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Hypoxic dilatation of porcine small coronary arteries: role of endothelium and K_{ATP}-channels

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1 The aim of the present study was to determine the cellular mechanims and potential mediators involved in hypoxic dilatation of porcine small coronary arteries.

2 Small coronary arteries were isolated from a branch of the left anterior descending artery of porcine hearts, cannulated with glass micropipettes and studied in a perfusion myograph system. At a transmural pressure of 40 mmHg, the arteries had an internal diameter of 167.8 ± 6.6 μm ($n=37$).

3 In arteries contracted with acetylcholine (ACh), hypoxia (0% O_2, 30 min) caused dilatation ($86.9 \pm 6.7\%$ relaxation, $n=6$) in vessels with endothelium but constriction in endothelium-denuded vessels.

4 Hypoxic vasodilatation occurring in arteries with endothelium was abolished by the K_{ATP} channel inhibitor, glibenclamide (0.44 μM), but was not affected by inhibition of nitric oxide synthase (L-NAME, 44 μM) or cyclo-oxygenase (indomethacin, 4.4 μM).

5 Bradykinin evoked endothelium-dependent relaxation that was inhibited by L-NAME (44 μM) but not glibenclamide 0.44 μM). Cromakalim (0.1–0.3 μM), a K_{ATP} channel opener, caused relaxation that was inhibited by glibenclamide, but was not affected by L-NAME (44 μM) and/or indomethacin (4.4 μM).

6 Endothelium-removal inhibited vasodilatation evoked by cromakalim, but increased vasodilator responses to the NO donor, SIN-1 (10^{-8} to 10^{-5} M).

7 These results indicate that hypoxia acted directly on vascular smooth muscle of small coronary arteries to cause contraction. However, this effect was overwhelmed by endothelium-dependent relaxation in response to hypoxia. This relaxation was most likely mediated by release of an endothelium-derived factor, distinct from nitric oxide or prostacyclin, that activated smooth muscle K_{ATP}-channels.

Keywords: Vascular smooth muscle; microcirculation; arteriole; nitric oxide

Introduction

Hypoxic dilatation of coronary arteries is an important physiological mechanism that helps match blood flow to oxygen demand. A decrease in oxygen tension causes vasodilatation throughout the coronary circulation (Daut *et al*., 1990; Graser & Rubanyi, 1992; Mellemkjaer & Nielsen-Kudsk, 1994). In isolated large coronary arteries, hypoxia caused direct relaxation of coronary vascular smooth muscle that was resistant to inhibition of ATP-sensitive K-channels (K_{ATP}) (Graser & Rubanyi, 1992; Mellemkjaer & Nielsen-Kudsk, 1994). Endothelial cells did not contribute to this hypoxic relaxation, but instead caused a transient hypoxic constriction that was mediated by a decreased activity of endothelium-derived NO (EDNO) (Graser & Vanhoutte, 1991; Muramatsu *et al*., 1992; Graser & Rubanyi, 1992; Mellemkjaer & Nielsen-Kudsk, 1994). The relevance of these responses to the hypoxic response of small coronary arteries and arterioles is unclear. In studies performed *in vivo* or in isolated hearts, hypoxia or ischaemia caused vasodilatation that was inhibited by glibenclamide, an antagonist of K_{ATP}-channels, with little or no contribution from EDNO (Daut *et al*., 1990; von Beckerath *et al*., 1991; Kanatsuka *et al*., 1992; Park *et al*., 1992; Komaru *et al*., 1993; Katsuda *et al*., 1995a,b). Because K_{ATP} channels are located on vascular smooth muscle and can mediate hyperpolarization and relaxation (Standen *et al*., 1989; Klieber & Daut, 1994), these studies of the intact circulation led to the conclusion that the K_{ATP}-dependent, hypoxic vasodilatation was mediated by a direct effect of hypoxia on smooth muscle with little or no contribution from the endothelium (Daut *et al*., 1990; von Beckerath *et al*., 1991; Kanatsuka, *et al*., Komaru *et al*., 1993; Katsuda *et al*., 1995b).

The aim of the present study was to analyse the cellular mechanisms and potential mediators involved in hypoxic responses of porcine isolated small coronary arteries.

Methods

Blood vessel chamber

Male pigs (about 25 kg) were anaesthetized with ketamine (700 mg i.m.) followed by pentobarbitone sodium (12.5 mg kg^{-1}, i.v.). The pigs were then killed by exsanguination through the femoral arteries. The heart was rapidly removed and placed in cold Krebs-Ringer bicarbonate solution (in mM): NaCl 118.3, KCl 4.7, $MgSO_4$ 1.2, KH_2PO_4 1.2., $CaCl_2$ 2.5, $NaHCO_3$ 25.0 and glucose 11.1 (control solution). Small coronary arteries about 50–150 μm in diameter and 1 mm long were isolated from branches of the left anterior descending (LAD) coronary artery under microscopy. In some arteries, the endothelial cells were disrupted by placing a wire (70 μm in diameter) into the vessel lumen. Endothelial denudation was confirmed during the course of the experiment by the absence of a relaxant response to the endothelium-dependent dilator, bradykinin. The small arteries were cannulated at both ends with glass micropipettes, secured with 12-0 nylon monfilament suture and placed in a microvascular chamber (Living Systems, Burlington, VT). The arteries were maintained in no-flow state and held at a constant transmural pressure of 40 mmHg. The chamber was superfused with

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control solution and maintained at 37°C, pH 7.4, and gassed with 16% O_2-5% CO_2-balance N_2. A custom-built plexiglass cover was placed over the chamber in order to stabilize the perfusate oxygen tension. An oxygen electrode (MI 730 electrode, OM-4 oxygen meter, Microelectrodes, Londonderry, NH, U.S.A.) was passed through a port in the cover into the perfusate, near the vessel, to provide continuous measurement of oxygen tension. Hypoxia was induced by changing the gas mixture to 0% O_2, 5% CO_2-balance N_2 (Kovitz *et al.*, 1993). Calibration of the electrode was performed in a custom-built-chamber before the electrode was placed in the bath. Hypoxia decreased superfusate oxygen content from 16 to 0%. In some experiments, superfusate was also removed from the chamber and the P_{O_2} determined with a blood gas analyser (model BMS 3Mk2, Radiometer, Copenhagen, Denmark). At 16% O_2, P_{O_2} was 120–127 mmHg, whereas at 0% O_2, P_{O_2}, was 8–12 mmHg. The chamber was placed on the stage of an inverted microscope (X20, Nikon TMS-F, Japan) connected to a video camera (Panasonic, CCTV camera, Japan). The vessel image was projected onto a video monitor and the intraluminal diameter continuously determined by a video dimension analyser (Living Systems Instrumentation, Burlington VT.). Oxygen tension, vessel diameter and pressure measurements were continuously monitored with a four channel recorder.

Experimental protocol

Small arteries were allowed to equilibrate for 20–30 min at a transmural pressure (P_{TM}) of 10 mmHg. In some experiments, arteries were then exposed to increasing and decreasing P_{TM} (10 to 80 mmHg, in 10 mmHg steps) by raising and lowering downstream pressure with a reservoir system (Kuo *et al.*, 1991). In all experiments, P_{TM} was then held constant at 40 mmHg throughout the remainder of the experiment. KCl (60 mM) was added to the chamber to evoke vasoconstriction. The chamber superfusate was then replaced with fresh control solution and vessel diameter allowed to return to baseline. In most experiments, the small arteries were constricted to approximately 40% baseline ID with acetylcholine before hypoxic or vasorelaxant responses were assessed: acetylcholine evokes endothelium-independent constriction of porcine coronary arteries (Nakayama *et al.*, 1988; Muller *et al.*, 1993). In experiments that assessed the influence of inhibitors on these responses, the inhibitors were added to the chamber superfusate 30 min before the arteries were constricted with acetylcholine. When responses to vasorelaxant agonists were analysed, the concentration of agonist was increased in half-log increments once the response to the preceding concentration had stabilized.

None of the inhibitors used including N^G-nitro-L-arginine methyl ester (L-NAME; 4.4×10^{-5} M), an inhibitor of NO synthase, indomethacin (4.4×10^{-6} M), an inhibitor of cyclo-oxygenase, or glibenclamide (4.4×10^{-7} M), an inhibitor of K_{ATP}-channels, had any significant effect on baseline ID. However, L-NAME (4.4×10^{-5} M) decreased the concentration of acetylcholine required to cause 40% constriction of the arteries (log [acetylcholine] values of -6.72 ± 0.10, $n=10$, and -7.29 ± 0.14, $n=7$, for control and L-NAME-treated arteries, respectively, $P<0.05$). Indomethacin (4.4×10^{-6} M) or glibenclamide (4.4×10^{-7} M) did not affect the sensitivity of the arteries to acetylcholine.

Drugs

Acetylcholine choloride, bradykinin (Calbiochem, CA), cromakalim (SmithKline Beecham, PA), glibenclamide, indomethacin, N^G-nitro-L-arginine-methyl ester (L-NAME), papaverine and sydnonimine-1 (SIN-1; Cassella, Frankfurt, Germany) were obtained from Sigma Chemical (St. Louis. MO) unless stated otherwise. Stock solutions of drugs were prepared fresh each day and stored at 4°C during the experiment. Drugs were dissolved in distilled water with the exception of: (i) glibenclamide, which was dissolved initially in methanol followed by dilution in distilled water (chamber concentration of methanol: 0.07% v/v), and (ii) cromakalim, which was dissolved in a small volume of dimethyl sulphoxide (DMSO) followed by dilution in distilled water (highest chamber concentration of DMSO: 0.0008% v/v). At these concentrations, DMSO or methanol did not alter reactivity of the small coronary arteries. All drug concentrations are expressed as final molar concentration (M, mol^{-1}) in the chamber superfusate.

Data analysis

Vasomotor responses were expressed either as a percentage change in intraluminal diameter (ΔID%), or as percentage inhibition of the acetylcholine-induced contraction. Data are expressed as means ± s.e.mean for n number of experiments, where n equals the number of animals from which blood vessels were studied. The effect of interventions on the concentration-effect curve to cromakalim was evaluated by comparing the concentration of cromakalim causing 25 or 75% relaxation of the contraction to acetylcholine (inhibitory concentration: IC_{25}, IC_{75}). These values were interpolated by regression analysis of the concentration-effect curve and are presented as log IC_{25}/IC_{75}. Statistical evaluation of the data was performed by Student's t test for either paired or unpaired observations. When more than two means were compared, anlaysis of variance was used. If a significant F value was found, Scheffe's test for multiple comparisons was employed to identify differences among groups. Values were considered to be statistically different when P was less than 0.05.

Results

Baseline characteristics

Baseline intraluminal diameter (ID) was 167.8 ± 6.6 μm ($n=37$) in small arteries with endothelium, at a transmural pressure of 40 mmHg. Increasing transmural pressure (10 to 80 mmHg) caused increases in ID with no development of vasoconstriction or 'myogenic reactivity' (Figure 1).

Endothelial denudation decreased lumen diameter from 189.5 ± 12.5 to 167.5 ± 12.5 μm ($\Delta ID-11.7\pm3.1\%$) ($n=12$, $P<0.05$) and increased the magnitude of constriction to KCl (60 mM) from $-57.6\%\pm3.0\%$ to $-77.6\pm4.4\%$ (ΔID%, $n=4$, $P<0.05$). Endothelial denudation also increased the dilator response to the NO-donor, SIN-1, causing an approximate 40 fold, leftward shift in the concentration-effect to the

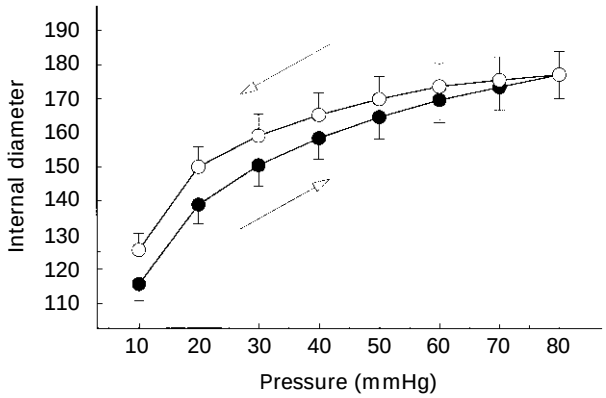

Figure 1 Relationship between intraluminal diameter and transmural pressure in porcine small coronary arteries with endothelium. Results are expressed as changes in diameter and are presented as means with vertical lines showing s.e.mean ($n=37$). Arrows indicate whether transmural pressure was increasing (●) or decreasing (○).

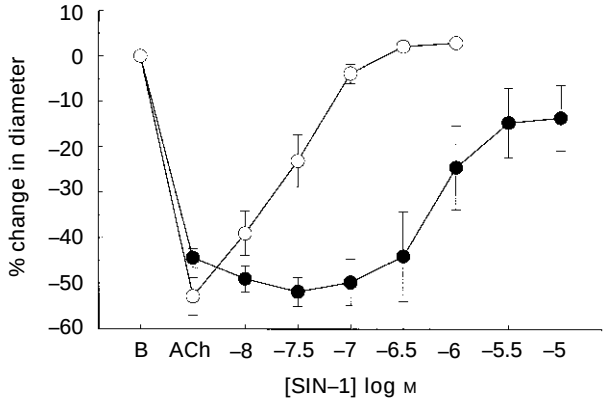

Figure 2 Vasodilator responses to the NO-donor, SIN-1 (10^{-8} to 10^{-5} M) in porcine small coronary arteries with and without endothelial cells. Coronary arteries were constricted with acetylcholine (ACh) to ~40% of baseline diameter before the responses to SIN-1 were determined. Results are expressed as % change in baseline diameter and are presented as means with vertical lines showing s.e.mean ($n=4$). Symbols (●) with endothelium; (○) without endothelium.

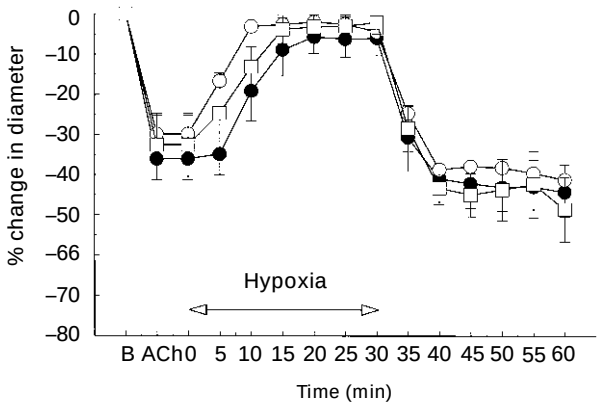

Figure 3 Effect of the NO synthase inhibitor, L-NAME ($4.4.\times10^{-5}$ M) or the cyclo-oxygenase inhibitor, indomethacin (4.4×10^{-6} M) on the vasodilator response to hypoxia (0% O_2) in porcine small coronary arteries with endothelium. Arteries were constricted with acetylcholine (ACh) to ~40% of baseline diameter before the hypoxic exposure. Results are expressed as % change in baseline diamater and are presented as means with vertical lines showing s.e.mean ($n=6$ (control) or 4 (L-NAME, indomethacin)). Symbols: (●) control; (○) indomethacin; (□) L-NAME.

agonist and increasing the maximal response from 67.8±19.8% to 105.9±2.1% relaxation of acetylcholine-induced contraction ($n=4$, $P<0.05$) (Figure 2).

Response to hypoxia

Hypoxia (0% O_2) had no effect in vessels with endothelium under quiescent conditions, i.e. in the absence of contractile tone. In subsequent experiments, arteries were constricted by approximately 40% with acetylcholine before the response to hypoxia was tested.

During constriction to acetylcholine (ACh), hypoxia (0% O_2) caused vasodilatation (86.9±6.7% relaxation of ACh-induced constriction, $n=6$, Figure 3) in small coronary arteries with endothelium that was not influenced by the inhibitor of nitric oxide synthase, L-NAME (4.4×10^{-5} M) (95.1±4.2% relaxation, $n=4$, Figure 3, $P>0.05$) or by the inhibitor of cyclo-oxygenase, indomethacin (4.4×10^{-6} M) (85.2±8.0% relaxation, $n=4$, Figure 3, $P>0.05$). However, inhibition of K_{ATP} channels with glibenclamide (4.4×10^{-7} M) abolished the hypoxic vasodilatation and the response was converted to hypoxic vasoconstriction (Figure 4).

In arteries without endothelium, hypoxia caused vasoconstriction under quiescent conditions (ΔID of −49.8±11.0%, $n=3$, $P<0.05$) or during constriction to acetylcholine (Figure 5).

Effect of reoxygenation

Following hypoxia (30 min), reoxygenation (to 16% O_2) caused a reversal of hypoxic vasodilatation in control arteries with endothelium and vasoconstrictor tone returned to pre-hypoxic levels (Figure 3). This reoxygenation response was not affected by L-NAME (4.4×10^{-5} M) or by indomethethacin (4.4×10^{-6} M) (Figure 3).

In arteries with endothelium in the presence of glibenclamide (4.4×10^{-7} M) the vasoconstriction evoked by hypoxia was maintained and was not reversed on reoxygenation (Figure 4). Indeed, 30 min after reoxygenation, arteries were still constricted to a significantly greater degree (ΔID −63.0±5.2%, $n=4$) compared to the prehypoxic level of tone (ΔID −32.1±6.1%, $n=4$, $P<0.05$) (Figure 4). As with glibenclamide-treated arteries, the hypoxic vasoconstriction observed in arteries without endothelium was not reversed by reoxygenation (Figure 5).

Superoxide dismutase (SOD, 150 μl ml^{-1}) prevented the maintained vasoconstriction associated with reoxygenation in

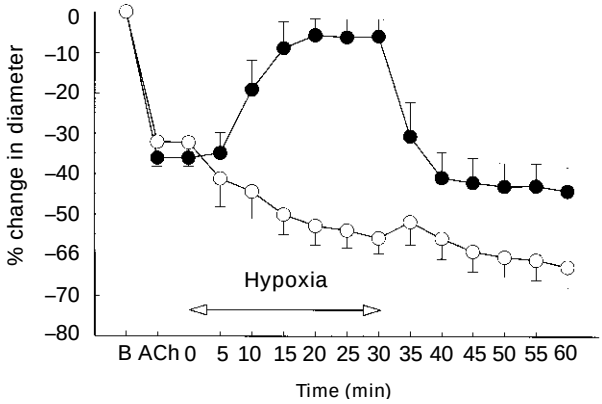

Figure 4 Effect of the K_{ATP} channel inhibitor, glibenclamide (4.4×10^{-7}M) on the vasodilator response to hypoxia (0% O_2) in porcine small coronary arteries with endothelium. Arteries were constricted with acetylcholine (ACh) to ~40% of baseline diamater before the hypoxic exposure. Results are expressed as % change in baseline diamater and are presented as means with vertical lines showing s.e.mean $n=6$ (control) or 4 (glibenclamide). Symbols: (●) control; (○) glibenclamide.

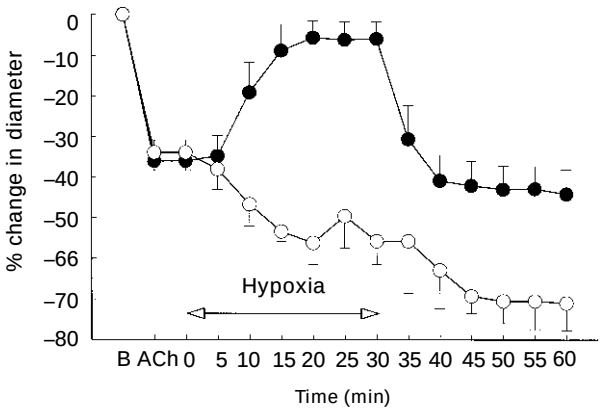

Figure 5 Vasodilator response to hypoxia (0% O_2) in porcine small coronary arteries with and without endothelium. Arteries were constricted with acetylcholine (ACh) to ~40% of baseline diameter before the hypoxic exposure. Results are expressed as % change in baseline diameter and are presented as means with vertical lines showing s.e.mean ($n=6$ (with endothelium) or 5 (without endothelium)). Symbols: (●) with endothelium; (○) without endothelium.

glibenclamide-treated arteries. Thus, in the presence of glibenclamide + SOD (150 u ml^{-1}), hypoxic vasoconstriction was reversed on reoxygenation and the level of tone was similar during prehypoxic and reoxygenation periods (ΔIDs of $-39.6 \pm 5.1\%$ and $-43.2 \pm 4.1\%$, respectively, $n=5$, $P > 0.05$). By contrast, SOD did not affect the vasoconstrictor response to hypoxia (ΔIDs of $-55.9 \pm 3.9\%$, $n=4$, and $-50.4 \pm 5.3\%$, $n=5$, for glibenclamide and glibenclamide + -SOD-treated arteries, respectively, $P > 0.05$).

Response to cromakalim

In arteries with endothelium, the K_{ATP} channel opener, cromakalim (10^{-8} to 10^{-6} M) caused concentration-dependent relaxation of acetylcholine-induced constriction that was inhibited by the K_{ATP} channel inhibitor, glibenclamide (4.4×10^{-7} M) (IC_{25} values of -7.31 ± 0.11, $n=5$, and -6.27 ± 0.15, $n=4$, for control and glibenclamide-treated arteries, respectively, $P < 0.05$, Figure 6). Relaxation to cromakalim was not affected by L-NAME (4.4×10^{-5} M) plus indomethacin (4.4×10^{-6} M) (Figure 6), but was inhibited in arteries without endothelium (IC_{75} values of -6.90 ± 0.11, $n=5$, and -6.34 ± 0.14, $n=4$, for arteries with and without endothelium, respectively, $P < 0.05$, Figure 7).

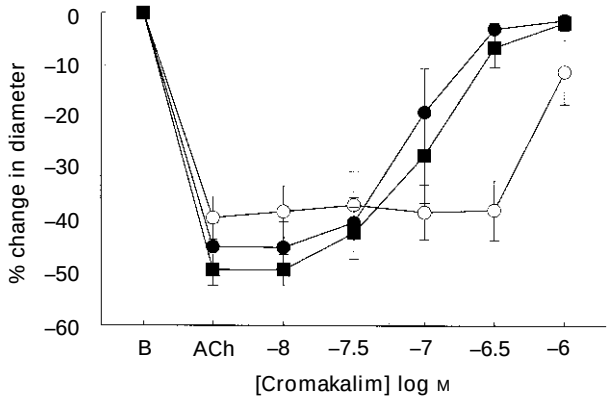

Figure 6 Effect of the K_{ATP} channel inhibitor, glibenclamide (4.4×10^{-7} M), or of the NO synthase/cyclo-oxygenase inhibitors, indomethacin (4.4×10^{-6} M) + L-NAME (4.4×10^{-5} M), on the vasodilator response to the K_{ATP} channel activator, cromakalim (10^{-8} to 10^{-6} M) in porcine small coronary arteries. Arteries were constricted with acetylcholine (ACh) to ~40% of baseline diameter before the dilator responses were assessed. Results are expressed as % change in baseline diameter and are presented as means with vertical lines showing s.e.mean ($n=5$ (control) or 4 (L-NAME + indomethacin, glibenclamide)). Symbols: (●) control; (○) glibenclamide; (■) indomethacin + L-NAME.

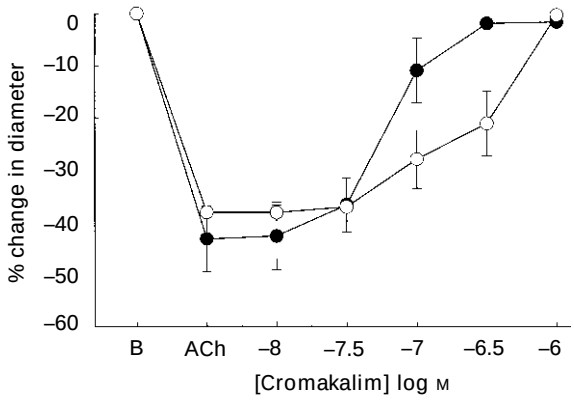

Figure 7 Vasodilator response to cromakalim (10^{-8} to 10^{-6} M) in porcine small coronary arteries with (●) and without (○) endothelium. Arteries were constricted with acetylcholine (ACh) to ~40% of baseline diameter before the dilator responses were assessed. Results are expressed as % change in baseline diameter and are presented as means with vertical lines showing s.e.mean (n = 5 (with endothelium) or 4 (without endothelium)). Symbols: (●) with endothelium; (○) without endothelium.

Response to bradykinin

In small coronary arteries constricted with acetylcholine, bradykinin (10^{-9} M) caused vasodilatation ($86.1 \pm 5.2\%$ relaxation of ACh-induced constriction, $n=5$, Figure 8) that was not affected by glibenclamide (4.4×10^{-7} M) ($95.6 \pm 2.0\%$ relaxation, $n = 4$, $P > 0.05$, Figure 8) but was markedly reduced by L-NAME (4.4×10^{-5} M)($31.0 \pm 18.5\%$ relaxation, n = 4, $P < 0.05$, Figure 8). Bradykinin was without effect in endothelium-denuded arteries (data not shown).

Discussion

In the present study, hypoxia caused marked vasodilatation of porcine isolated small coronary arteries. This hypoxic response was not affected by L-NAME, an inhibitor of nitric oxide synthase, or by indomethacin, an inhibitor of cyclooxygenase, suggesting that the endothelium-derived mediators, NO or prostacyclin, are not involved in mediating the vasodilatation. However, the hypoxic vasodilatation was abolished by glibenclamide, an inhibitor of K_{ATP}-channels, suggesting that the response is mediated by activation of K_{ATP} channels. Similar results have been obtained in studies of the intact coronary circulation (Daut *et al.*, 1990; von Beckerath *et al.*, 1991; Kanatsuka *et al.*, 1992; Komaru *et al.*, 1993). Because K_{ATP} channels are located on and can mediate smooth muscle relaxation (e.g. Standen *et al.*, 1989; Klieber & Daut, 1994), studies of the intact circulation have proposed that hypoxic vasodilatation is mediated by direct activation of smooth muscle K_{ATP} channels by hypoxia (Daut *et al.*, 1990; von Beckerath *et al.*, 1991; Kanatsuka *et al.*, 1992; Komaru *et al.*, 1993). However, in the present study, hypoxic dilatation of isolated small arteries was abolished by endothelial denudation and in the absence of the endothelium, hypoxia caused vasoconstriction. Therefore, hypoxia did not act in a simple manner to cause vasodilatation by stimulating smooth muscle K_{ATP} channels. Rather, it seems that hypoxic dilatation of small coronary arteries is dependent on the activity of K_{ATP} channels, but is mediated by hypoxic modulation of endothelial: smooth muscle cell interaction.

This conclusion is dependent upon the specificity of glib-

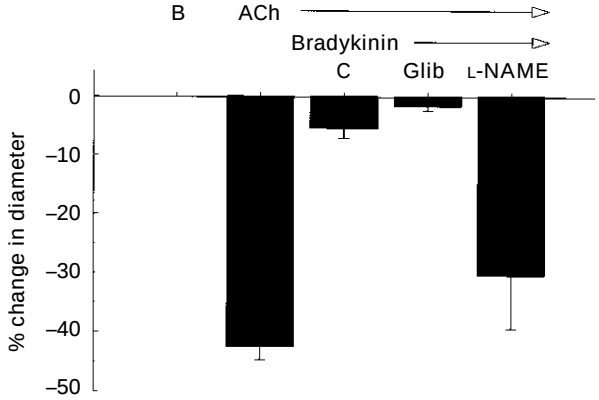

Figure 8 Effect of glibenclamide (4.4×10^{-7} M) or L-NAME (4.4×10^{-5} M) on the response to the endothelium-dependent vasodilator bradykinin (10^{-9} M) in porcine small coronary arteries with endothelium. Arteries were constricted with acetylcholine (ACh) to ~40% of baseline diameter before the dilator responses were assessed. Results are expressed as % change in baseline diameter and are presented as means ± s.e.mean ($n=5$ (control) or 4 (L-NAME, glibenclamide)).

enclamide. The present results suggest that glibenclamide acts selectively to inhibit K_{ATP} channels and does not disrupt endothelium-dependent relaxation in a non-specific manner. Thus, bradykinin caused endothelium-dependent relaxation that was inhibited by L-NAME, suggesting that this response is mediated by endothelium-derived NO. This endothelium-dependent, NO-mediated response was not influenced by glibenclamide (0.44 μM). However, at this concentration, glibenclamide markedly inhibited relaxation evoked by the K_{ATP} channel opener, cromakalim. Based on the rightward shift in the concentration-effect curve to cromakalim, the calculated dissociated constant (K_B) for glibenclamide (65 nM) is consistent with an interaction of the antagonist with K_{ATP}-channels (Quast & Cook, 1989).

K_{ATP} channels have been localized to vascular smooth muscle with opening of the channel leading to hyperpolarization and vasodilatation (Standen *et al*., 1989; Klieber & Daut, 1994). In the present study, cromakalim evoked vasodilatation in endothelium-denuded vessels confirming that these channels are functional in smooth muscle of porcine small coronary arteries (e.g. Klieber & Daut, 1994; Dart & Standen, 1995). K_{ATP} channels have also been localized to certain endothelial cells (Janigro *et al*., 1993) suggesting that K_{ATP} channels may contribute to endothelial cell regulation of vascular tone. Because endothelial cells generally lack voltage-operated calcium channels, hyperpolarization mediated by opening of endothelial K_{ATP} channels could increase cytosolic levels of calcium leading to increased production of endothelium-derived mediators (Adams *et al*., 1989). Some of the mediators released from endothelial cells can diffuse to the underlying muscle and cause activation of K_{ATP} channels located on smooth muscle cells (Standen *et al*., 1989; Parkington *et al*., 1995; Murphy & Brayden, 1995). Therefore, hypoxia could conceivably inititate an endothelium-dependent and K_{ATP}-channel-dependent vasodilatation by directly activating channels located on either cell type, or by stimulating endothelial cells to generate an endogenous K_{ATP} channel opener (Figure 9).

In our view, the hypoxic dilatation of small coronary arteries is most probably mediated by hypoxic stimulation of endothelial cells to release a vasodilator mediator(s) (mechanisms 1 and 2, Figure 9). Indeed, hypoxia caused endothelium-dependent relaxation of femoral and pulmonary arteries (Busse *et al*., 1984; Kovitz *et al*., 1993) and of skeletal muscle arterioles (Messina *et al*., 1992) by stimulating endothelial cells to produce NO and prostacyclin. However, in the present study, the endothelium-dependent hypoxic relaxation was unaffected by inhibition of NO or prostacyclin production. These results suggest that hypoxia modulates the activity of an endothelium-derived dilator mediator distinct from NO or prostacyclin (HRF, hypoxic relaxing factor, Figure 9). Although NO and prostacylcin can activate K_{ATP} channels

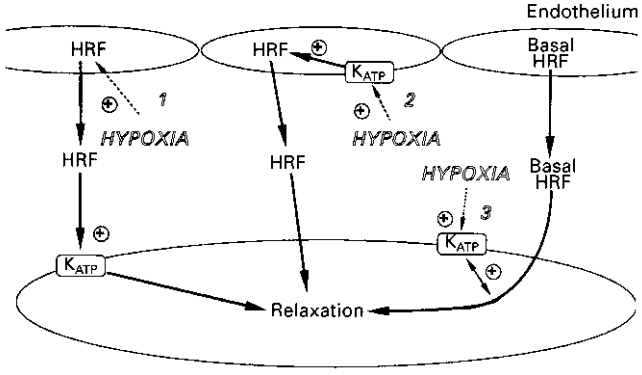

Figure 9 Postulated mechanisms to explain the vasodilator response to hypoxia in porcine small coronary arteries. HRF: hypoxic relaxing factor (distinct from NO and prostacyclin).

(Jackson *et al*., 1993; Kubo *et al*., 1994; Murphy & Brayden, 1995), endothelial cells also generate dilator mediators, termed endotheilium-derived hyperpolarizing factors (EDHFs), that cause smooth muscle hyperpolarization by activating K_{ATP} or K_{Ca} channels (calcium-activated K-channels) (Vanhoutte, 1993; Flavahan & Vanhoutte, 1995). One EDHF has been tentatively identified as a cytochrome P450 metabolite of arachidonic acid (Hecker *et al*., 1994; Campbell *et al*., 1996; Corriu *et al*., 1996). Thus, hypoxic activation of the coronary endothelium may cause the release of a glibenclamide-sensitive, K_{ATP}-dependent EDHF (Figure 9, mechanism 1). The finding that the response to hypoxia was not NO- or prostacyclin-dependent could reflect (i) increased susceptibility of these oxygenase-dependent mediators to a decrease in oxygen tension (e.g. Myers *et al*., 1991; Rengasamy & Johns, 1991), or (ii) a selective coupling of a hypoxic signal transduction pathway to the generation of a K_{ATP}-dependent EDHF (e.g. Parsaee *et al*., 1992; Flavahan & Vanhoutte, 1995). Hypoxic stimulation of endothelial cells could be mediated by modulation of an endothelial heme-protein oxygen sensor (e.g. Goldberg *et al*., 1988) or by activation of endothelial K_{ATP}-channels (Janigro *et al*., 1993) (mechanisms 1 and 2, respectively, Figure 9). In the latter case, the vasodilatation need not be dependent on an EDHF, but could be mediated by a distinct factor (e.g. CO, Zachary *et al*, 1996).

Hypoxia could also evoke an endothelium-dependent and K_{ATP}-dependent dilatation by acting on smooth muscle K_{ATP}-channels to enhance the activity of a relaxant mediator(s) released under basal conditions from endothelial cells (or vice-versa, mechanism 3, Figure 9). For example, the activity of K_{ATP}-channels is increased by concurrent stimulation of protein kinase A (e.g. Linde & Quast, 1995). We consider this mechanism to be less likely because in endothelial-denuded arteries, hypoxia caused constriction not dilatation of arterial smooth muscle. Hypoxia may therefore have failed to activate directly K_{ATP}-channels located on smooth muscle or the effect may have been negated by the hypoxic constriction. The mechanism underlying the constriction was not examined in the present study. However, in pulmonary arterial smooth muscle cells, hypoxia causes constriction by inhibiting K-channel activity (Murray *et al*., 1990; Post *et al*., 1992; Yuan *et al*., 1993).

Previous studies, with more proximal coronary arteries from the pig (200 1000 μm, non-pressurized diameter), demonstrated hypoxic activation of smooth muscle K_{ATP}-channels (Dart & Standen, 1995). Although these authors did not assess the effects of hypoxia on smooth muscle contractility, previous studies of porcine proximal coronary arteries suggest that K_{ATP}-independent mechanisms may normally predominate in mediating vasomotor responses to hypoxia (Mellemkjaer & Nielsen-Kudsk, 1994). The pattern of hypoxic responses is clearly different between proximal and distal coronary arteries. In proximal porcine coronary arteries, hypoxia evoked a transient endothelium-dependent contraction, followed by sustained, glibenclamide-insensitive smooth muscle relaxation (Mellemkjaer & Nielsen-Kudsk, unpublished observations), whereas in porcine small coronary arteries hypoxia stimulated smooth muscle constriction and endothelium-dependent dilatation. The mechanism(s) underlying this variation in response to hypoxia and whether it reflects altered activity of hypoxic sensors or hypoxic effectors (including K-channels) is not known.

As with hypoxia, the K_{ATP} channel opener, cromakalim also caused dilatation of small coronary arteries that was not altered by inhibition of NO or prostacylclin production, but was inhibited by endothelial denudation. This effect of endothelial denudation does not represent non-specific depression of vasodilator responsiveness. Endothelial-denudation increased the vasodilatation evoked by the NO donor, SIN-1, and also increased the vasoconstrictor response to depolarization with KCl. These observations are consistent with loss of the basal production of endothelium-derived dilators (e.g. Flavahan & Vanhoutte, 1989). These results suggests that the K_{ATP} channel opener may initiate similar cellular mechanisms as those dis-

cussed for hypoxia (e.g. Figure 9). However, the modulating influence of the endothelium was more prominent in the response to hypoxia than in the response to cromakalim. This may result from differences in the mechanism of endothelial modulation of these responses. For example, hypoxia but not cromakalim may activate endothelial cells (via a K_{ATP}-independent mechanism #1, Figure 9), whereas cromakalim but not hypoxia may cause relaxation by activating smooth muscle K_{ATP} channels (mechanism #3, Figure 9). Alternatively, the potent smooth muscle relaxant effect of cromakalim may simply be masking our ability to observe the full extent of the endothelium-dependent component.

Reoxygenation (to 16% O_2) reversed the hypoxic vasodilatation and microvascular tone returned to prehypoxic levels. This recovery was not altered by inhibition of NO or prostacyclin production. In contrast, the hypoxic vasoconstriction occurring in the presence of glibenclamide or in endothelium-denuded arteries was not reversible. Reoxygenation, after even a brief period of hypoxia, increases the production of superoxide from endothelial and vascular smooth muscle cells (Mohazzab-H & Wolin, 1994; Zweier *et al*., 1994). This radical can evoke constriction either by a direct action on smooth muscle or indirectly by inactivating NO (Katusic & Vanhoutte, 1991). Indeed, superoxide dismutase, a scavenger of superoxide anion, inhibited the maintained constriction associated with reoxygenation and enabled the hypoxic vasoconstriction to be reversed during reoxygenation. This suggests that the maintained constriction was mediated by superoxide produced by smooth muscle cells in response to reoxygenation. Because the reoxygenation response was observed only when the K_{ATP}-channel-dependent mechanism was inhibited (in the presence of glibenclamide or after endothelial-denudation,), activation of K_{ATP}-channels during hypoxia may constitute a protective mechanism to attenuate the deleterious effects of reoxygenation.

The porcine coronary arteries investigated in the present study demonstrated little inherent myogenic activity and responded passively to increases in transmural pressure with no active myogenic vasoconstrictor response. This is in agreement with results obtained by Nakayama *et al*. (1988) who analysed similar-sized porcine coronary arteries and who used a similar methodological approach. By contrast, Kuo and colleagues (Kuo *et al*., 1988; 1990; 1991) have consistently observed myogenic activity and myogenic responses in smaller porcine coronary arteries (ID $\leqslant$ 100 μM). Responses evoked by other vasoconstrictor or vasodilator stimuli were similar in these different studies. These variations in myogenic reactivity could result from the use of different buffer systems (e.g. Muller *et al*., 1993) or from differences in artery size (e.g. Kuo *et al*., 1988). A bicarbonate/CO_2 buffer was used in the present study to ensure normal physiological conditions for the analysis of hypoxic responses (e.g. Shirahata & Fitzgerald, 1991).

In summary, the present study demonstrated that hypoxia caused vasodilatation of porcine isolated small coronary arteries. Although hypoxia caused direct constriction of vascular smooth muscle, this response was overcome by a powerful endothelium-dependent relaxation in response to hypoxia. The endothelium-dependent vasodilatation was not mediated by endothelium-derived NO or prostacyclin but was dependent on the activity of K_{ATP} channels. The dilatation was most likely mediated by hypoxic stimulation of endothelial cells and the release of a K_{ATP}-dependent, endothelium-derived hyperpolarizing factor.

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