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Effect of chronic hypoxia on adrenoceptor responses of ovine foetal umbilical vessels

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1 The effects of chronic hypoxia on α_1-adrenoceptor-mediated contractions were investigated in foetal umbilical vessels obtained from near-term (~140 day gestation) pregnant sheep maintained near sea level (~300 m) and at high altitude (3820 m) from 30 day gestation.

2 Chronic hypoxia significantly decreased contractile sensitivity of the umbilical vein to noradrenaline (pD_2: 6.22 ± 0.19 vs 5.67 ± 0.09) and reduced the maximum response by 43%. Noradrenaline-induced contraction of the umbilical artery was abolished. In contrast, contractions to KCl were not affected by chronic hypoxia.

3 In umbilical vein, the apparent dissociation constant (K_A) of noradrenaline to α_1-adrenoceptors was increased from 0.54 ± 0.06 μM in control animals to 1.35 ± 0.14 μM in chronically hypoxic animals. In accordance, radioligand binding of agonist showed high and low affinity binding sites for noradrenaline in both normoxic and chronically hypoxic tissues. Addition of GTPγS (100 μM) abolished apparent high affinity binding sites. Whereas proportional binding sites were not changed by chronic hypoxia, the apparent high affinity of noradrenaline was significantly decreased (pK_i: 7.80 ± 0.17 vs 7.20 ± 0.16).

4 Chronic hypoxia significantly decreased α_1-adrenoceptor density (fmol mg protein^{-1}) in umbilical vein (24.6 ± 3.2 vs 12.3 ± 3.1) and the artery (7.1 ± 0.4 vs 3.1 ± 0.9) with no change in [^{3}H]-prazosin binding affinity. There was a linear correlation of the maximum contractions to noradrenaline and α_1-adrenoceptor density.

5 We conclude that chronically hypoxic-induced depression in contractions of ovine foetal umbilical vessels to noradrenaline is mediated predominantly by decreases in α_1-adrenoceptor density and the agonist binding affinity.

Keywords: Sheep; umbilical vessels; adrenoceptors; hypoxia

Introduction

Chronic hypoxia during the course of pregnancy is one of the most common insults to the maternal cardiovascular system and foetal development, and is thought to be associated with increased risk of preeclampsia and foetal intrauterine growth restriction (Moore *et al*., 1982a,b; Zamudio *et al*., 1995a,b). Whereas the pathogenesis of chronic hypoxia-induced complications in the mother and her developing foetus is not well understood at present, the uteroplacental circulation is a likely target for chronically hypoxic-mediated effects. Changes in either uterine or umbilical blood flow would be expected to seriously alter normal maternal-foetal exchange of nutrients and waste products.

It has been demonstrated that noradrenaline, by acting on α_1-adrenoceptors, plays an important role in regulating contractile responses and vascular tone of the uterine artery in near-term pregnant sheep and foetal ovine umbilical vessels (Zhang & Dyer, 1991; Zhang *et al*., 1995). The physiological significance of α_1-adrenoceptors in the umbilical vessels is not entirely clear at present. It is thought that they do not play an important role in the moment-to-moment regulation of the umbilical circulation (Meschia, 1983; Oakes *et al*., 1980). However, they could become important since they may respond to circulating catecholamines resulting from conditions of stress. In a recent study, we have demonstrated that chronic hypoxia decreases noradrenaline-induced contractions in the uterine artery of near-term pregnant sheep, and the suppressed adrenergic responses are mediated in part by decreases in noradrenaline affinity to α_1-adrenoceptors and receptor numbers (Hu *et al*., 1996). Similarly, chronic hypoxia inhibited serotonin affinity to $5HT_2$ receptors and suppressed serotonin-induced contraction of the uterine arteries (Hu & Zhang, 1997). However, chronic hypoxia showed no effect on $5HT_2$ receptor numbers (Hu & Zhang, 1997). On the foetal side little is known about the effect of chronic hypoxia on adrenergic responses in the umbilical vessels, despite the finding that chronic hypoxia decreased arterial Po_2 and increased arterial pressure and circulating adrenaline and noradrenaline levels in foetal lamb (Kitanaka *et al*., 1989).

The primary purpose of the present study was to test the hypothesis that chronic hypoxia attenuates noradrenaline-mediated vasoconstriction of ovine foetal umbilical vessels by inhibiting noradrenaline affinity and down-regulating α_1-adrenoceptors. To test our hypothesis, we characterized noradrenaline-induced contractions of foetal umbilical vessels obtained from near-term (~140 day gestation) pregnant sheep maintained near sea level (~300 m) and at high altitude (3820 m) from 30 day gestation. The apparent affinity of noradrenaline to α_1-adrenoceptors was determined using methods of partial irreversible blockade of agonist-mediated contractions (Furchgott, 1966) and of agonist competitive radioligand binding in membranes. The effect of chronic hypoxia on α_1-adrenoceptors in these vessels was determined by evaluating the binding characteristics of the α_1-adrenoceptor antagonist radioligand [^{3}H]-prazosin.

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Methods

Tissue preparation

Time-dated pregnant sheep were obtained from Nebeker Ranch (Lancaster, California, U.S.A., altitude: ~300 m) with maternal and foetal arterial Po_2 (Pa,o_2) being 102 ± 2, and 23 ± 1 mmHg, respectively. To induce chronic hypoxia, the animals were transported at 30 days gestation to Barcroft Laboratory, White Mountain Research Station, Bishop, CA, U.S.A. (altitude: 3820 m, maternal and foetal Pa,o_2: 60 ± 2 and 19 ± 1 mmHg, respectively, $P < 0.05$ as compared with the control values), whereas the control animals were maintained near sea level (~300 m) throughout the gestation period. Although it is widely recognized that acclimatization to high altitude is a multifaceted process of cellular responses to external signals, numerous studies have demonstrated that lowered arterial O_2 tension is the crucial factor (Kitanaka *et al*., 1989; Moore *et al*., 1982b; Hu *et al*., 1996). With its relatively low arterial O_2 tension and steep oxyhemoglobin saturation curve, the foetus is particularly vulnerable to hypoxia. The animals (~140 days gestation) were transported to our laboratory immediately before the studies. Anaesthesia of the animals was rapidly induced with intravenous injection of thiamylal (10 mg kg^{-1}). The ewes were then intubated and anaesthesia was maintained on 2.0% halothane in oxygen throughout surgery. An incision was made in the abdomen and the uterus exposed. The umbilical cord was sectioned at least 3 inches from the foetus, and placed into a modified Krebs' solution (pH 7.4) of the following composition (mM): NaCl, 115.21, KCl, 4.70, $CaCl_2$, 1.80; $MgSO_4$, 1.16; KH_2PO_4, 1.18; $NaHCO_3$, 22.14; and dextrose, 7.88. EDTA (0.03 mM) was added to suppress oxidation of amines. The Krebs' solution was oxygenated with a mixture of oxygen-carbon dioxide (95:5%). After removal of the tissues, animals were killed with T-61 (euthanasia solution, Hoechst-Roussel, Somerville, NJ, U.S.A.).

The umbilical artery and vein were carefully cleaned of surrounding connective tissue and cut into rings of ~2 mm in length. To exclude the influence of the endothelium, endothelial cells were removed by gentle rotation of the artery rings on an approximately sized, rough-surfaced blunt hypodermic needle as described previously (Zhang & Hu, 1995). Validation of endothelium removal was demonstrated by the examination of endothelial integrity using en-face silver staining. All procedures and protocols used in the present studies were approved by the Animal Research Committee of Loma Linda University and followed the guidelines put forward in the NIH Guide for the Care and Use of Laboratory Animals.

Contractile studies

Contractile responses of the rings of umbilical vessels were quantified in Krebs' solution in tissue baths at 37°C as described previously (Zhang & Hu, 1995). Isometric tensions were measured. After 60 min equilibration in the tissue bath, each ring was stretched to the optimal resting tension as determined by the tension developed in response to KCl (120 mM) added at each stretch level. Concentration-response curves were obtained by cumulative addition of the agonist in approximate one-half log increments. EC_{50} values for the agonist in each experiment were taken as the molar concentration at which the contraction-response curve intersected 50% of the maximum response, and were expressed as pD_2 values. As previously described (Zhang *et al*., 1995), in all experiments, cocaine (3 μM) was added to block amine uptake, propranolol (1 μM) to inhibit β-adrenoceptors, and iproniazid (0.36 mM) to block monoamine oxidase. Iproniazid was added for 40 min and tissues were then washed four times over 30 min with fresh Krebs' solution. Cocaine and propranolol were added 15 min before addition of the agonist.

The agonist apparent dissociation constant (K_A) of noradrenaline was determined as previously described (Zhang *et al*., 1995). Briefly, the concentration-response curves to noradrenaline were determined before and after treating the tissues with phenoxybenzamine (60 nM for 20 min) to inactivate a fraction of the receptors and reduce the maximal response to noradrenaline by ~50%. The reciprocal of the concentration of noradrenaline before phenoxybenzamine treatment (1/[A]) was then plotted against the reciprocal of the corresponding equieffective concentration after the treatment (1/[A′]). The values for K_A and for the fraction of active receptors remaining (q) were calculated as follows (Furchgott, 1966): $1/[A] = (1-q)/qK_A + 1/q[A']$, where K_A = (slope − 1)/intercept and q = 1/slope. The fractional occupancy of α_1-adrenoceptor at each concentration of noradrenaline was calculated from the equation (Furchgott & Bursztyn, 1967): $[RA]/[R_T] = [A]/([A] + K_A)$, where [RA] is the concentration of the receptor-agonist complex, $[R_T]$ is the total concentration of the receptors, and [A] is the concentration of noradrenaline.

Radioligand binding studies

Saturation binding of [^{3}H]prazosin, an α_1-adrenoceptor antagonist radioligand, was performed by a rapid filtration method as described previously (Hu *et al*., 1996). Briefly, umbilical vessels were homogenized with a Brinkman polytron PT10/35 homogenizer (speed setting 5, 5×15 s) in ice-cold 50 mM Tris HCl buffer containing 1 mM EGTA (pH 7.4). Nuclei and cell debris were removed by low speed centrifugation at $1086 \times g$ for 10 min. The supernatant was centrifuged at $50{,}000 \times g$ for 60 min. The microsomal pellet was resuspended in the same Tris buffer to yield approximate 0.2 mg ml^{-1} protein as determined by the method of Bradford (Bradford, 1976). Equilibrium binding was carried out at 30°C for 45 min in a 500 μl volume, consisting of 440 μl of membrane suspension, 50 μl of radioligand, and 10 μl of drug or diluent. The concentrations of [^{3}H]-prazosin employed were from 0.002 to 4 nM. Nonspecific binding was determined by the addition of 10 μM phentolamine. All determinations were performed in triplicate. Bound and free radioligand was separated by rapid filtration of the membrane suspension over polyethylenimine (0.5%)-pretreated filters (Whatman GF/C) with a Brandel cell harvester. Filters were rinsed with two 5-ml aliquots of the ice-cold Tris buffer, and counted for radioactivity at 45% efficiency in Packard 1900CA Tri-Carb liquid scintillation analyzer (Packard Instrument Company, Downers Grove, IL, U.S.A.).

Agonist competition binding was performed by competition of binding of [^{3}H]-prazosin in membranes prepared from umbilical veins. The concentration of [^{3}H]-prazosin used was $2 \times$ the antagonist apparent dissociation constant (K_D) as determined in the saturation binding. Increased concentrations (1 nM to 0.1 mM) of noradrenaline were employed to compete for binding of [^{3}H]-prazosin. Total binding was determined in the absence of noradrenaline, whereas nonspecific binding was determined in the presence of 10 μM phentolamine. Conditions of incubation and separation of bound from free [^{3}H]-prazosin were the same as those in the saturation binding. Two characteristics of agonist binding were determined: agonist binding affinity and relative portions of high and low affinity

binding sites. To examine the role of G proteins in noradrenaline binding, the experiments were performed in the absence and/or presence of GTPγS (100 μM), a stable analogue of GTP.

Materials

Noradrenaline, phentolamine, propranolol, phenoxybenzamine, iproniazid, and guanosine-5′-O-(3-thio)triphosphate (GTPγS) were obtained from Research Biomedicals Inc. (Natick, MA). [^{3}H]-Prazosin (78.0 Ci/mmol) was purchased from DuPont NEN (Boston, MA, U.S.A.).

Data analysis

[^{3}H]-Prazosin saturation binding data and noradrenaline concentration-response curves were analysed by computer assisted non-linear regression to fit the data and to determine dissociation constant (K_D), receptor density (B_{max}), and pD_2 (GraphPad Prism, GraphPad Software, San Diego, CA, U.S.A.). Linear regression analysis was performed using the method of symmetry (Brace, 1977). Results were expressed as means ± s.e.mean, and the differences were evaluated for statistical significance ($P<0.05$) by the Student's *t*-test.

Results

Noradrenaline produced concentration-dependent contractions in both umbilical artery and vein of foetal lamb. The tissue contractile sensitivity to noradrenaline (pD_2) was similar in both vessels (5.91 ± 0.01 *vs* 6.22 ± 0.19, $P>0.05$), but the maximal response to noradrenaline normalized relative to the potassium maximum was significantly higher in the umbilical vein (108.2 ± 1.8%) than that in the umbilical artery (22.1 ± 2.4%) ($P<0.01$, $n=4$). The potassium maximum contractile response was also significantly higher in the umbilical vein (5.96 ± 1.10 g mm$^{2-1}$) than that in the umbilical artery (2.80 ± 0.23 g mm$^{2-1}$) ($P<0.05$, $n=4$).

Chronic hypoxia significantly decreased contractile sensitivity of the umbilical vein to noradrenaline and shifted noradrenaline concentration-contraction curves to the right (pD_2: 6.22 ± 0.19 *vs* 5.67 ± 0.09, $P<0.05$, $n=6$). The maximal response was also decreased from 108.2 ± 1.8 to 58.3 ± 6.8% of the KCl maximum ($P<0.05$) (Figure 1, left panel). Noradrenaline-induced contractions of the umbilical artery were completely abolished after chronic hypoxia (Figure 1, right panel). In contrast, chronic hypoxia showed no effect on the potassium maximal response in either umbilical artery (2.80 ± 0.23 *vs* 2.63 ± 0.08 g mm$^{2-1}$) or umbilical vein (5.96 ± 1.10 *vs* 5.48 ± 0.41 g mm$^{2-1}$).

As shown in Figure 2, pre-treatment with phenoxybenzamine (60 nM for 20 min) produced an approximately 50% reduction in the noradrenaline-induced maximal response of the umbilical vein. The inset (Figure 2) illustrates double reciprocal plots of equieffective concentrations of noradrenaline before (1/[A]) and after (1/[A′]) phenoxybenzamine treatment. The dissociation constant (K_A) of noradrenaline calculated was 0.54 ± 0.06 μM. The relationship between respective α_1-adrenoceptor occupancy ([RA]/[R_T]) and noradrenaline-induced contraction was then constructed using noradrenaline dissociation constant (see Methods). The linear relationship with the slope of 1 shown in Figure 3 indicates that there is no α_1-adrenoceptor reserve in this vessel. Chronic hypoxia significantly increased the dissociation constant of noradrenaline in the umbilical vein (0.54 ± 0.06 *vs* 1.35 ± 0.14 μM, $P<0.05$, $n=5$), suggesting that noradrenaline binding affinity to α_1-adrenoceptors was significantly decreased.

The effect of chronic hypoxia on α_1-adrenoceptor density in the umbilical artery and vein was determined by evaluating the saturation binding of [^{3}H]-prazosin, a selective α_1-adrenoceptor antagonist radioligand. The binding of [^{3}H]-prazosin to α_1-adrenoceptors was specific and saturable, and was best described by an interaction of the radioligand with a single class of high affinity binding sites in both umbilical artery and vein (Figure 4). Although the dissociation constant (K_D, nM) of [^{3}H]-prazosin was not different in the umbilical artery

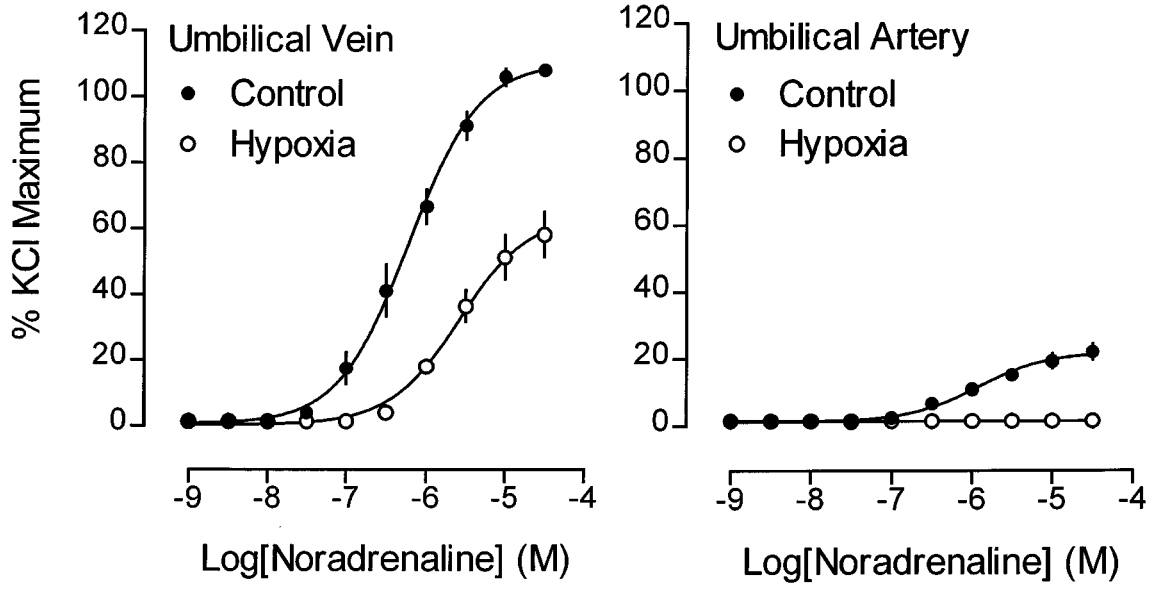

Figure 1 Effect of chronic hypoxia on noradrenaline-induced contractions of the umbilical vein and artery. Cumulative concentration-response curves to noradrenaline were obtained with the vessel rings from the control and high altitude animals. Data are means ± s.e.mean of the tissues from four to six animals. Mean values of the EC_{50}s and the maximal responses to noradrenaline normalized to the KCl maximum are presented in the text.

(0.04 ± 0.01) and vein (0.06 ± 0.01), the density (B_{max}, fmol (mg protein)$^{-1}$) of α_1-adrenoceptors was significantly higher in the umbilical vein (24.6 ± 3.2, $n=6$) than that in the umbilical artery (7.1 ± 0.4, $n=5$) ($P<0.05$). Chronic hypoxia significantly decreased α_1-adrenoceptor density (B_{max}) in both umbilical artery (3.1 ± 0.9, $n=3$, $P<0.05$,) and umbilical vein (12.3 ± 3.1, $n=6$, $P<0.05$). In contrast, the dissociation constant (K_D) of [^{3}H]-prazosin was not changed by chronic hypoxia in either umbilical artery (0.04 ± 0.01) or the vein (0.04 ± 0.01).

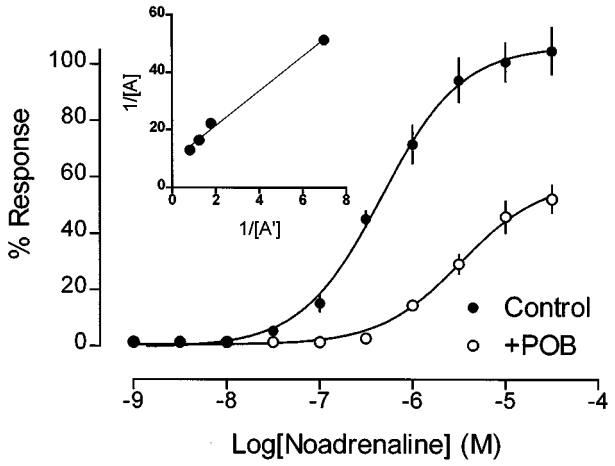

Figure 2 Effect of phenoxybenzamine on noradrenaline-induced contractions of the umbilical vein. Contractions to noradrenaline were obtained before and after exposure of the tissue to 60 nM phenoxybenzamine (POB) for 20 min. POB was washed out of the tissue before obtaining the second concentration-response curve to noradrenaline. Results are expressed as percentage of the maximal contraction produced by noradrenaline before the treatment with POB. Inset: plot of the reciprocals of equieffective concentrations of noradrenaline before (1/[A]) and after (1/[A']) POB treatment. The dissociation constant (K_A) of noradrenaline was calculated as described in Methods and presented in the text.

The ability of noradrenaline to compete for specific [^{3}H]-prazosin binding to α_1-adrenoceptors in normoxic and chronically hypoxic umbilical vein was studied in the absence and presence of the stable GTP analogue GTPγS. As shown in Table 1, in the absence of GTPγS competition curves for noradrenaline were best fit by computer as a sum of binding to two classes of sites in both normoxic and chronically hypoxic tissues. GTPγS (100 μM) abolished the high affinity binding sites for noradrenaline in both groups, suggesting a role for G proteins in coupling of α_1-adrenoceptors in umbilical veins. In the absence of GTPγS, whereas proportional binding sites for high and low affinities were not different between normoxic and chronically hypoxic umbilical veins, the apparent high affinity for noradrenaline (pK_i) was significantly decreased in chronically hypoxic tissues (7.20 ± 0.16) compared with normoxic ones (7.80 ± 0.17).

Using the number of α_1-adrenoceptors obtained by [^{3}H]-prazosin and fractional occupancy of α_1-adrenoceptors determined by its K_A, the α_1-adrenoceptor number occupied by each concentration of noradrenaline was calculated. There was a linear relationship between α_1-adrenoceptors and contractile tension in both control and chronically hypoxic umbilical veins with the slope of 0.2855 ± 0.04 and 0.3153 ± 0.01 (g tension) (fmol receptor (mg protein)$^{-1}$)$^{-1}$, respectively.

Correlation of the parameters derived from contractile and radioligand binding studies among all four groups of the vessels, i.e. control umbilical vein, artery, hypoxic umbilical vein, artery was analysed by linear regression performed using the method of symmetry (Brace, 1977). As shown in Figure 5, the correlation coefficient for relating the α_1-adrenoceptor density (B_{max}) to the maximal response (E_{max}) and potency (EC_{50}) of noradrenaline in stimulating contractions among the four groups was 0.98 ($P<0.01$) and 0.22 ($P=0.69$), respectively.

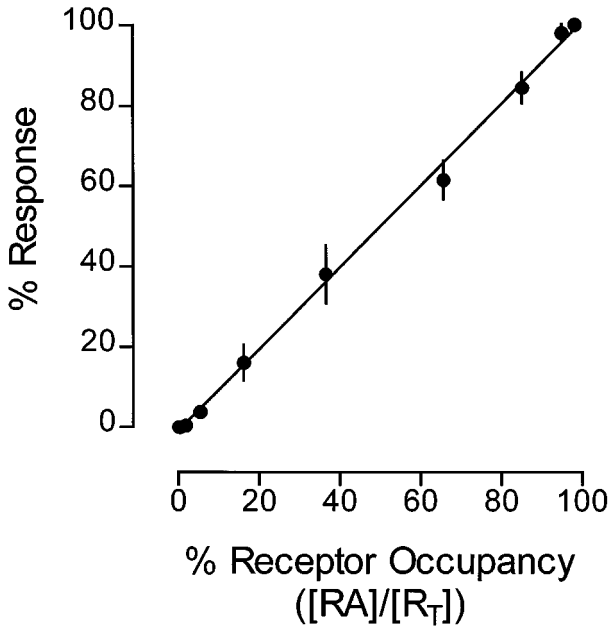

Figure 3 Relationship of noradrenaline-induced contractions of the umbilical vein and α_1-adrenoceptor occupancy ([RA]/[R_T]). The fraction of α_1-adrenoceptors occupied at each concentration of noradrenaline was calculated as described in Methods. The slope of the line is not different from unity indicating a one-to-one ratio in α_1-adrenoceptor occupancy and noradrenaline-induced contraction.

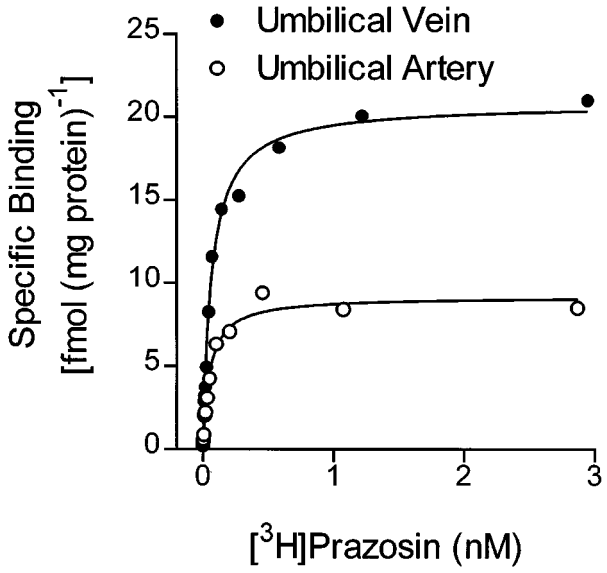

Figure 4 Saturation binding of [^{3}H]-prazosin to membranes prepared from the umbilical vein and artery. Increasing concentrations of [^{3}H]-prazosin were incubated with the membranes as described in Methods. Specific binding was defined as the arithmetic difference between total binding and nonspecific binding observed in the presence of 10 μM phentolamine. Analysis of the specific binding data by nonlinear computer-based methods (fit to a rectangular hyperbola) confirmed that [^{3}H]-prazosin bound to a single class of binding sites in both vessels studied. Mean values of dissociation constant (K_D) and receptor density (B_{max}) are presented in the text.

Table 1 Characteristics of noradrenaline binding to α_1-adrenoceptors in ovine foetal umbilical veins

	pK_{iH}	pK_{iL}	$R_H(\%)$	$R_L(\%)$
−GTPγS				
Normoxia	7.80±0.17	5.62±0.10	27.2±3.8	72.8±3.8
Hypoxia	7.20±0.16*	5.59±0.21	24.8±4.8	75.2±4.8
+GTPγS 100 μM				
Normoxia		5.84±0.08		100
Hypoxia		5.82±0.10		100

High and low affinities (pK_{iH} and pK_{iL}, respectively) of noradrenaline and proportional binding sites (R_H and R_L, respectively) in competition for [^{3}H]-prazosin binding in membranes prepared from normoxic and chronically hypoxic umbilical veins were determined in the presence and absence of 100 μM GTPγS. In both groups, addition of GTPγS abolished agonist high affinity binding sites. Data are means±s.e.mean of four to five independent experiments performed in triplicate. *Value significantly different from that of normoxic ($P<0.05$).

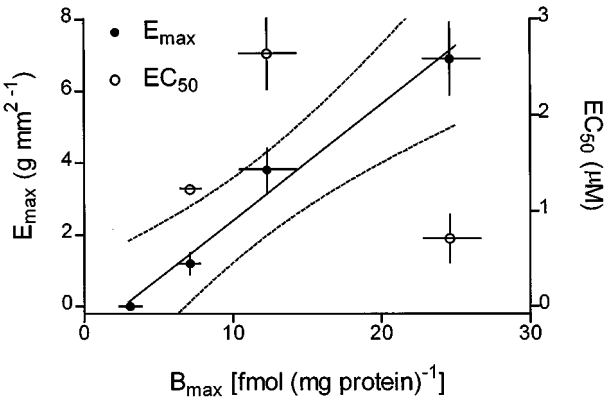

Figure 5 Correlation of α_1-adrenoceptor density (B_{max}) to noradrenaline potency (EC_{50}) and the maximal response (E_{max}). The correlation was obtained among four groups of vessels, i.e. control umbilical vein, artery, hypoxic umbilical vein, artery. The correlation coefficient for relating the α_1-adrenoceptor density (B_{max}) to the maximal response (E_{max}) and potency (EC_{50}) of noradrenaline in stimulating contractions among four groups is 0.98 ($P<0.01$) and 0.22 ($P=0.69$), respectively.

Discussion

The major finding of the present study is that chronic hypoxia significantly suppressed the noradrenaline-mediated vasoconstriction of the umbilical artery and vein in near-term foetal lamb. Consistent with the contractile data is the finding that α_1-adrenoceptor density and agonist binding affinity in the umbilical vessels were significantly decreased in response to chronic hypoxia. In light of the finding that chronic hypoxia increased foetal circulating noradrenaline and adrenaline levels (Kitanaka *et al.* 1989), the attenuated reactivity of the umbilical vessels is likely to represent a foetal adaptive mechanism to the stress of chronic hypoxia, and may play an important role in the acclimatization of the foetus to moderate chronic hypoxia.

It has been demonstrated that noradrenaline-induced contractions of foetal ovine umbilical vessels are mediated predominantly by α_1-adrenoceptors (Zhang & Dyer, 1991). These results are supported by the radioligand binding data in the present study. The dissociation constant of [^{3}H]-prazosin obtained in the present study is comparable to those determined in other studies in which α_1-adrenoceptors were demonstrated (Jones *et al.*, 1987; Hu *et al.*, 1996; Ueno *et al.*, 1997). The finding that the [^{3}H]-prazosin binding affinity was not different between the umbilical vein and artery indicates that the type of α_1-adrenoceptors are the same in both vessels studied. The significantly decreased efficacy of noradrenaline in stimulating contractile responses in the umbilical artery, compared with the vein, is likely due to, at least in part, the lower density of α_1-adrenoceptors in this vessel. Not only did the umbilical artery respond less to the agonist-stimulation it also contracted less to potassium than the vein, suggesting some other more fundamental differences in excitation/contraction coupling between the artery and vein. Although the physiological significance of these differences between the umbilical vein and artery is not entirely clear at the present, it is speculated that the umbilical vein is prone to physiological and/or pathophysiological modulations. The finding of the linear relationship with the slope of one between respective α_1-adrenoceptor occupancy and noradrenaline-induced contraction in the umbilical vein indicates a lack of an appreciable effective α_1-adrenoceptor reserve in this vessel. This is in contrast with the previous finding in ovine uterine artery in which a hyperbolic relationship between noradrenaline-induced contractions and α_1-adrenoceptor occupancy was demonstrated (Zhang *et al.*, 1995), and suggests a low efficiency of α_1-adrenoceptor coupling in the umbilical vein (Kenakin, 1984).

In response to chronic hypoxia, the tissue contractile sensitivity and the maximal response to noradrenaline were significantly decreased in the umbilical vessels. The result is in agreement with previous studies in rats in which chronic hypoxia produced a sustained attenuation of systemic and pulmonary vasoreactivity to a variety of agonists such as noradrenaline, phenylephrine, angiotensin II, and arginine vasopressin (Marriott, 1989; Doyle & Walker, 1991; Wanstall *et al.*, 1995). In the present study, the experiments were performed in tissue bath bubbled with 95% oxygen, and thus the sustained effects of chronic hypoxia which were not immediately reversible upon re-oxygenation in the baths were studied. That the blunted vascular response to vasoconstrictors following chronic hypoxia is not reversed upon restoration of oxygen in the baths was demonstrated in sheep uterine artery (Hu *et al.*, 1996; Hu & Zhang, 1997) and cerebral artery (Longo *et al.*, 1993; Ueno *et al.*, 1997), as well as in rat systemic vessels (Doyle & Walker, 1991). Similar findings were also obtained with the oxytocin-induced contraction in pregnant rat myometrium, which showed a sustained reduction in response to chronic hypoxia (Rhee *et al.*, 1996, 1997).

The cellular and biochemical mechanisms underlying chronic hypoxia-mediated vascular responses are not well understood. While the endothelium is likely to play a role (Le Cras *et al.*, 1996; Resta & Walker, 1996), part of the mechanisms relies on changes in receptor-mediated excitation-contraction coupling and/or signal transduction in the

vascular smooth muscle. Previous studies have suggested that chronic hypoxia suppresses vascular smooth muscle pharmacomechanical coupling by altering the function of cell membrane receptors as well as the post-receptor mechanisms (Hu *et al*., 1996; Hu & Zhang, 1997; Ueno *et al*., 1997; Zhou *et al*., 1997). The suppressed noradrenaline-induced contraction of the umbilical vessels in the present study may be mediated by multiple mechanisms including noradrenaline affinity to α_1-adrenoceptors, the receptor density, and the intrinsic ability of the receptor in mediating the response. In the present study, the validity of the determination of the apparent dissociation constant (K_A, as a measurement of agonist affinity) carried out for noradrenaline in umbilical vein depends on the acceptance of theoretical assumptions of receptor-agonist interactions after fractional irreversible receptor inactivation, as outlined by Furchgott (1966). The finding that the dissociation constant in umbilical vein was increased in response to chronic hypoxia indicates a decrease in noradrenaline affinity to α_1-adrenoceptors, which is likely to play an important role in hypoxic-mediated decrease in tissue contractile sensitivity to noradrenaline. Similar findings were demonstrated for noradrenaline- and serotonin-induced contractions of sheep uterine arteries (Hu *et al*., 1996; Hu & Zhang, 1997). It is postulated that a decrease in agonist binding affinity to its receptors in vascular smooth muscle may be generalized effect of chronic hypoxia.

It has been well established that agonist binding affinity to G-protein coupled receptors is regulated by G-proteins. Chronic hypoxia may impair interactions of G-proteins and receptors by changing G-protein levels (Hammond *et al*., 1993; Kacimi *et al*., 1995). The agonist competition binding in the present study exhibited a biphasic nature in both normoxic and chronically hypoxic umbilical veins, which likely reflects the state of interaction of α_1-adrenoceptors with endogenous G proteins. This was confirmed by the addition of non-hydrolyzable GTP analogue GTPγS, which abolished the agonist high affinity binding and produced a proportional increase in the number of binding sites with low affinity for noradrenaline. In the absence of GTPγS, although proportional high and low affinity binding sites were not different between normoxic and chronically hypoxic tissues, the apparent high affinity of noradrenaline to α_1-adrenoceptors was significantly decreased in hypoxic tissues compared with normoxic ones. This result is in agreement with that found in contractile studies, and suggests that hypoxia changes the interaction of α_1-adrenoceptors with G proteins in ovine foetal umbilical veins. Whether this hypoxic-associated change is a result of a decrease in expression of the pertinent G proteins, or rather of a decreased association of the receptors with existing G proteins, is unclear. Because coupling of α_1-adrenoceptors with G proteins is required for physiological activity, impairment of the coupling is likely to play an important role in hypoxic-mediated decrease in vascular reactivity of ovine foetal umbilical veins.

The finding that [^{3}H]-prazosin binding sites were significantly lower in the umbilical vessels of chronically hypoxic animals than those of the normoxic animals indicates that chronic hypoxia decreased α_1-adrenoceptor numbers in these vessels. In contrast, the dissociation constant of [^{3}H]-prazosin in binding to α_1-adrenoceptors was not changed, suggesting that chronic hypoxia did not affect the type of α_1-adrenoceptors. Similar findings were obtained in the uterine artery (Hu *et al*., 1996) and the cerebral artery (Ueno *et al*. 1997) in which chronic hypoxia caused a significant decrease in α_1-adrenoceptors. In contrast, chronic hypoxia showed no effect on serotonin receptor density in sheep uterine artery (Hu & Zhang, 1997), suggesting a heterogeneous effect of chronic hypoxia on the turnover of cell membrane receptors. While the mechanisms underlying the decreased α_1-adrenoceptors are not entirely clear at the present, it may be due to the down-regulation of α_1-adrenoceptors in response to the increased sympathetic activity and elevated circulating levels of noradrenaline and adrenaline seen in the foetal lamb in response to chronic hypoxia (Kitanaka *et al*., 1989). In light of the observation that there was no α_1-adrenoceptor reserve and a low efficiency in noradrenaline-α_1-adrenoceptor coupling in the umbilical vessels, the decrease of α_1-adrenoceptor numbers is likely to play a key role in chronically hypoxic-mediated attenuation of noradrenaline-induced contractions in these vessels. This notion is supported by the close correlation between noradrenaline-induced maximal contraction and α_1-adrenoceptor density demonstrated in the present study. On the other hand, hypoxic-mediated decrease in contractile sensitivity of umbilical vein to noradrenaline is likely due to the decrease in noradrenaline affinity to α_1-adrenoceptors. Indeed, it has been shown that the contractile sensitivity of sheep uterine arteries (Hu *et al*., 1996) and rabbit arteries (Oriowo *et al*., 1989) to noradrenaline is primarily dependent on the affinity of noradrenaline to α_1-adrenoceptors.

The effect of chronic hypoxia on the mechanisms beyond the agonist-receptor interaction in the umbilical vessels is not clear at present. In a recent study, we demonstrated that chronic hypoxia significantly inhibited serotonin receptor-vasoconstriction coupling in the uterine artery by decreasing the coupling efficiency of serotonin receptor-inositol 1,4,5-triphosphate synthesis and that of inositol 1,4,5-trisphosphate-Ca^{2+} mobilization (Hu & Zhang, 1997; Zhang & Xiao, 1998). It has been well known that inositol 1,4,5-trisphosphate plays a key role in mediating α_1-adrenoceptor-mediated vasoconstriction (Minneman, 1988; Zhang *et al*., 1995). In the present study, the finding that for a given number of α_1-adrenoceptors occupied, noradrenaline produced the same contractile tension in the umbilical vessels of control and chronically hypoxic animals suggests that the coupling efficiency of receptor-second messenger-contraction is not affected by chronic hypoxia in these vessels.

In conclusion, the present study provides, for the first time, the evidence that in the umbilical vessels of near-term foetus moderate chronic hypoxia produces direct effect on vascular smooth muscle and depresses the contractile response to noradrenaline. This chronically hypoxic-mediated attenuation of noradrenaline-induced contractions was mediated predominantly by decreases in α_1-adrenoceptor numbers and the agonist binding affinity. In addition to the umbilical vessels, chronic hypoxia has been shown to suppress the receptor-effector-contraction coupling in the uterine artery of late pregnancy (Hu *et al*., 1996; Hu & Zhang, 1997; Zhang & Xiao, 1998). Thus, chronic hypoxia has the propensity to change uteroplacental circulation from both the maternal and foetal sides of the placenta, which may play an important role in the pathogenesis of chronically hypoxic-induced complications in the mother and her developing foetus.

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