Receptor-Effecter Coupling Pathway for 
\(\alpha_1\)-Adrenergic Modulation of Abnormal Automaticity in ‘Ischemic’ Canine Purkinje Fibers

Evgeny P. Anyukhovsky, Susan F. Steinberg, Ira S. Cohen, Michael R. Rosen

Abstract We studied the receptor-effector coupling mechanism responsible for \(\alpha_1\)-adrenergic receptor-induced increases in abnormal automaticity (AA) occurring at low membrane potentials in “ischemic” Purkinje fibers, superfused with Tyrode’s solution containing [K\(^+\)], 10 mmol/L, pH 6.8, P\(_\text{O}_2\) < 25 mm Hg. To exclude \(\beta\)-adrenergic actions, propranolol was added to all solutions. We derived membrane slope resistance (\(R_m\)) from the current-voltage relation obtained with two microelectrodes for intracellular current injection and transmembrane voltage recording. We also measured the membrane time constant, \(\tau_m\), to assess changes in membrane resistance. Phenylephrine effects on \(R_m\) in simulated ischemia were studied in the absence or presence of the \(\alpha_1\)-subtype blockers WB 4101 (WB) or chloroethylclonidine (CEC), both 0.1 \(\mu\)mol/L, and in Purkinje fibers from dogs injected with pertussis toxin (PTX) (30 \(\mu\)g/kg IV, 60 to 72 hours before study). There were no significant differences in mean values of \(R_m\) before phenylephrine superfusion among all groups of Purkinje fibers. \(\tau_m\) increased by 23% during phenylephrine 0.1 \(\mu\)mol/L superfusion, and \(R_m\) increased by 11%. These two results suggest a 23% increase in \(R_m\) with no concordant change in longitudinal resistance. In the presence of CEC, phenylephrine increased \(R_m\) by 12%. In contrast, WB blocked phenylephrine effects on \(R_m\) (0.3%). In PTX-treated Purkinje fibers, the levels of PTX-sensitive G protein as well as phenylephrine effects on \(R_m\) (3%) were significantly reduced. In the absence of WB and of CEC, the phenylephrine effects both on \(R_m\) and on the incidence of AA were directly related to the level of PTX-sensitive substrate. BaCl\(_2\) 10 \(\mu\)mol/L increased \(R_m\) by 22% and augmented phenylephrine effects on AA. Hence, an \(\alpha_1\)-receptor subtype that is blocked specifically by WB and inhibits K conductance via a PTX-sensitive G protein underlies the \(\alpha_1\)-adrenergic stimulation of AA during ischemia. (Circ Res. 1994;74:937-944.)

Key Words • \(\alpha\)-adrenergic receptors • GTP regulatory proteins • ischemic arrhythmias • abnormal automaticity • potassium conductance

In normally polarized Purkinje fibers, catecholamines induce two different effects on automaticity via actions at distinct \(\alpha_1\)-adrenergic receptor subtypes coupled to different effector mechanisms. An \(\alpha_1\)-induced decrease in automaticity results from activation of a chloroethylclonidine (CEC)-sensitive \(\alpha_1\)-receptor linked to stimulation of Na-K pump current via a pertussis toxin (PTX)-sensitive G protein. An \(\alpha_1\)-induced increase in automaticity results from activation of a WB 4101-sensitive \(\alpha_1\)-receptor subtype and a PTX-insensitive signal transduction mechanism. The WB 4101-sensitive \(\alpha_1\)-receptor subtype has been shown to stimulate phosphoinositide hydrolysis in ventricular myocytes via a GTP-dependent process that is not PTX sensitive.

In contrast to the above, the role of \(\alpha_1\)-adrenergic receptor stimulation in the development of abnormal automaticity (ie, which occurs at depolarized membrane potentials) has been explored in Purkinje fibers subjected to simulated ischemia. Subtype-specific \(\alpha_1\)-adrenergic effects on these arrhythmias have been demonstrated: stimulation of the CEC-antagonized \(\alpha_1\)-adrenergic receptor inhibits abnormal automaticity, whereas stimulation of the WB 4101-antagonized \(\alpha_1\)-receptor enhances abnormal automaticity.

Our most recent study indicates that \(\alpha_1\)-adrenergic receptor coupling to an increase in automaticity occurs via distinct signal transduction mechanisms in both normal and “ischemic” Purkinje fibers. In normal Purkinje fibers, the \(\alpha_1\)-induced increase in automaticity is attenuated by ryanodine but not by PTX. Ryanodine presumably blocks calcium release from sarcoplasmic reticulum. In contrast, the \(\alpha_1\)-induced increase in abnormal automaticity of ischemic fibers is not suppressed by ryanodine but fails to occur in animals pretreated with PTX. Moreover, a PTX-sensitive effect of \(\alpha_1\)-receptor stimulation on potassium conductance (gK) of normoxic Purkinje myocytes has been shown by Shah et al. On the basis of these results, we hypothesized that the \(\alpha_1\)-adrenergic stimulation of abnormal automaticity during ischemia depends on an \(\alpha_1\)-adrenergic receptor subtype blocked by WB 4101 that might inhibit gK via a PTX-sensitive G protein. Hence, we designed the

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present study to test whether stimulation of α₁-adrenergic receptors can change membrane resistance while increasing abnormal automaticity in Purkinje fibers subjected to simulated ischemia. We also studied the ability of barium, which decreases gK in normoxic Purkinje fibers, to change the membrane resistance and the incidence of abnormal automaticity in the ischemic setting.

Materials and Methods

Mongrel dogs weighing 10 to 15 kg were anesthetized with sodium pentobarbital (30 mg/kg IV). Their hearts were rapidly removed through a left lateral thoracotomy and immersed in cold Tyrode’s solution equilibrated with 95% O₂/5% CO₂, and containing (mmol/L): NaCl 131, NaHCO₃ 18, KCl 2.7, CaCl₂ 2.7, MgCl₂ 0.5, NaH₂PO₄ 1.8, and dextrose 5.5. Adult dogs weighing 6 to 8 kg were used for the experiments with PTX-treated fibers. Hearts were removed 60 to 72 hours after injection of PTX 30 μg/kg IV. Free-running Purkinje fibers were dissected from the right and left ventricles, placed in a tissue bath, and superfused with Tyrode’s solution warmed to 37°C. The pH of the control solution was 7.3±0.05. Solutions were pumped through the tissue bath at a rate of 12 mL/min, changing chamber content three times each minute in both the control and ischemic settings. The bath was connected by a 3 mol/L KCl/Ag/AgCl junction.

Electrophysiological Studies

Membrane resistance was estimated from the voltage change produced by square pulses of anodal or cathodal current. All fibers were impaled with two 3 mol/L KCl-filled glass capillary microelectrodes coupled by Ag/AgCl junctions to a two-channel WPI amplifier (model Duo 773, World Precision Instruments, New Haven, Conn). Inter-electrode distance, measured with a microscope having an ocular micrometer, was <50 μm in all experiments. One microelectrode (tip resistance, 15 to 30 MΩ) was coupled to the A channel of an amplifier having high input impedance and capacity neutralization and was used for recording of transmembrane voltage. A second microelectrode (tip resistance, 3 to 5 MΩ) was coupled to the B channel, which housed a constant-current generator, and was used for intracellular current injection. The current polarity, amplitude, and waveform were linearly proportional to an external voltage command produced by a WPI pulse generator (model A310, Accupulsar). Hyperpolarizing and depolarizing square pulses of 100-millisecond duration were injected into the fiber every 2 seconds. The maximum strength of the current was adjusted to produce a 6- to 9-mV hyperpolarizing or depolarizing change in transmembrane potential at the recording microelectrode site. The currents and transmembrane potentials were displayed on an oscilloscope (model 4074, Gould, Cleveland, Ohio) and recorded on a plotter (model 7470A, Hewlett-Packard, Calif).

Our aim in using this method was to determine membrane resistance (Rᵢ) and longitudinal resistance (Rₓ) to understand whether changes in gK, the major component of Rᵢ, or junctional conductance, which contributes to Rₓ, was dominant. We measured membrane slope resistance (Rₓ) as (dVᵢ/ dI), where Vᵢ is the change in membrane potential measured and I is the magnitude of current inducing the change in membrane potential. The use of Rₓ as an indicator of membrane resistance requires some comment: after the intracellular application of a long constant current, the transmembrane voltage along the fiber declines exponentially, with a length constant of 1.2 to 3.5 mm. The distance between microelectrodes for current injection and recording in our experiments was <50 μm, considerably less than the length constant. This means that the changes in transmembrane voltage recorded in our study were very close to the changes in voltage at the site of current application and that Rₓ was practically equal to input resistance (Rᵢ). According to cable theory, Rᵢ (or Rₓ in our experiments) is proportional to (RₓRᵢ)¹/², where Rₓ and Rᵢ are the membrane and longitudinal resistances of a cable. Therefore, changes in Rₓ might be induced not only by changes in Rᵢ but also by changes in Rₓ, which, in turn, is determined normally by gap junctional resistance.

To additionally monitor Rₓ, we took advantage of cable theory as follows: for an infinite cable that is point polarized at x=0, the time constant occurs at 84% of the maximum response. Thus, the membrane time constant, τ, can be calculated as T=τ=τᵢRᵢCᵢ, where membrane current, Cᵢ, should not change. Based on this formula, any percent change in τ reflects a comparable change in Rᵢ. We therefore calculated τ by noting the time after discontinuing the current pulse at which membrane potential, Vᵢ, had decayed to 84% of its initial value. Note that only the time-dependent portion of the voltage decay is used for this measurement, since the initial instantaneous change in voltage is due to a point source effect of three-dimensional current spread from the current electrode. Knowing the change in Rᵢ, we then used the change in Rₓ to estimate the change in Rₓ.

Protocols

After stable microelectrode impalements were obtained and after equilibration in control Tyrode’s solution for 60 minutes, simulated ischemia was commenced. The simulation of an ischemic environment has been described in detail. The ischemic solution contained (mmol/L): NaCl 137, NaHCO₃ 5, CaCl₂ 2.7, MgCl₂ 0.5, NaH₂PO₄ 1.8, dextrose 5.5, and KCl 10, equilibrated with 95% N₂/5% CO₂. pH was approximately 6.7. Bath PO₂ was maintained at 10 to 25 mm Hg for the duration of the ischemic period. To minimize β-adrenergic receptor stimulation, propranolol 2x10⁻⁷ mol/L was added to all solutions.

A diagram of the pharmacological interventions performed during simulated ischemia and the time of exposure to the agents is shown in Fig 1A. Twenty minutes after the onset of ischemia, current-voltage relations were determined. Then fibers were superfused with ischemic Tyrode’s solution containing phenylephrine 1x10⁻⁷ mol/L, and current-voltage relations were determined at 15 minutes. In experiments using the α₁-subtype-selective blockers CEC and WB 4101, these drugs (both 1x10⁻⁷ mol/L) were included in all control and ischemic solutions.

If periods of spontaneous beating occurred during ischemic perfusion, the current-voltage relations were obtained between these periods. In those fibers in which depolarizing current pulses provoked an action potential, only pulses that did not reach threshold were used. At the end of each phenylephrine superfusion protocol, fiber bundles were frozen in liquid N₂ and kept at −70°C for biochemical assay.

The effects of BaCl₂ (1x10⁻⁵ mol/L) on current-voltage relations in the ischemic setting were studied with the same experimental protocol (Fig 1A). We also studied the effects of barium on the normal automaticity of Purkinje fibers and on the incidence of abnormal automaticity during simulated ischemia (Fig 1B). Barium was added to the control solutions at the end of a 45-minute stabilization period and equilibrated for 15 minutes before simulated ischemia was started. It also was added to the “ischemic” solutions.

Biochemical Studies

PTX catalyzes the ADP ribosylation and inactivation of a family of G proteins. Dogs were injected with PTX (30 μg/kg IV) to manipulate functional G protein levels in Purkinje fibers. Membranes were prepared from individual Purkinje fibers from control and PTX-treated dogs, and PTX-catalyzed...
When more than two means were compared, an analysis of variance with Bonferroni’s test for multiple comparisons was used. For the experiments on the incidence of abnormal automaticity, Fisher’s exact test was used. Data are expressed as mean±SEM. Significance was determined at P<.05.

Results

The effects of phenylephrine on membrane potential during simulated ischemia in all groups of fibers are presented in Table 1. The greatest membrane depolarization occurred in the presence of CEC, whereas hyperpolarization was seen in the presence of WB 4101. Phenylephrine had no effects on membrane potential in PTX-treated fibers.

Fig 2 is a representative record, illustrating the changes in $V_m$ in response to current injection. In simulated ischemia, the membrane potential was $-61$ mV (Fig 2A). Application of phenylephrine induced a 2-mV shift of $V_m$ in the hyperpolarizing direction (Fig 2B) and an increase in $V_m$ deflections in response to current pulses of the same magnitude as in Fig 2A. In addition, there was an attenuation of the change in $V_m$ after current offset (Fig 2). Because the changes in $V_m$ were small (not exceeding $\pm 9$ mV), data points for the current-voltage relation were fit by linear regression (Fig 2C). There was a strong correlation between I and $V_m$; correlation coefficients in individual experiments ranged from 0.977 to 0.999.

$R_d$ can be approximated by the relation of $dV_m/dI$. In the experiment in Fig 2, phenylephrine increased $R_d$ from 112 to 129.3 kΩ and increased T from 18 to 22 milliseconds (which reflects a 22% increase in $R_m$). In 19 experiments, the mean value of $R_d$ before phenylephrine superfusion was $103.5 \pm 11.2$ kΩ, and phenylephrine significantly increased $R_d$ by $11.0 \pm 2.0\%$ (P<.05). Recognizing that $R_d$ is proportional to $(R_m, R_e)^{1/2}$, this predicts a 23% increase in the product $R_m \cdot R_e$. In the same 19 experiments, the mean value of the membrane time constant, T, before phenylephrine superfusion was $20.5 \pm 1.2$ milliseconds. Phenylephrine induced an increase in T by $22.9 \pm 4.7\%$ and therefore (given that $C_m$ is unlikely to change) in $R_m$ by $22.9 \pm 4.7\%$ (P<.05). Taken together, these measurements suggest that $R_e$ is unaltered by phenylephrine and that $R_m$ increases by 23%.

Consistent with our previous results,21 phenylephrine also increased the incidence of abnormal automaticity: before phenylephrine, abnormal automaticity was seen in 2 of 19 fibers (11%); after phenylephrine superfusion, it was seen in 7 of 19 (37%). It is interesting to note that in the 5 fibers in which phenylephrine induced abnormal automaticity, the changes in slope resistance were significantly greater ($20.2 \pm 4.1\%$) than in the 14

![Diagram](http://circres.ahajournals.org/)

### Table 1. Effects of Phenylephrine on Membrane Potential During Simulated Ischemia

<table>
<thead>
<tr>
<th></th>
<th>Without Blockers</th>
<th>CEC (n=9)</th>
<th>WB (n=8)</th>
<th>PTX (n=9)</th>
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<tbody>
<tr>
<td>A. Before PE superfusion</td>
<td>$-60.3 \pm 0.5$</td>
<td>$-60.3 \pm 1.2$</td>
<td>$-60.4 \pm 0.6$</td>
<td>$-61.0 \pm 0.8$</td>
</tr>
<tr>
<td>B. After PE superfusion</td>
<td>$-62.1 \pm 0.5^*$</td>
<td>$-58.0 \pm 1.2^*$</td>
<td>$-63.8 \pm 0.5^*$</td>
<td>$-60.7 \pm 0.8$</td>
</tr>
</tbody>
</table>

CEC indicates chloroethylcystinidone ($10^{-7}$ mol/L); WB, WB 4101 ($10^{-7}$ mol/L); PTX, fibers from pertussis toxin–injected dogs; and PE, phenylephrine ($10^{-7}$ mol/L). Values are mean±SEM. Membrane potentials (mV) were determined 20 minutes after the onset of ischemia (A) and 20 minutes after addition of PE to the ischemic Tyrode’s solutions (B).

*P<.05 compared with the respective value before PE superfusion.
The effects of phenylephrine on the incidence of abnormal automaticity during simulated ischemia are shown in Fig 4. Consistent with our previous studies,\textsuperscript{11,12} phenylephrine increased the incidence of abnormal automaticity to 89% in the presence of CEC. Phenylephrine decreased the incidence of abnormal automaticity to 0% in the presence of WB 4101. Phenylephrine had no effect on abnormal automaticity in PTX-treated fibers.

To further consider the relation between \(\alpha\)-adrenergic-induced changes in \(R_d\) and abnormal automaticity, we reasoned that such a relation should be best demonstrable in any subset of fibers in which a change of \(g_K\) was the sole or major modulator of this \(\alpha\)-adrenergic effect. Since \(\alpha\)-agonists not only decrease \(g_K\) but also stimulate Na-K pump function\textsuperscript{4,5} we initially tested the relation in a setting in which the Na-K pump-stimulating actions of \(\alpha\)-agonist are blocked, ie, in the presence of CEC. As shown in Fig 5A, there is a strong correla-
tion between the change in abnormal automaticity and that in $R_a$. In contrast, this relation is not apparent when CEC is not present and $\alpha$-adrenergic effects on both $gK$ and the Na-K pump are permitted (Fig 5B).

PTX-sensitive G protein levels in Purkinje fiber membranes were measured on completion of the electrophysiological studies. Although PTX-sensitive G protein values varied among Purkinje fibers from dogs studied without blockers (0.179±0.032 pmol/mg) or in the presence of CEC (0.245±0.057 pmol/mg) or WB 4101 (0.112±0.020 pmol/mg), these differences were not significant ($P>.05$). In contrast, the level of G protein substrate available for subsequent in vitro [$^{32}$P]ADP ribosylation by PTX and [$^{32}$P]NAD was markedly decreased in Purkinje fibers from dogs injected with PTX (0.065±0.018 pmol/mg, $P<.05$). The effect of phenylephrine on $R_a$ is plotted against PTX-sensitive G protein levels for each fiber in Fig 6. The strong correlation between these values in fibers studied in the absence of $\alpha$-subtype blockers as well as in the presence of CEC. Although PTX-sensitive G protein levels were markedly reduced in Purkinje fibers from PTX-treated dogs, the strong positive correlation between percent change in $R_a$ and G protein level was maintained in this group. In contrast, no correlation between percent change in $R_a$ and G protein level was apparent in the fibers superfused with WB 4101, which blocks the phenylephrine-dependent increase in $R_a$.

Fig 7 presents the relation of fibers that did and those that did not develop abnormal automaticity in response to phenylephrine during simulated ischemia as a function of their PTX-sensitive G protein levels. Only 1 of 10 fibers with G protein levels <0.137 pmol/mg (40% of the maximal value) showed abnormal automaticity, whereas 6 of 7 fibers with G protein levels >40% of the maximal value developed abnormal automaticity ($P<.05$).

During simulated ischemia, barium ($10^{-5}$ mol/L) induced an increase in $R_a$ by 22±2.7% from a control of 87.3±16.2 kΩ ($P<.05$, n=5). The ability of barium to promote the development of abnormal automaticity and to potentiate the stimulatory effect of phenylephrine on abnormal automaticity in the ischemic setting is shown in Table 2. In control Tyrode’s solution, barium induced a significant increase in spontaneous rate. During simulated ischemia in the presence of barium (Table 2A), the incidence of abnormal automaticity (70%) was significantly ($P<.05$) greater than in fibers superfused with...
propranolol alone (11%, see Fig 4). Similar effects of barium were observed in Tyrode’s solution containing phenylephrine and CEC: the incidence of abnormal automaticity in these conditions in the absence of barium was 89% (Fig 4), whereas in the presence of barium, abnormal automaticity was seen in 100% of fibers (Table 2B).

Discussion

We previously showed that positive chronotropic responses induced by α1-adrenergic stimulation of normal and “ischemic” Purkinje fibers are blocked by WB 4101.3,11 On the basis of these results, we suggested that the mechanism responsible for α1-receptor–induced increases of abnormal automaticity in ischemia might be the same as for modulation of the increase in automaticity in normal fibers. However, we recently found two differences between these mechanisms: (1) PTX, which ADP-ribosylates and inactivates a family of G proteins, blocks the α1-adrenergic enhancement of automaticity in the setting of ischemia12 but not in normal fibers.6 (2) Ryanodine, which blocks sarcoplasmic reticulum Ca2+ release, attenuates the increase in normal automaticity but has no effect on abnormal automaticity in ischemic fibers.12 These results suggested that α1-adrenergic receptors increase automaticity at high membrane potentials in normal Purkinje fibers and increase abnormal automaticity at low membrane potentials in ischemic Purkinje fibers via different effector mechanisms. On the basis of these results and taking into account the report of Shah et al14 that there is a PTX-sensitive effect of α1-receptor stimulation to decrease gK of normoxic Purkinje myocytes, we suggested that the α1-adrenergic–induced increase in abnormal automaticity in ischemic Purkinje fibers results from a decrease in gK, and this depends on a WB 4101–sensitive receptor subtype whose actions are transduced by a PTX-sensitive G protein.12 This hypothesis was tested in the present study.

A major aspect of this study was the testing of different interventions on Purkinje fiber Rm. One might speculate that α1-receptor agonists affect Rm via activation of phosphoinositide hydrolysis.7,23,24 The resultant intracellular second messengers IP3 and DAG would be predicted to increase [Ca2+]i, via sarcoplasmic reticulum Ca2+ release and/or phosphorylation of transsarcomemal Ca2+ channels25,26 and activation of protein kinase C. These events all can decrease junctional conductance,27,28 which increases Rm and, with this, Rm.29 However, our results in Purkinje fibers from dogs injected with PTX argue against this hypothesis, since α1-adrenergic–induced increases in phosphatidylinositol metabolism are not blocked by PTX,6,30 whereas the effects of phenylephrine to increase Rm are significantly attenuated by PTX. Moreover, a strong linear correlation between phenylephrine effects on Rm and PTX-sensitive G protein levels was observed. The regression lines cross the ordinate (Rm axis) at zero, which suggests that the phenylephrine effect on Rm depends completely on the PTX-sensitive G protein. Finally, and most importantly, the calculation of changes in Rm based on changes in T implicate Rm rather than R, as the important factor. If T equals CmRm,20,21 where Cm remains unchanged, then changes in T reflect the changes in Rm.

### Table 2. Effects of Barium on Normal Automaticity and on the Incidence of Abnormal Automaticity During Simulated Ischemia

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Ischemia</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>MDP, mV</td>
<td>Rate, bpm</td>
</tr>
<tr>
<td>A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prop</td>
<td>-93±0.9</td>
<td>17±4</td>
</tr>
<tr>
<td>B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prop+C/EC+PE</td>
<td>-91±0.7</td>
<td>21±3</td>
</tr>
</tbody>
</table>

MDP indicates maximum diastolic potential; Rate, spontaneous rate; bpm, beats per minute; AA, abnormal automaticity (percent of fibers in which abnormal automaticity was observed); Prop, propranolol (2×10−7 mol/L); CEC, chloroethyl- clonidine (10−7 mol/L); and PE, phenylephrine (10−7 mol/L). Values are mean±SEM, n=10 per group.

*p<.05 compared with the respective control values before barium 10−5 mol/L superfusion.
Our measurements of T indicate a change in $R_m$ of 23%. Our studies of $R_m$ indicate that $R_m$ is unaltered, since the total change in the product $R_m R_s$ equals 23%. The major membrane conductance at rest is $g_K$. Therefore, to the limits of the accuracy of our cable analysis (and some uncertainties exist when voltage recording and current passing electrodes are placed close together [see Reference 31]), we conclude that the changes we observed with $\alpha_1$-agonists are due to a reduction in $g_K$.

Hence, the present study supports our hypothesis that an $\alpha_1$-adrenergic receptor subtype that is blocked specifically by WB 4101 and reduces membrane resistance via a PTX-sensitive $G$ protein underlies the $\alpha_1$-adrenergic stimulation of abnormal automaticity during ischemia. Moreover, it provides two new observations that strengthen this hypothesis: (1) There is an important role for membrane resistance changes in development of abnormal automaticity. (2) The phenylephrine effects on $R_m$ and on the incidence of abnormal automaticity are directly related to PTX-sensitive $G$ protein levels. In fact, the striking correlation between PTX-sensitive $G$ protein levels and the effect of phenylephrine on $R_m$ suggests that physiological variations in $G$ protein expression may contribute to the susceptibility to $\alpha_1$-agonist–induced increases in abnormal automaticity during ischemia. These results also can explain the observed differences in the effects of phenylephrine on membrane potential in simulated ischemia in the absence or presence of specific $\alpha_1$-receptor subtype blockers. The two $\alpha_1$-receptor subtypes have opposing effects on membrane potential: CEC-sensitive $\alpha_1$-receptors activate the electrogenic $Na-K$ pump and move potential in a hyperpolarizing direction; WB 4101–sensitive $\alpha_1$-receptors induce membrane depolarization because of a decrease in membrane conductance. Thus, in the presence of phenylephrine during simulated ischemia, the greatest membrane depolarization was seen in the presence of CEC and the least in the presence of WB 4101. Both of these $\alpha_1$-adrenergic receptor subtypes act through a PTX-sensitive $G$ protein, and, as expected, phenylephrine had no effects on membrane potential in fibers from PTX-treated dogs.

If, in fact, changes in membrane conductance are important to abnormal automaticity, it would be expected that interventions that decrease $g_K$ should increase $\alpha_1$-adrenergic–induced abnormal automaticity in simulated ischemia. We studied barium, which decreases the inwardly rectifying potassium current and thereby $g_K$.14 We used a concentration of barium (10$^{-4}$ mol/L) that did not depolarize Purkinje fibers in control Tyrode’s solution. In the ischemic setting, however, this barium concentration significantly increased $R_m$ and membrane depolarization as well as the incidence of abnormal automaticity in the absence of $\alpha_1$-agonist and potentiated the positive chronotropic effects of phenylephrine on abnormal automaticity. Thus, the experiments performed with barium also support the view that a decrease in $g_K$ is important for $\alpha_1$-induced increases in abnormal automaticity.

In conclusion, the results of the present study are important to our understanding of the mechanisms underlying $\alpha_1$-adrenergic receptor modulation of abnormal rhythm in ischemia. Taken together with our previous data,11,12 they establish that $\alpha_1$-adrenergic agonists induce abnormal automaticity during ischemia through actions at an $\alpha_1$-adrenergic receptor subtype that is specifically blocked by WB 4101 and inhibits $g_K$ via a PTX-sensitive $G$ protein.

Acknowledgments

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