Increased coronary blood flow signals growth of coronary resistance vessels in near-term ovine fetuses

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Received 8 February 2001; accepted in final form 11 September 2001

Wothe, D., A. Hohimer, M. Morton, K. Thornburg, G. Giraud, and L. Davis. Increased coronary blood flow signals growth of coronary resistance vessels in near-term ovine fetuses. Am J Physiol Regulatory Integrative Comp Physiol 282: R295–R302, 2002.—We measured maximal coronary artery conductance in near-term fetal sheep before and after chronic infusion with adenosine to determine whether an increase in coronary flow without hypoxemia results in increased coronary vascular growth. Adenosine was infused into the circumflex coronary artery for 12 h each day for 4 days. Coronary flow was maintained at double the resting level by regulating the infusion of adenosine via a computerized servocontrol device signaled by a Doppler flow-velocity sensor. Total arterial hemoglobin, oxygen content, and hemo-dynamics were unchanged. Resting circumflex coronary blood flow increased from control of 250 ± 111 to 530 ± 216 ml·min⁻¹·100 g left ventricle⁻¹ with adenosine on day 1 and from 194 ± 74 to 878 ± 210 ml·min⁻¹·100 g left ventricle⁻¹ with adenosine on the last day (P < 0.01). Coronary conductance, determined during maximal vasodilatation, increased from 14.0 ± 5.0 to 26.9 ± 3.9 ml·min⁻¹·100 g⁻¹·mmHg⁻¹ over the 4 days (P < 0.001). Coronary flow reserve, the difference between resting and maximal myocardial blood flow interpolated at 40 mmHg, increased from 299 ± 196 to 672 ± 266 ml·min⁻¹·100 g⁻¹ (P < 0.001). Maximal coronary conductance was unchanged in control saline-infused fetuses (18.5 ± 5.1 vs. 18.5 ± 8.7 ml·min⁻¹·100 g⁻¹·mmHg⁻¹). We conclude that chronic intracoronary adenosine administration to the fetal myocardium modulates coronary vascular growth, even in the absence of tissue hypoxia.

THE MYOCARDIUM OF FETAL SHEEP adapts to chronic anemia by increasing resting coronary blood flow nearly sixfold, doubling maximal coronary conductance, and increasing ventricular capillary volume density by 40% (3, 22). This increase in myocardial blood flow supports augmented cardiac work as stroke volume and cardiac output increase by ∼50% (2). The slope of the relationship between myocardial blood flow during maximal vasodilatation with adenosine infusion and coronary perfusion pressure (coronary conductance) is a physiological measure of the total cross-sectional area of the myocardial resistance vessels (15, 30). Approximately half of the increase in coronary conductance in hearts from anemic fetuses can be explained by decreases in blood viscosity. The other half is, in part, attributed to vascular remodeling and angiogenesis secondary to decreased tissue oxygen tension (3). The latter stimulates hypoxia-inducible factor 1, a heterodimeric basic helix-loop-helix transcription factor that binds to a hypoxia response element 5′ to the start site of the human vascular endothelial growth factor (VEGF) gene (9, 22). However, the role of increased flow during anemia in developing myocardium, independent of hypoxia, as a stimulus of vascular growth has not been defined.

Blood flow is known to regulate the growth of the vessel through which it flows via endothelial signals (5). Mechanical stimuli that are known to modulate vascular endothelial cell growth in vivo and in vitro include shear stress (23), stretch (20), and pulsatile strain (26). These factors can induce vascular growth by several known endothelial mechanisms, including nitric oxide (NO)-stimulated induction of basic fibroblast growth factor (35), adenosine-modulated increases in NO (21), and/or adenosine-induced increases in VEGF expression (11). In addition, adenosine may also directly promote angiogenesis independent of flow-related mechanical stimuli. Adenosine has been shown to increase endothelial cell proliferation in cell culture (7), activating G protein receptors and increasing VEGF (10). Inhibitors of adenosine kinase, which increase adenosine levels, also increase VEGF in cultured myocardial myoblasts (12).

Adenosine, when infused chronically in adult rabbits, has been shown to increase capillary density in the heart (34). The chronic administration of dipyridamole, a blocker of adenosine uptake that increases tissue adenosine concentration, has also been shown to enhance collateral coronary flow in adult swine after coronary artery constriction (29). Changes in capillary density, however, are not necessarily accompanied by increases in larger-resistance vessels. When dipyridamole was administered chronically to adult rabbits, cardiac capillary density increased but maximal coronary vascular resistance during chemical vasodilation was not altered (32). Thus it appears that dipyridamole...
induced capillary growth in the adult but did not increase growth in arteriolar-like resistance vessels.

In contrast, maximal coronary blood flow, when expressed per unit weight of heart, has been shown to decrease with age, being higher in the fetus than in the newborn and lower in the adult (3, 8, 31). This suggests that extensive remodeling of the coronary circulation occurs in the perinatal period. We previously showed that coronary reserve is increased with chronic hypoxemia in immature animals (24) and that coronary conductance is increased under the stimulus of fetal anemia. However, virtually nothing is known regarding the separate importance of hypoxia alone compared with blood flow and adenosine as growth signals to the coronary endothelium in the fetal heart. Therefore, the purpose of the present study was to determine the role of coronary intravascular adenosine administration in altering fetal coronary conductance in the absence of the hypoxic and viscosity alterations that accompany anemia. We tested the hypothesis that the intermittent intracoronary administration of adenosine would augment coronary conductance over a 4-day period. Maximal coronary conductance was measured to determine changes in resistance vessel area and, thereby, index vascular growth.

MATERIALS AND METHODS

General anesthesia was induced in 10 ewes at 118–121 days of gestation with intravenous pentobarbital sodium and maintained with 1.5% halothane and nitrous oxide-oxygen (1:1), as previously described (2, 3). All animal procedures were reviewed and approved by the Oregon Health Sciences University Institutional Animal Care and Use Committee. After a midline peritoneal incision, the right side of the fetal neck was exposed through a uterine incision. Polyvinyl catheters, V5 (0.86 mm ID; Bolab) and V8 (1.19 mm ID), were placed in the carotid artery and advanced into the ascending aorta. Two V8 catheters were placed in the jugular vein and advanced to the right atrium. The descending aorta was mobilized by blunt dissection through a left fourth intercostal thoracotomy, and a 10-mm inflatable vascular occluder (In Vivo Metric Systems) was placed around the descending aorta distal to the ductus arteriosus. A V5 catheter with a 2-mm Silastic tip was inserted into the left hemizygous vein and advanced to the coronary sinus for sampling. A second inflatable vascular occluder (8 mm) was placed around the inferior vena cava above the diaphragm. The pericardial sac was opened, and two V5 catheters with 4-mm-long V8 tips were placed in the left atrial appendage. A Doppler crystal with a Silastic cuff (Crystal Biotech, Hopkinton, MA) was placed on the proximal left circumflex coronary artery. For the intracoronary infusion of adenosine, a V5 catheter tipped with 0.5-mm-ID Silastic tubing was attached to a 26-gauge needle and then brought through the proximal left circumflex artery. The Silastic tubing was cut and allowed to retract with the Silastic tip in the artery. In closing, a pericardial catheter was left in place, the ribs were reapproximated with suture, and the thoracotomy was closed in layers. An amniotic fluid catheter was attached to the thorax, the uterine incision was closed, and the catheters were tunneled to a pouch on the ewe's flank. Penicillin (1 × 106 U) was given in the amniotic space.

After 4 days of postoperative recovery, the fetus was studied. Baseline hydrostatic pressures, including carotid arterial, right atrial, left atrial, pericardial, and amniotic fluid pressures, were measured with Transpac transducers (Abboott Critical Care Systems, Chicago, IL) calibrated before use. Doppler flow signals (System 6, Triton, San Diego, CA) and heart rate were recorded with a chart recorder (model TA-6000, Gould, Valley View, OH) and stored on-line using a MacADIOS-equipped Apple Macintosh 8100-100AV computer and Superscope II software (GW Instruments, Somerville, MA). Atrial and carotid pressures were referenced to pericardial pressure. The computer was used to control the rate of adenosine infusion with the input voltage, the Doppler hertz signal, and the output signal connected to a roller pump (Cole-Parmer, Vernon Hills, IL).

Blood gases and oxygen content were measured from simultaneously drawn blood from the carotid artery and coronary sinus by a CO-oximeter (model 482, Instrumentation Laboratories) and a pH blood-gas analyzer (model 1610, Instrumentation Laboratories) calibrated at 39°C. Propranolol (1 mg/kg) and atropine (0.5 mg/kg estimated fetal wt) were given intravenously to the fetus, and measurements of coronary arterial blood gases, oxygen content, and hemodynamics were repeated. Propranolol and atropine blockades minimize baroreceptor-induced heart rate changes during the subsequent pressure-flow studies.

Left circumflex arterial blood flow was first measured in response to coronary arterial pressure changes without adenosine as follows: Pressure was changed over the range of 20–65 mmHg by transiently inflating first the inferior vena cava and then the aortic occluder, or vice versa, over ~10 s in a randomized order. During the experiment, mean blood pressures and the Doppler flow signal were sampled every 10 ms, averaged every 250 ms, and recorded over ~30 s. After blood pressures and Doppler flow returned to baseline levels, a dose-response curve was obtained by increasing the adenosine infusion (3 mg/ml) in steps until maximal vasodilation was reached. The lowest infusion rate that produced maximal steady-state vasodilation was then selected for use in determining coronary conductance relationships. A steady-state coronary blood flow response was generally reached within 2 min. Arterial blood gas was sampled and hemodynamics were measured during the adenosine infusion as previously described. The adenosine infusion was stopped, and the fetus was allowed to recover for 20 min.

A coronary conductance relationship was then determined as adenosine was infused. To determine whether the dose of adenosine that was chosen maximally dilated the perfused coronary arterial segment, a coronary conductance relationship was measured under conditions of hypoxic hypoxemia combined with adenosine. A spacious plastic bag attached to a canister of 10% O2-4% CO2-86% N2 was placed over the ewe's head. After the ewe breathed the mixture for 10 min, the adenosine infusion was restarted and fetal arterial blood gases were determined and a coronary conductance relationship was measured. The bag was then removed, and the ewe breathed room air during the remainder of the experiment.

During each of the 4 subsequent days, adenosine was infused into the left circumflex coronary artery for 12 h; a computer was used to control a roller infusion pump. An infusion rate was chosen to double flow beyond the baseline Doppler hertz signal. The Doppler flow signal was averaged at 100 Hz for the last 2 min of a 4-min control cycle; this permitted changes in adenosine to reach a steady state. Step increases or decreases in infusion rates were programmed to occur when the Doppler flow signal was ~20% outside the chosen target level. The 12-h adenosine infusion period was followed by a similar 12-h period without infusion, during which Doppler flow was also recorded. Each day, after the...
12-h recovery period, a dose-response curve to adenosine was repeated to establish that day’s target infusion rate. The lowest dose that resulted in twice the mean flow velocity hertz signal of the previous 12-h non-adenosine-infusion period was chosen as the starting dose.

On day 4, ~10 h after the last 12-h adenosine infusion was finished, the final experiment was started. Hemodynamic data were collected before and after blockade with propranolol and atropine. A dose-response curve was again generated, and coronary blood flow vs. coronary pressure studies were repeated before and during adenosine infusion. Coronary conductance relationships were again determined under conditions of hypoxic hypoxemia. To calibrate the Doppler flow, radiolabeled microspheres (1.5 × 10⁶, 15 μm diameter, ⁹⁵Nb or ¹⁴¹Ce) were injected into the left atrium under steady-state conditions while adenosine was infused into the circumflex artery at a rate that maximized flow as a carotid artery reference sample was withdrawn for 2.5 min at a rate of 4.26 ml/min (2). Finally, to determine the effects of inhibition of NO on conductance, 200 mg of nitro-L-arginine methyl ester (L-NAME; Sigma, St. Louis, MO) dissolved in 60 ml of normal saline were infused into the left atrium over a period of 40 min in four of the six chronic adenosine-infused fetuses (24). After 20 min, hemodynamic parameters, blood gases, and conductance relationships were measured as previously described for adenosine.

At the conclusion of the coronary conductance studies, the ewe and fetus were killed with an intravenous injection of pentobarbital sodium. The left and right atria, interventricular septum, and ventricles were dissected free and separately weighed, and the radioactivity of the tissues and reference arterial samples was determined with a Wallac 1470 Wizard gamma counter. Blood flow was calculated as follows: (radioactive cpm of 100 g tissue/cpm of arterial reference) × withdrawal rate (where cpm is counts per minute).

In each fetus, linear regression analysis (zero flow and maximal flow with adenosine) was used to define the relationship between the Doppler hertz signal and circumflex coronary artery flow as measured by radiolabeled microspheres. Pressure-flow relationships were described by linear regression to interpolate resting blood flow at 40 mmHg. Coronary reserve at 40 mmHg was determined as the difference between left ventricular blood flow interpolated at 40 mmHg under resting conditions and that during maximal adenosine infusion. Data such as coronary reserve, slope of the adenosine conductance, and maximal adenosine flow during the 12-h infusion of adenosine. Thus six fetuses were studied with chronic infusions of adenosine and four with chronic infusions of saline.

**RESULTS**

Experiments were begun at 127.8 ± 3.2 days of gestation in adenosine-treated fetuses and 126.7 ± 2.2 days in saline-infused controls. Fetal weights for the experimental and saline control groups were 3,607 ± 608 and 3,531 ± 1,413 g, respectively. The fetal heart-to-body weight ratios were 7.8 ± 1.0 and 6.9 ± 0.5 g/kg fetal wt. Baseline physiological data on day 1 of the study (Table 1) were similar to controls in chronically instrumented fetal sheep at a similar gestational age in our laboratory (2, 3, 24). The hematocrits were similar: 29.2 ± 4.7 and 32.1 ± 3.1% for adenosine-treated and control animals, respectively. Blood pressures, blood gases, oxygen content, heart rate, and carotid arterial pH were unaffected by blockade with atropine-propranolol or by adenosine infusion (Table 1). On day 1 of the study, resting left ventricular coronary blood flow (250 ± 111 ml/min⁻¹ 100 g⁻¹) increased with adenosine infusion to 530 ± 216 ml/min⁻¹ 100 g⁻¹ (P < 0.01). After 4 days of periodic adenosine infusion, blood pressures and heart rate were unchanged from the initial values. On day 4,
unperturbed circumflex coronary blood flow (194 ± 74 ml·min⁻¹·100 g⁻¹) was similar to the basal flow measured on day 1 of the study, but the maximal circumflex coronary artery flow was increased to 878 ± 210 ml·min⁻¹·100 g⁻¹ (P < 0.001) in response to adenosine administration. This maximal flow was significantly greater than could be achieved on day 1 (P < 0.001). As expected, oxygen delivery to the left ventricle supplied by the circumflex coronary artery increased during maximal adenosine infusion. On day 1 of the study, oxygen delivery increased from 15.3 ± 3.2 to 38.2 ± 21.7 ml O₂·min⁻¹·100 g⁻¹ (P < 0.03), and on the final day of the experiment, oxygen delivery increased from 13.0 ± 7.0 to 58.4 ± 24 ml O₂·min⁻¹·100 g⁻¹ (P < 0.01). The heart rate-peak systolic pressure product was unchanged during the 4-day experiment among all conditions.

Figure 1 illustrates the average coronary blood flow during the 12 h of controlled infusion of adenosine and the recovery period without infusion. The average increase in circumflex coronary artery blood flow during adenosine infusion for the 4-day experiment was 189 ± 32%. Circumflex coronary artery blood flow averaged 223 ± 101 ml·min⁻¹·100 g⁻¹ during the recovery period for each of the 4 days between infusion periods and 424 ± 204 ml·min⁻¹·100 g⁻¹ during adenosine infusion. Over the 4 days, the average infusion rate of adenosine was 14.8 ± 5.3 and 91.6 ± 73 μg·min⁻¹·kg fetus⁻¹ during the first 2 h and last 2 h, respectively (P = 0.007). Because target Doppler flow rates could change each day, the design of the experiment was not suitable for comparing pharmacological responsiveness to adenosine. However, the adenosine infusion rate required to maintain a doubling of the Doppler flow signal generally increased after 8 h. Generally, the adenosine infusion rate was reset to lower levels after the 12-h recovery.

Figure 2 shows a representative example of the coronary perfusion pressure-coronary flow relationship in one fetus in which adenosine was infused for 12 h each day for 4 days. Resting coronary blood flow without adenosine during saline infusion showed normal regulation of blood flow over a range of pressures and was unchanged at the end of the study. The slope of the coronary conductance relationship obtained after maximal vasodilation with adenosine on day 1 (18.3 ml·min⁻¹·100 g⁻¹·mmHg⁻¹) rose to 29.5 ml·min⁻¹·100 g⁻¹·mmHg⁻¹ by the end of the study. Thus left ventricular coronary blood flow at 40 mmHg increased from 897 ml·min⁻¹·100 g⁻¹ to a maximum of 1,255 ml·min⁻¹·100 g⁻¹ during adenosine infusion. The coronary flow reserve in the circumflex distribution (flow above baseline at 40 mmHg) increased from 668 to 1,023 ml·min⁻¹·100 g⁻¹.

Changes in coronary conductance are shown in Fig. 3. In adenosine-infused fetuses, maximal coronary conductance increased from 14.0 ± 5.0 to 26.9 ± 3.9 ml·min⁻¹·100 g⁻¹·mmHg⁻¹ (P < 0.001). Coronary conductance relationships during conditions of hypoxia were generated in four of the six fetuses in which adenosine was infused. Hypoxic challenge did not stimulate further increases in slope in adenosine-infused fetuses: 10.8 ± 5.1 and 23.5 ± 9.6 ml·min⁻¹·100 g⁻¹·mmHg⁻¹ on days 0 and 4, respectively. In these animals, 10 min of hypoxia reduced arterial oxygen content from 6.5 ± 1.5 to 4.4 ± 0.9 ml/dl on day 1 of the study and decreased pH from 7.31 ± 0.03 to 7.27 ± 0.05. Similar results were obtained on day 4. Coronary conductance was unchanged in the four saline-infused control animals: 18.5 ± 10.9 and 18.5 ± 8.7 ml·min⁻¹·100 g⁻¹·mmHg⁻¹ on days 0 and 4, respectively.

Figure 4 shows circumflex coronary artery blood flow interpolated from pressure-flow relationships at 40 mmHg. Because blood pressure did not change, these data are similar to uninterpolated data presented in Table 1. Resting coronary blood flows at 40 mmHg without adenosine at the start and end of the study were similar: 252 ± 124 and 235 ± 62 ml·min⁻¹·100 g⁻¹, respectively. Adenosine stimulation increased the
interpolated blood flow to \(591 \pm 214 \text{ ml min}^{-1} \cdot 100 \text{ g}^{-1}\) on the initial day \((P < 0.03)\) and to \(907 \pm 204 \text{ ml min}^{-1} \cdot 100 \text{ g}^{-1}\) after 4 days \((P < 0.001; \text{Fig. 4})\). Fetuses exposed to maternal hypoxic hypoxemia demonstrated no further rise in coronary blood flow.

Coronary flow reserve in the circumflex coronary artery distribution is shown in Fig. 5. Because resting coronary flow did not increase but coronary conductance did increase in response to adenosine, coronary flow reserve increased from \(299 \pm 196 \text{ ml min}^{-1} \cdot 100 \text{ g}^{-1}\) on day 1 to \(672 \pm 266 \text{ ml min}^{-1} \cdot 100 \text{ g}^{-1}\) \((P < 0.001)\) on the final day of the study. Fetal hypoxia resulted in no additional change in coronary reserve.

Figure 6 illustrates data obtained from one fetus instrumented with a flow probe placed around the ascending aorta in addition to the standard preparation. We were concerned that the increase in adenosine concentration required to maintain the Doppler flow signal at twice basal levels at the end of each 12-h infusion period might have resulted in a systemic response that could influence cardiac growth. Average aortic blood flow measured \(165 \text{ ml min}^{-1}\) before adenosine infusion and \(163 \text{ ml min}^{-1}\) after 10 h of adenosine infusion with coronary flow controlled at \(789 \text{ ml min}^{-1} \cdot 100 \text{ g left ventricle}^{-1}\). For comparison, the arrow indicates the average initial dose of adenosine infused in the study. Despite an increase in the dose of adenosine required to maintain the increase in coronary flow, there was no change in aortic blood flow.

To investigate the role of NO in mediating the increase in coronary conductance after chronic adenosine infusion, \(L\)-NAME was infused into the left atrium, and 1 h later a maximal response to adenosine was deter-

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**Fig. 3.** Coronary conductance on days 0 and 4. For animals treated with adenosine and subjected to hypoxia and for those treated with adenosine + \(L\)-NAME \((\text{L-NAME}), n = 4\); for all others, \(n = 6\). LV, left ventricle. *Significantly different from baseline \((P < 0.001\) for adenosine; \(P < 0.05\) for adenosine hypoxia).

**Fig. 4.** Coronary blood flow at 40 mmHg before and after chronic adenosine (Aden) infusion. For animals treated with adenosine and subjected to hypoxia and for animals treated with adenosine + \(L\)-NAME \((\text{L-NAME}), n = 4\); for all others, \(n = 6\). *Significantly different from saline on the day of the study.

**Fig. 5.** Coronary reserve at 40 mmHg before and after chronic adenosine infusion. For animals treated with adenosine and subjected to hypoxia and for animals treated with adenosine + \(L\)-NAME \((\text{L-NAME}), n = 4\); for all others, \(n = 6\). *Significantly different from baseline \((P < 0.001\) for adenosine; \(P < 0.02\) for adenosine hypoxia).

**Fig. 6.** Systemic effects of intracoronary adenosine infusion.
The basal measurement of coronary conductance in the circumflex distribution on day 1 of the study was similar to previous data published in fetal sheep (3) and approximately twice that determined in adult sheep (8). This decrease in coronary conductance with maturation is consistent with the fivefold increase in myocyte cross-sectional area and 40% decrease in vascular volume in the left ventricle that occurs as the fetus transitions to the adult (28). We observed that conductance did not change in saline-infused controls over the 4 days of the study but doubled in adenosine-infused fetuses. This suggests that the normal means of signaling growth in the normoxic fetus may well be flow-mediated shear stress, and our pharmacological challenge may have merely stimulated a normal existing pathway. However, we were not able to distinguish between the effect of adenosine acting directly at a G protein receptor to stimulate angiogenesis and indirectly through increased flow and subsequent mechanical signals. Indeed, adenosine may be important in signaling alterations in vessel architecture separate from its vasodilatory effect. However, we hypothesize that flow is a powerful stimulant of vessel remodeling in the fetus. We therefore speculate that, in the developing fetus, the daily increase in fetal weight would lead to an increase in cardiac work, adenosine release, an increase in coronary flow and shear stress, and subsequent angiogenesis.

To our knowledge, adenosine kinetics have not been studied in the fetal heart. However, in the adult dog, <1% of [3H]adenosine injected into the left anterior descending artery appears in the coronary sinus (18). These investigators found that the adenosine dose-coronary blood flow response curve was quite steep. A 90% increase in arterial plasma correlated with a 60% increase in cardiac interstitial adenosine concentration, and this change was found to increase coronary flow from a threshold of 5% of maximal flow to 50% of maximal flow (28a). In this study, injection of adenosine directly into the proximal circumflex artery showed no systemic effect. Selective transport of adenosine into the endothelium and smooth muscle (25) as well as adenosine deaminase additionally may have limited extracardiac effects. Intracoronary infusions of adenosine in a fetus instrumented with an aortic flow probe demonstrated no change in blood pressure, heart rate, or aortic flow, even though adenosine was infused at levels greater than those used in the rest of the experiments. In addition, peak systolic pressure-heart rate product was not different with adenosine, suggesting that increased external work did not mediate the changes in conductance. To test whether maximal vasodilation was achieved with adenosine, we determined conductance relationships under conditions of moderate hypoxic hypoxemia with no effect on the slope of the coronary conductance relationship or the left circumflex coronary maximal blood flow. However, we did not test whether extreme hypoxemia would increase coronary conductance to a greater degree than observed with adenosine. Indeed, studies of fetal sheep under conditions of acute hypoxic hypoxemia in which...
the carotid arterial oxygen content was reduced to 1.7 ml/dl have reported coronary blood flow rates that exceed those of adenosine infusion (24).

Remarkably, tolerance to adenosine appeared to develop and regress rapidly. Over the 4 days of the study, the dose of adenosine required in the first 2 h of the constant infusion to maintain coronary blood flow at the selected rate averaged 14.8 ± 5.3 μg·kg⁻¹·min⁻¹. Over the same period of time, the dose required during the last 2 h of the 12-h infusion averaged 91.6 ± 73 μg·kg⁻¹·min⁻¹. After each 12-h rest, the response to adenosine rapidly reset to the previous period, requiring as little initially as 1/30th of the dose delivered 12 h earlier. This suggests that adenosine is capable of rapid reversible feedback regulation. This could represent regulation of the adenosine type 2A receptor, adenosine reuptake, adenosine deaminase, or adenosine kinase activity (18, 25, 27). We also observed that, after L-NAME administration, coronary conductance, left ventricular blood flow, and coronary reserve were similar to baseline values on day 1 of the study. In culture systems, adenosine has been shown to increase the NO production of endothelial cells (21) as well as VEGF expression (10, 11). Furthermore, VEGF has been shown to increase endothelial cell production of endothelial constitutive NO synthase mRNA, protein, and NO release (16). Because the conductance and flow responses to adenosine infusion after L-NAME were not less than responses to adenosine alone, the data are consistent with differential regulation of larger proximal resistance vessels by NO and more distal smaller arterioles by adenosine, as suggested by Kuo et al. (19). These investigators found that, in adult dogs, small (<100-μm) arterioles dilated more to adenosine than large (>100-μm) arterioles. Furthermore, arterioles dilated in response to L-NAME, whereas small arteries contracted. Thus large arteries were more responsive to shear stress, preserving distal metabolic regulation of arterioles to adenosine (17). Because the site of resistance in the coronary vasculature has not been determined in the developing fetus, further studies that delineate the response to flow-modulated vs. metabolic regulation are needed to understand the mechanisms involved. The site and geometry of these changes remain to be determined. Another possibility that cannot be excluded is that increased sensitization of NO production during adenosine infusion could have resulted in augmented vasodilation. To confirm that increases in coronary conductance correlate with increases in arteriole volume density, histological studies are needed.

In summary, we have demonstrated in fetal sheep that an increase in coronary blood flow brought about by adenosine, in the absence of changes in blood pressure or oxygen content, is sufficient to alter coronary conductance and, therefore, affect the geometry of resistance vessels. Our preliminary data suggest that these changes persist in the adult (unpublished observations).

This study was supported by National Institutes of Health Grants HL-45043 and P01 HD-34430.

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