Enhanced β-receptor-mediated vasorelaxation in hypoxic porcine coronary artery

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Fukuda, Satoru, Takashi Toriumi, Hui Xu, Hidenori Kinoshita, Hironobu Nishimaki, Seiichiro Kokubun, Naoshi Fujiwara, Hideyoshi Fujihara, and Koki Shimoji. Enhanced β-receptor-mediated vasorelaxation in hypoxic porcine coronary artery. Am. J. Physiol. 277 (Heart Circ. Physiol. 46): H1447–H1452, 1999.—To investigate the β-adrenoceptor-mediated responses in hypoxic coronary arteries, we studied the effect of isoproterenol (Iso) on isolated porcine coronary arteries contracted with endothelin-1 in media aerated with 0, 5, 7.5, and 95% O2. The concentration-response curve of Iso was significantly shifted to the left by hypoxia (0 and 5% O2). In oxygenated and hypoxic arteries, 3 × 10⁻⁸, 10⁻⁹, and 10⁻¹⁰ M Iso significantly increased the contents of cAMP. However, there was no difference in the increase of cAMP content induced by 3 × 10⁻⁸ M Iso between oxygenated and hypoxic arteries. The content of cAMP induced by high concentrations of Iso (10⁻⁶ and 10⁻⁵ M) was significantly larger in hypoxic than in oxygenated arteries.

METHODS

Experimental Protocol

Tension measurement. Porcine hearts, obtained immediately after slaughter, were immersed in cold modified Krebs solution. Ring segments of the middle portion of the left anterior descending coronary arteries (2.0–3.0 mm in diameter, 3 mm long) were isolated. The methods used in this study were similar to those we have reported previously (7).

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RESULTS

The Po2 values of the solutions aerated with 95, 7.5, 5, and 0% O2 were 615 ± 15 (n = 129), 61 ± 2 (n = 8), 50 ± 1 (n = 13), and 24 ± 1 mmHg (n = 112), respectively.

Table 1. ED50 values and maximum responses to isoproterenol in untreated, endothelium-denuded, indomethacin-treated, and glibenclamide-treated preparations

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>ED50, ×10^(-6) M</th>
<th>Maximum Response, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>32</td>
<td>5.2 ± 0.5</td>
<td>98.5 ± 1.4</td>
</tr>
<tr>
<td>Oxygenated</td>
<td>13</td>
<td>1.3 ± 0.3†</td>
<td>101.8 ± 2.7</td>
</tr>
<tr>
<td>Endothelium denuded</td>
<td>5</td>
<td>7.4 ± 1.1</td>
<td>101.1 ± 4.2</td>
</tr>
<tr>
<td>Hypoxic</td>
<td>5</td>
<td>1.8 ± 0.5†</td>
<td>109.9 ± 2.6</td>
</tr>
<tr>
<td>Oxygenated</td>
<td>6</td>
<td>6.6 ± 2.1</td>
<td>99.5 ± 3.9</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>5</td>
<td>2.0 ± 0.4†</td>
<td>102.1 ± 4.3</td>
</tr>
<tr>
<td>Hypoxic</td>
<td>8</td>
<td>5.0 ± 0.8</td>
<td>84.9 ± 2.3†</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>8</td>
<td>3.4 ± 0.5</td>
<td>93.8 ± 2.2†</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, number of experiments. ED50, half-maximal effective dose. Significantly different from oxygenated preparation in each group: *P < 0.05; †P < 0.01. Significantly different from untreated preparation in oxygenated or hypoxic condition: ‡P < 0.05; ††P < 0.01.

The basal tone was slightly decreased by 5 × 10^(-6) M indomethacin by 0.06 ± 0.01 g (1.2 ± 0.3% of 50 mM KCl contraction, n = 11). Glibenclamide (10^(-8) M) did not alter the basal tone in 11 of 16 preparations, but in the other 5 preparations it increased the basal tone by 0.09 ± 0.01 g (2.3 ± 0.3% of 50 mM KCl contraction, n = 5). Hypoxia (0% O2) induced a contraction followed by a decrease in its tone. The tone of the vessels returned to basal value within 15 min. The transient contractions by hypoxia in untreated, glibenclamide-treated, and endothelium-denuded preparations were 0.21 ± 0.03 g (4.6 ± 0.6% of the 50 mM KCl contraction, n = 34), 0.17 ± 0.03 g (4.2 ± 0.7% of the 50 mM KCl contraction, n = 20), and 0.18 ± 0.04 g (4.8 ± 1.0% of the 50 mM KCl contraction, n = 5), respectively. In indomethacin-treated preparations the hypoxia-induced transient contraction was not observed. Hypoxia (5% O2) induced a transient contraction (0.02 ± 0.01 g; 0.5 ± 0.2% of the 50 mM KCl contraction, n = 13) that was less than that induced by 0% O2. Hypoxia (7.5% O2) did not alter the basal tone.

Iso (>=3 × 10^(-9) M) relaxed the endothelium-intact arteries in solution aerated with 95% O2. The relaxant response curve of ISO was significantly shifted to the left by aeration with 0 and 5% O2, but not by aeration with 7.5% O2 (Fig. 1). There was no significant difference in the response to ISO between the groups aerated with 0 and 5% O2 and between the groups aerated with 7.5 and 95% O2.

In oxygenated (95% O2) and hypoxic (0% O2) conditions, glibenclamide significantly decreased the maximum response to ISO (Table 1), and in the hypoxic condition (0% O2), glibenclamide significantly restored the response of hypoxic coronary arteries to ISO to that observed in oxygenated untreated preparations (Fig. 2A, Table 1). The relaxations of coronary arteries contracted with KCl to ISO were not different between oxygenated and hypoxic conditions (Fig. 2B). Removal
of the endothelium and treatment with indomethacin did not prevent the potentiation of Iso-induced relaxation under the hypoxic (0% O2) condition (Table 1).

Forskolin (10^{-8} M) relaxed the porcine coronary arteries in oxygenated and hypoxic conditions. Glibenclamide did not alter the response to forskolin, and hypoxia did not influence the response to forskolin in untreated and glibenclamide-treated preparations (Fig. 3A). Dibutyryl cAMP (>10^{-7} M) relaxed the porcine coronary arteries in oxygenated and hypoxic conditions. Hypoxia did not potentiate the relaxant response to dibutyryl cAMP (Fig. 3B).

Iso (3 \times 10^{-8}, 10^{-6}, and 10^{-5} M) significantly increased the cAMP content in oxygenated and hypoxic conditions. There was no difference in the increase in the cAMP content induced by 3 \times 10^{-8} M Iso between the hypoxic and the oxygenated condition. However, the content of cAMP in response to 10^{-6} and 10^{-5} M Iso was significantly larger in the hypoxic than in the oxygenated condition (Fig. 4A). Hypoxia significantly reduced the cGMP content in control and 10^{-5} M Iso-treated preparations. However, Iso did not influence the content of cGMP in oxygenated and hypoxic conditions (Fig. 4B).

**DISCUSSION**

The present study demonstrated that the K_{ATP} channels may be involved in the potentiation of the response to Iso by hypoxia independently of the increase in the production of cAMP. It has been reported that Iso-induced relaxation is through an agonist, trimeric G protein (G_{s}) stimulation of adenylate cyclase (10), leading to an increase of cAMP and activation of A kinase, the phosphorylated substrates of which induce relaxation by decreasing intracellular Ca^{2+}. Shaul et al. (33) reported that chronic hypoxia for 3 and 7 days in rats resulted in increased G_{s} activity in systemic arteries. However, they did not show the relationship between the receptor (G protein) and the K_{ATP} channel. In the present study the potentiation of the response to Iso in the hypoxic condition was abolished in preparations treated with glibenclamide, a selective K_{ATP} channel blocker (26), or with KCl, suggesting that the K_{ATP} channel may be involved in the potentiation of the response to Iso. In addition, there was no difference in the contents of cAMP between oxygenated and hypoxic arteries in responses to 3 \times 10^{-8} M Iso, which induced a greater relaxation in the hypoxic than in the oxygenated arteries. Furthermore, the dilatation by forskolin, which directly activates adenylate cyclase (32), was not influenced by hypoxia. These findings suggest that...
direct coupling between the β-receptor and the KATP channel independently of intracellular cAMP may play an important role in the vasodilatation of hypoxic coronary arteries. The direct coupling between receptor-associated G protein and the KATP channel was originally speculated in porcine coronary arteries by Dart and Standen (1). However, it remains to be elucidated whether the activation of G protein by Iso is receptor mediated, since some substances, such as substance P, histamine, and bradykinin, exert their effects not only via a receptor-operated mechanism but also via the direct activation of G protein (21). It is interesting to note that acidosis-induced coronary arteriolar dilation is mediated by the opening of smooth muscle KATP channels through the activation of pertussis toxin-sensitive G proteins (Gt) (14). Further study is necessary to elucidate the relationship between Iso and Gt protein in porcine coronary smooth muscle under hypoxic conditions.

The KATP channels have been shown to be regulated by intracellular factors, such as a decrease in intracellular acidosis (2, 14, 15) or a fall in the submembrane concentration of ATP (34). It seems that these changes may modify the activity of the KATP channel. The possibility of a decrease in intracellular pH may be excluded, since hypoxia did not alter the intracellular pH of porcine coronary smooth muscle cells (6). Von Beckerath et al. (34) reported that the inhibition of ATP generation in coronary arteries caused a glibenclamide-sensitive dilatation and hyperpolarization. However, hypoxia has been reported to cause little change in ATP

![Graph](image1)

Fig. 3. Responses to forskolin (A) and dibutyryl cAMP (B) in oxygenated and hypoxic conditions. Mean absolute values of contractions produced with ET-1 were 2.1 ± 0.3 g (n = 9) in oxygenated preparations, 1.8 ± 0.3 g (n = 9) in hypoxic untreated preparations, 2.0 ± 0.2 g (n = 12) in oxygenated glibenclamide-treated preparations, 2.2 ± 0.3 g (n = 12) in hypoxic glibenclamide-treated preparations in response to forskolin, and 1.9 ± 0.1 g (n = 7) in oxygenated untreated preparations in response to dibutyryl cAMP. Hypoxia influenced response to neither forskolin nor dibutyryl cAMP. Values in parentheses represent number of experiments.

![Graph](image2)

Fig. 4. Contents of cAMP (A) and cGMP (B) in response to Iso. A: 0.3 ± 10^{-5} M Iso increased cAMP in oxygenated and hypoxic conditions. Hypoxia did not influence increase in cAMP induced by 0.3 ± 10^{-5} M Iso, whereas it enhanced increase in cAMP by 10 ± 5 M Iso. Kruskal-Wallis test was used for comparison among groups, because homogeneity of variance among groups was not obtained with Bartlett test. For comparison between 2 groups, Mann-Whitney U test was used. B: hypoxia significantly decreased cGMP content in response to 10^{-5} M Iso. Iso did not influence cGMP content in oxygenated or hypoxic condition. Values in parentheses represent number of experiments.
content in rabbit aorta (24). We do not know whether hypoxia will induce a fall in intracellular ATP content to the extent that the open probability of the K_{ATP} channel in these porcine coronary arteries is increased. In this experiment we could not obtain a clear concentration-dependent effect of hypoxia; effects of 0 and 5% O_2 were the same but different from effects of 7.5 and 95% O_2. There might be a critical P_O2 for activation of the K_{ATP} channel or a steep vasodilatory response to Iso between the 0 and 7.5% O_2 groups. It remains to be resolved how the ATP content in porcine coronary vascular smooth cells may be influenced by the degree of hypoxia.

Using the patch-clamp technique under oxygenated conditions, Miyoshi and Nakaya (19) reported that a high concentration of Iso (10^{-3} M) increased the open probability of the K_{ATP} channel in enzyme-dispersed porcine coronary vascular smooth muscle cells. This finding is consistent with our result that glibenclamide attenuated the maximum responses to a high concentration of Iso in oxygenated preparations. In our study the increase in the cAMP content in response to high concentrations of Iso that induced maximum dilatation in the hypoxic condition was larger than that in the oxygenated condition. We cannot explain the action and mechanism of the enhanced accumulation of cAMP in hypoxic arteries in response to high concentrations of Iso.

There is controversy concerning the involvement of NO in β-adrenergic-induced relaxation. In rat and porcine newborn pial arteries, the relaxant response to Iso was inhibited by methylene blue and by the NO synthase inhibitor NG-monomethyl-L-arginine (11, 12, 29). In contrast, removal of the endothelium or use of NO synthase inhibitors did not influence the relaxation induced by Iso (4, 20). In the present experiment, removal of the endothelium did not alter the relaxant response to Iso, and cGMP production was not increased by Iso. Thus, in porcine coronary arteries, NO may not be involved in the relaxation induced by Iso in the oxygenated condition or in potentiation of the Iso response in the hypoxic condition.

Rubanyi and Paul (31) reported that anoxia (0% O_2) transiently increased the tension, which began to fall and reached its lowest level within 20 min (relaxation). However, 12% O_2 hypoxia induced a sustained contraction. They suggested that the relaxation induced by anoxia may be related to limitation of oxidative energy metabolism. In our experiment, aeration with 95% N_2-5% CO_2 induced a transient increase in the tone that returned to basal level within 15 min. The bath P_O2 in our experiment was ~24 mmHg and scarcely reached 0 mmHg in our system. The difference between their experiment and ours may be different bath P_O2.

In indomethacin-treated preparations the transient contraction was not observed, suggesting that the transient contraction induced by hypoxia is mediated by vascular prostaglandin synthesis.

It has been reported that a decrease of bath O_2 concentration from 95 to 40% significantly reduced β-receptor responsiveness in porcine coronary preparations precontracted with KCl or histamine (30). Indomethacin augmented this β-adrenergic responsiveness in the presence of 95% O_2 and prevented the inhibitory effects of the decrease in bath O_2 concentration. It appears that changes in P_O2 may modify the relaxant response to Iso under these O_2-rich conditions through endothelium production via cyclooxygenase. In contrast, it has been reported that hypoxia (9 mmHg) suppressed prostaglandin production in isolated bovine coronary arteries (16). In the present study the cyclooxygenase inhibitor indomethacin did not modify the response to Iso in oxygenated or hypoxic conditions, suggesting that cyclooxygenase-related prostaglandin may not be involved in the potentiation of the response to Iso in the hypoxic condition.

Hypoxemia is common in congestive heart failure due to myocardial ischemia or infarction (9, 22), because the intrapulmonary shunt increases as a result of pulmonary edema derived from left ventricular dysfunction. Our study showed that hypoxia potentiated the response to a low concentration of Iso through the K_{ATP} channel independently of the increase in cAMP, and it greatly enhanced the accumulation of cAMP induced by a high concentration of Iso. In our previous study, hypoxia also potentiated the relaxation of endothelium-intact arteries induced by nitroglycerin and PGE_2, whereas it attenuated the hydralazine-induced relaxation (7). From these findings we conclude that hypoxia may greatly modify the action of vasoactive agents on porcine coronary arteries. Further study is necessary to determine how hypoxia modifies the action of these vasoactive agents in coronary resistance vessels, since this study was performed in large conduit coronary arteries, which might not mainly contribute to coronary flow regulation.

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