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Glibenclamide prevents coronary vasodilation induced by \( \beta_1 \)-adrenoceptor stimulation in dogs. Am. J. Physiol. 266 (Heart Circ. Physiol. 35): H184-H192, 1994.—This study aimed to determine whether a putative ATP-sensitive K⁺-channel blocker, glibenclamide (Glb), prevents metabolic coronary vasodilation associated with increased myocardial oxygen consumption (MVO₂) caused by \( \beta_1 \)-adrenoceptor stimulation in anesthetized open-chest dogs. Isoproterenol (Iso) was infused selectively into the left circumflex coronary artery before and after Glb. Coronary blood flow (CBF) by an electromagnetic flowmeter, regional myocardial function by sonomicrometers, and left ventricular and arterial pressures were continuously measured. An intra-coronary infusion of Iso (150 ng·kg⁻¹·min⁻¹) resulted in the sustained increase in CBF as well as in the myocardial inotropic and chronotropic state. Glb (10, 30, and 100 \( \mu \)g·min⁻¹) attenuated the Iso-induced increase in CBF in a dose-dependent manner, whereas inotropic and chronotropic responses to Iso were not affected by Glb. After \( \beta_1 \)-blockade with hexapropranol (0.3 mg/kg), which completely inhibited inotropic and chronotropic responses to Iso, the Iso-induced increase in CBF, presumably mediated by vascular \( \beta_2 \)-receptor stimulation, was not affected by Glb. Intracoronary denopamine (0.1 \( \mu \)g·kg⁻¹·min⁻¹), a \( \beta_2 \)-selective agonist, increased CBF, which was almost completely abolished by Glb. The increases in MVO₂ induced by Iso or denopamine were similar before and after Glb, indicating that attenuation of the Iso- or denopamine-induced increase in CBF by Glb did not result from the decrease in MVO₂. These results indicate that Glb prevents the increase in CBF associated with increased MVO₂ caused by \( \beta_1 \)-adrenoceptor stimulation. It is suggested that ATP-sensitive K⁺-channels may play an important role in metabolic coronary vasodilation in dogs.

adeno-sine triphosphate-sensitive potassium channels; metabolic coronary vasodilation; isoproterenol; denopamine; pinacol

**METHODS**

Experimental Preparation

Adult mongrel dogs (16-23 kg) of either sex were anesthetized with an intravenous administration of pentobarbital sodium (25 mg/kg), intubated, and ventilated with a positive-pressure respirator. Arterial pH, PaO₂, and PaCO₂ were maintained within the physiological range (pH = 7.30-7.50, PaO₂ = 80-110 mmHg, PaCO₂ = 25-45 mmHg). Supplemental oxygen was given if needed. Left thoracotomy was performed in the fourth intercostal space and the pericardial cradle was made. An electromagnetic flow probe of an appropriate size was placed at the proximal segment of the left circumflex coronary artery (LCX) or at midportion of the left anterior descending coronary artery (LAD). For the measurement of regional myocardial function, a pair of miniature piezoelectric ultrasonic crystals (5 MHz, Murata, Kyoto) were placed in the subendocardial portion of myocardium in the region perfused by LCX as described previously (21). A pneumatic cuff occluder was also placed distal to the flow probe for calibration of zero flow. A heparin-filled miniature needle was cannulated into the study artery just distal to the flow probe for drug infusion. A polyethylene catheter was inserted into the aortic arch through the right carotid artery for the measurement of aortic pressure, and a 7-Fr catheter-tipped pressure transducer was inserted into the left ventricular (LV) cavity through the left carotid artery for the measurement of LV pressure (LVP).

Aortic pressure (Ap) was measured using a strain-gauge transducer (Statham P23-D8, Statham Instruments). A cardio-tachometer triggered by Ap pulse was utilized to monitor
heart rate (HR). LVP was measured with a catheter-tipped pressure transducer (PC 350, Millar Instruments, Houston, TX), and the positive first derivative of LVP (LV dP/dt) was obtained by electronic differentiation. Coronary blood flow (CBF) was measured with a calibrated square-wave electromagnetic flowmeter (MVF-2000, Nihon-Kohden, Tokyo, Japan). The zero-level flow signal was obtained both in normal saline solution and during coronary occlusion before and at the end of each experiment. The electromagnetic flow measurement system was calibrated by perfusing blood at known flow rates through a coronary arterial branch with the flow probes.

The end-systolic segment length was measured at 20 s before peak negative LV dP/dt, and the end-diastolic segment length at the onset of rapid upstroke of positive LV dP/dt. Percent systolic segment shortening (SSS) was calculated as follows:

\[
\text{SSS} = \frac{\text{end-diastolic length} - \text{end-systolic length}}{\text{end-diastolic length}} \times 100
\]

Averaged SSS of five beats was used for analysis. All variables were continuously monitored and recorded with use of a polygraph system (Polygraph 360, NEC San-Ei, Tokyo, Japan). The data were stored on tape using a digital audiotape recorder (PC-108M, Sony).

In some animals, to determine myocardial oxygen consumption (MV\text{O}_2), oxygen saturation of arterial and coronary venous blood was measured (see Experimental Protocol). Oxygen saturation of paired blood samples from the aortic arch and the coronary venous was measured by a calibrated oxygen analyzer (Unistat Oxymeter, American Optical, Buffalo, NY). MV\text{O}_2 was calculated by the following formula:

\[
\text{MV\text{O}_2 (ml/min) = CBF (ml/min) \times 0.0136} \\
\text{Hb(g/dl)} \times \text{(SaO}_2(\%) - \text{SvO}_2(\%)} \div \text{100}
\]

where Hb is hemoglobin, SaO_2 is arterial blood oxygen saturation, and SvO_2 is coronary venous oxygen saturation.

Experimental Protocol

After completion of surgical preparations, dogs were allowed to stabilize for 30 min. Then, the following protocols were performed in five groups of animals.

**Protocol 1.** In nine dogs, isoproterenol at a dose of 10 \( \mu \)g \text{·kg}^{-1} \text{·min}^{-1} \ was infused selectively into LCx for 2 min (1 ml/min) by an infusion pump, while CBF at LCx, AoP, HR, LVP, LV dP/dt, and the segment length were continuously monitored and recorded. Then, isoproterenol infusion was repeated with simultaneous infusion of vehicle (4% glucose with 0.01 N NaOH) or glibenclamide at graded doses of 10, 30, and 100 \( \mu \)g/min. In the latter experiments, isoproterenol infusion was begun 1 min after the onset of vehicle or glibenclamide infusion at each dose. The order of vehicle and glibenclamide was randomized. After completion of the experiments with a dose of glibenclamide, we waited for 30 min before and during simultaneous infusion of glibenclamide at a dose of 30 \( \mu \)g/min, while CBF at LCx, AoP, HR, LVP, LV dP/dt, and the segment length were continuously monitored and recorded.

**Protocol 2.** In six dogs, after the responses to isoproterenol alone were examined in the same manner as in protocol 1, a selective \( \beta \)-blocker, bisoprolol (0.3 mg/kg) was administered intravenously. Ten minutes later, when hemodynamic variables were stabilized, isoproterenol was infused into LCx before and during simultaneous infusion of glibenclamide at a dose of 30 \( \mu \)g/min, while CBF at LCx, AoP, HR, LVP, LV dP/dt, and the segment length were continuously monitored and recorded.

**Protocol 3.** In six dogs, a selective \( \beta \)-agonist, denopamine (344), was infused selectively into LCx at a dose of 0.1 mg/kg per min before and during simultaneous infusion of glibenclamide at a dose of 30 \( \mu \)g/min, while CBF at LCx, AoP, HR, LVP, LV dP/dt and the segment length were continuously monitored and recorded.

**Protocol 4.** In six dogs, isoproterenol (20 mg kg \text{·}^{-1} \text{·min}^{-1} \) was infused into the left main coronary artery before and during simultaneous infusion of glibenclamide at a dose of 100 \( \mu \)g/min. The doses of the drugs in this study were selected, assuming that blood flow rate at the left main coronary artery is two- to threefold greater than that at LCx. To determine MV\text{O}_2, oxygen saturation of arterial and great cardiac venous blood was measured. Coronary venous blood was sampled via a miniature tube inserted into the great cardiac vein, which drained blood largely from myocardium perfused via LAD. CBF was measured as a percentage of LAD: AoP, HR, LVP, and LV dP/dt were also monitored and recorded. Hemoglobin concentration was measured before and at the end of isoproterenol infusion in these dogs.

**Protocol 5.** In five dogs, denopamine (0.2–0.5 mg kg \text{·}^{-1} \text{·min}^{-1} \) was infused into the left main coronary artery before and during simultaneous infusion of glibenclamide (100 \( \mu \)g/min). CBF at the midportion of LAD and MV\text{O}_2 in myocardium perfused by LAD were determined as described in protocol 4. CBF, AoP, HR, LVP, and LV dP/dt were also measured.

**Protocol 6.** The effect of glibenclamide on the coronary vasodilating responses to pinacidil, a specific ATP-sensitive \( K^+ \)-channel opener, was examined in six dogs. Pinacidil was infused into LCx at graded doses of 3 and 30 \( \mu \)g/min (1 ml/min over 2 min) before and during glibenclamide infusion at a dose of 30 \( \mu \)g/min, while CBF at LCx, AoP, HR, LVP, LV dP/dt, and the segment length were continuously monitored and recorded.

**Drugs**

Isoproterenol (Nikken Kapaku, Tokyo, Japan), glibenclamide (Sigma Chemicals, St. Louis, MO), pinacidil (N'-cyanopropyl-N'-(2,3-dimethylphenyl)biguanide; Tanabe Pharmaceutical, Tokyo, Japan), and bisoprolol and denopamine (Tanabe Pharmaceutical, Tokyo, Japan) were used. Glibenclamide was dissolved in 4% glucose solution containing 0.01 N NaOH. Pinacidil was dissolved in 0.1 N HCl and neutralized by adding equimolar NaOH. Other drugs were dissolved in normal saline.

**Statistical Analysis**

Data are shown as means \( \pm \) SE. Paired data were compared using a paired Student's \( t \)-test. Hemodynamic responses to isoproterenol before and during glibenclamide at three doses were compared using analysis of variance (ANOVA) for repeated measures followed by Bonferroni's multiple-comparison tests. The effects of glibenclamide on the responses to pinacidil were also analyzed using ANOVA. Probability of \( p < 0.05 \) was considered statistically significant.

**RESULTS**

Effects of Glibenclamide on Isoproterenol-Induced Coronary Vasodilation (Protocol 1)

Representative recordings of hemodynamic variables during isoproterenol infusion into LCx before and during simultaneous infusion of glibenclamide (30 \( \mu \)g/min) are shown in Fig. 1. During the first 30 s of isoproterenol infusion, CBF at LCx, LV dP/dt, and HbSS increased,
Because glibenclamide at graded doses inhibited the CBF response to isoproterenol similarly at the early and late phase, the responses to isoproterenol at the late phase were analyzed in the following experiments.

**Fig. 1.** Representative experimental recordings during infusion of isoproterenol (10 ng·kg⁻¹·min⁻¹) into LCx before (A) and after (B) pretreatment with glibenclamide at dose of 30 µg·min⁻¹. AoP, mean aortic pressure; LV, left ventricle; LV dP/dt, first derivative of LV pressure; HR, heart rate.

**Effects of Glibenclamide on Isoproterenol-Induced Coronary Vasodilation after the Treatment with Bisoprolol (Protocol 3)**

Table 2 summarizes the effects of isoproterenol infused into LCx (10 ng·kg⁻¹·min⁻¹) on hemodynamic variables before and during glibenclamide in the absence and presence of a β-blocker bisoprolol (0.3 mg/kg). Glibenclamide decreased baseline CBF at LCx, AoP, HR, peak positive dP/dt, and 9SS at (P < 0.01). Bisoprolol attenuated the isoproterenol-induced increase in CBF (P < 0.01) and abolished the isoproterenol-induced change in AoP, HR, peak positive dP/dt, and 9SS. After the treatment with bisoprolol, glibenclamide did not affect baseline variables and did not alter the isoproterenol-induced increase in CBF.

**Effects of Glibenclamide on Denopamine-Induced Coronary Vasodilation (Protocol 4)**

Table 3 summarizes the effect of denopamine, a β₂-selective agonist, on hemodynamic variables before and during glibenclamide. Before glibenclamide, denopamine (0.1 µg·kg⁻¹·min⁻¹) infused into LCx increased CBF at LCx (P < 0.01), HR (P < 0.05), LV dP/dt (P < 0.01), and decreased the arteriovenous oxygen difference (P < 0.05). After glibenclamide, the denopamine-induced increase in CBF was abolished by glibenclamide (Fig. 2C). The denopamine-induced increase in CBF was not altered by glibenclamide alone, which was not altered by glibenclamide (Fig. 2B). The denopamine-induced increase in CBF was significantly increased in LV dP/dt and AoP, HR, and oxygen saturation before and during glibenclamide. Glibenclamide attenuated the isoproterenol-induced increase in CBF (P < 0.01) but did not alter the percentage increase in MVo₂, evolved by isoproterenol. Isoproterenol increased coronary venous oxygen saturation (P < 0.05) and decreased the arteriovenous oxygen difference (P < 0.05) before glibenclamide.

**Table 1. Effects of glibenclamide on isoproterenol-induced changes in coronary blood flow and hemodynamic variables**

<table>
<thead>
<tr>
<th>CBF/AoP/HR</th>
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<th>CBF/AoP/HR</th>
</tr>
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<tbody>
<tr>
<td>baseline</td>
<td>before</td>
<td>after</td>
</tr>
<tr>
<td>54 ± 5</td>
<td>108 ± 8</td>
<td>222 ± 11</td>
</tr>
<tr>
<td>116 ± 7</td>
<td>195 ± 13</td>
<td>222 ± 11</td>
</tr>
<tr>
<td>108 ± 12</td>
<td>190 ± 4</td>
<td>222 ± 11</td>
</tr>
</tbody>
</table>

**Table 2. Effects of glibenclamide on CBF and hemodynamic variables in presence of β₂-blockade with bisoprolol**

<table>
<thead>
<tr>
<th>CBF/AoP/HR</th>
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</tr>
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</table>

**Table 3. Effects of denopamine on hemodynamic variables**

<table>
<thead>
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<tbody>
<tr>
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<td>108 ± 12</td>
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</tbody>
</table>

**Table 4. Effects of glibenclamide on the Isoproterenol-Induced Increase in MVo₂ (Protocol 4)**

<table>
<thead>
<tr>
<th>CBF/AoP/HR</th>
<th>CBF/AoP/HR</th>
<th>CBF/AoP/HR</th>
</tr>
</thead>
<tbody>
<tr>
<td>baseline</td>
<td>before</td>
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</tr>
<tr>
<td>54 ± 5</td>
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<tr>
<td>108 ± 12</td>
<td>190 ± 4</td>
<td>222 ± 11</td>
</tr>
</tbody>
</table>
Effects of Glibenclamide on the Denopamine-Induced Increase in MVO₂ (Protocol 5)

Table 3. Effects of glibenclamide on denopamine (a β₂-selective agonist)-induced increase in CBF and hemodynamic variables

<table>
<thead>
<tr>
<th>Condition</th>
<th>CBF (ml/min)</th>
<th>MVO₂ (μmol/min)</th>
<th>HR (beats/min)</th>
<th>dP/dtmax (mmHg/s)</th>
<th>dP/dtmin (mmHg/s)</th>
<th>(a-v)O₂ (mL/min)</th>
<th>AoP (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before glibenclamide</td>
<td>116 ± 9</td>
<td>2,140 ± 248</td>
<td>112 ± 10</td>
<td>2,560 ± 134</td>
<td>188 ± 14</td>
<td>200 ± 10</td>
<td>14 ± 3</td>
</tr>
<tr>
<td>Glibenclamide (30 μg/min)</td>
<td>54 ± 8</td>
<td>1,935 ± 195</td>
<td>122 ± 11</td>
<td>2,500 ± 131</td>
<td>154 ± 9</td>
<td>205 ± 11</td>
<td>12 ± 3</td>
</tr>
</tbody>
</table>
| Values are means ± SE. (n = 5); *P < 0.01 vs. baseline; †P < 0.05 vs. control response to denopamine.

Fig. 2. Effect of glibenclamide (30 μg/min) on CBF and other variables

ATP-K⁺ CHANNELS AND METABOLIC CORONARY VASODILATION

Table 4. Effects of isoproterenol-induced increase in CBF, S VO₂, and S VO₂ without and with glibenclamide

<table>
<thead>
<tr>
<th>Condition</th>
<th>Baseline Isoproterenol</th>
<th>%Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>CBF (ml/min)</td>
<td>37 ± 6</td>
<td>55 ± 8</td>
</tr>
<tr>
<td>S VO₂ (μmol/min)</td>
<td>1,200 ± 140</td>
<td>1,240 ± 170</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>120 ± 11</td>
<td>122 ± 11</td>
</tr>
<tr>
<td>dP/dtmax (mmHg/s)</td>
<td>13 ± 2</td>
<td>15 ± 2</td>
</tr>
</tbody>
</table>

Fig. 3. Effect of glibenclamide on CBF, S VO₂, and S VO₂ in dogs

CONCLUSION

This study indicates that glibenclamide, a potentiating blocker of ATP-sensitive K⁺ channels, inhibited the increase in CBF associated with increased MVO₂ caused by β₂-adrenoceptor stimulation. This conclusion is based on the following findings. First, glibenclamide attenuated in a dose-dependent manner the isoproterenol-induced increase in CBF without altering the inotropic and chronotropic effect of isoproterenol. Second, after selective β₂-blockade with bisoprolol, glibenclamide did not alter the isoproterenol-induced increase in CBF.
5). These results indicate that inhibition of the isoproterenol- or denopamine-induced increase in CBF by glibenclamide did not result from the decrease in $M \text{VO}_2$. We calculated the ratio of the increase in CBF to the increase in estimated $M \text{VO}_2$ during isoproterenol or denopamine infusion. The ratio during glibenclamide was markedly reduced compared with that before glibenclamide (Fig. 2). These results strongly suggest that glibenclamide inhibited the mechanisms by which increased $M \text{VO}_2$ due to $\beta$-stimulation caused metabolic coronary vasodilation, so that metabolic coronary vasodilatation proportional to the increase in $M \text{VO}_2$ did not occur after glibenclamide.

We considered the magnitude of contribution of ATP-sensitive $K^+$ channels to metabolic coronary vasodilatation evoked with $\alpha_1$-adrenoceptor blockade. Glibenclamide at the dose of $30 \mu g/min$ reduced the isoproterenol-induced increase in CBF by $\approx 30\%$ (Fig. 2). The results of the experiments with isoproterenol suggest that $\beta_1$-adrenoceptor-mediated coronary vasodilatation was $\approx 70\%$ of the increase in CBF induced by isoproterenol in our experimental setting (Table 2). The increase in CBF evoked with denopamine was nearly abolished by glibenclamide (Fig. 2). These results suggest that ATP-sensitive $K^+$ channels contribute very importantly to metabolic coronary vasodilatation evoked with $\beta_1$-adrenoceptor stimulation. Furthermore, we examined the effect of glibenclamide on the pinacidil-induced increase in CBF. Glibenclamide at the dose of $30 \mu g/min$ markedly reduced by $\approx 80\%$ the increase in CBF induced by pinacidil of $3 \mu g/min$, which suggests that the dose of glibenclamide used in this study was sufficient to inhibit ATP-sensitive $K^+$ channels. The increase in CBF induced by pinacidil ($\mu g/min$) was comparable to that evoked with denopamine. Based on these findings, we consider that metabolic coronary vasodilatation evoked with $\beta_1$-adrenoceptor stimulation may be largely mediated by opening of ATP-sensitive $K^+$ channels of coronary vascular smooth muscle.

The effect of glibenclamide on the isoproterenol- or denopamine-induced increase in $M \text{VO}_2$ was examined in an experimental setting in which oxygen consumption of myocardium perfused via LAD and CBF at LAD were examined, whereas isoproterenol or denopamine was infused into the left main coronary artery before and during simultaneous infusion of glibenclamide ($\mu g$ protocols 4 and 5). This preparation was employed because regional CBF can be reasonably assessed only at the region perfused by LAD. However, the results of the studies with drug infusion into LCX (protocols 1–3) were consistent with those with drug infusion into the left main coronary artery (protocols 4 and 5), since glibenclamide infused into LCX attenuated the isoproterenol- or denopamine-induced increase in CBF at LCX with no alterations in the increase in HR, peak positive LV dp/dt and $98\%$ (Table 1). Inhibition of the denopamine-induced increase in $M \text{VO}_2$ by glibenclamide was associated with the smaller increase in $98\%$ (Table 5). We consider that reduction in the denopamine-induced increase in $98\%$ by glibenclamide was not the cause but the result of the inadequate increases in CBF, since the denopamine-induced increase in $M \text{VO}_2$ was not affected by glibenclamide. It is likely that glibenclamide did not alter regional myocardial function ($98\%$) during isoproterenol infusion, because isoproterenol increased CBF even after glibenclamide by stimulating vascular $\beta_1$-receptors.

We need to consider a possibility that glibenclamide was acting as a $\beta_1$-adrenoceptor antagonist in our prepa­


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