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Short communication

Inhibition of prostanoid-mediated contraction to endothelin-1 after hypoxia in rat aorta

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Abstract

The role of the thromboxane A_2/prostaglandin H_2 receptor in endothelin-1 contraction was investigated in aortic rings from rats exposed to normoxia (21% O_2) or hypoxia (10% O_2) for 12 h. Indomethacin (10 μM) and SQ 29,548 (0.1 μM, thromboxane A_2/prostaglandin H_2 receptor antagonist) reduced maximum tension and increased EC_{50} in endothelium-intact and -denuded rings from normoxic animals. Neither inhibitor had any effect on rings from hypoxic rats. Thromboxane A_2 and/or prostaglandin H_2 contribute to the response to endothelin-1 in aortas from normoxic rats but not from rats exposed to hypoxia. Loss of prostanoid-enhancement of endothelin-1 contraction contributes to impair vascular reactivity after hypoxia. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Hypoxia; Endothelin-1; Thromboxane; Cyclooxygenase

1. Introduction

Hypoxia occurs frequently in patients with cardiopulmonary disease and in normal individuals at high altitudes. The associated reduction in systemic oxygen availability elicits circulatory responses that redistribute blood flow to maintain vital organ oxygen supply (Doherty and Liang, 1984; Kuwahira et al., 1993). The endothelium plays a central role in these responses since endothelium-dependent flow-dilation is necessary to maximize perfusion (Kurjiaka and Segal, 1995; Pohl et al., 2000) and hypoxic vasodilation is mediated by endothelium-derived relaxing factor release in a number of vital circulations (Liu and Flavahan, 1997; Wilderman and Armstead, 1998; Ward, 1999). If hypoxia is prolonged, however, endothelium-dependent relaxation is impaired (Auer and Ward, 1998; Toporsian et al., 2000) and the endothelium becomes a site of tonic vasoconstrictor release (Auer and Ward, 1998; Zacour et al., 1998), changes that will limit the efficacy of the compensatory responses.

Hypoxia is associated with elevated levels of endothelin-1 in plasma (Elton et al., 1992) and aorta (Zacour et al., 1998) and endothelin-1 protein and mRNA expression are increased in endothelial cells cultured under hypoxic conditions (Kourembanas et al., 1991). Moreover, the endothelin-A receptor antagonist, BQ123 (cyclo{-D-Asp-L-Pro-D-Val-L-Leu-D-Trp-}) blocks endothelial enhancement of phenylephrine contraction in aortic rings from rats exposed to hypoxia (Zacour et al., 1998) indicating that the hypoxia-induced change in endothelial function is, in part, mediated by endothelin(s). Thromboxane A_2 and/or its precursor, prostaglandin H_2, have been shown to contribute to endothelin-1 contraction in rat aorta through the activation of their shared receptor (Reynolds and Mok, 1990; Lin and Nasjletti, 1992; Asano et al., 1994). Since hypoxic incubation increases the production of vasoconstrictor prostaglandins by endothelial and smooth muscle cells in culture (Madden et al., 1986; Takayasu-Okishio et al., 1990; Hu et al., 1997; Xu et al., 2000; Rowe et al., 2000) we hypothesized that increased endothelin-stimulated production of thromboxane A_2 and/or prostaglandin H_2 may play a role in the alteration in endothelial function induced by hypoxia. Accordingly, the current study was carried out to determine the role of thromboxane A_2/prostaglandin H_2 receptor-mediated activation of aor-

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tic contraction during stimulation with endothelin-1 after exposure to hypoxia in vivo.

2. Materials and methods

2.1. Functional studies

Male Sprague–Dawley rats (200–250 g) were exposed for 12 h to either normoxia (21% O_2) or hypoxia (10% O_2) before decapitation. The thoracic aorta of each rat was excised, cleaned and divided into two. The endothelium was removed from one half by gentle abrasion of the lumenal surface. The segments were then sectioned into 4-mm rings and mounted in jacketed organ baths containing Krebs–Henseleit solution (composition, in mM: 120 NaCl, 25 $NaHCO_3$, 11.1 glucose, 4.76 KCl, 1.18 $MgSO_4 \cdot 7H_2O$, 1.18 KH_2PO_4 and 2.5 $CaCl_2$; KHS) aerated with 95% O_2/5% CO_2 and maintained at 37 °C. During the 60-min equilibration, the bathing medium was changed periodically and the rings stabilized at a baseline tension of 2 g. The presence of a functional endothelium was tested by ascertaining that acetylcholine (1 μM) was effective in relaxing aortic rings precontracted by phenylephrine (1 μM). Failure of acetylcholine to elicit the relaxation of rings previously subjected to abrasion of the lumenal surface was taken as evidence of functional endothelial ablation. Phenylephrine and acetylcholine were then washed out and the tension allowed to return to resting values. The tissues were incubated for 20 min with a final bath concentration of 0.1% dimethyl sulfoxide vehicle (DMSO), 10 μM indomethacin (Sigma, USA) or 0.1 μM SQ 29,548 [1S-[1α,2α(Z),3α,4α]]-7-[3-[[2-[(phenylmino)carbonyl]hydrazine]methyl]-7-oxabicyclo[2.2.1]hept--yl]-5-heptanoic acid, thromboxane A_2/prostaglandin H_2-receptor antagonist, Cayman Chemicals, USA). Concentration–response curves to porcine endothelin-1 (1 pM–0.1 μM, Sigma) were then constructed. At the end of each experiment, the rings were removed from the myograph, dried overnight and weighed.

2.2. Data and statistical analysis

Tension was expressed as g/mg dry weight and data represent the mean ± standard error of the mean, for *n* number of animals. Maximum tension was calculated by averaging readings taken from the highest plateau region of each concentration–response curve. The concentration of endothelin-1 that produced 50% of the maximal response (EC_{50}) was calculated for each ring by nonlinear least-squares regression analysis. Comparisons of multiple means were performed by analysis of variance (ANOVA)

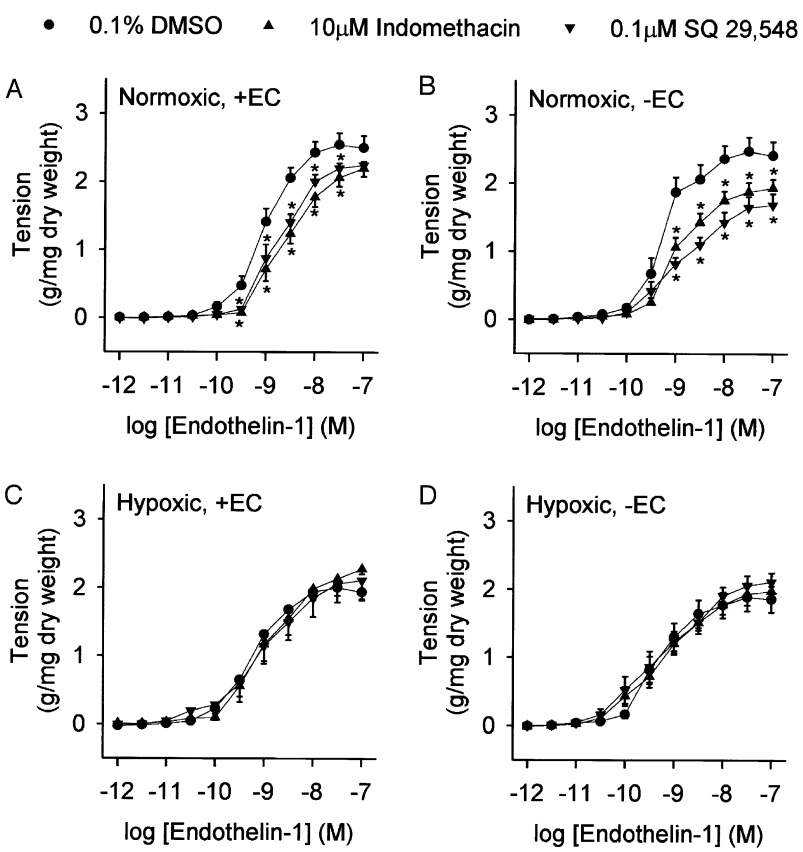

Fig. 1. Endothelium-intact (+EC) and -denuded (−EC) aortic rings from normoxic and hypoxic rats were incubated for 20 min with DMSO, indomethacin or SQ 29,548 before endothelin-1 concentration–response curves were constructed. (*), $P < 0.05$ vs. the corresponding DMSO group.

corrected for repeated measures when appropriate. When overall differences were detected, variations among individual means were evaluated post hoc by the Dunnett's test. Differences in maximum tension and EC_{50} values were evaluated by two-tailed Student's *t*-test. $P < 0.05$ was considered significant.

3. Results

Acetylcholine elicited $74.2 \pm 5\%$ and $38.5 \pm 5\%$ reversal of phenylephrine-induced contraction in endothelium-intact rings from normoxic rats and from rats exposed to hypoxia for 48 h, respectively ($P < 0.05$ for both). In contrast, the change in tension following treatment with acetylcholine in endothelium-denuded rings did not differ from zero ($-2.3 \pm 2\%$ and $-0.7 \pm 0.1\%$ in rings from normoxic and hypoxic animals, respectively). Comparable responses have been observed previously (Auer and Ward, 1998).

Maximum tension during endothelin-1 contraction in the absence of antagonists was reduced in both endothelium-intact and -denuded rings from rats exposed to hypoxia compared to the normoxic controls (Fig. 1, Table 1). The maximum responses elicited in both endothelium-intact and -denuded aortic rings from normoxic animals were reduced by 10 μM indomethacin and by 0.1 μM SQ 29,548 (Fig. 1; Table 1). Both inhibitors produced a significant rightward shift of the response curve in rings from rats in the normoxic group, as reflected by reductions in the $-\log EC_{50}$ values (Fig. 1; Table 1). In endothelium-denuded rings from normoxic rats treated with indomethacin and with SQ 29,548, but not in those exposed to vehicle alone, maximum tension is reduced compared with the corresponding endothelium-intact group. In contrast, in aortas from rats exposed to hypoxia, SQ 29,548 had no effect on endothelin-1 contraction, whereas indomethacin enhanced maximum tension, compared to vehicle alone, in endothelium-intact but not -denuded rings (Fig. 1; Table 1).

4. Discussion

The results of this study demonstrate that endothelin-1-stimulated contractions are reduced in aortic segments from rats exposed to hypoxia for 12 h, compared with aortas from normoxic rats. Since this is apparent in both endothelium-intact and -denuded rings, the effect results primarily from a change in smooth muscle function rather than the release of endothelium-derived relaxing factors. This finding extends those of previous studies in which prior in vivo exposure to hypoxia diminished contractions of arterial segments to phenylephrine and KCl in an endothelium-independent manner (Toporsian and Ward, 1997; Auer and Ward, 1998).

Our data further demonstrate that in both endothelium-intact and -denuded aortic rings from normoxic rats, indomethacin and SQ 29,548 decrease the sensitivity to endothelin-1 and the maximum tension achieved during endothelin-1-induced contraction. These findings are also in agreement with earlier reports (Reynolds and Mok, 1990; Lin and Nasjletti, 1992) and indicate that a cyclooxygenase-derived product and the thromboxane A_2/prostaglandin H_2 receptor, in part, mediate endothelin-1 contraction in rat aorta. In contrast, neither indomethacin nor SQ 29,548 had any inhibitory action in aortas from rats exposed to hypoxia. The component of the contractile response to endothelin-1 which is dependent on activation of the thromboxane A_2/prostaglandin H_2 receptor is, therefore, eliminated following hypoxia, contributing to the reduction in systemic vascular reactivity.

Table 1.
Effects of indomethacin and SQ 29,548 on the $-\log EC_{50}$ values and maximum contractions elicited by endothelin-1

			Maximum tension (g/mg dry weight)	$-\log EC_{50}$ (M)
Normoxia	endothelium-intact	0.1% DMSO	2.52 ± 0.10	9.05 ± 0.02
		10 μM indomethacin	2.15 ± 0.07[a]	8.62 ± 0.05[a]
		0.1 μM SQ 29,548	2.23 ± 0.04[a]	8.74 ± 0.05[a]
	endothelium-denuded	0.1% DMSO	2.43 ± 0.12	9.28 ± 0.04
		10 μM indomethacin	1.91 ± 0.07[a,b]	9.00 ± 0.05[a]
		0.1 μM SQ 29,548	1.68 ± 0.09[a,b]	8.87 ± 0.06[a]
Hypoxia	endothelium-intact	0.1% DMSO	1.97 ± 0.07[c]	9.25 ± 0.02
		10 μM indomethacin	2.22 ± 0.05[a]	8.96 ± 0.06
		0.1 μM SQ 29,548	2.10 ± 0.15	9.00 ± 0.04
	endothelium-denuded	0.1% DMSO	1.87 ± 0.11[c]	9.35 ± 0.04
		10 μM indomethacin	1.96 ± 0.09[b]	9.21 ± 0.04
		0.1 μM SQ 29,548	2.10 ± 0.08[c]	9.19 ± 0.07

($n = 7$–10 per group).
[a] $P < 0.05$ vs. the corresponding DMSO group.
[b] $P < 0.05$ vs. the corresponding endothelium-intact group.
[c] $P < 0.05$ vs. the corresponding normoxic group.

The inhibitory effects of SQ 29,548 and indomethacin on reactivity of aortas from normoxic rats to endothelin-1 do not require an intact endothelium. The prostanoid vasoconstrictor(s) involved in the endothelin-1 response therefore derive, at least in part, from the medial layer and are likely of smooth muscle cell origin. This supports the physiological relevance of previous studies in which endothelin-1 was found to activate the protein kinase C-phospholipase A_2 pathway (Resink et al., 1989) liberating arachidonic acid and stimulating eicosanoid release from vascular smooth muscle cells in culture (Takayasu-Okishio et al., 1990).

Endothelin-1 also activates the protein kinase C-phospholipase A_2 pathway and thromboxane A_2 release in cultured endothelial cells (Hollenberg et al., 1994; Oriji, 1999) suggesting that endothelial release of vasoconstrictor eicosanoids may contribute to the contractile response. In support of this notion, Reynolds and Mok (1990) observed consistently greater relaxation of rat aortic rings precontracted with endothelin-1 by indomethacin and SQ 29,548 when the endothelium was present. Our current finding that, in the presence of these agents, maximum contraction to endothelin-1 is decreased in endothelium-denuded compared to -intact aortic rings from normoxic rats, therefore, provides further support for a role for endothelial release of vasoconstrictor prostanoids in the aortic response to endothelin-1 under normal conditions.

Increased endothelin-1-stimulated release of vasoconstrictor prostaglandins from the endothelium has been reported to enhance vascular reactivity in rats with hypertension, aortic coarctation and estrogen deficiency (Asano et al., 1994; Taddei and Vanhoutte, 1993; Dellipizzi et al., 1997; Rubanyi et al., 1997). Hypoxic incubation has been shown to increase phospholipase A_2 activity (Michiels et al., 1993), cyclooxygenase (isoform 2) mRNA transcription (Xu et al., 2000) and thromboxane A_2 synthetase protein expression (Rowe et al., 2000) in cultured endothelial cells, suggesting that both substrate availability and the capacity to catalyze thromboxane A_2 and prostaglandin H_2 production should be greater in the systemic circulation following prolonged hypoxia in vivo. Accordingly, we proposed that hypoxia is among those pathological conditions in which enhanced production of vasoconstrictor prostaglandins contributes to altered endothelial function. In aortas from rats exposed to hypoxia, however, SQ 29,548 had no effect on endothelin-1-stimulated contraction whether the endothelium was intact or not. Moreover, in endothelium-intact rings from hypoxic rats treated with indomethacin, endothelin-1 contraction was increased, not inhibited as would be expected if our hypothesis were correct. Therefore, although the release of vasoconstrictor prostanoids may be suppressed following hypoxia, endothelium-derived products of cyclooxygenase continue to exert a vasorelaxant effect.

Hypoxia may impede endothelin-1-induced formation of prostaglandin H_2 and thromboxane A_2 either through its' direct effect on cyclooxygenase and/or thromboxane A_2 synthetase (Daley et al., 1996; Bonazzi et al., 2000) or through the release of an endogenous agent that inhibits the activities of these enzymes (Wade and Fitzpatrick, 1997). Alternatively, such an agent could act as an antagonist at the prostaglandin H_2/thromboxane A_2 receptor. Further studies are now indicated to evaluate the potential roles of these mechanisms. Although our present results provide evidence against cyclooxygenase as a pathway for endothelium-derived vasoconstricting factor production after hypoxia, other vasoactive substances are formed from arachiconic acid through the actions of lipoxygenases and *P*450 epoxygenases in vascular tissues that may participate in hypoxia-induced changes in smooth muscle tone (Nally et al., 1996; Harder et al., 1996). In view of the known effects of hypoxia on arachidonic acid release in vascular smooth muscle and endothelial cells (Resink et al., 1989; Oriji, 1999), further investigation is warranted to determine whether these other eicosanoids, alone or in concert with endothelin-1, may contribute to the change in endothelial function and systemic vascular reactivity that occur after exposure to hypoxia in vivo.

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