate concentration with the infusion of vehicle. Fetal arterial bicarbonate (HCO_3) concentration

Fig. 1. Fetal pH, base excess (BE), and P_{CO_2} before (time 0) and during the infusion of vehicle (n = 4), norepinephrine (n = 6), and epinephrine (n = 5). Results are mean values ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001, compared with time 0; †p < 0.05, ††p < 0.01, †††p < 0.001, compared with vehicle treatment.

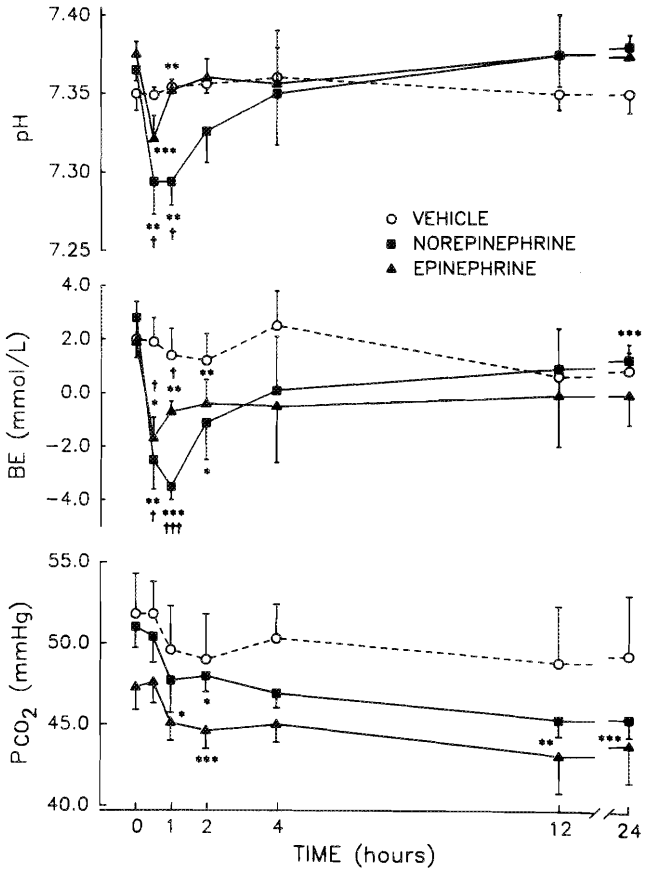
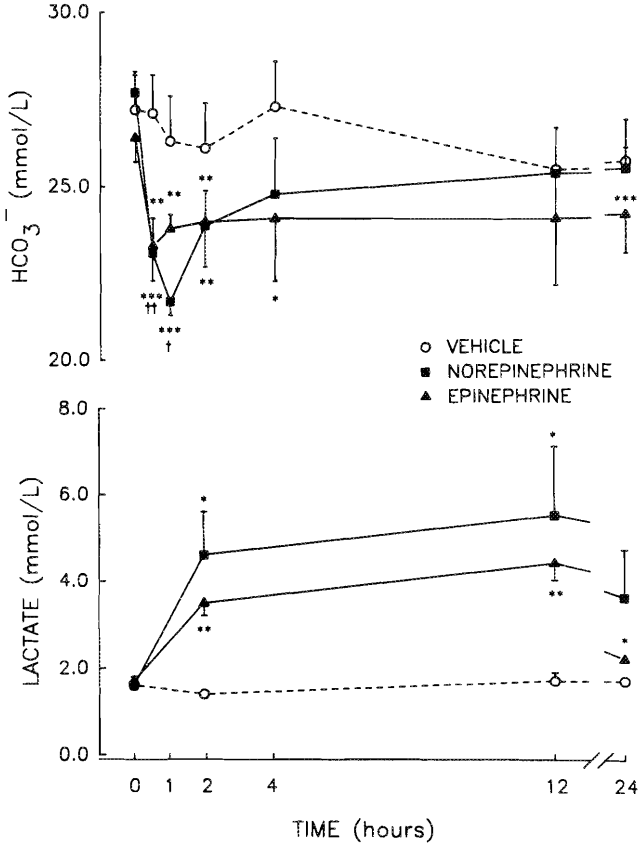


Fig. 2. Fetal whole-blood HCO_3 and lactate concentrations before (time 0) and during the infusion of vehicle (n = 4), norepinephrine (n = 6), and epinephrine (n = 5). Results are mean values ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001, compared with time 0; †p < 0.05, ††p < 0.01, compared with vehicle treatment.



decreased from 27.7 ± 0.6 to 21.7 ± 0.4 mmol/L at 1 h of the norepinephrine infusion and from 26.4 ± 0.7 to 23.3 ± 0.8 mmol/L at 0.5 h of the epinephrine infusion, followed by a return to control values by 12 h. There was no change in fetal arterial HCO_3 with vehicle infusion alone (Fig. 2).

Fetal heart rate and blood pressure

Mean FHR increased (p < 0.001) from 176 ± 5 to 246 ± 6 beats/min during the 1st h of the norepinephrine infusion, with a maximum rise of 60% above control values at 30 min. Mean FHR then gradually fell to 194 ± 8 beats/min by 12 h of the norepinephrine infusion, remaining significantly higher than preinfusion values (Fig. 3). There was a trend for mean FHR to decrease initially from 174 ± 4 to 167 ± 13 beats/min at 5 min of epinephrine infusion (data not shown), following which it increased (p < 0.05) to a maximum of 220 ± 6 beats/min during the 1st h. This was followed by a return to preinfusion values by 4 h (Fig. 3). There was no change in mean FHR with the infusion of vehicle alone.

Mean FBP increased (p < 0.05) from 43 ± 3 before to 53 ± 3 mmHg during the 1st h of norepinephrine infusion and remained significantly elevated (p < 0.01) for the dura-

Fig. 3. Mean hourly fetal heart rate (FHR) before (time 0) and during the infusion of vehicle (n = 4), norepinephrine (n = 6), and epinephrine (n = 5). *p < 0.05, **p < 0.01, ***p < 0.001, compared with time 0. Results are mean values ± SEM.

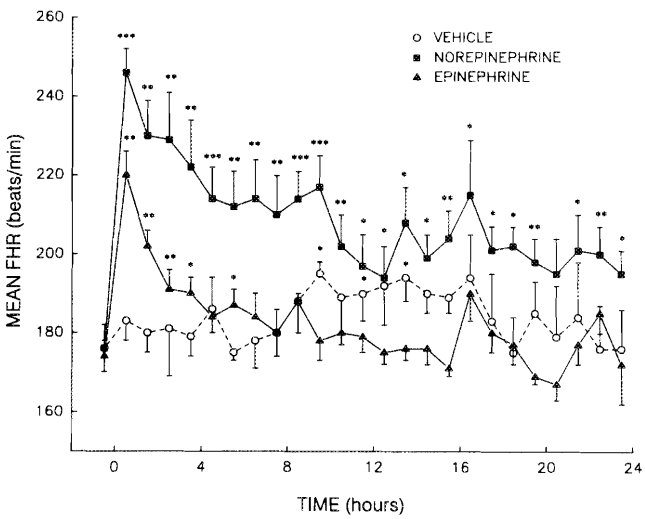
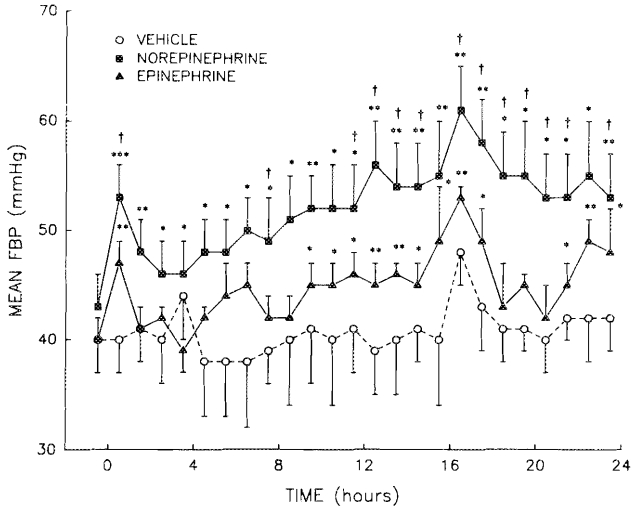


Fig. 4. Mean hourly fetal blood pressure (FBP) before (time 0) and during the infusion of vehicle ($n = 4$), norepinephrine ($n = 6$), and epinephrine ($n = 5$). Results are mean values ± SEM. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, compared with time 0; $\dagger p < 0.05$, compared with vehicle treatment.



tion of infusion at 20–30% above control for the last 16 h (Fig. 4). The greatest increase in FBP was at 5 min of infusion, with peak values of 90% above preinfusion FBP. FBP also increased from 40 ± 2 to 47 ± 2 mmHg during the 1st h of epinephrine infusion and remained at 5–10% above control values for the duration of infusion, although this was not statistically significant (Fig. 4). There was no change in FBP with the infusion of vehicle alone.

Fetal breathing movements

FBMs occurred 55 ± 4, 55 ± 12, and 49 ± 4% of the time during the 2 h before the infusion of norepinephrine, epinephrine, and vehicle, respectively. The mean amplitude of FBM was 3.2 ± 0.2, 4.4 ± 0.3, and 4.5 ± 1.0 mmHg for the hour before the infusion of vehicle, norepinephrine, and epinephrine, respectively. There was no significant change in the incidence or amplitude of FBM during the infusion of norepinephrine, epinephrine, or vehicle.

Fetal body and organ weights

Fetal body weights at the time of autopsy were not different between animals that received norepinephrine (2675 ± 78 g), epinephrine (3000 ± 108 g), or vehicle (3081 ± 436 g). Fetal liver/body weight ratio was decreased ($p < 0.05$) in epinephrine-infused animals (0.030 ± 0.001) compared with vehicle-infused animals (0.036 ± 0.002) and was also decreased in norepinephrine-infused animals (0.032 ± 0.001), although this was not statistically significant (Table 1). There was no significant change in the organ/body weight ratio for any of the other tissues studied (Table 1).

Fetal protein and DNA content

Liver

Total protein content of the fetal liver was not significantly different between any of the infusion groups (Table 1). However, there was a significant decrease ($p < 0.05$) in the amount of DNA when corrected for fetal body weight in the livers of fetuses that received epinephrine compared with those that received vehicle. A decrease in fetal liver DNA content was also observed in animals that received norepinephrine, although this was not statistically significant (Table 2). The ratio of DNA to protein in hepatic tissue was significantly decreased ($p < 0.05$) in norepinephrine-infused animals compared with vehicle-infused animals and was also decreased in fetuses that received epinephrine, although this was not statistically significant (Fig. 5).

Heart

Protein concentration was significantly greater ($p < 0.001$) in the fetal right ventricle (131.26 ± 3.08 mg/g tissue) compared with the left ventricle (72.41 ± 1.66 mg/g tissue) of vehicle-infused animals. Similar results were observed for fetuses infused with norepinephrine and epinephrine, and there was no effect of infusions on protein concentration in either ventricle. DNA concentration was not significantly different between the right ventricle (3.23 ± 0.29 mg/g tissue) and left ventricle (3.91 ± 0.16 mg/g tissue) of vehicle-infused animals and was unchanged with infusion of either norepinephrine or epinephrine. The ratio of tissue DNA to protein was significantly greater ($p < 0.01$) in the left ventricle compared with the right ventricle of vehicle-infused animals (Fig. 5). A similar difference in ventricular DNA/protein ratio was observed for both norepinephrine- and epinephrine-infused animals (Fig. 5). There was no effect of either catecholamine infusion on the DNA/protein ratio compared with vehicle-infused animals for either the left or the right ventricle.

Kidney

Protein content of the kidney was significantly increased ($p < 0.05$) in fetuses infused with epinephrine compared with those receiving vehicle (Table 1). Protein content was also increased in those fetuses receiving norepinephrine, although this was not statistically significant. DNA content was, however, significantly greater ($p < 0.05$) in the kidneys of fetuses infused with norepinephrine but not with epinephrine (Table 2). The ratio of DNA to protein was 85.57 ± 1.35 in the kidneys of fetuses receiving vehicle alone and was significantly lower ($p < 0.001$) in those infused with epinephrine but not norepinephrine (Fig. 5).

Lung, adrenal, and thymus glands

Protein and DNA content of the lung and adrenal glands were unchanged with either infusion (Table 1). However, the ratio of tissue DNA to protein in the lungs of epinephrine-infused animals was significantly greater ($p < 0.05$) than that for the lungs of vehicle-infused fetuses (Fig. 5). The ratio of DNA to protein in the adrenal gland was also significantly greater ($p < 0.05$) in epinephrine-infused fetuses compared with those receiving vehicle alone (Fig. 5).

Protein concentration of the thymus gland was 130.19 ± 1.21 mg/g tissue in vehicle-infused fetuses and was unchanged in those that received norepinephrine (119.47 ± 5.66 mg/g tissue) or epinephrine (130.67 ± 7.51 mg/g tissue). Thymus DNA concentration was 20.18 ± 0.19 mg/g

Table 1. Organ/body weight and total tissue protein content/fetal body weight values for fetuses infused with vehicle (n = 4), norepinephrine (n = 5), and epinephrine (n = 5) at 120 days of gestation.

	Organ/body weight			Total protein/body weight (mg/g)		
	Vehicle	Norepinephrine	Epinephrine	Vehicle	Norepinephrine	Epinephrine
Brain	0.014 ± 0.002	0.014 ± 0.001	0.012 ± 0.001	0.525 ± 0.083	0.592 ± 0.045	0.506 ± 0.035
Placenta	0.131 ± 0.012	0.112 ± 0.009	0.120 ± 0.017	11.66 ± 1.38	11.72 ± 1.39	11.86 ± 1.69
Lung	0.027 ± 0.001	0.028 ± 0.002	0.030 ± 0.002	0.900 ± 0.100	1.265 ± 0.203	0.746 ± 0.175
Liver	0.036 ± 0.002	0.032 ± 0.001	0.030 ± 0.001*	3.426 ± 0.119	3.667 ± 0.191	3.529 ± 0.147
Adrenals	0.000 12 ± 0.000 02	0.000 11 ± 0.000 01	0.000 12 ± 0.000 01	0.012 ± 0.002	0.010 ± 0.002	0.010 ± 0.001
Kidney	0.006 ± 0.001	0.007 ± 0.000	0.007 ± 0.000	0.286 ± 0.012	0.348 ± 0.026	0.378 ± 0.022*
Heart	0.007 ± 0.000	0.008 ± 0.000	0.008 ± 0.000	—	—	—

Note: Results are means ± SEM.
*p < 0.05.

Table 2. Total tissue DNA content/fetal body weight and DNA synthesis rate for fetuses infused with vehicle (n = 4), norepinephrine (n = 5), and epinephrine (n = 5) at 120 days gestation.

	Total DNA/body weight (mg/g)			DNA synthesis rate ([^{3}H]thymidine dpm/μg DNA)		
	Vehicle	Norepinephrine	Epinephrine	Vehicle	Norepinephrine	Epinephrine
Brain	0.011±0.001	0.011±0.001	0.009±0.000	167.20±20.91	152.97±36.84	171.23±21.68
Placenta	0.484±0.055	0.438±0.027	0.446±0.073	175.42±20.92	215.07±31.77	194.44±11.73
Lung	0.158±0.016	0.183±0.011	0.163±0.024	114.85±27.84	78.24±10.51	96.14±25.78
Liver	0.212±0.014	0.193±0.016	0.179±0.014*	692.76±124.04	619.64±82.09	763.98±159.27
Adrenals	0.0006±0.0001	0.0006±0.0000	0.0006±0.0000	112.66±15.73	152.65±20.14	89.60±11.36
Kidney	0.024±0.001	0.029±0.001*	0.024±0.002	134.57±15.90	128.24±22.73	127.65±22.57
Heart	—	—	—	131.00±22.98	161.04±45.86	178.03±16.62
Thymus	—	—	—	75.18±17.17	92.00±11.97	69.56±10.58
Quadriceps	—	—	—	119.89±34.52	72.67±19.54	59.65±13.98

Note: Results are means ± SEM.
*p < 0.05.

tissue in vehicle-infused fetuses and was significantly less (p < 0.01) in those that received epinephrine (18.00 ± 0.38 mg/g tissue).

Cerebral cortex, quadriceps, and placenta

There was no change in the protein or DNA content or DNA/protein ratio in the cerebral cortex, quadriceps muscle, or placental cotyledons with either infusion (Tables 1 and 2).

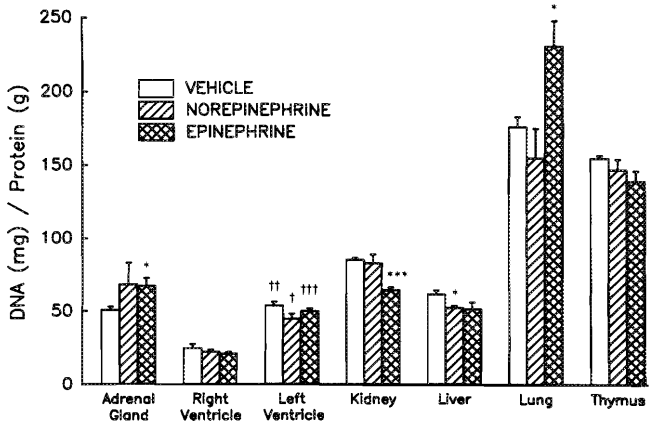
DNA synthesis rate

There was no significant difference in the rate of DNA synthesis in any of the tissues examined between any of the infusion groups (Table 2).

Discussion

In this study, we have increased fetal plasma catecholamine concentrations to levels that occur with prolonged RUBF (Hooper et al. 1990). Prolonged infusion of either norepinephrine or epinephrine did not affect fetal Pao_2 or Sao_2, although pHa and base excess decreased transiently, as a result of an increase in lactate concentration. Mean FHR increased markedly in the 1st h of both infusions and subsequently declined. In contrast, blood pressure remained consistently elevated for the duration of both infusions and there was no change in the incidence or amplitude of FBMs. There was no change in the DNA synthesis rate over the last 8 h in any of the fetal organs examined, despite organ-specific changes in DNA and protein content.

Fig. 5. Tissue DNA/protein ratio for selected organs in fetuses infused with vehicle (n = 4), norepinephrine (n = 5), and epinephrine (n = 5). Results are mean values ± SEM. *p < 0.05, ***p < 0.001, compared with vehicle; †p < 0.05, ††p < 0.01, †††p < 0.001, compared with right ventricle.



Short-term administration of epinephrine and norepinephrine to fetal sheep has been shown previously to increase circulating lactate concentrations, although the mechanism underlying this increase has not been investigated in the fetus (Jones and Ritchie 1978*b*). It is possible that it results from an increased conversion of glucose to lactate, as the associated fetal hyperglycemia (Hooper et al. 1994) would promote increased placental uptake of glucose from the fetus (DiGiacomo and Hay 1989). Alternatively, it may result from an increase in anerobic metabolism in skeletal muscle due to peripheral vasoconstriction. Fetal arterial pH, however, returned to control values by 1 h in epinephrine- and 4 h in norepinephrine-infused fetuses and remained within the normal range for the duration of the infusion, despite the sustained elevation of lactate concentrations. A similar return to normal pH with elevated lactate concentrations has been reported with prolonged RUBF by ourselves (Bocking et al. 1992; Hooper et al. 1991) and others (Wilkening et al. 1993). With RUBF, the increase in fetal blood lactate concentrations likely results from increased lactate production secondary to increased anerobic metabolism (Boyle et al. 1992). Whether the same changes in fetal pH and lactate concentrations would occur with prolonged hypoxemia in the absence of elevated catecholamine concentrations is unknown. Further investigations are required into the mechanism whereby this acid–base adaptation of the fetus occurs, in the presence of elevated lactate concentrations with both RUBF and elevated catecholamine concentrations.

Short-term infusions of norepinephrine have previously been reported to result in an initial bradycardia (Lorijn and Longo 1980*a*, 1980*b*) followed by a tachycardia (Cheung and Brace 1987, 1988) or tachycardia alone (Jones and Ritchie 1978*a*). In our study, the initial heart rate response to the infusion of norepinephrine was an increase, followed by a gradual decrease to 10–20% above preinfusion values during the last 12 h of the infusion, similar to what occurs with prolonged RUBF.

In contrast with norepinephrine, the initial heart rate response to the infusion of epinephrine was a decrease of 6% at 5 min (data not shown), which was followed by a tachycardia with a maximal increase in FHR of 40% at 15 min and subsequent return to preinfusion values at 4 h. A similar decrease in FHR followed by a tachycardia has been reported previously, with short-term infusions of epinephrine (Jones and Ritchie 1978*a*). It is possible that the initial decrease in FHR seen with the infusion of epinephrine was secondary to activation of baroreceptors, in association with the rapid rise in mean arterial pressure (Hanson 1988). It is of interest, however, that mean FHR did not decrease initially with the infusion of norepinephrine despite an even greater rise in arterial pressure. This could be explained by a greater inotropic effect of epinephrine on the cardiac β-receptor than would occur with norepinephrine, giving rise to an increase in stroke volume. In the norepinephrine-infused animals, it is possible that the chronotropic effect was sufficiently large to override any vagal stimulatory effect of baroreceptor activation, with the net effect being an increase in mean FHR. Further experiments are required to investigate in more detail this possible differential effect of norepinephrine and epinephrine on fetal cardiac function.

The return in FHR to near control values with sustained infusions of both norepinephrine and epinephrine is in keeping with either a decrease in number or sensitivity of β-receptors in the heart. A downregulation of β-receptors with prolonged infusions of β-agonists has been observed in the lung of fetal sheep (Warburton et al. 1988). In addition, the development of tachyphylaxis to prolonged infusion in fetal lambs of a β-agonist (Ritodrine) has also been previously reported (Bassett et al. 1989, 1990). It is of interest that the increase in FBP was sustained with the infusion of norepinephrine in keeping with this being predominantly an α-adrenergic-mediated effect. In contrast with β-receptors there appears to be no effect of prolonged infusion of either norepinephrine or epinephrine on α-receptor function. It is of interest that the sustained increase in FBP with prolonged catecholamine infusion is greater than that seen with prolonged RUBF, suggesting that other adaptive mechanisms are present with prolonged hypoxemia.

In this study, there was no significant effect of either norepinephrine or epinephrine infusion on either the incidence or the amplitude of FBMs. This is in contrast with previous studies in which short-term infusions of both epinephrine and norepinephrine were reported to produce variable effects on FBMs (Jansen et al. 1986). Jones and Ritchie (1978*a*) reported an increase in the incidence of FBMs with a 1-h infusion of epinephrine and no change with norepinephrine. Murata et al. (1981) reported a decrease in the incidence of FBMs in fetal rhesus monkeys with a 30-min infusion of norepinephrine and no change with epinephrine. Although peripheral infusions of α-antagonists have led to an increase in FBMs during both normoxia and hypoxia (Giussani et al. 1992), it is likely that central α-adrenergic mechanisms are more important in regulating FBMs (Bamford et al. 1990), which would not be identified in our experiments. We can conclude from these studies, however, that the previously shown initial inhibition in FBMs with RUBF is not related to an increase in circulating catecholamine concentrations.

In contrast with our previous study of prolonged hypoxemia secondary to RUBF (Hooper et al. 1991), we found no change in the rate of DNA synthesis in any of the organs studied, suggesting that those changes were not a direct consequence of elevated circulating catecholamine concentrations. However, we did observe a decrease in the ratio of the liver to body weight of fetuses infused with epinephrine, which is likely due to a decrease in glycogen content resulting from increased glycogenolysis (White et al. 1973). In addition, we found a decrease in hepatic DNA content with a decrease in the DNA/protein ratio. These findings suggest that epinephrine infusion decreases the number of cells in the fetal liver, although we cannot determine from these studies whether it is hepatocyte or hemopoietic cells, or both, which are primarily affected. Because of the short duration (24 h) of infusion, it is likely that the decrease in cell number is due to fewer hemopoietic cells. Since DNA synthesis rate was unchanged over the last 8 h of infusion, the decrease in DNA content must have occurred earlier in the infusion of epinephrine and the explanation for this temporal effect remains unknown. It is possible that epinephrine infusion may stimulate hematopoietic cell migration away from the liver, complicating the interpretation of any effects of epinephrine on liver DNA content.

With the infusion of epinephrine, there was a decrease in the DNA concentration of the fetal thymus gland, but no change in protein concentration (data not shown), in keeping with a decrease in the number of cells. This is also in contrast with what is observed with prolonged RUBF, when there is a decrease in DNA synthesis rate over the last 8 h with no change in thymus DNA concentration. It is possible that the elevation of plasma epinephrine concentrations leads to a release of thymocytes into the circulation, whereas prolonged RUBF alters DNA synthesis within thymocytes through a different mechanism such as changes in blood flow.

Although neither norepinephrine nor epinephrine altered myocardial DNA or protein content, protein content was significantly greater in the right ventricle than the left in all animals. This is consistent with previous detailed morphometric studies of the heart in fetal sheep, which showed that right ventricle myocytes are larger in cross section than left ventricle myocytes (Smolich et al. 1989).

There was no change in DNA or protein content and DNA synthesis rate within the lung, placenta, or quadriceps muscle with infusion of either catecholamine. These findings indicate that the previously observed effect of prolonged RUBF on skeletal muscle growth is not mediated through the elevation of circulating catecholamine concentrations alone. Other changes that occur concomitantly with RUBF, such as distribution of blood flow and tissue oxygenation, must occur in order for these tissues to be affected.

In summary, in these experiments we have shown that there are differential effects of prolonged infusion of epinephrine and norepinephrine on α- and β-receptor-mediated cardiovascular function. Further studies on the effects of prolonged RUBF on fetal myocardial and vascular smooth muscle adrenergic-receptor numbers and function would be of interest. We observed no significant effect of either infusion on FBMs and, therefore, suggest that the fetal behavioural changes that occur with prolonged RUBF are not as a consequence of elevated circulating catecholamine concentrations. In addition, we have observed organ-specific changes in both DNA and protein content primarily with the infusion of epinephrine. These changes are different from those seen with prolonged RUBF, in keeping with different mechanisms mediating the alterations in tissue growth seen under these two experimental conditions.

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