Positive Chronotropic Responses Induced by 
α₁-Adrenergic Stimulation of Normal and
“Ischemic” Purkinje Fibers Have Different
Receptor–Effector Coupling Mechanisms

Eugeny P. Anyukhovsky, Vitalyi O. Rybin, Alexei V. Nikashin,
Olga P. Budanova, and Michael R. Rosen

We studied the mechanisms underlying the increase in automaticity induced by α₁-adrenergic stimulation of normal and “ischemic” canine Purkinje fibers. Fibers were superfused with a control Tyrode’s solution, followed by an ischemic superfusate that included 10 mM KCl, 5 mM NaHCO₃, P0₂ of 10–25 mm Hg, and pH 6.7. To exclude β-adrenergic actions, propranolol was added to all solutions. In the presence of phenylephrine, normal automaticity at high membrane potentials usually decreased, whereas the incidence of abnormal automaticity during ischemia was increased from a control value of 10% to 30%. Block of an α₁-receptor subtype with chloroethylenolidine in the presence of phenylephrine caused normal automaticity to increase in all fibers studied and significantly increased abnormal automaticity to 70%. The α₁-adrenergic–induced increase in automaticity did not occur in ischemic fibers from animals pretreated with pertussis toxin (PTX), which ADP-ribosylated and functionally inactivated the 41-kd family of GTP regulatory proteins. In contrast, the use of PTX enhanced the increase in automaticity induced by phenylephrine in normally polarized Purkinje fibers. Ryanodine, which blocks sarcoplasmic reticulum Ca²⁺ release, attenuated the increase in normal automaticity in nonischemic fibers but had no effect on abnormal automaticity in ischemic fibers. The increase in abnormal automaticity was, however, blocked by the α₁ subtype blocker WB 4101, which also blocks the increase in automaticity in normal fibers. In conclusion, the increase in abnormal automaticity in ischemic Purkinje fibers depends on a WB 4101–sensitive α₁-adrenergic receptor subtype whose actions are transduced by a PTX-sensitive 41-kd G protein and are not blocked by ryanodine. This is clearly different from the mechanism underlying the increase in automaticity in normal Purkinje fibers, which is independent of the PTX substrate but is suppressed by ryanodine. (Circulation Research 1992;71:526–534)

Key Words • GTP regulatory proteins • sarcoplasmic reticulum Ca²⁺ release • potassium conductance • ischemic arrhythmias • abnormal automaticity

In normally polarized canine Purkinje fibers, α₁-adrenergic agonists may decrease or increase automaticity.¹–³ The α₁-induced decrease in automaticity appears to result from stimulation of Na-K pump current via a pathway transduced by a member of the 41-kd family of GTP regulatory proteins that are pertussis toxin (PTX) substrates.⁴–⁵ In contrast, the increase in automaticity appears unassociated with the PTX substrate.⁶–⁸ It has been suggested that the positive chronotropic response to α₁-agonists is associated with enhanced phosphoinositide metabolism (via a GTP-dependent process that is not PTX sensitive) and resultant increases in free intracellular calcium via mobilization from the sarcoplasmic reticulum.⁹–¹³ This view is supported by the finding that α₁-adrenergic stimulation increases phosphoinositide metabolism in rat⁶–⁸ and canine¹⁰,¹¹ hearts, and this response is magnified in hypoxia.¹¹ Moreover, increases in phosphoinositide metabolism secondary to interventions other than α₁-agonists increase free intracellular Ca²⁺ levels in canine Purkinje fibers,¹³,¹² and α₁-agonists have been shown to induce small increases in free intracellular calcium in rabbit myocardium.¹³

The effects of α₁-agonists on normal automaticity of canine Purkinje fibers have been linked to pharmacologically distinct α₁-adrenergic receptor subtypes. The alkylation agent chloroethylenolidine (CEC) blocks the α₁-induced decrease in automaticity and does not influence activation of phosphoinositide metabolism. In contrast, the competitive antagonist WB 4101 inhibits both the increase in automaticity and activation of phosphoinositide hydrolysis.⁶

The role of α₁-adrenergic stimulation in the development of abnormal rhythmic activity after ischemia and
reperfusion has been studied in the feline heart in situ as well as in isolated canine Purkinje fibers. During simulated ischemia in the presence of phenylephrine, CEC block of an α-receptor subtype increases membrane depolarization and the incidence of abnormal automaticity. In contrast, WB 4101 block of a different α-receptor subtype decreases membrane depolarization and suppresses abnormal automaticity. This type of abnormal automaticity is also blocked by prazosin. The following tentative explanation of these results has been suggested: CEC-sensitive α-receptors activate the electrogenic Na-K pump and prevent the development of abnormal automaticity during simulated ischemia, whereas WB 4101-sensitive α-renergic receptors induce the hydrolysis of membrane phosphoinositides, increase intracellular calcium, and tend to enhance the incidence of abnormal automaticity via a nonspecific cation conductance. Validation of this explanation depends, first, on the demonstration that α-agonists induce phosphoinositide metabolism in canine heart, which has previously been reported. It also requires that α-agonists increase free intracellular calcium levels in canine myocytes, which has not been demonstrated. In contrast, studies with aequorin in rabbit heart have shown only small increases in [Ca^2+] in response to concentrations of α-agonists that markedly increase the force of contraction and have suggested that the mechanism for this behavior might be an increase in myofibrillar sensitivity to Ca^2+.

Our goal in the present study was first to test whether the same mechanism is responsible for the α-adrenergic increase in automaticity in normal and ischemic Purkinje fibers. Hence, we tested the relation between the presence of the 41-kd family of G proteins that are PTX substrates and the response to phenylephrine of normal, ischemic, and reperfused Purkinje fibers from control dogs and from dogs pretreated with PTX. As shall be demonstrated, the PTX substrate maintains an essential role in α-adrenergic stimulation of abnormal automaticity during ischemia. We then asked whether the increase in automaticity in the control and ischemic settings might be related to α-adrenergic-induced sarcoplasmic reticulum calcium release. For these studies, we used ryanodine, which blocks calcium release from sarcoplasmic reticulum, and verapamil, which blocks transsarcolemmal calcium current (although it is a weak α-blocker and modifies potassium currents as well).

Materials and Methods

Healthy adult mongrel dogs weighing 10–20 kg were anesthetized with sodium pentobarbital (30 mg/kg i.v.). The hearts were removed quickly through a right lateral thoracotomy and immersed in cold Tyrode’s solution equilibrated with 95% O2–5% CO2 and containing (mM) NaCl 131, NaHCO3 18, 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 10–25 mm Hg for the duration of the ischemic period. To determine pH, PO2, and PO2 of the superfusate, a blood gas analyzer (model 158, Corning Glass Inc., Corning, N.Y.) was used.

Electrophysiological Protocols

All Purkinje fiber bundles were stimulated at a cycle length of 500 msec. To exclude the influence of β-adrenergic receptor stimulation, propranolol (2×10^-7 M) was added to all solutions. This concentration has no effect on the transmembrane potential or automaticity in control, ischemic, and reperfused fibers. After 20 minutes, the control action potentials were recorded, and fibers were permitted to beat spontaneously.

Studies of effects of ischemia on PTX substrate and on automaticity. Figure 1 summarizes all protocols for the experiments in which the relation between the PTX substrate and the response to phenylephrine of normal, ischemic, and reperfused Purkinje fibers was studied. Fiber bundles were divided into four groups as follows: 1) 40 minutes in control solution, 2) 40 minutes in control solution plus 10 minutes in “ischemic” solution, 3) 40 minutes in control solution plus 40 minutes in ischemic solution, and 4) 40 minutes in control solution plus 40 minutes in ischemic solution plus 40 minutes in control solution. The last protocol was also used in experiments on Purkinje fiber bundles from dogs pretreated with PTX. Each group was divided into two subgroups (a and b in Figure 1) superfused in the absence (subgroup a) or presence (subgroup b) of phenylephrine (10^-7 M). This concentration range was chosen because the α-adrenergic effects of phenylephrine on automaticity are maximal. In contrast, the...
FIGURE 1. Protocols for studying pertussis toxin-sensitive G protein coupling in ischemic and reperfused Purkinje fibers. Prop, propranolol (2 x 10^{-7} M); PE, phenylephrine (1 x 10^{-7} M). From six to nine fibers were used in each protocol (exact numbers of fibers in each group are indicated in “Results”). See text for description.

Effects of phenylephrine on repolarization tend to occur at concentrations of 10^{-6} M and greater.21 At the end of each superfusion protocol, fiber bundles were frozen at -70°C for biochemical assay.

Studies of ryanodine actions. The effects of ryanodine on the phenylephrine-induced increases in normal automaticity and in the incidence of abnormal automaticity were studied in different Purkinje fiber bundles subjected to the same control–ischemia–reperfusion protocol. Control and ischemic solutions contained 1 x 10^{-7} M CEC, because in this setting a_{1}-adrenergic agonists induce increases in normal automaticity and the highest incidence of abnormal automaticity during simulated ischemia.17 In the first group of experiments, fiber bundles were superfused with phenylephrine (10^{-9}–10^{-7} M) after stabilization. Action potential characteristics and spontaneous rates were recorded after 15 minutes of superfusion with each concentration of phenylephrine (a steady-state response was attained within 5 minutes after the onset of superfusion). Then fiber bundles were superfused for 40 minutes with ischemic Tyrode’s solution containing 10^{-7} M phenylephrine and reperfused for 40 minutes with control Tyrode’s solution. The same protocol was used in the second group of experiments, but the control and the ischemic Tyrode’s solutions contained 10^{-7} M ryanodine.

Two additional control experiments were performed. In the first experimental group, control and ischemic solutions contained CEC and ryanodine. In the second group, both Tyrode’s solutions contained phenylephrine, ryanodine, and the a_{1} subtype–selective blockers CEC and WB 4101 (both 10^{-7} M). Finally, we used verapamil (10^{-6} M) to compare the actions of a transsarcolemmal Ca^{2+} channel–blocking drug with those of ryanodine. After equilibration of fibers in propranolol, CEC, and phenylephrine for a 40-minute control period, verapamil was added for an additional 15 minutes of control and throughout the ischemic period.

In all experiments, at the end of the ischemic superfusion, fibers were stimulated for 1 minute at cycle lengths of 500 and 300 msec; then stimulation was discontinued to observe whether delayed afterdepolarizations or triggered activity occurred.

**Biochemical Studies**

Each Purkinje fiber was homogenized in 1.5 ml buffer A (20 mM Tris-Cl, pH 8.0, 1 mM EDTA, and 2 mM β-mercaptoethanol). The homogenate was sonicated for 30 seconds. The crude membrane fraction was pelleted by centrifugation of the suspension for 60 minutes at 50,000g; the resultant pellet was suspended in 120 μl buffer A containing 1% Lubrol on ice for 1 hour. After centrifugation at 50,000g for 60 minutes, the supernatant was extracted.

The ADP-ribosylation mixture (40 μl) contained 25 mM HEPES, pH 7.5, 1 mM EDTA, 2 mM MgCl_{2}, 0.1 mM GTP, 1 mM ATP, 200 μM NAD, 10 mM thymidine, 10 mM dithiothreitol, 10 μM [32P]NAD (2 μCi per assay), 10 μg/ml PTX, and 1–3 μg supernatant extract from Purkinje fiber membranes. Samples were incubated for 90 minutes at 37°C. Forty microliters of sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) sample buffer (∗2 stock) was added to stop the reaction. Samples were then subjected to SDS-PAGE. Eleven percent polyacrylamide gels were used. The SDS-PAGE gels were stained with Comassie R250, destained, dried, and autoradiographed. Regions of the gels that corresponded to bands on the autoradiograms were excised, and the amount of radioactivity was quantified.

**Statistical Analysis**

Microelectrode data were analyzed only from impalements that were maintained throughout the course of each experimental protocol. Automaticity is reported only for those experiments in which the control automatic rates showed a variance not greater than 10%. Data are expressed as mean±SEM. The statistical technique used was analysis of variance, with Scheffe’s test when the F value permitted this.22 For experiments on the incidence of abnormal automaticity, Fisher’s exact test was used. Significance was determined at p<0.05.

**Materials**

We purchased phenylephrine and propranolol from Sigma Chemical Co., St. Louis, Mo.; CEC and WB 4101 from Research Biochemicals Inc., Natick, Mass.; ryanodine from Progressive Agri-Systems, Inc., Wind Gap, Pa.; and PTX from List Biological Laboratories, Inc., Campbell, Calif. Verapamil HCl was a gift from Knoll Pharmaceutical Co., Orange, N.J.

**Results**

**Relation Between the Levels of 41-kd G Protein and the Response to Phenylephrine of Normal, Ischemic, and Reperfused Purkinje Fibers**

When fibers were superfused with control Tyrode’s solution and driven at a basic cycle length of 500 msec (Table 1), there were no significant differences in action potential characteristics between control groups superfused in the absence or presence of phenylephrine. The action potential characteristics for PTX-treated fibers are also shown in Table 1. No statistically significant
difference in any variable was seen in the presence or absence of phenylephrine.

As previously reported, the normal automatic rates of spontaneously beating Purkinje fiber bundles were significantly lower in those superfused with phenylephrine than in those that were not treated (Table 2). The opposite relation was observed in fibers from dogs injected with PTX: in the phenylephrine-treated group, the automatic rate was significantly faster than it was in the nontreated group (Table 2).

Figure 2 shows the effects of simulated ischemia and reperfusion on an automatic Purkinje fiber in the absence of phenylephrine. During control there was a high membrane potential and regular automatic rhythm. Superfusion with ischemic Tyrode's solution led to depolarization of the membrane and the cessation of automaticity. Repolarization and gradual reemergence of normal automaticity were observed with the onset of reperfusion.

A contrasting pattern of behavior is shown in Figure 3, recorded in the presence of phenylephrine. The depolarization and cessation of normal automaticity during ischemia and the repolarization and restoration of normal automaticity on reperfusion demonstrated in Figure 2 were seen here as well. However, the quiescence during ischemia was interrupted by a spontaneous rhythm. Modulation of the automatic rhythm at the low level of membrane potential during simulated ischemia was a major focus of this study.

Membrane potential, the incidence of abnormal automaticity during simulated ischemia, and the levels of 41-kd G proteins for all groups of fibers are summarized in Table 3. When all ischemic fibers (groups 2–4 in Table 3) were grouped based on the presence (subgroup b) or the absence (subgroup a) of phenylephrine, there was a significant difference in membrane potential between the phenylephrine plus propranolol group (−61±0.6 mV, n=23) and the group receiving propranolol alone (−57±1.2 mV, n=19) (p<0.05). In contrast, phenylephrine had no effect on membrane potential during simulated ischemia in Purkinje fibers from the PTX-treated dogs (group 5).

Although there was marked variability in the G protein values in groups 1–4 of Table 3, only Purkinje fibers obtained from PTX-injected dogs (group 5) showed significantly decreased levels of the protein. Thus, the level of PTX substrate did not depend on the setting of ischemia or reperfusion or on the presence of phenylephrine.

The incidence of abnormal automaticity during simulated ischemia in Purkinje fibers from control dogs (14–17%) (Table 3) is comparable to the 20–21% reported in our previous studies. In the presence of phenylephrine, the incidence of abnormal automaticity increased to 33–38% during 40 minutes of simulated ischemia (groups 3b and 4b). In contrast, abnormal automaticity was not observed in Purkinje fibers from PTX-treated dogs in the absence or in the presence of phenylephrine (groups 5a and 5b). Hence, the functional availability of the 41-kd PTX substrate appeared to be essential for the expression of abnormal automaticity.

Effects of Ryanodine on the Phenylephrine-Induced Increases in Normal Automaticity and on the Incidence of Abnormal Automaticity During Simulated Ischemia

In our previous studies, α1-adrenergic receptor stimulation induced an increase in normal automaticity in

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**Table 1. Action Potential Characteristics of Purkinje Fibers Superfused With Control Tyrode's Solution During Drive at a Basic Cycle Length of 500 msec**

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>MDP (mV)</th>
<th>V_max (V/sec)</th>
<th>O_s (mV)</th>
<th>APD_50 (msec)</th>
<th>APD_90 (msec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Purkinje fibers</td>
<td>Prop</td>
<td>27</td>
<td>96±0.8</td>
<td>558±15</td>
<td>31±0.8</td>
<td>196±4.5</td>
</tr>
<tr>
<td></td>
<td>Prop+PE</td>
<td>29</td>
<td>97±0.8</td>
<td>539±16</td>
<td>30±1.0</td>
<td>206±6</td>
</tr>
<tr>
<td>Pertussis toxin–treated Purkinje fibers</td>
<td>Prop</td>
<td>7</td>
<td>94±2.2</td>
<td>477±27</td>
<td>27±2.0</td>
<td>174±12</td>
</tr>
<tr>
<td></td>
<td>Prop+PE</td>
<td>9</td>
<td>93±1.1</td>
<td>466±39</td>
<td>29±1.6</td>
<td>188±3</td>
</tr>
</tbody>
</table>

n, Number of fibers; MDP, maximum diastolic potential; V_max, maximum rate of rise of phase 0; O_s, overshoot; APD_50 and APD_90, action potential duration at 50% and 90% of full repolarization, respectively; Prop, propranolol (2×10⁻⁷ M); PE, phenylephrine (1×10⁻⁷ M). Values are mean±SEM.

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**Table 2. Automatic Action Potential Characteristics of Purkinje Fiber Bundles Superfused With Control Tyrode's Solution**

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Rate (bpm)</th>
<th>MDP (mV)</th>
<th>AV (mV)</th>
<th>Slope (mV/sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Purkinje fibers</td>
<td>Prop</td>
<td>27</td>
<td>24±2.3</td>
<td>93±0.8</td>
<td>84±1.4</td>
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<tr>
<td></td>
<td>Prop+PE</td>
<td>29</td>
<td>15±2.5*</td>
<td>95±0.8</td>
<td>86±0.9</td>
</tr>
<tr>
<td>Pertussis toxin–treated Purkinje fibers</td>
<td>Prop</td>
<td>7</td>
<td>5±3.0</td>
<td>91±1.5</td>
<td>83±1.6</td>
</tr>
<tr>
<td></td>
<td>Prop+PE</td>
<td>9</td>
<td>17±4.3*</td>
<td>90±1.2</td>
<td>82±1.5</td>
</tr>
</tbody>
</table>

n, Number of fibers; rate, spontaneous rate; MDP, maximum diastolic potential; AV, activation voltage; slope, slope of phase 4; Prop, propranolol (2×10⁻⁷ M); PE, phenylephrine (1×10⁻⁷ M). Values are mean±SEM.

* p<0.05 compared with the corresponding groups without PE.
potentiaL and the normal activity simulated to according

Panel B: With

FIGURE 2. Chart recordings of typical simulated ischemia and reperfusion experiment performed according to protocol 4a (Figure 1). Panel A: During control, there is an automatic rhythm occurring at a high level of membrane potential. Superfusion with “ischemic” Tyrode’s solution leads to depolarization and loss of normal automaticity. Panel B: With the onset of reperfusion, the membrane repolarizes, and the normal automatic rhythm recommences. In this figure as well as in Figures 3, 5, and 6, the horizontal line across the top of the panels indicates the period of simulated ischemia.

data show that all Purkinje fibers exposed to CEC and significantly increased the incidence of abnormal automaticity during simulated ischemia. In the experiments described in this section, CEC was added to all control and ischemic solutions in addition to propranolol to maximize α1-adrenergic increases in normal and abnormal automaticity.

Figure 4 is a representative experiment in which effects of phenylephrine on normal automaticity and on the incidence of abnormal automaticity during simulated ischemia were studied in the same Purkinje fiber bundle. Phenylephrine induced a concentration-dependent increase in normal automatic rate (panels A, B, C, and the left part of D). During simulated ischemia, isolated spontaneous beats and bursts of ectopic activity at the low level of membrane potential occurred. The normal automatic rhythm returned on reperfusion.

Figure 5 shows a typical experiment in which the effects of phenylephrine were studied in the presence of ryanodine. In contrast to the example in Figure 5, phenylephrine had no effect on the rate of normal automaticity in the presence of ryanodine (panels A, B, C, D, and the left part of E). However, bursts of ectopic activity persisted at the low level of membrane potential during simulated ischemia.

Figure 6 summarizes the effects of phenylephrine on normal automatic rate in the absence and presence of ryanodine. Ryanodine completely eliminated the phenylephrine-induced increase in normal automaticity occurring in CEC-superfused fibers (see Figure 6 legend). Ryanodine itself had no effect on the normal automatic rate (15±3.0 beats per minute before and 15±2.9 beats per minute after ryanodine action). Maximum diastolic potentials remained practically unchanged in the presence of both drugs. In the absence of ryanodine, the maximum diastolic potential was −91±0.8 mV in the control condition and −92±0.6 mV in the presence of 10−7 M phenylephrine. In the other groups of fibers, the maximum diastolic potential was −93±1.0 mV in the control condition, −92±0.7 mV in the presence of 10−7 M ryanodine, and −93±1.0 mV in the presence of ryanodine plus 10−7 M phenylephrine.

Table 4 incorporates two control groups (groups 1 and 2) and experimental groups in which the effects of ryanodine on phenylephrine-induced arrhythmias in the ischemic setting are quantified (groups 3 and 4). No significant differences among all groups of fibers occurred in membrane potential during ischemia. However, consistent with our previous study, the least membrane depolarization was seen in the presence of phenylephrine (group 2) and the most with phenylephrine plus CEC (group 3). As previously reported, the maximum diastolic potential of normal automatic fibers was highest and the rate was lowest in the presence of phenylephrine (group 2); the lowest maximum diastolic potential and the fastest rate were seen in the presence of phenylephrine plus CEC (group 3).

Table 4 further shows that with respect to abnormal automaticity during ischemia, a 10% incidence was observed with propranolol alone (group 1). In accordance with our previous results, the incidence of abnormal automaticity increased to 30% in the presence of phenylephrine (group 2) and to 70% in the presence of phenylephrine plus CEC (group 3). The stimulatory action of phenylephrine on abnormal automaticity during simulated ischemia was not affected by ryanodine: in the presence of CEC, phenylephrine, and ryanodine, there was a 60% incidence of abnormal automaticity (group 4). Ryanodine had no effect on abnormal automaticity in the absence of phenylephrine (group 5). However, in the presence of phenylephrine and ryanodine plus both subtype selective blockers of α1-adrenergic receptors (group 6), the incidence of abnormal automaticity was essentially the same as with propranolol alone. Finally, we compared the effects of ryanodine with those of verapamil (group 7). Neither maximum diastolic potential nor automatic rate of normal fibers was altered by verapamil. However, abnormal automaticity during simulated ischemia was completely abolished. The membrane potential in the ischemic setting was practically the same in the presence of verapamil as in its absence (compare with group 3).

Discussion

We previously have established the subtype specificity of α1-adrenergic induction of arrhythmias in canine.
Purkinje fibers subjected to simulated ischemia. In brief, α-adrenergic stimulation of CEC-antagonized receptors lessened membrane depolarization and inhibited abnormal automaticity, whereas stimulation of WB 4101-antagonized receptors increased membrane depolarization and enhanced abnormal automaticity. Similarly, in normally polarized fibers, activation of CEC-sensitive α1-receptors increases maximum diastolic potential and decreases automaticity, whereas stimulation of WB 4101-sensitive α1-receptors decreases maximum diastolic potential and increases automaticity. Based on this similarity of effects in the normal and ischemic settings, our premise in the present study was that the mechanism responsible for α1-receptor-induced increases of abnormal automaticity in ischemia might be the same as that for modulation of the increase in automaticity in normal fibers. If this were the case, we would anticipate abnormal automaticity during ischemia to be blocked by WB 4101 (which in fact occurs) and to be independent of the 41-kd PTX substrate.

In the present experiments, the PTX substrate did not vary with the duration of ischemia and reperfusion or in the presence or absence of phenylephrine. That phenylephrine hyperpolarized the fibers during the control condition, as well as during ischemia, suggests a continued functional role for this family of G proteins. In contrast, in fibers obtained from dogs pretreated with intravenous PTX, in which the substrate was ADP-ribosylated, phenylephrine had no effects on membrane potential in control or in ischemic Tyrode’s solution and no longer induced abnormal automaticity during ischemia.

Since the presence of the PTX substrate is not necessary for the positive chronotropc response of Purkinje fibers having normal automaticity, the present study emphasizes that the increase in normal automaticity by α1-agonists is via a different signal transduction mechanism than the increase in abnormal automaticity occurring during ischemia. We state this because, if the α1-induced increase in abnormal automaticity involved the same receptor-effector pathway as the α1-induced

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**TABLE 3.** Membrane Potential, Spontaneous Rate, the Incidence of Abnormal Activity, and the ADP-Ribosylatable Substrate Level in All Groups of Fibers Presented in Figure 1

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>MDP (−mV)</th>
<th>Rate (bpm)</th>
<th>MDP (−mV)</th>
<th>Fibers</th>
<th>MDP (−mV)</th>
<th>Rate (bpm)</th>
<th>G protein (pmol/mg)</th>
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<tr>
<td></td>
<td></td>
<td>Control</td>
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<td>Reperfusion</td>
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<td>Control Purkinje fibers</td>
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</tr>
<tr>
<td>1a</td>
<td>8</td>
<td>92±1.3</td>
<td>26±3</td>
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<td>...</td>
<td>34.9±4.3</td>
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<tr>
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<td>...</td>
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<td>...</td>
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<td>3b</td>
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<td>12±3</td>
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<td>25±4</td>
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<td>8</td>
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<td>14±3</td>
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<td>3 38</td>
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<td>14±3</td>
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<td>Pertussis toxin-treated Purkinje fibers</td>
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</tbody>
</table>

Group, number of each group corresponding to the protocol number in Figure 1; N, number of fibers per group; MDP, maximum diastolic potential; rate, spontaneous rate; n, %, number and percent of fibers showing abnormal activity during ischemia. Values are mean±SEM.

* p<0.05 compared with G protein values for all groups of control Purkinje fibers.

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**FIGURE 4.** Recordings showing the effects of phenylephrine (PE) on normal automaticity and the incidence of abnormal automaticity during simulated ischemia. All solutions contain propranolol and chloroethylclonidine (10−7 M). Panel A: Automatic rhythm after 40 minutes of adaptation in control Tyrode's solution. Panels B, C, and the left part of D: 15 minutes of action of three PE concentrations. Spontaneous rate in beats per minute is indicated in the top of each panel. There is a concentration-dependent increase in automaticity. Maximum diastolic potential remains practically unchanged. Panel D: Imposition of ischemia. The membrane depolarizes and normal automaticity ceases. Isolated spontaneous beats occur. Panel E: A self-initiating and self-terminating burst of ectopic activity at the low level of membrane potential during "ischemia." Panel F: Reperfusion resulting in the repolarization and reinitiation of the normal automatic rhythm. Control and ischemic Tyrode's solutions contained 2×10−7 M propranolol and 1×10−7 M chloroethylclonidine. Ischemic solution also contained 1×10−7 M PE.
increase in normal automaticity, we would anticipate an increase in abnormal automaticity in the isometric Purkinje fibers from PTX-treated dogs. These results support two conclusions: 1) The receptor-effector coupling mechanisms by which α₁-adrenergic receptors increase automaticity at high membrane potentials in normal Purkinje fibers and abnormal automaticity at low membrane potentials in ischemic Purkinje fibers are different. 2) The PTX substrate plays an important role in the α₁-adrenergic enhancement of automaticity in the setting of ischemia but not in the enhancement of automaticity in normal fibers.

Our pharmacological experiments with ryanodine provide further insights into the mechanisms involved in the control of automaticity in normal and ischemic fibers. That ryanodine prevented the phenylephrine-induced increase in normal automaticity in fibers treated with CEC suggests that either the mobilization of free intracellular calcium or, in consonance with the work of Endoh and Blinks, a sensitization of a component of the receptor-effector coupling pathway to the effects of calcium is essential for the α₁-induced positive chronotropic response of normal fibers.

That the effects of ryanodine differed from those of verapamil supports the contention that a specific action of ryanodine was necessary rather than a transsarcolemmal calcium channel-blocking action. Invoking a pathway that involves an increase in free intracellular Ca²⁺ in the induction of normal automaticity requires the identities of the intermediary steps involved. We have previously suggested that it is the stimulation of phosphatidylinositol metabolism by α₁-agonists that induces increased automaticity.6,9 Heathers and colleagues10,11 have demonstrated that α₁-agonists do increase phosphatidylinositol metabolism in the canine heart. Experiments on rat heart have shown that increases in phosphatidylinositol metabolism are not dependent on a PTX substrate.8,9 The major problem here is that studies by Endoh and Blinks13 in the rabbit heart and Terzic et al.23 in the rat heart have suggested there is little or no increase in free [Ca²⁺], in response to α₁-agonists, even though a physiological response consistent with a Ca²⁺-dependent mechanism (i.e., contractility) is enhanced. This led Endoh and Blinks to suggest that sensitization to the actions of Ca²⁺ in the receptor-effector pathway (including the contractile apparatus) was involved. It is important in relating these various studies to one another and to our own work to understand that these are important interspecies differences in α₁-adrenergic receptor-effector coupling.24 Complete resolution of the α₁-adrenergic receptor-effector coupling pathway with respect to normal automaticity awaits answers to the following questions: Is [Ca²⁺]
increased as a result of the α-adrenergic effect on phosphatidylinositol metabolism? Is the increase in phosphatidylinositol metabolism essential to the increase in automaticity or merely a parallel event?

In contrast to normal automaticity, the phenylephrine-induced increase in abnormal automaticity during ischemia was not altered in the presence of ryanodine, suggesting that changes in intracellular calcium do not play a key role here. Earlier experiments and preliminary data permit some speculation about the mechanism involved. We have previously shown that potassium conductance (gK) of disaggregated Purkinje myocytes is reduced by phenylephrine and that the mechanism is abolished by pretreatment with PTX and antagonized by prazosin.4 In the present studies, the α1-adrenergic effect to increase automaticity in ischemic fibers did not occur with PTX pretreatment. Moreover, the α1-adrenergic effect on ischemic fibers is blocked by the α1 subtype-selective antagonist WB 4101.17 If, in fact, changes in gK are important to this type of abnormal automaticity, it would be expected that interventions that increase or decrease gK should respectively decrease or increase α1-adrenergic–induced abnormal automaticity in simulated ischemia. Preliminary experiments25 have demonstrated that Ba2+, which decreases gK,26,27 and acetylcholine, which increases gK,28,29 respectively enhance and suppress the action of phenylephrine on abnormal automaticity. These results, if borne out, would support a hypothesis that a decrease in gK is an important mechanism for α1-induced increases in abnormal automaticity in ischemic fibers.

In conclusion, we propose that different receptor–effector pathways induce the α1-adrenergic positive chronotropic responses of normal and ischemic Purkinje fibers. For normal fibers, calcium release from sarcoplasmic reticulum via a WB 4101–blocked pathway independent of the 41-kd family of PTX-sensitive G proteins appears to be a principal component. In contrast, α1-adrenergic effects on abnormal automaticity are blocked by WB 4101, are PTX substrate dependent, and are not suppressed by ryanodine. Preliminary data suggest that a decrease in gK may be important here.25

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References

Table 4. Effects of Ryanodine on Phenylephrine-Induced Abnormal Automaticity During Simulated Ischemia

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>MDP (mV)</th>
<th>Automatic rate (bpm)</th>
<th>AA (%)</th>
<th>MDP (mV)</th>
<th>Automatic rate (bpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Prop</td>
<td>94±1.7</td>
<td>20±4</td>
<td>10</td>
<td>91±1.9</td>
<td>20±4</td>
</tr>
<tr>
<td>2</td>
<td>Prop+PE</td>
<td>95±1.4</td>
<td>12±3</td>
<td>30</td>
<td>94±1.3</td>
<td>12±3</td>
</tr>
<tr>
<td>3</td>
<td>Prop+CEC+PE</td>
<td>92±0.6</td>
<td>23±2</td>
<td>70</td>
<td>93±0.7</td>
<td>10±1</td>
</tr>
<tr>
<td>4</td>
<td>Prop+CEC+Ry+PE</td>
<td>93±1.0</td>
<td>16±3</td>
<td>60</td>
<td>93±1.0</td>
<td>13±3</td>
</tr>
<tr>
<td>5</td>
<td>Prop+C+Ry</td>
<td>94±1.0</td>
<td>19±4</td>
<td>60</td>
<td>94±0.9</td>
<td>19±5</td>
</tr>
<tr>
<td>6</td>
<td>Prop+C+WB+Ry+PE</td>
<td>92±0.9</td>
<td>17±4</td>
<td>60</td>
<td>92±0.9</td>
<td>15±4</td>
</tr>
<tr>
<td>7</td>
<td>Prop+CEC+Verap+PE</td>
<td>90±1.6</td>
<td>23±3</td>
<td>0</td>
<td>90±1.3</td>
<td>21±3</td>
</tr>
</tbody>
</table>

MDP, maximum diastolic potential; bpm, beats per minute; AA, abnormal automaticity (percentage of fibers in which abnormal automaticity was observed); Prop, propranolol (2×10−7 M); PE, phenylephrine (10−7 M); CEC, chloroethylclonidine (10−7 M); Ry, ryanodine (10−7 M); WB, WB 4101 (10−7 M); Verap, verapamil (10−8 M). Values are mean±SEM; n=10 per group.

*p<0.05 compared with the corresponding control values.
†p<0.05 compared with corresponding values for group 1.
Positive chronotropic responses induced by alpha 1-adrenergic stimulation of normal and "ischemic" Purkinje fibers have different receptor-effector coupling mechanisms. E P Anyukhovsky, V O Rybin, A V Nikashin, O P Budanova and M R Rosen

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