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Development of vasomotor responses in fetal mesenteric arteries

E. V. ROUWET,[1] J. G. R. DE MEY,[2] D. W. SLAAF,[3] E. HEINEMAN,[1]
G. RAMSAY,[1] AND F. A. C. LE NOBLE[4]
Department of Surgery[1], University Hospital Maastricht, and Departments of [2]Pharmacology, [3]Biophysics, and [4]Physiology, Cardiovascular Research Institute Maastricht, Maastricht University, 6200 MD Maastricht, the Netherlands

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Rouwet, E. V., J. G. R. De Mey, D. W. Slaaf, E. Heineman, G. Ramsay, and F. A. C. le Noble. Development of vasomotor responses in fetal mesenteric arteries. *Am J Physiol Heart Circ Physiol* 279: H1097–H1105, 2000.—Changes in mesenteric arterial diameters were studied using intravital microscopy in chick fetuses at *days 13* and *17* of incubation, corresponding to 0.6 and 0.8 fetal incubation time, both during 5 min of hypoxia followed by 5 min of reoxygenation and after topical administration of increasing concentrations (10^{-6}–10^{-2} M) of norepinephrine (NE) and acetylcholine (ACh). Baseline diameters of second-order mesenteric arteries increased from 56 μm at 0.6 incubation to 75 μm at 0.8 incubation. Acute hypoxia induced a reduction in arterial diameter to 87 ± 4.4% of baseline at 0.6 incubation and to 44 ± 6.7% at 0.8 incubation ($P < 0.01$). During reoxygenation, mesenteric arteries dilated to 118 ± 6.5% and 121 ± 7.5% of baseline at 0.6 and 0.8 fetal incubation time, respectively. Phentolamine did not affect the vasoconstriction during hypoxia at 0.6 incubation, whereas this α-adrenergic antagonist significantly attenuated the vasoconstrictor response at 0.8 incubation (to 93 ± 2.7% of baseline, $P < 0.01$). Topical NE induced maximal vasoconstriction to 71 ± 3% of baseline at 0.6 incubation and to 35 ± 3.8% at 0.8 incubation ($P < 0.01$). Maximal vasodilation to topical ACh was 113 ± 4.4% and 122 ± 4.8% of baseline at 0.6 and 0.8 incubation, respectively. These in vivo findings show that fetal mesenteric arteries constrict in response to acute hypoxia and that the increase in magnitude of this vasoconstrictor response from 0.6 to 0.8 of fetal development results from an increase in adrenergic constrictor capacity.

cardiovascular development; hypoxia; necrotizing enterocolitis; norepinephrine; acetylcholine

NEONATAL NECROTIZING ENTEROCOLITIS is a clinical condition characterized by necrosis of the neonatal intestine. An imbalance between oxygen consumption of and arterial oxygen supply to the intestine has been implicated in the pathophysiology of this disease (1, 10). Previous studies with regard to circulatory physiology of the neonatal intestine were conducted in piglets during the first month after birth. However, necrotizing enterocolitis is predominantly observed in preterm neonates, with an increased incidence with decreasing gestational age at birth. Because arterial oxygen supply depends in part on the capability to regulate arterial diameter, insight into the regulation of mesenteric arterial tone in the fetal developing intestine may contribute to our understanding of the pathophysiology of this disease.

Information regarding onset and nature of the regulation of arterial tone during fetal development may be derived from experimental studies conducted in fetal sheep and chick fetuses, which addressed the redistribution of the cardiac output during an acute reduction of the arterial oxygen content at consecutive stages of fetal gestation (5, 9). It was demonstrated that acute hypoxia induced a decrease in intestinal blood flow at 0.9 of fetal gestation in both species (6, 9). Because both administration of the α-adrenergic antagonist phenoxybenzamine (11) and chemical sympathectomy using 6-hydroxydopamine (6) blunted this reduction in intestinal blood flow, it was postulated that circulating catecholamines or sympathetic nerves may be involved in the control of intestinal arterial tone at this stage of fetal gestation. However, interpretation of these observations with respect to the regulation of intestinal arterial tone is hampered by the fact that in these studies intestinal blood flow was measured by means of a microsphere technique or flow probe instead of measuring the actual arterial diameter. According to Poiseuille's law, blood flow is determined by vascular resistance and blood pressure. Hence, a reduction in intestinal blood flow during hypoxia may be caused by an increase in intestinal arterial resistance and/or by a decrease in arterial pressure. The latter may be due to a reduction of cardiac output or a resistance decrease in cerebral or myocardial vascular beds (shunting). Additionally, changes in blood pressure may also directly influence arterial diameter through alteration of the myogenic tone of the vessel. To discern between these mechanisms responsible for a reduction in intestinal blood flow, it is necessary to measure both intestinal arterial diameter and blood pressure during acute hypoxia.

Address for reprint requests and other correspondence: F. A. C. le Noble, Dept. of Physiology, Maastricht Univ., PO Box 616, 6200 MD Maastricht, the Netherlands (E-mail: flenoble@hotmail.com).

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We designed an experimental setup using intravital microscopy to investigate changes in mesenteric arterial diameters in vivo in response to physiological and pharmacological stimuli in the developing chick fetus. The intestine of the chick fetus is partly located outside the abdomen in the naturally occurring omphalocele; hence, the mesenteric arteries can be studied without extensive invasive surgery and general anesthesia, thereby avoiding any influence of anesthetics on arterial tone (7). The study was conducted at *days 13* and *17* of incubation, which corresponds to 0.6 and 0.8 fetal incubation time. It has been suggested that regulation of arterial tone may be initiated in this period of fetal development (4). First, we determined the changes in mesenteric arterial diameter during an acute reduction in fetal oxygen supply (hypoxia) and the subsequent response after restoring oxygen delivery (reoxygenation). To elucidate the possible role of α-adrenoceptors in this response, experiments were also performed during α-adrenergic blockade. Because acute hypoxia in the chick fetus is associated with a rise in plasma levels of norepinephrine (NE) (14), we subsequently investigated the effect of exogenously applied NE on mesenteric arterial diameter. The vasodilator capacity of the mesenteric arteries at 0.6 and 0.8 fetal incubation time was evaluated by measuring changes in vascular diameter in response to acetylcholine (ACh).

MATERIALS AND METHODS

Surgical Preparation

Experimental procedures were in accordance with the Dutch law on the use of laboratory animals. Fertile Lohman selected White Leghorn eggs were incubated at 37°C, a relative air humidity of 60%, and rotated once every hour (Polyhatch incubator; Brinsea Products, Sandford, UK). Incubation time until hatching for these eggs is 21 days. In this study we used chick fetuses at *days 13* and *17* of incubation (0.6 and 0.8 of fetal incubation time, respectively), corresponding to stages 39 and 42 according to Hamburger and Hamilton (2).

Surgical preparations were performed in a clinical intensive care incubator (type 7510; Drögerwerk, Lübeck, Germany) equipped with a dissecting microscope (model MS5; Leica, Rijswijk, the Netherlands) while temperature and relative air humidity were maintained at 37°C and 60%, respectively. The eggs were opened at the blunt end containing the air cell. After part of the egg shell and outer shell membrane was removed, the inner shell membrane was moistened with 0.9% NaCl to visualize the vessels of the underlying chorioallantoic membrane (CAM). After the penetration of CAM in an area with sparse vascularization, avoiding bleeding, the omphalocele was localized and centered at the level of the CAM by means of two sutures through the connective tissue (Ethicon, Prolene 6-0). The omphalocele was opened by careful blunt dissection, and the intestine was exposed. One segment of the umbilical loop of the ileum with its mesentery was placed on a 1-cm piece of cotton tape, which was attached to the egg shell (Fig. 1). Only preparations that were completed within 15 min after opening the egg and that required minimal manipulation during positioning were included in the experimental protocol. For further microscopic evaluation in vivo, the egg was transferred to a single-egg chamber, in which temperature (37°C) and air flow (4 l/min) were controlled, then subsequently placed in an intravital microscope setup (Fig. 1).

Intravital Videomicroscopy

All in vivo observations were made with an intravital microscope (Leitz Orthoplan 946627). Visualization of the vasculature was performed with a Leitz ×20 long-working-

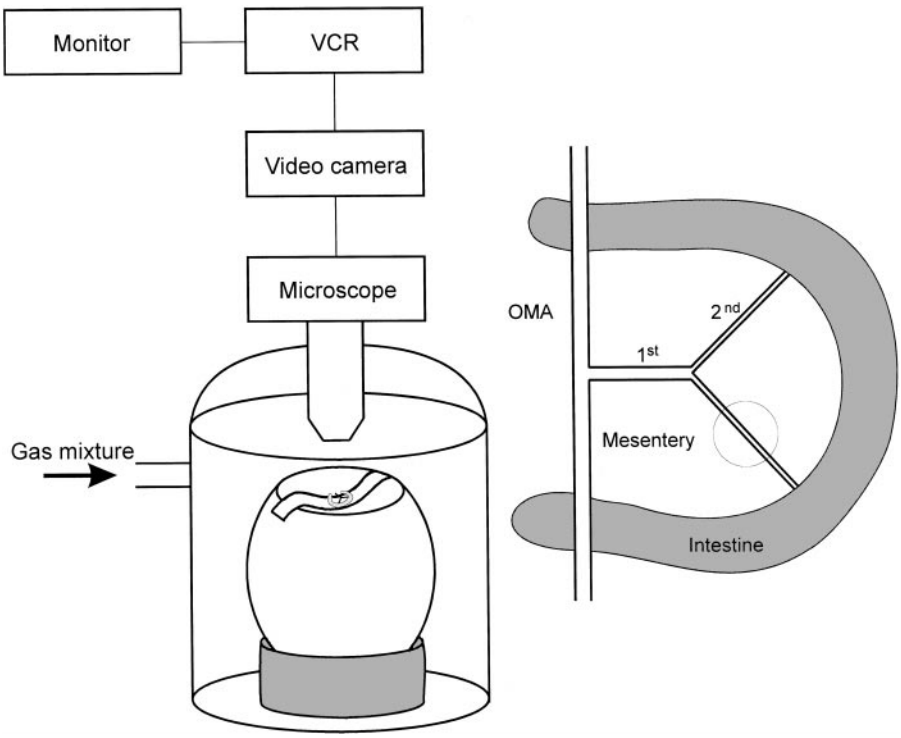

Fig. 1. *Left*: schematic representation of the intravital microscopy setup. *Right*: top view of fetal mesenteric preparation with site of observation in circle. OMA, omphalomesenteric artery; 1st and 2nd, branch order of mesenteric artery.

distance objective (numerical aperture 0.32) via a Leitz Ploemopak illuminator 2.1 (×1.25), equipped with a Leitz POL polarizer-analyzer cube (12). The intestine was epi-illuminated using a 75-W xenon lamp. Images were projected onto a charge-coupled device camera (Hamamatsu Photonics, Hamamatsu, Japan), connected to a Super-VHS video recorder (Panasonic model NV-FS100HQ), and stored on videotape (Sony Super VHS VXSE-180Vf). Images were displayed on a monitor screen (Sony model PVM-122CE). Final resolution was 1 μm. Arterial diameters were analyzed off-line using an image-shearing device (model 908; IPM, San Diego, CA). All experiments were performed on anatomically similar locations of second order branches of the omphalomesenteric artery.

Drugs and Solutions

NE (L-arterenol bitartrate), ACh, sodium nitroprusside, adenosine, papaverine, and phentolamine were obtained from Sigma Chemical. All drugs were dissolved in HEPES-buffered Krebs with the following composition (in mM): 143.3 NaCl, 4.7 KCl, 1.2 $MgSO_4$, 2.5 $CaCl_2$, 5.6 glucose, and 15 HEPES. The pH of the buffer was KH_2PO_4 adjusted to 7.4. Final concentrations of the solutions ranged from 10^{-6} to 10^{-2} M. All drugs were freshly prepared on the day of the experiment; temperature of the solutions at the time of administration was 37°C.

Experimental Protocol

Effect of acute hypoxia. Evaluation of the effect of acute hypoxia on second-order mesenteric arterial diameters was performed at 0.6 (n = 8) and 0.8 (n = 7) fetal incubation time. After an equilibration period of 15 min following surgical preparation, a 2-min baseline recording was made. Subsequently, hypoxia was induced by replacing the ambient air (21% O_2) in the egg chamber by 100% N_2 (4 l/min) for 5 min, according to a method described previously (9). After completion of the hypoxic period, reoxygenation was achieved by replacing the N_2 with ambient air. During this period one mesenteric artery was continuously kept in focus and recorded.

To verify whether this protocol actually reduced blood oxygen content, i.e., resulted in hypoxemia, blood samples were collected at the end of the 5-min period of hypoxia in a separate series of chick fetuses at 0.6 (n = 6) and 0.8 (n = 7) fetal incubation time, and blood gas values were compared with control groups under normoxic conditions. Samples (0.2 ml) were obtained by withdrawing blood from the chorioallantoic vein using a 1-ml syringe attached to a 21-gauge needle and analyzed at 37°C using a blood gas analyzer (model ABL510; Radiometer, Copenhagen, Denmark). The chorioallantoic vein, being the avian equivalent of the mammalian umbilical vein, transports blood from the CAM (where gas exchange takes place) to the fetus. Therefore, changes in chorioallantoic vein blood gas values reflect changes in fetal arterial blood gas values.

Blood pressure measurements. In an additional series of experiments at 0.6 (n = 5) and 0.8 (n = 6) fetal incubation time, mean arterial pressure and heart rate were determined under normoxic conditions as well as during a 5-min period of hypoxia. The technique for measuring blood pressure in the chick fetus was adapted from a method previously described by Tazawa and colleagues (3), which has been demonstrated to provide reliable blood pressure measurements while maintaining fetal gas exchange. Briefly, after opening of the egg in the clinical incubator, one of the two branches of the chorioallantoic artery was catheterized with a 10-cm-long nylon catheter (internal diameter 0.5 mm) that was filled with 0.9% NaCl. The catheter was inserted into the artery with its tip pointing upstream. The free end of the catheter was connected to a pressure transducer (Baxter Uniflow; Baxter, Uden, the Netherlands), which was placed at the same height as the egg. Pressure signals were recorded on a computer using a data acquisition system and the heart rate was calculated.

Effect of topically applied phentolamine. The effect of α-adrenergic blockade on vasomotor responses to acute hypoxia was assessed in chick fetuses at 0.6 (n = 8) and 0.8 (n = 8) fetal incubation time. After equilibration and baseline recording, a 20-μl aliquot of a 10^{-3} M phentolamine solution, corresponding to a single dose of 6.35 μg, was applied to the mesenteric arteries. During the 5 min after application of phentolamine, a recording was made to assess the effect of α-adrenergic blockade on baseline arterial diameter. Subsequently, hypoxia was induced for 5 min followed by 5 min of reoxygenation. During this period, the same mesenteric artery was continuously kept in focus and recorded.

Effect of topically applied NE. In a separate series of chick fetuses at 0.6 (n = 8) and 0.8 (n = 10) fetal incubation time, we assessed constrictor responses of the mesenteric arteries to NE. After equilibration and baseline recording a cumulative log molar concentration-response curve was constructed by applying 20-μl aliquots of increasing concentrations (10^{-6}–10^{-2} M) of NE to the mesenteric arteries, corresponding to single doses of 6.38 ng to 63.8 μg. After application of each dose, a 2-min recording was made.

To verify the efficacy of the α-adrenergic blockade with 10^{-3} M phentolamine, we also constructed a concentration-response curve for NE 5 min after topical administration of a 20-μl aliquot of a 10^{-3} M phentolamine solution to the mesenteric arteries in a separate series of chick fetuses at 0.6 (n = 6) and 0.8 (n = 6) fetal incubation time.

Effect of topically applied ACh. To assess the vasodilator capacity of second-order mesenteric arteries both under baseline conditions and after induction of arterial tone using 10^{-2} M topically applied NE, we measured changes in mesenteric arterial diameters in response to topically applied ACh. Dilator responses under baseline conditions were assessed in chick fetuses at 0.6 (n = 6) and 0.8 (n = 7) fetal incubation time. After equilibration and baseline recording, a cumulative log molar concentration-response curve was constructed by applying 20-μl aliquots of increasing concentrations (10^{-6}–10^{-2} M) of ACh to the mesenteric arteries, corresponding to single doses of 3.63 ng to 36.3 μg. After each dose a 2-min recording was made. Dilator responses in NE-constricted arteries were assessed in a similar way by topical application of 20-μl aliquots of 10^{-6}–10^{-2} M ACh after administration of 10^{-2} M NE to the mesenteric arteries.

In a separate series of chick fetuses at 0.8 fetal incubation time (n = 5), we investigated whether topical application of a 20-μl aliquot of 10^{-2} M ACh induced maximal vasodilation. To this end, the vasodilator effect of 10^{-2} M ACh was compared with a cocktail of 10^{-2} M each of sodium nitroprusside, adenosine, and papaverine. No additional increment in arterial diameter was observed after application of this cocktail on top of ACh (data not shown), indicating that a 20-μl aliquot of 10^{-2} M ACh induced maximal vasodilation.

The effect of repetitive application of the buffer solution was assessed in a separate series of chick fetuses at 0.8 fetal incubation time (n = 5). To this end, 20-μl aliquots of HEPES-buffered Krebs solution were applied to the mesenteric arteries 10 times, with a 2-min interval. No significant changes in arterial diameters were observed during 10 suc-

cessive applications of 20 μl of HEPES-buffered Krebs solution within a 30-min period (data not shown).

Quantification of Arterial Responses

Luminal diameter of a second-order mesenteric artery was measured at about 50 μm from the bifurcation of the first order mesenteric artery, a site with clear distinction of the inner margins of the vessel wall (8). During the course of an experiment, all measurements were performed at this site of the second-order mesenteric artery. Changes in diameter are presented as percentage of baseline diameter, with baseline being 100%. Thus an increase in arterial diameter to 150% of baseline indicates that the diameter is 1.5 times baseline diameter. Similarly, a decrease in arterial diameter to 50% of baseline indicates that the diameter is 0.5 times baseline diameter.

Data Analysis

Data are expressed as means ± SE. The term n refers to the number of individual arteries in which observations were made; one artery per fetus was selected. Statistical comparisons between groups were made using the Mann-Whitney U test. Statistical comparisons within groups were made using the Wilcoxon signed-rank test. Statistical significance was defined as a $P < 0.05$.

RESULTS

Effect of Acute Hypoxia

Figure 2 illustrates a diameter tracing of the effect of acute hypoxia on a second order mesenteric artery at 0.8 fetal incubation time. The artery was recorded continuously, and arterial diameter was measured every 15 s. The response to hypoxia was characterized by an initial transient constriction with a peak after about 100 s, followed by a relaxation to baseline level that continued until the end of the 5-min hypoxic period. Reoxygenation resulted in a vasodilation above baseline, which was maintained until the end of the 5-min period of reoxygenation. In subsequent experiments, mesenteric arterial diameters were measured at four time points: before hypoxia (base), at maximal vasoconstriction during hypoxia (T_N), at the end of the hypoxic period (5 min), and at the end of reoxygenation (10 min). Baseline diameters increased from 56 ± 2.9 μm at 0.6 incubation to 75 ± 2.4 μm at 0.8 fetal incubation time. During hypoxia, arterial diameters significantly decreased to 87 ± 4.4% of baseline (from 56 to 49 μm, $P < 0.05$) at 0.6 fetal incubation time and to 44 ± 6.7% (from 64 to 28 μm, $P < 0.05$) at 0.8 fetal incubation time (Fig. 3). The magnitude of this vasoconstrictor response was fourfold larger at 0.8 compared with 0.6 fetal incubation time ($P < 0.01$). Maximal vasoconstriction during the 5-min period of hypoxia was observed at 106 s (range 51–207) and 105 s (range 79–137) from the start of hypoxia at 0.6 and 0.8 fetal incubation time, respectively, and lasted for ~30 s. During reoxygenation, a significant vasodilation above baseline was observed in both groups (to 118 ± 6.5% and 121 ± 7.5% of baseline, $P < 0.05$, at 0.6 and 0.8 fetal incubation time, respectively).

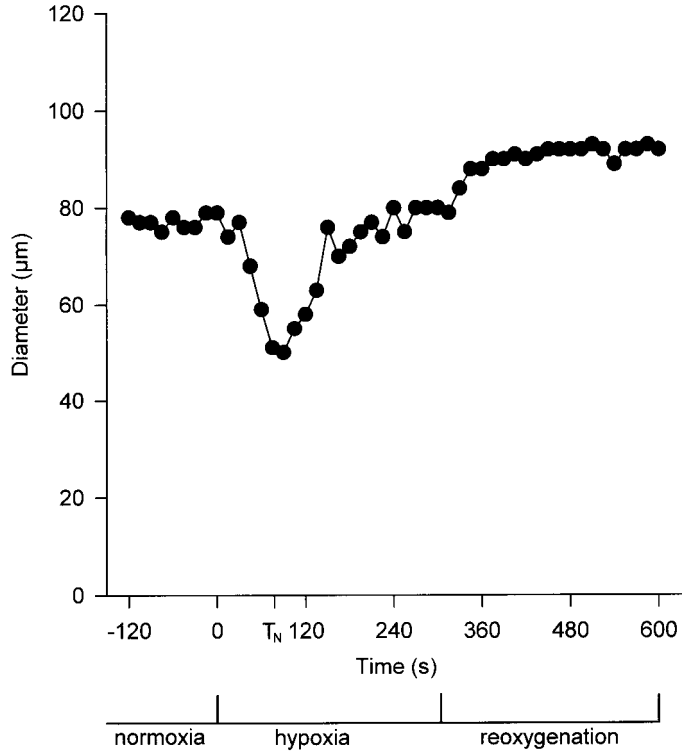

Fig. 2. Diameter tracing of the effect of hypoxia and reoxygenation on a second-order mesenteric artery at 0.8 fetal incubation time (0.8 reflects *day 17* of chick incubation). Arterial diameter was measured every 15 s, under baseline conditions (−120–0 s), during hypoxia (0–300 s), and during reoxygenation (300–600 s). T_N, time point of maximal vasoconstriction.

Blood gas analysis demonstrated that 5 min of hypoxia induced a significant decrease of arterial Po_2 from 11.7 to 3 kPa at 0.6 fetal incubation time and a reduction of arterial Po_2 from 7.2 to 1.6 kPa at 0.8 fetal incubation time. The absolute level of the arterial Po_2 at the end of the 5-min period of hypoxia was higher at 0.6 compared with 0.8 fetal incubation time. However, when expressed as a percentage of control (normoxic) oxygen tension, arterial Po_2 was reduced to a similar extent at 0.6 and 0.8 fetal incubation time (74% and 78%, respectively, Table 1).

Mean arterial pressure under baseline conditions significantly increased from 11 ± 0.4 mmHg at 0.6 fetal incubation time to 21 ± 0.6 mmHg at 0.8 fetal incubation time ($P < 0.01$). During acute hypoxia, mean arterial pressure significantly decreased to 4 ± 1.1 mmHg ($P < 0.05$) at 0.6 and 11.8 ± 2.8 mmHg ($P < 0.05$) at 0.8 fetal incubation time (Fig. 4*A*). Heart rate under baseline conditions was not significantly different between 0.6 and 0.8 fetal incubation time (216 ± 3 and 210 ± 14 beats/min, respectively). During hypoxia, heart rate significantly decreased to 68 ± 31 beats/min ($P < 0.05$) at 0.6 and 132 ± 20 beats/min ($P < 0.05$) at 0.8 fetal incubation time (Fig. 4*B*).

Effect of Topically Applied Phentolamine

Topical application of phentolamine alone did not significantly alter baseline mesenteric arterial diame-

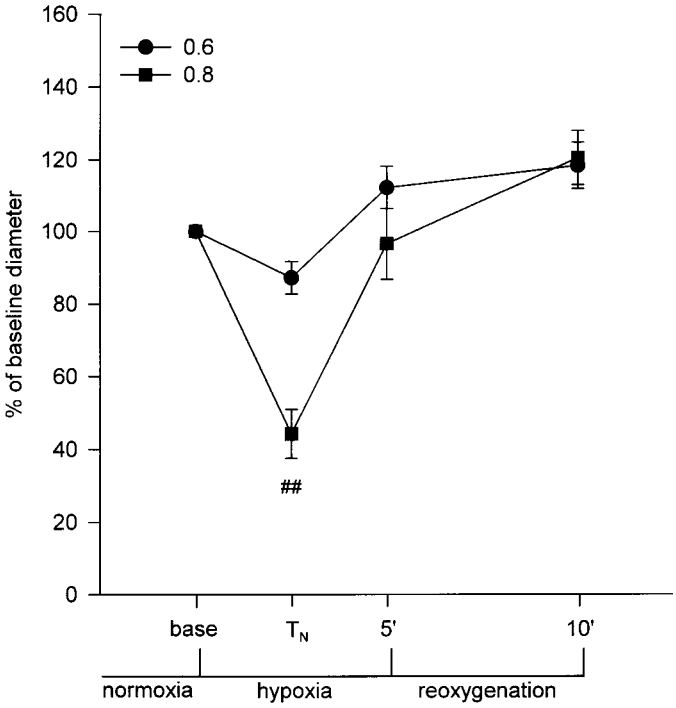

Fig. 3. Effect of 5 min of hypoxia followed by 5 min of reoxygenation on mesenteric arterial diameter at 0.6 and 0.8 fetal incubation time. Acute hypoxia induced a significant reduction in arterial diameter at both 0.6 and 0.8 fetal incubation time. The magnitude of the vasoconstriction was significantly larger in the older fetuses. During reoxygenation, a similar level of vasodilation above baseline was observed at both stages of fetal incubation. $^{\#\#}P < 0.01$, for 0.6 compared with 0.8 fetal incubation time.

ters at 0.6 and 0.8 fetal incubation time (97 ± 2.5% and 98 ± 3%, respectively). At 0.6 fetal incubation time, neither the hypoxia-associated vasoconstriction (to 89 ± 4% of baseline) nor the vasodilation during reoxygenation (to 116 ± 3.2% of baseline) was significantly affected by α-adrenergic blockade (Fig. 5*A*). In contrast, at 0.8 fetal incubation time the vasoconstrictor response during hypoxia was significantly attenuated by α-adrenergic blockade ($P < 0.01$, Fig. 5*B*). In the presence of phentolamine, acute hypoxia induced only a slight decrease in mesenteric arterial diameter to 93 ± 2.7% of baseline (from 66 to 62 μm, $P = 0.05$).

Table 1. *Blood gas values at 0.6 and 0.8 fetal incubation time at the end of the 5-min period of hypoxia (100% N_2) compared with control conditions (21% O_2)*

	0.6 Fetal Incubation Time		0.8 Fetal Incubation Time	
	Control	Hypoxia	Control	Hypoxia
n	5	5	7	7
P_{O_2}, kPa	11.65 ± 0.63	2.98 ± 0.26*	7.18 ± 0.59	1.57 ± 0.18†
P_{CO_2}, kPa	1.98 ± 0.07	1.43 ± 0.04*	4.57 ± 0.41	4.45 ± 0.25
pH	7.57 ± 0.02	7.63 ± 0.03	7.56 ± 0.03	7.53 ± 0.03

Values are means ± SE; *n* = no. of individuals. *Days 13* and *17* of incubation correspond to 0.6 and 0.8 fetal incubation times, respectively. *$P < 0.05$ and †$P < 0.01$ at the end of 5 min of hypoxia vs. control.

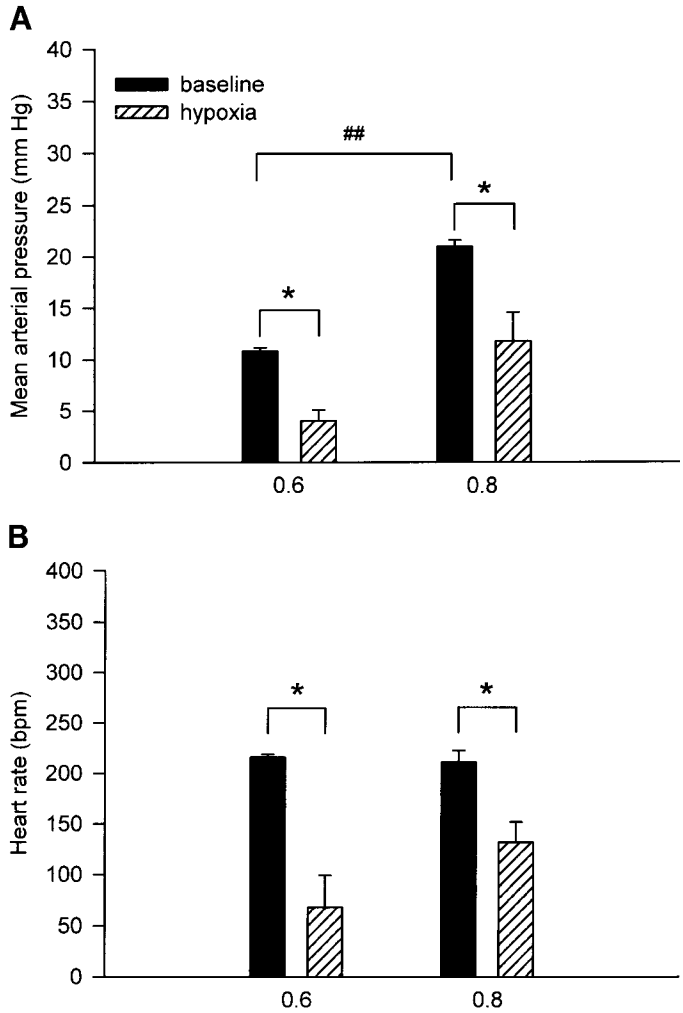

Fig. 4. Effect of 5 min of hypoxia on mean arterial pressure and heart rate at 0.6 and 0.8 fetal incubation time. Baseline mean arterial pressure was significantly higher at 0.8 compared with 0.6 fetal incubation time and decreased during acute hypoxia at both stages of fetal incubation (*A*). Baseline heart rates were similar at 0.6 and 0.8 fetal incubation time and significantly decreased during hypoxia in both groups (*B*). *$P < 0.05$ compared with baseline. $^{\#\#}P < 0.01$, for 0.6 compared with 0.8 fetal incubation time.

During the second part of the hypoxic period, diameters increased to 117 ± 3.3% above baseline ($P < 0.05$) and further increased to 122 ± 2.7% ($P < 0.05$) during reoxygenation.

Effect of Topically Applied NE

Cumulative application of increasing concentrations of NE in control normoxia state caused a decrease in arterial diameter in both age groups (Fig. 6). Maximal vasoconstriction was observed after application of 10^{-4} M (0.6 incubation) or 10^{-3} M (0.8 incubation) NE. At these applied concentrations, arterial diameters significantly decreased to 71 ± 3% ($P < 0.05$) and 35 ± 3.8% ($P < 0.01$) of baseline at 0.6 and 0.8 fetal incubation time, respectively. The magnitude of maximal constriction to NE was approximately twofold larger at 0.8 compared with 0.6 fetal incubation time ($P < 0.01$).

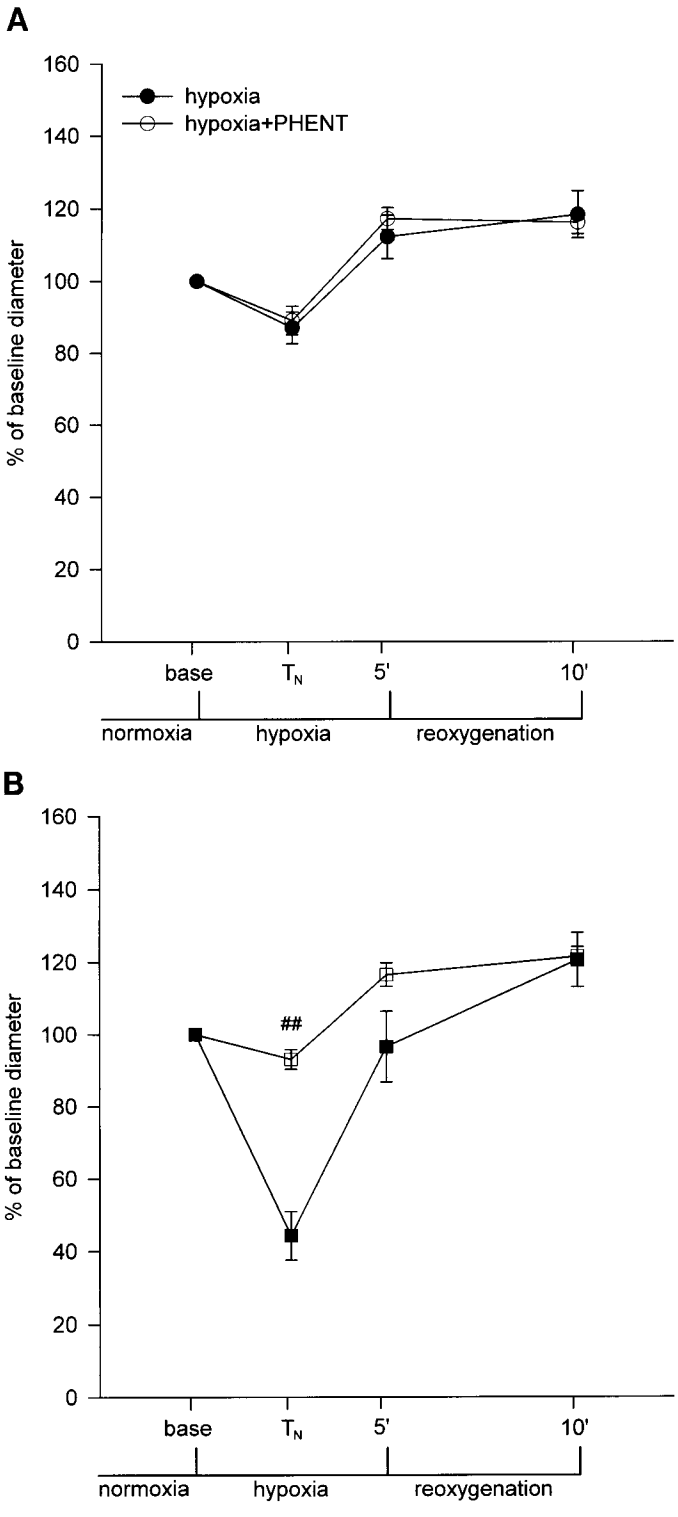

Fig. 5. Effect of topically applied phentolamine (PHENT) on vasomotor responses during acute hypoxia and reoxygenation at 0.6 and 0.8 fetal incubation time. α-Adrenergic blockade did not affect vasomotor responses at 0.6 incubation (*A*) but significantly reduced the hypoxia-associated vasoconstriction at 0.8 fetal incubation time (*B*). $^{\#\#}P < 0.01$, for hypoxia in the presence of PHENT compared with hypoxia alone.

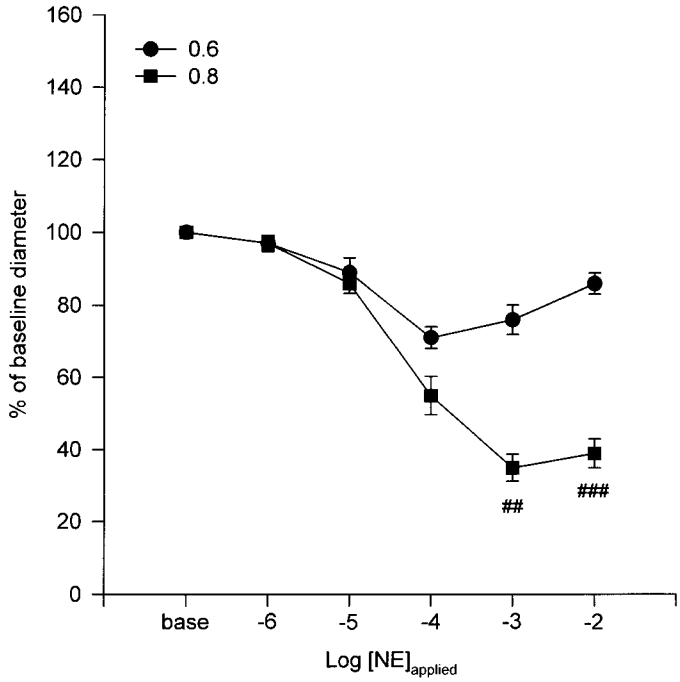

Fig. 6. Effect of topically applied norepinephrine (NE) on mesenteric arterial diameter at 0.6 and 0.8 fetal incubation time. Maximal constriction to NE was significantly greater at 0.8 compared with 0.6 incubation. $^{\#\#}P < 0.01$ and $^{\#\#\#}P < 0.001$, for 0.6 compared with 0.8 fetal incubation time.

Topical administration of a 20-μl aliquot of 10^{-3} M phentolamine to the mesenteric arteries completely prevented the reduction in arterial diameter by NE at 0.6 fetal incubation time (Fig. 7*A*). At 0.8 fetal incubation time, the concentration-response curve for NE shifted to the right in the presence of phentolamine, indicating a reduction in NE sensitivity (Fig. 7*B*).

Effect of Topically Applied ACh

Subsequent application of increasing concentrations of ACh to the same arteries resulted in a gradual vasodilation at both 0.6 and 0.8 fetal incubation time. Maximal arterial diameters were observed after application of 10^{-2} M ACh and were significantly different between 0.6 and 0.8 fetal incubation times (63 ± 3.5 vs. 91 ± 2.3 μm, $P < 0.001$). However, expressed as percentage change from baseline, maximal levels of vasodilation were not significantly different between the two stages of fetal development (to 113 ± 4.4% and 122 ± 4.8% of baseline at 0.6 and 0.8 fetal incubation time, respectively; Fig. 8*A*).

Topical application of increasing doses of ACh under baseline conditions, i.e., without prior constriction with NE, also caused a concentration-dependent dilation of the mesenteric arteries at 0.6 and 0.8 fetal incubation time. Maximal levels of vasodilation in both groups were not significantly different (to 125 ± 2.6% and 133 ± 5.4% of baseline at 0.6 and 0.8 fetal incubation time, respectively; Fig. 8*B*).

At both stages of fetal development, maximal levels of vasodilation after application of 10^{-2} M ACh were

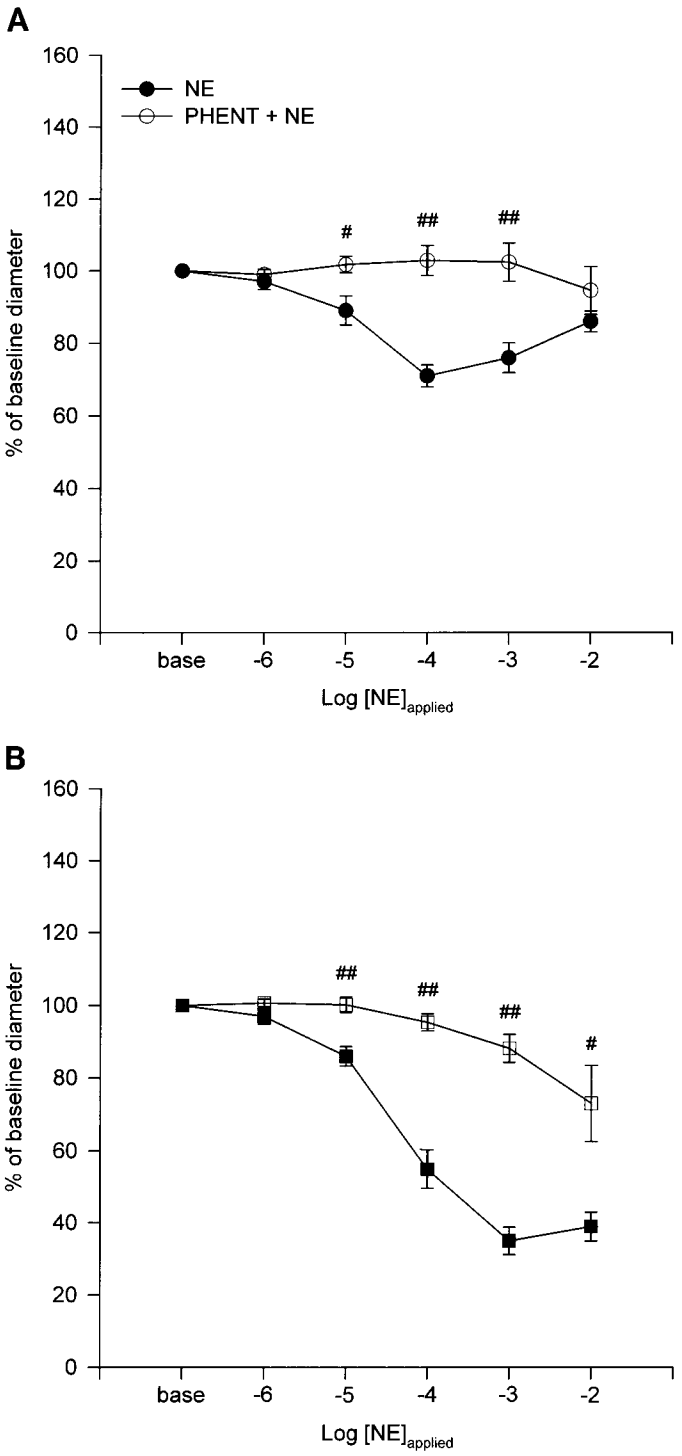

Fig. 7. Efficacy of α-adrenergic blockade with PHENT. The vasoconstrictor response to NE was significantly reduced by phentolamine at both 0.6 (*A*) and 0.8 (*B*) fetal incubation time. $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$, for NE in the presence of PHENT compared with NE alone.

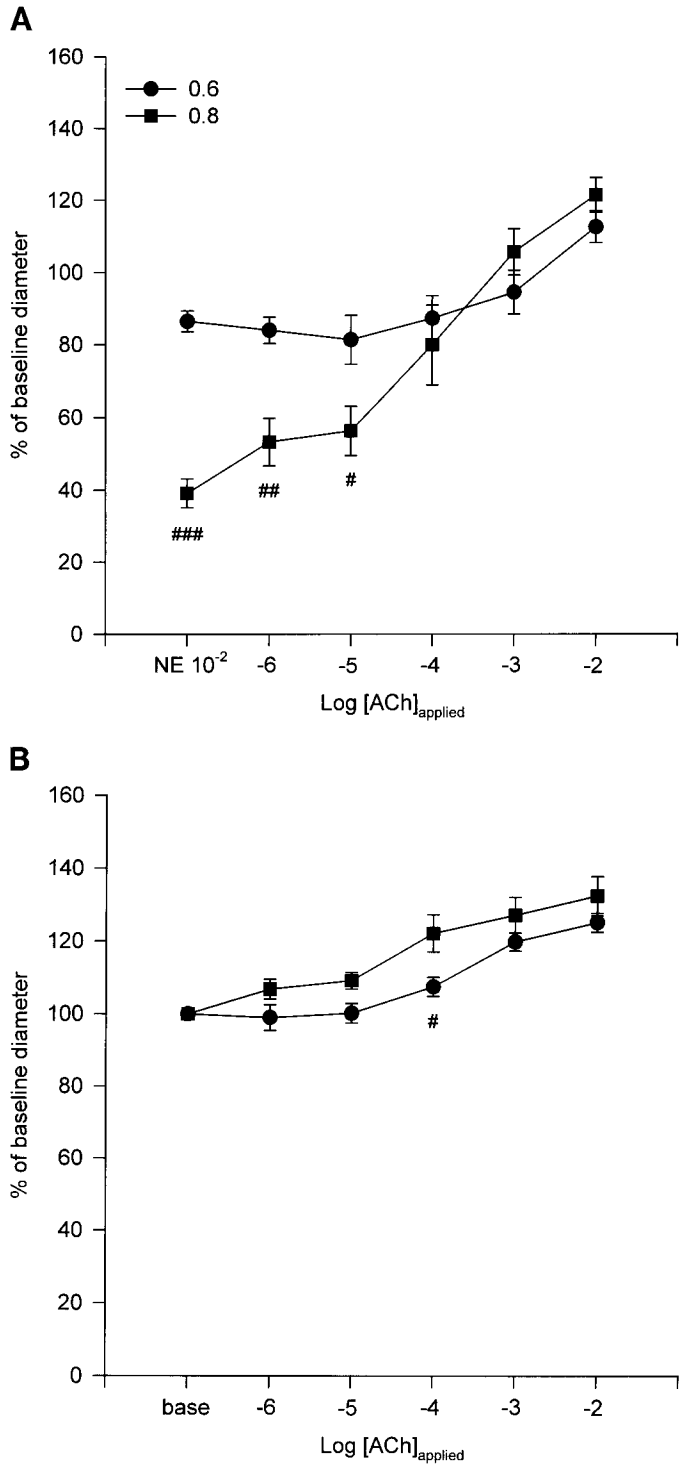

Fig. 8. Effect of topically applied acetylcholine (ACh) on mesenteric arterial diameter at 0.6 and 0.8 fetal incubation time. ACh induced a significant increase in diameter above baseline level both in arteries subjected to NE-induced constriction (*A*) and in arteries under baseline conditions (*B*). Note that maximal vasodilation to ACh was similar at 0.6 and 0.8 fetal incubation time. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, and $^{\#\#\#}P < 0.001$, for 0.6 compared with 0.8 fetal incubation time.

similar in NE-constricted arteries and in arteries under baseline conditions. Furthermore, maximal diameters in response to topical application of ACh were not significantly different from maximal diameters observed during reoxygenation after 5 min of hypoxia (Fig. 9).

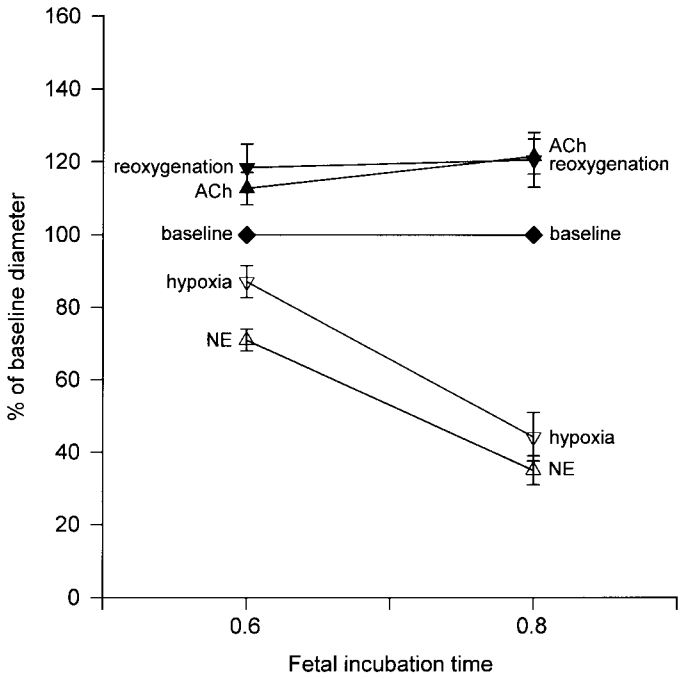

Fig. 9. Vasomotor responses of fetal mesenteric arteries at 0.6 and 0.8 fetal incubation time. Vasoconstrictor responses to acute hypoxia and to topically applied NE increased during fetal development, whereas vasodilator responses during reoxygenation and to topically applied ACh were similar at 0.6 and 0.8 fetal incubation time. Note the significant difference ($P < 0.05$) between hypoxia- and NE-induced vasoconstrictor levels at 0.6 fetal incubation time.

DISCUSSION

To obtain information with regard to the regulation of vascular tone in mesenteric arteries during fetal development, we designed a novel experimental setup for measurements of mesenteric arterial diameter in the intact chick fetus at different stages of fetal development. During the period 0.6–0.8 of chick fetal incubation time, baseline luminal diameters of second order mesenteric arteries increased from 56 to 75 μm. Because topical administration of the vasodilator substance ACh induced an increase in arterial diameter of about 20% above baseline at both 0.6 and 0.8 fetal incubation time, it may be concluded that the mesenteric arteries already exhibit a degree of vascular tone under baseline conditions. Topical administration of the nonselective α-adrenergic antagonist phentolamine did not affect baseline arterial diameter. This suggests that during the observed period of fetal development, α-adrenergic mechanisms are not involved in the establishment of baseline arterial tone at this level of the arterial tree.

The mesenteric arteries constricted in response to topically applied NE at both 0.6 and 0.8 fetal incubation time. Furthermore, this constriction was significantly attenuated in the presence of phentolamine. This indicates that functional α-adrenergic pharmacomechanical coupling is present in the mesenteric arteries as early as 0.6 fetal incubation time. The maximal constrictor response to NE increased about twofold in the period 0.6–0.8 fetal incubation time. This may be due to developmental changes in the efficacy of the α-adrenergic signal transduction pathway, the amount of adrenoceptors, as well as maturation of the vascular smooth muscle contractile apparatus. Interestingly, the mean arterial pressure increased about twofold during this period of development, from 11 mmHg at 0.6 to 21 mmHg at 0.8 fetal incubation time. Assuming that the perfusion pressure at the level of the second order mesenteric arteries also increased, the arteries at 0.8 fetal incubation time were subjected to a higher transmural pressure compared with 0.6 fetal incubation time. Hence, to obtain the observed decreases in luminal diameter, the contractile force generated by the mesenteric arteries at 0.8 fetal incubation time must be substantially larger compared with 0.6 fetal incubation time.

A 5-min period of hypoxia induced a transient decrease in mesenteric arterial diameter at both stages of fetal development. The magnitude of the hypoxia-associated vasoconstrictor response increased from a 13% reduction of arterial diameter at 0.6 incubation to a reduction of 56% at 0.8 fetal incubation time. At 0.6 fetal incubation time, application of phentolamine did not significantly affect the vasoconstriction. This suggests that the constrictor response is not mediated by α-adrenergic receptors at this stage of fetal development. In contrast, at 0.8 fetal incubation time the vasoconstriction during hypoxia was substantially attenuated in the presence of phentolamine. This suggests that it is at least in part mediated by α-adrenoceptors at this stage of fetal development.

At 0.8 fetal incubation time, the concentration-response curve for NE shifted to the right in the presence of phentolamine. This indicates a reduction in NE sensitivity, which is typical of a competitive antagonist such as phentolamine. Based on these results it cannot be ruled out that the 7% constriction that remained during acute hypoxia in the presence of phentolamine is related to incomplete α-adrenergic blockade at 0.8 fetal incubation time.

Central hemodynamic measurements showed that during acute hypoxia, both heart rate and blood pressure decreased considerably at 0.6 as well as at 0.8 fetal incubation time. Assuming a reduction of perfusion pressure at the level of the mesenteric arteries, luminal diameter may passively decrease, due to elastic recoil of the arteries subjected to a lower perfusion pressure. This may alternatively explain the observed small diameter reduction in the presence of phentolamine.

Finally, it may be argued that the difference in the vasoconstrictor response during acute hypoxia between 0.6 and 0.8 fetal incubation time is due to a difference in the level of hypoxia. Indeed, arterial Po_2 during acute hypoxia is lower at 0.8 compared with 0.6 fetal incubation time. However, arterial Po_2 under normoxic conditions is also proportionally lower at 0.8 compared with 0.6 fetal incubation time, thus the relative decrease in arterial Po_2 is comparable. The mechanisms underlying the developmental change in arterial Po_2 under normoxic conditions are beyond the

scope of this article but are discussed in detail by Tazawa et al. (13). Briefly, it has been demonstrated that during the third trimester of chick fetal incubation under normoxic conditions, there is a gradual decrease in arterial P_{O_2} and an increase in P_{CO_2} that is, in part, metabolically compensated. According to these authors, the changes in blood gas levels during late fetal development are related to a rise in metabolic rate of the fetus during this period of incubation. Oxygen concentration of the arterial blood, however, is maintained by concomitant increases in hematocrit and oxygen affinity of hemoglobin. Because it has been postulated that oxygen-sensitive cells sense the oxygen concentration of the blood (14), it seems unlikely that the difference in the vasoconstrictor response during acute hypoxia between 0.6 and 0.8 fetal incubation time is due to the difference in arterial P_{O_2}.

The current study shows that fetal mesenteric arteries constrict in response to acute hypoxia and suggests that the increase in magnitude of this vasoconstrictor response during 0.6–0.8 of fetal development results from an increase in adrenergic constrictor capacity.

Interestingly, during the reoxygenation period, the mesenteric arterial diameters increased above baseline levels, suggesting a hyperemic response at both 0.6 and 0.8 fetal incubation time. The maximal arterial diameters obtained during the reoxygenation period were comparable to the levels of vasodilation obtained with topically applied 10^{-2} M ACh, a concentration sufficient to obtain maximal vasodilation. The vasodilator response actually started during the hypoxic phase and progressed during the reoxygenation phase. Maximal vasoconstriction during the 5-min period of hypoxia was observed at ~100 s from the start of hypoxia and lasted for about 30 s. By the end of the 5-min hypoxic period, arterial diameters returned almost to baseline level. In contrast, the vasoconstriction observed after topical application of NE under normoxic conditions lasted as long as 30 min (data not shown). Because acute hypoxia in the chick fetus is associated with a rise in plasma levels of NE (15), it is interesting to observe a vasodilator response already during the hypoxic phase. This suggests that secondary to the initial constriction in response to hypoxia a potent vasodilator substance is released locally.

In conclusion, the chick fetal mesenteric vasculature is a readily accessible vascular bed for studying the development of vasomotor control in small-resistance-type arteries. In the current study we showed that α-adrenergic pharmacomechanical coupling is already present in chick fetal mesenteric arteries as early as 0.6 fetal incubation time. The ability of mesenteric arteries to constrict in response to both physiological and pharmacological stimuli increases during the period 0.6–0.8 fetal incubation time. In contrast, vasodilator responses of the mesenteric arteries remained constant during this period of fetal development. This may suggest that maturation of vasodilator mechanisms precedes that of vasoconstrictor mechanisms during fetal development.

REFERENCES

1. **Crissinger KD.** Regulation of hemodynamics and oxygenation in developing intestine: insight into the pathogenesis of necrotizing enterocolitis. *Acta Paediatr Suppl* 396: 8–10, 1994.
2. **Hamburger V and Hamilton HL.** A series of normal stages in the development of the chick embryo. *J Morphol* 88: 49–98, 1951.
3. **Höchel J, Akiyama R, Masuko T, Pearson JT, Nichelmann M, and Tazawa H.** Development of heart rate irregularities in chick embryos. *Am J Physiol Heart Circ Physiol* 275: H527–H533, 1998.
4. **Iwamoto HS, Kaufman T, Keil LC, and Rudolph AM.** Responses to acute hypoxemia in fetal sheep at 0.6–0.7 gestation. *Am J Physiol Heart Circ Physiol* 256: H613–H620, 1989.
5. **Jensen A and Berger R.** Fetal circulatory responses to oxygen lack. *J Dev Physiol* 16: 181–207, 1991.
6. **Jensen A and Lang U.** Foetal circulatory responses to arrest of uterine blood flow in sheep: effects of chemical sympathectomy. *J Dev Physiol* 17: 75–86, 1992.
7. **Le Noble JL, Struyker-Boudier HA, and Smits JF.** Differential effects of general anesthetics on regional vasoconstrictor responses in the rat. *Arch Int Pharmacodyn Ther* 289: 82–92, 1987.
8. **Le Noble JL, Tangelder GJ, Slaaf DW, Van Essen H, Reneman RS, and Struyker-Boudier HA.** A functional morphometric study of the cremaster muscle microcirculation in young spontaneously hypertensive rats. *J Hypertens* 8: 741–748, 1990.
9. **Mulder ALM, Van Golde JC, Prinzen FW, and Blanco CE.** Cardiac output distribution in response to hypoxia in the chick embryo in the second half of the incubation time. *J Physiol (Lond)* 508: 281–287, 1998.
10. **Nowicki PT and Nankervis CA.** The role of the circulation in the pathogenesis of necrotizing enterocolitis. *Clin Perinatol* 21: 219–233, 1994.
11. **Reuss ML, Parer JT, Harris JL, and Krueger TR.** Hemodynamic effects of alpha-adrenergic blockade during hypoxia in fetal sheep. *Am J Obstet Gynecol* 142: 410–415, 1982.
12. **Slaaf DW, Tangelder GJ, Reneman RS, Jager K, and Bollinger A.** A versatile incident illuminator for intravital microscopy. *Int J Microcirc Clin Exp* 6: 391–397, 1987.
13. **Tazawa H, Visschedijk AHJ, Wittmann J, and Piiper J.** Gas exchange, blood gases and acid-base status in the chick before, during and after hatching. *Respir Physiol* 53: 173–185, 1983.
14. **Semenza GL.** Perspectives on oxygen sensing. *Cell* 98: 281–284, 1999.
15. **Wittmann J and Prechtl J.** Respiratory function of catecholamines during the late period of avian development. *Respir Physiol* 83: 375–386, 1991.