Direct effects of acute hypoxia on the reactivity of peripheral arteries of the chicken embryo


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Ruijtenbeek, K., C. G. A. Kessels, E. Villamor, C. E. Blanco, and J. G. R. De Mey. Direct effects of acute hypoxia on the reactivity of peripheral arteries of the chicken embryo. Am J Physiol Regulatory Integrative Comp Physiol 283: R331–R338, 2002. First published April 4, 2002; 10.1152/ajpregu.00675.2001.—In the chicken embryo, acute hypoxemia results in cardiovascular responses, including an increased peripheral resistance. We investigated whether local direct effects of decreased oxygen tension might participate in the arterial response to hypoxemia in the chicken embryo. Femoral arteries of chicken embryos were isolated at 0.9 of incubation time, and the effects of acute hypoxia on contraction and relaxation were determined in vitro. While hypoxia reduced contraction induced by high K⁺ to a small extent (−21.8 ± 5.7%), contractile responses to exogenous norepinephrine (NE) were markedly reduced (−51.1 ± 3.2%) in 80% of the arterial segments. This effect of hypoxia was not altered by removal of the endothelium, inhibition of NO synthase or cyclooxygenase, or by depolarization plus Ca²⁺ channel blockade. When arteries were simultaneously exposed to NE and ACh, hypoxia resulted in contraction (+49.8 ± 9.3%). Also, relaxing responses to ACh were abolished during acute hypoxia, while the vessels became more sensitive to the relaxing effect of the NO donor sodium nitroprusside (pD₂: 5.81 ± 0.21 vs. 5.31 ± 0.27). Thus, in chicken embryo femoral arteries, acute hypoxia blunts agonist-induced contraction of the smooth muscle and inhibits stimulated endothelium-derived relaxation factor release. The consequences of this for in vivo fetal hemodynamics during acute hypoxemia depend on the balance between vasomotor influences of circulating catecholamines and those of the endothelium.

catecholamine; endothelium-derived relaxation factor

IN THE FETUS, AN ACUTE DECREASE in arterial oxygen tension leads to cardiovascular responses, involving an elevation in blood pressure and redistribution of the cardiac output in favor of vital organs. In fetal lambs (10), fetal llamas (9), and chicken embryos (24, 25), increased levels of circulating catecholamines take part in this response. Early in gestation, the chromaffin cells in the primitive adrenal medulla are directly sensitive to low oxygen tension. Later in gestation, activation of efferent sympathetic nerves also contributes to the response (38). Antagonists of α-adrenergic receptors blunt the hypoxia-induced increase in fetal total peripheral resistance (9, 10, 25).

Neurohumoral mechanisms have been proposed to be important regulators of blood flow in the hypoxic fetus, but it remains to be established whether local and direct effects of decreased oxygen tension participate in the fetal cardiovascular response to hypoxemia. In previous studies, we have shown that chronic exposure to hypoxia affects both sympathetic innervation (34) and endothelium-dependent relaxation (33) of femoral arteries of the chicken embryo, but acute effects of hypoxia in isolated systemic arteries were not studied. Moreover, acute effects of low oxygen tension have been studied in fetal pulmonary (35, 42) and cerebral and carotid arteries (3, 8, 43), but few studies have addressed this in isolated systemic peripheral arteries of fetuses.

In adult animals, a broad variety of local responses to acute hypoxia has been observed in systemic arteries. Contraction, relaxation, and even biphasic responses have been described (47). Reduction of relaxation and augmentation of contractile responses during hypoxia/anoxia have been mainly attributed to an inhibition of endothelium-derived relaxation factor (EDRF)/nitric oxide (NO) release (26, 44). Vasorelaxation in response to a decrease in oxygen tension has been shown to involve NO (12, 17), prostaglandins (12, 22), K⁺ channels (19, 41), Ca²⁺ channels (15), and/or adenosine (41). The role of the endothelium in relaxing responses to hypoxia is a subject of discussion. The different types of responses seem to depend not only on the vascular bed and the species studied, but also on the degree of hypoxia (15) and on the developmental stage of the animal studied (27).

In the present study, we investigated the acute effect of low oxygen tension on isolated femoral arteries of the chicken embryo near the end of incubation. In previous studies, we showed that at this time point in...
the chicken embryo neurohumoral mechanisms are important in vivo response to acute hypoxia (23) and that vasoconstrictor and vasodilator responses are detectable using the wire myograph technique (18). Therefore, we studied the effect of acute hypoxia in the presence of mediators that play a role in the sympathetic nervous system and in the regulation of vascular tone during fetal hemodynamic responses to hypoxemia.

**METHODS**

Fertilized eggs of White Leghorn chickens (‘t Anker, Ochten, The Netherlands) were incubated at 37°C and 21% O₂ with a relative humidity of 60% and were rotated hourly. After 19 days of the 21-day incubation time, the eggs were opened. The embryos were taken out and immediately killed by decapititation. Ring segments of the femoral artery (2 mm long) were isolated and mounted in a myograph organ bath (model 610M, J. P. Trading, Aarhus, Denmark) for recording of isometric force development. The organ bath was filled with Krebs-Ringer bicarbonate solution (KRB), which was maintained at 37°C and aerated with 95% O₂-5% CO₂. The experiments complied with the Dutch law for animal experimentation.

**Study Protocol**

After an equilibration period of 30 min, the vessel segments were stretched to their optimal diameter, i.e., the diameter at which the largest contraction in response to a high-K⁺ solution (63 mmol/l K⁺) was observed (494 ± 4 μm). Then, either no stimulus was given or contraction was induced by a single concentration of norepinephrine (NE) or K⁺. An acute decrease in oxygen tension was induced after 10 min (when contraction was stable) by switching the gas mixture (aerating the organ bath) from 95% O₂-5% CO₂ to 95% N₂-5% CO₂ as has been described by others studying fetal preparations (3, 8, 43). The PO₂ in the organ chambers was measured with an ISO2 dissolved oxygen meter and oxygen electrode (World Precision Instruments, Berlin, Germany) and reduced rapidly (PO₂ became <25 mmHg after 4 min and was 16.7 ± 2.7 mmHg after 8 min).

Different concentrations of NE (1 and 5 μmol/l) were used to induce receptor-mediated contraction. Depolarization-induced contraction was obtained by raising the K⁺ concentration of the KRB (63, 94, and 125 mmol/l) in exchange for Na⁺. Arterial responses to a decrease in oxygen tension were also studied during electrical field stimulation, which was previously shown to activate periarterial sympathetic nerves of the chicken embryo femoral artery (18). Because neurogenic contractile responses to nerve stimulation were not stable, the above-mentioned protocol had to be adjusted. Transient responses to 4 Hz (2 ms, 85 mA) field stimulation were studied during hypoxia (after 10 min) and under control conditions.

To evaluate the role of changes in membrane potential and of L-type Ca²⁺ channels, the following protocol was used. Vessels were exposed to 75 mmol/l K⁺ in the presence of nifedipine (1 μmol/l), a blocker of voltage-operated Ca²⁺ channels, and were then stimulated with 1 μmol/l NE. Hypoxia was induced and maintained for 10 min. In addition, experiments were performed with Bay-K8644, which stimulates L-type Ca²⁺ channels. As 300 nmol/l Bay-K8644 did not change basal tone, it was added during contraction with 25 mM K⁺ or 1 μmol/l NE. When contraction was stable, a 10-min period of hypoxia was induced. The response to low oxygen tension was also studied in vessel segments with and without endothelium. The endothelium was removed by rubbing the inside of the mounted vessel with a human hair or by perfusing the vessel segment for 90 s with 0.1% Triton X-100 (perfusion pressure = 40 mmHg) before mounting.

The response to ACh (1 μmol/l) during contraction with high K⁺ was used to check whether the vessel was successfully denuded. For a number of vessels this was also checked histologically by means of scanning electron microscopy. The effect of hypoxia in denuded and intact vessels was studied in unstimulated vessel segments and during NE-induced contraction.

**Drugs and Solutions**

KRB contained (in mmol/l) 118.5 NaCl, 1.2 MgSO₄·7H₂O, 1.2 KH₂PO₄, 25.0 NaHCO₃, 2.5 CaCl₂, and 5.5 glucose. Solutions containing different concentrations of K⁺ were prepared by replacing part of the NaCl by an equimolar amount of KCl. Arterenol bitartrate (NE), indomethacin, and L-NAME were obtained from Sigma Chemical (St. Louis, MO), nifedipine from Bayer (Leverkusen, Germany), ACh chloride from Janssen Chimica (Beersen, Belgium), and SNP from Acros (Geel, Belgium). Bay-K8644 was kindly supplied by Dr. S. Kazda (Bayer). Indomethacin and nifedipine were dissolved in 100% ethanol, Bay-K8644 in DMSO, and all other agents in distilled water.

**Data Analysis**

Active wall tension (AWT) was calculated by dividing force by two times the length of the vessel segment (N/m). Responses to acute hypoxia were expressed as percent change of AWT. Whenever possible, two vessel segments were taken from one artery of the same chicken embryo (femoral artery segment of 4 mm cut in two) to study the effect of acute hypoxia under the different circumstances. In this case, paired t-test or the nonparametric variant (Wilcoxon signed rank test) was used for statistical analysis. Otherwise, data were analyzed with t-test for two groups or the nonparametric variant (Mann-Whitney U-test), when normality test failed (Sigmastat 2.0, Jandel Scientific). Data are presented as means ± SE of n embryos, and P < 0.05 was considered statistically significant.

**RESULTS**

**Hypoxia-Induced Increase in Arterial Tone**

In unstimulated vessels, acute hypoxia induced a small transient increase in tone (3.8 ± 0.9% (n = 18) of
the contraction induced by 63 mmol/l K\(^+\)]. Endothelial removal seemed to increase this contraction (+8.9 ± 2.5 vs. 2.1 ± 1.0% of K\(^+\)-induced contraction, \(n = 6\), Wilcoxon signed rank test, \(P = 0.03\), but blockade of NO synthase did not induce changes (+6.3 ± 2.9% vs. +4.2 ± 2.0% of K\(^+\)-induced contraction, Wilcoxon signed rank test, \(n = 6\), \(P = 0.44\)). When an acute decrease in oxygen tension was induced during contraction stimulated by 1 μmol/l NE, no effect was observed in 12 of 59 vessel segments. In 28 artery segments, hypoxia induced a transient increase in tension (+12.7 ± 2.4% increase of NE-induced contraction, Fig. 2), which was not modified by the presence of L-NAME (+7.3 ± 1.8% vs. +8.9 ± 2.6%, \(n = 7\), paired t-test, \(P = 0.52\)) or by denudation (+17.6 ± 4.5% vs. +8.6 ± 1.8%, \(n = 7\), paired t-test, \(P = 0.06\)).

**Hypoxia-Induced Decrease of Contraction in Stimulated Arteries**

**NE-induced contraction.** In 80% of the studied vessel segments, hypoxia ultimately induced a decrease in the contraction induced by 1 μmol/l NE (−51.1 ± 3.2%, \(n = 47\)) (Fig. 1). Using 5% O\(_2\) instead of 95% O\(_2\) to aerate the organ bath did not modify NE-induced (1 μmol/l) contraction (1.92 ± 0.15 vs. 2.16 ± 0.24 N/m, \(n = 5\), Wilcoxon signed rank test, \(P = 0.25\)). Hypoxia (0% O\(_2\)) had similar effects on NE-induced contraction in artery segments that were equilibrated in 95% O\(_2\) and in 5% O\(_2\) (−42.0 ± 7.3% vs. −51.0 ± 11.2%, \(n = 5\), Wilcoxon signed rank test, \(P = 0.63\)).

The decrease in contraction during biphasic responses to hypoxia was not different from hypoxic relaxation that was not preceded by hypoxic contraction (−47.6 ± 3.0% vs. −56.2 ± 6.5%, \(n = 19–28\), Mann-Whitney U-test, \(P = 0.14\)). Initial NE-induced contraction did not differ either (1.77 ± 0.12 vs. 1.98 ± 0.09 N/m, \(n = 19–28\), t-test, \(P = 0.16\)).

Under control conditions, contraction induced by 5 μmol/l NE (2.08 ± 0.18 N/m) was comparable to that induced by 1 μmol/l NE (2.06 ± 0.19 N/m, t-test, \(n = 5–7\)). The hypoxia-induced decreases in contraction did not differ between vessel segments stimulated by 5 μmol/l NE (−44.3 ± 3.9%) or 1 μmol/l NE (\(n = 5–8\), t-test, \(P = 0.43\)).

Neurogenic sympathetic contraction induced by 4-Hz electrical field stimulation was reduced by half during hypoxia (0.69 ± 0.10 N/m vs. 1.45 ± 0.24 N/m during normoxia, \(n = 6\), Wilcoxon signed rank test, \(P = 0.03\)).

**Contraction induced by depolarization.** Under control conditions, contractile responses to high K\(^+\) (63 mmol/l: 1.57 ± 0.22 N/m; 94 mmol/l: 1.54 ± 0.23 N/m; and 125 mmol/l: 1.79 ± 0.16 N/m) did not significantly differ from those to 1 μmol/l NE (Wilcoxon signed rank test and paired t-test, \(n = 6\), \(P = 0.44\), \(P = 0.48\), and \(P = 0.95\), respectively). The decrease of contraction in response to hypoxia (−21.8 ± 5.7%, −19.0 ± 4.5%, and −11.7 ± 13.2%, respectively, Fig. 2) was significantly smaller during contraction stimulated by depolarization than during α-adrenergic contraction (Mann-Whitney U-test, \(n = 5–8\), \(P < 0.01\) for all concentrations of K\(^+\)).

Blockade of voltage-operated Ca\(^{2+}\) channels (Ca\(_\text{V}^\text{b}\)) by nifedipine markedly reduced contractile responses to 75 mmol/l K\(^+\) (remaining contraction: 20 ± 3.1% of initial contraction). Contraction to NE was decreased by 40% in the continuous presence of 75 mmol/l K\(^+\) and 1 μmol/l nifedipine (0.98 ± 0.23 vs. 1.71 ± 0.32 mN/mm, paired t-test, \(n = 5\), \(P = 0.02\)). Figure 3 illustrates that the amplitude of the hypoxic relaxation was not reduced under these circumstances (−81.0 ± 11.9% vs. −51.5 ± 6.6%, paired t-test, \(n = 5\), \(P = 0.28\)).

Bay-K8644 (300 nmol/l), which activates L-type Ca\(^{2+}\) channels, did not change AWT in unstimulated artery segments. However, contractile responses to 25

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**Fig. 1.** A: typical tracing of isometric force as a function of time illustrating the effect of hypoxia (N\(_2\)) on the contraction induced by 1 μmol/l norepinephrine (NE). B: bar graph shows mean ± SE of 47 artery segments.
mmol/l K⁺ were increased by 50% (1.61 ± 0.16 vs. 1.07 ± 0.10 N/m, paired t-test, n = 7, P < 0.01). Responses to 1 μmol/l NE were slightly altered in the presence of Bay-K8644 (2.05 ± 0.14 vs. 1.85 ± 0.14 N/m, paired t-test, n = 8, P < 0.01). Induction of hypoxia reduced contraction in response to 25 mmol/l K⁺ and 300 mmol/l Bay-K8644 by 46.1 ± 4.4% and contraction stimulated with 1 μmol/l NE and 300 mmol/l Bay-K8644 by 61.7 ± 9.3%.

Effect of endothelium removal. Endothelium removal abolished relaxing responses to ACh (1 μmol/l: −4.5 ± 1.3% vs. −74.2 ± 2.1%, n = 25). Mechanical and chemical removal of endothelium resulted in significant and comparable decreases of contractile responses to NE (1 μmol/l: 1.06 ± 0.08 vs. 1.91 ± 0.13 N/m, n = 20, paired t-test, P < 0.001) and K⁺ (125 mmol/l: 0.87 ± 0.07 vs. 1.71 ± 0.09 N/m, n = 25, paired t-test, P < 0.001). In denuded arteries contracted with 1 μmol/l NE, the hypoxic change in contraction tended to be less pronounced (−16.5 ± 10.1 vs. −43.3 ± 3.8%, paired t-test, n = 8, P = 0.08). However, in denuded vessels contracted with higher concentrations of NE (3 and 10 μmol/l, contraction: 1.55 ± 0.06 N/m), the hypoxic response was not significantly different compared with intact vessels stimulated with 1 μmol/l NE (hypoxic relaxation: −56.4 ± 5.0% vs. −39.7 ± 6.4%, n = 7, paired t-test, P = 0.07).

Effect of NO synthase and cyclooxygenase inhibition. At 95% O₂, contraction induced by 1 μmol/l NE was not modified by 100 mmol/l L-NAME (2.0 ± 0.1 vs. 2.0 ± 0.1, Wilcoxon signed rank test, n = 11, P = 0.88). NO synthase inhibition during NE-induced contraction did not affect the hypoxia-induced decrease in AWT (−47.0 ± 7.0% vs. −54.1 ± 7.2%, n = 11, paired t-test, P = 0.49). During incubation with the cyclooxygenase inhibitor indomethacin (3 μmol/l), hypoxia reduced contraction induced by 1 μmol/l NE by −43.5 ± 7.6% (n = 4).

Effect of Hypoxia on ACh-Induced Relaxation

Under control conditions, ACh induced dose-dependent relaxation (n = 6, pD₂ = 6.82 ± 0.09, Eₘₐₓ = −85.3 ± 2.8%, Fig. 4). This relaxation was completely abolished by hypoxia (Fig. 4). However, low oxygen tension did not reduce relaxation induced by the NO donor SNP; sensitivity was even increased during hypoxia (pD₂: 5.81 ± 0.21 vs. 5.31 ± 0.27, paired t-test, n = 6, P = 0.04, Eₘₐₓ: −95.1 ± 2.4% vs. −91.7 ± 3.8%, paired t-test, n = 6, P = 0.47, Fig. 4).

Hypoxia reversed relaxing responses to 300 nmol/l M ACh during NE (1 μmol/l)-induced contraction (Fig. 5). The resulting contraction (49.80 ± 9.27% of NE contraction) was comparable to the remaining contraction observed in vessel segments that were exposed to hypoxia during NE-induced contraction without ACh (paired t-test, n = 7, P = 0.77).

DISCUSSION

Our findings indicate that hypoxia reduces agonist-induced contraction and at the same time inhibits the release of EDRFs in isolated femoral arteries of chicken embryos at 0.9 of the incubation time. The net effect, namely vasocontraction, may contribute to the total arterial contractile response in hypoxemic chicken embryos.

We have previously shown that the chicken embryo is a useful model to study the development of cardiovascular control (11, 23). It has advantages over mammalian models, including the possibility to evaluate...
Effects of isolated environmental factors, such as hypoxia and malnutrition. We described effects of hypoxia on cardiac output distribution, circulating catecholamines (acute), and on the development of cardiovascular sympathetic nerves in the chicken embryo (chronic) (24, 25, 34). The present study was undertaken to investigate whether low oxygen tension directly influences peripheral arterial reactivity.

Effects of Acute Hypoxia on Contraction

In 80% of all vessels studied, NE-induced contraction was ultimately reduced during 10-min exposure to hypoxia. In 50% of these vessels, the hypoxic relaxation was preceded by a small significant increase in tone. In some artery segments, however, reduction of oxygen tension did not have any effect. Variability in the response to hypoxia may be due to subtle developmental differences between embryos, contractile effects of intermediate levels of oxygen tension, or multiple effects of hypoxia on the arterial wall. A limitation of the present study may be introduced by the PO2 levels that we used under control conditions. Aerating the organ bath with 95% O2-5% CO2 is standard procedure in studies using the wire myograph technique to examine adult vessels and in the limited number of studies investigating the effects of acute hypoxia on fetal systemic arteries (3, 8, 43). However, oxygen levels in these conditions largely exceed in vivo fetal PO2 (20–30 mmHg) (4, 16). We therefore performed additional experiments with 5% O2 to check whether the effect of acute hypoxia was dependent on the starting PO2 levels. However, contraction stimulated with NE and subsequent responses to acute hypoxia were comparable using 95% O2 or 5% O2 as control conditions.

In the majority of arteries stimulated with NE, induction of acute hypoxia ultimately resulted in a partial decrease in tone. A reduction in tone in response to hypoxia may be the result of energy limitation, hypoxia-induced release of vasodilators, or interruption of pharmacomechanical coupling.

Hypoxia did not attenuate contractile responses to high K+ to a large extent. Thus femoral arteries of chicken embryos, like adult arteries of mammalian species (7), can derive energy from anaerobic glycolysis in hypoxic/anoxic conditions. Energy limitations may therefore not be responsible for the reduction of the contractile response to NE by low oxygen tension.

Many studies in adult species show that hypoxia stimulates the release of EDRFs, such as NO (12, 17),
prostaglandins (12, 22), and endothelium-derived hyperpolarizing factor (EDHF) (19). Removal of the endothelium did not abolish the hypoxic response in the femoral artery of the chicken embryo. It should be mentioned that endothelium removal decreased contractions to NE and K⁺ up to 50%. This could indicate that the denudation procedure damaged the vascular smooth muscle cells. However, inhibition of NO synthase and cyclooxygenase did not blunt the effects of hypoxia. Combined depolarization and Ca²⁺ channel blockade also failed to inhibit the hypoxia-induced decrease in contraction in artery segments contracted with NE. Thus the reduction of contractile force during acute hypoxia does not seem to be caused by the release of EDRFs like NO, prostaglandins, and hyperpolarizing factors. This is in agreement with studies that demonstrate that the role of the endothelium in hypoxic relaxation only becomes evident in mature carotid and cerebral ovine arteries (48). Persistence of hypoxia-induced decrease of contraction in the presence of Bay-K8644 demonstrates that inactivation of voltage-operated Ca²⁺ channels is not involved in the response either.

In previous experiments, we have shown that NE-induced contraction can be blocked by the α₁-adrenergic antagonist prazosin and that agonists of α₂- and β-adrenergic receptors have no significant effects in chicken embryo femoral arteries (18, 34). In the present study we show that, although nifedipine severely reduced contraction induced by high K⁺, almost 60% of NE-induced contraction persisted during depolarization and Caᵥ channel blockade. This indicates that in the chicken embryo, α₁-adrenergic receptors stimulate contraction at least partly by pharmacomechanical coupling as has been documented for mammalian arteries (39). This coupling seems to be more sensitive to low oxygen tension than electromechanical coupling induced by high K⁺. In adult mammalian arteries, α₁-adrenergic contraction involves phospholipase C, protein kinase C, Ca²⁺ release from intracellular stores, and an increase in Ca²⁺ sensitivity of the contractile apparatus (for review, see Ref. 32). The relative importance of these processes in arteries of chicken embryos is currently unknown but may be of interest to study because acute hypoxia has been shown to modulate the intracellular Ca²⁺ concentration ([Ca²⁺ᵢ])³⁻¹-force relationship (5, 37, 40) in adult mammalian arteries and may interfere with the ability of d-myoinositol 1,4,5-trisphosphate to induce contraction in fetal arteries (2) and possibly with Ca²⁺ handling in arteries of neonates (48). Studies in sheep suggest that fetal cerebral arteries display increased Ca²⁺ sensitivity compared with those of the adult (20). The role of Ca²⁺ sensitization in contraction induced by depolarization with high K⁺ is proposed to be smaller than in agonist-induced contraction (29, 39, 45). This could explain why hypoxia in arteries of the chicken embryo appears to interfere with pharmacomechanical coupling in response to NE rather than electromechanical coupling stimulated by high K⁺. The effect of acute hypoxia on Ca²⁺ sensitiveness in these arteries would therefore be an interesting topic for future research.

Effects of Acute Hypoxia on Relaxation

While α-adrenergic contraction was partially reduced, ACh-induced relaxation of chicken embryo femoral arteries was completely abolished by acute hypoxia. We and others have previously shown that responses to ACh in chicken arteries are endothelium dependent (14, 46) and, in the femoral artery, are mediated by the release of endothelium-derived NO, EDHF, and a factor the nature of which remains to be established (18). Hypoxia abolished ACh-induced relaxation in arteries contracted with K⁺. Blockade of ACh-induced relaxation during hypoxia was not due to reduced responsiveness of the vascular smooth muscle cells to NO, as maximal relaxation to the exogenous NO donor SNP was unchanged and sensitivity was even increased. Reduced ACh-induced relaxation during exposure to low oxygen tension has been described in arteries of adult animals (6, 44), and it has been proposed that oxygen is rate limiting in the regulation of endothelial NO synthase. Similar mechanisms seem to play a role in fetal pulmonary (36) and carotid (43) arteries. As ACh-stimulated cGMP levels in fetal pulmonary arteries decrease when PO₂ in the tissue bath is lowered (36) and fetal arteries may be more sensitive to cGMP than adult arteries (28), it is interesting to note that ODQ, a blocker of guanylate cyclase, completely blocks relaxation in response to ACh in the femoral artery of the chicken embryo (data not shown). However, in view of the complexity of endothelium-dependent relaxations in the chicken embryo femoral artery, the exact nature of the EDRFs and/or signaling pathways influenced by hypoxia remains unclear for now.

Relevance for In Vivo Hemodynamics During Acute Hypoxemia

We show that in isolated femoral arteries contracted with 1 µM NE and those in which periarterial sympathetic nerve endings were stimulated to release NE, contraction is partly counteracted by acute hypoxia. This may seem at variance with in vivo findings. However, Fig. 5 illustrates that our in vitro findings might be relevant for the hemodynamics in prenatal life in vivo. In the mammalian fetus, an acute decrease in oxygen results in cardiovascular responses involving an elevation in blood pressure and redistribution of the cardiac output mediated by increased levels of circulating catecholamines (9, 10), which are released from the adrenals and later in gestation from the sympathetic nerves (38). During acute hypoxia, NE levels rise from 0.1 µM up to 0.8 µM in the chicken embryo (24). α₁-Adrenergic stimulation in response to acute hypoxia resulting in peripheral vasoconstriction has directly and indirectly been shown in the intact chicken embryo (25, 31). In the intact embryo, the arterial system may not only be exposed to vasoconstrictors such as NE, but also to a tonic endothelial dilator influence. Vasodilator
substances including endothelium-derived NO can be released under the influence of shear stress offered by flow in adult systems (1, 21), and it has also been shown to play a role in the fetal circulation (13, 30). In the experiment shown in Fig. 5, we tried to mimic conditions that play a role during in vivo hypoxemia, namely α-adrenergic stimulation and NO release. During simultaneous agonist-induced stimulation of the smooth muscle with NE and of the endothelium with ACh, we found that hypoxia resulted in contraction. Provided that the effects of low oxygen tension on agonist-induced, endothelium-dependent relaxation and those mediated by shear stress involve the same mechanism, this local contraction might contribute to the hemodynamic response to hypoxemia.

It is interesting to note that the effects of acute hypoxia on isolated arteries of the chicken embryo do not seem to be very different from those observed in arteries of adult animals. However, because in vivo fetal PO2 is low, a small reduction in oxygen levels may directly influence vascular tone. Furthermore, as mentioned, certain signal transduction pathways that are potential targets during acute hypoxia may be relatively more important in fetal arteries than in adult arteries (2, 20, 28).

In conclusion, hypoxia was found to partially counteract α1-adrenergic contraction and to inhibit endothelium-dependent relaxation in femoral arteries of the chicken embryo. The net result of these local direct effects may contribute to the peripheral arterial response to acute hypoxemia in the chicken embryo.

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