Enhanced \( \beta \)-receptor-mediated vasorelaxation in hypoxic porcine coronary artery

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\textbf{Fukuda, Satoru, Takashi Toriumi, Hui Xu, Hidenori Kinoshita, Hironobu Nishimaki, Seiichiro Kokubun, Naoshi Fujiwara, Hideyoshi Fujihara, and Koki Shimoji.} Enhanced \( \beta \)-receptor-mediated vasorelaxation in hypoxic porcine coronary artery. Am. J. Physiol. 277 (Heart Circ. Physiol. 46): H1447–H1452, 1999.—To investigate the \( \beta \)-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% \( \text{O}_2 \). The concentration-response curve of Iso was significantly shifted to the left by hypoxia (0 and 5% \( \text{O}_2 \)). In oxygenated and hypoxic arteries, 3 \( \times \) \( 10^{-8} \), 3 \( \times \) \( 10^{-7} \), and 3 \( \times \) \( 10^{-6} \) M Iso significantly increased the contents of cAMP. However, there was no difference in the increases of cAMP content induced by 3 \( \times \) \( 10^{-8} \) 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. Furthermore, the potentiation by hypoxia of the Iso-induced vasorelaxation was inhibited by glibenclamide and depolarization by KCl, but not by removal of endothelium and indomethacin. The vasodilatory response to forskolin and dibutyryl cAMP was unaffected by hypoxia. We conclude that activation of the ATP-sensitive K\(^+\) channel may account for the potentiation of the response to Iso in hypoxic coronary arteries.

\begin{itemize}
  \item \textbf{isoproterenol; cyclic nucleotides; ATP-sensitive potassium channel; glibenclamide}
  \item \textbf{\( \beta \)-RECEPTOR AGONISTS such as isoproterenol (Iso), dobutamine, and dopamine have been used for inotropic support for patients with congestive heart failure due to myocardial ischemia or myocardial infarction. Arterial \( \text{O}_2 \) desaturation has sometimes been seen in these patients, and episodic and constant hypoxemia are common during the 1st wk after acute myocardial infarction (9, 22). However, the mechanism by which these \( \beta \)-inotropics influence coronary vascular tone in a hypoxic environment has not been reported. In our previous study we found that low \( \text{PO}_2 \) greatly enhanced the prostaglandin E\(_1\) (PGE\(_1\))-induced coronary relaxation in porcine coronary arteries (8). Iso, like PGE\(_1\), has been reported to induce vasodilatation mainly through formation of cAMP via \( \beta \)-adrenoceptors (10). Besides this mechanism, Iso has been reported to have other vasodilatory actions: nitric oxide (NO) formation (3, 27, 29) and activation of ATP-sensitive K\(^+\) (K\(_{\text{ATP}}\)) channels (13, 23, 25, 28). In addition, it has been reported that changes in \( \text{PO}_2 \) may produce prostaglandins, which reduce the \( \beta \)-adrenergic responsiveness in bovine and porcine coronary arteries (31). In this study we examined the vasodilatory action of Iso at various \( \text{O}_2 \) concentrations and explored the vasodilatory mechanism of Iso in a hypoxic condition. We used endothelin-1 (ET-1) as a constrictor agent for observation of the vasodilatory response to Iso, because hypoxia induces ET-1 production in blood vessels (5, 8, 17).
  \item METHODS
  \item Experimental Protocol
  \begin{itemize}
    \item \textbf{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). Briefly, the preparations were fixed vertically between hooks, at a resting tone of 2.0 g, in 20-m siliconized glass tissue baths containing modified Krebs solution. In some preparations, the endothelium was mechanically rubbed off by using wooden sticks. The composition of the modified Krebs solution (mM) was 143.0 \( \text{Na}^+ \), 5.9 \( \text{K}^+ \), 2.5 \( \text{Ca}^{2+} \), 1.2 \( \text{Mg}^{2+} \), 153.9 \( \text{Cl}^- \), 25.0 HCO\(_3^-\), 1.2 SO\(_4^{2-}\), 1.2 \( \text{H}_2\text{PO}_4^-\), and 10.0 dextrose. The solution was maintained at 37.0 ± 0.2°C and aerated with 95% \( \text{O}_2\)-5% \( \text{CO}_2\) (pH 7.37–7.43). Arterial segments were connected to a transducer (model TB-612T, Nihon Kohden), and changes in their isometric force were measured. After 1 h of equilibration, 5 \( \times \) \( 10^{-2} \) M KCl was administered repeatedly until a stable contraction was obtained (usually 2 or 3 times). After the preparations were washed several times and the basal tone was adjusted, we administered \( 10^{-7} \) M bradykinin to the stabilized 2 \( \times \) \( 10^{-2} \) M KCl contraction to determine the presence or absence of functioning endothelial cells. Bradykinin (10\(^{-7}\) M) induced maximal relaxation in rings with endothelium, whereas it caused no relaxation in rings without endothelium. After they were washed with Krebs solution, the coronary arteries were exposed to 0, 5, or 7.5% \( \text{O}_2\)-\( \text{N}_2\) with 5% \( \text{CO}_2\) or 95% \( \text{O}_2\)-5% \( \text{CO}_2\) for 30 min before titration with 10\(^{-9}\)–10\(^{-6}\) M ET-1 to induce a contraction equivalent to 40–60% of the high-K\(^+\) contraction. After the tone of the coronary arteries contracted with ET-1 had been stabilized, we administered Iso (10\(^{-7}\)–10\(^{-5}\) M) in a cumulative fashion to endothelium-intact preparations. The relaxation was expressed as a percentage of the ET-1-induced contraction. The responses to Iso in medium aerated with 0, 5, and 7.5% \( \text{O}_2\) were compared with those in medium aerated with 95% \( \text{O}_2\). In the second set of experiments we tested the vasorelaxing effects of Iso (10\(^{-9}\)–10\(^{-5}\) M) in endothelium-denuded preparations treated with indomethacin (5 \( \times \) \( 10^{-6} \) M) and glibenclamide (10\(^{-6}\) M) constricted with ET-1 under oxygenated (95% \( \text{O}_2\)-5% \( \text{CO}_2\)) or hypoxic conditions (95% \( \text{N}_2\)-5% \( \text{CO}_2\)). The protocol was similar to that outlined above. Indomethacin was administered 40 min before hypoxic gas introduction or…}
  \end{itemize}
\end{itemize}
respectively.

In the third set of experiments, relaxation by Iso in preparations contracted with KCl was investigated under oxygenated (95% O2-5% CO2) or hypoxic conditions (95% N2-5% CO2). The KCl contraction was adjusted to induce a contraction of ~50% of the magnitude produced by 5 × 10⁻² M KCl obtained previously.

In the last set of experiments we examined the responses to forskolin (10⁻⁹–10⁻⁵ M) in untreated and glibenclamide-treated preparations under oxygenated (95% O2-5% CO2) or hypoxic conditions (95% N2-5% CO2). The response to dibutyryl cAMP (10⁻⁸–10⁻⁴ M) was also examined in preparations contracted with ET-1 under oxygenated (95% O2-5% CO2) or hypoxic conditions (95% N2-5% CO2).

Measurement of cyclic nucleotides. For measurement of cAMP in responses to Iso, endothelium-intact porcine coronary arteries were at a resting tone of 2 g. The arteries were divided into five groups: 2 × 10⁻⁹ M ET-1 (control), ET-1 + 10⁻⁹ M Iso, ET-1 + 3 × 10⁻⁸ M Iso, ET-1 + 10⁻⁶ M Iso, and ET-1 + 10⁻⁵ M Iso in oxygenated (95% O2-5% CO2) and hypoxic (95% N2-5% CO2) conditions. For the measurement of cGMP in response to Iso, the arteries were divided into two groups: ET-1 (control) and ET-1 + 10⁻⁵ M Iso in oxygenated and hypoxic conditions. In all the groups, hypoxic gas was administered 30 min before 2 × 10⁻⁹ M ET-1 administration. Inasmuch as the contraction by ET-1 was stabilized within 10 min and the relaxation by Iso after ET-1 was stabilized within 10 min, we obtained frozen preparations with liquid N2. 20 min after ET-1 administration. Frozen tissues were homogenized in ice-cold 6% TCA, and the extracts were assayed for cAMP and cGMP by RIA with use of cAMP and cGMP kits (New England Nuclear). Protein levels in the tissues were assayed by the method of Lowry et al. (18). The cAMP and cGMP levels were expressed as picomoles per milligram of protein. Throughout the experiments, PO2 in the bath solution was measured with an O2 monitor (model POG-5500, MT-Gikken).

Drugs

Iso was obtained from Nikken Chemical Pharmaceutical, dibutyryl cAMP, forskolin, glibenclamide, and indomethacin from Sigma Chemical (St. Louis, MO), and ET-1 from the Peptide Institute (Osaka, J. Japan).

Statistics

Values are means ± SE. Statistical analysis for concentration-response curves of Iso was performed using two-way ANOVA. The half-maximal effective doses and maximum responses in Table 1 and the cyclic nucleotide contents among groups were compared using one-way ANOVA followed by the least significant difference test for multiple comparisons. Before one-way ANOVA was used, the Bartlett test was used for homogeneity of variance of the data. If homogeneity of variance among groups was not obtained, we used the Kruskal-Wallis test for comparison among groups. For comparison between two groups, we used the Mann-Whitney U test. Differences were considered significant at P < 0.05.

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>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>ED50 (μM)</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>Oxidant</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>Oxidant</td>
<td>6</td>
<td>6.6 ± 2.1</td>
<td>99.5 ± 3.9</td>
</tr>
<tr>
<td>Hypoxic</td>
<td>5</td>
<td>2.0 ± 0.4†</td>
<td>102.1 ± 4.3</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>8</td>
<td>5.0 ± 0.8</td>
<td>84.9 ± 2.3§</td>
</tr>
<tr>
<td>Hypoxic</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 (10⁻⁶ 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 K<sub>ATP</sub> 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 K<sub>ATP</sub> 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 K<sub>ATP</sub> channels through the activation of pertussis toxin-sensitive G proteins (G<sub>i</sub>) (14). Further study is necessary to elucidate the relationship between Iso and G<sub>i</sub> protein in porcine coronary smooth muscle under hypoxic conditions.

The K<sub>ATP</sub> 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 K<sub>ATP</sub> 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
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<sub>ATP</sub> 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<sub>2</sub> were the same but different from effects of 7.5 and 95% O<sub>2</sub>. There might be a critical P<sub>O2</sub> for activation of the K<sub>ATP</sub> channel or a steep vasodilatory response to Iso between the 0 and 7.5% O<sub>2</sub> 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<sup>-3</sup> M) increased the open probability of the K<sub>ATP</sub> 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<sub>2</sub>) transiently increased the tension, which began to fall and reached its lowest level within 20 min (relaxation). However, 12% O<sub>2</sub> 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<sub>2</sub>-5% CO<sub>2</sub> induced a transient increase in the tone that returned to basal level within 15 min. The bath P<sub>O2</sub> 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<sub>O2</sub>. 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<sub>2</sub> concentration from 95 to 40% significantly reduced β-receptor responsiveness in porcine coronary prepara-

REFERENCES


