Abnormal Automatic Rhythms in Ischemic Purkinje Fibers Are Modulated by a Specific $\alpha_1$-Adrenergic Receptor Subtype

Evgeny P. Anyukhovsky, PhD and Michael R. Rosen, MD

Background. Recent advances in adrenergic pharmacology have made possible the identification of $\alpha_1$-adrenergic receptor subtypes using the specific blockers chloroethylclonidine and WB 4101.

Methods and Results. In the present study, we used these two blockers to determine the mechanisms responsible for automatic rhythms occurring during simulated ischemia and reperfusion of isolated canine Purkinje fibers. Experiments were done in the presence of propranolol to minimize $\beta$-adrenergic contributions to the rhythms studied. In the control situation, all fibers showed membrane potentials greater than $-90$ mV and normal automatic rhythms. During simulated ischemia, membrane potential depolarized to the $-60$ mV range. Abnormal automaticity was seen in 20% of fibers not treated with phenylephrine and in 50% of those superfused with $1 \times 10^{-7}$ M phenylephrine. The incidence of abnormal automaticity was reduced to 0% by WB 4101 (which blocks phosphoinositide metabolic effects of $\alpha_1$-adrenergic stimulation in the heart) and was increased to 90% by chloroethylclonidine (which blocks Na-K pump-stimulating effects of $\alpha$-agonists). Moreover, the ischemic fibers were significantly more hyperpolarized during superfusion with WB 4101 than with chloroethylclonidine. Triggered activity induced by delayed or early afterdepolarizations was not seen in any experiment.

Conclusions. Automatic arrhythmias induced by $\alpha_1$-adrenergic stimulation during simulated ischemia may be attributed to a specific $\alpha_1$-adrenergic receptor subtype that is blocked by WB 4101. These results have important implications with respect to the induction of arrhythmias in the setting of ischemia and the means for their prevention or treatment. (Circulation 1991;83:2076–2082)

In normal canine Purkinje fibers, $\alpha_1$-adrenergic stimulation induces either a decrease or an increase in automaticity.1 The decrease appears to result from the linkage of the $\alpha_1$-receptor to the Na-K pump.2 3 This receptor-effector pathway is transduced by a GTP regulatory protein that is a pertussis toxin substrate.1 In contrast, the increase in automaticity appears to be associated with enhanced phosphoinositide metabolism and resultant increases in intracellular $\text{Ca}^{2+}$ concentration.1 The transduction pathway is a GTP-dependent process, but the regulatory protein appears not to be a pertussis toxin substrate.

The complexity of the $\alpha_1$-adrenergic responsiveness begins at the level of the receptors themselves, which consist of at least two different subtypes that are identifiable through the use of selective blockers.4 One of these is the alkylating agent, chloroethylclonidine; the other, the competitive $\alpha_1$-adrenergic antagonist, WB 4101. In normal canine Purkinje fibers, chloroethylclonidine blocks the decrease in automaticity induced by $\alpha_1$-adrenergic stimulation.1 In contrast, WB 4101 is a competitive antagonist of the $\alpha_1$-adrenergic increase in automaticity in normal Purkinje fibers that is associated with enhanced phosphatidylinositol metabolism.1

The fact that $\alpha_1$-adrenergic stimulation is subtype specific may have important implications with respect to arrhythmias occurring during cardiac ischemia, because $\alpha_1$-adrenergic receptor stimulation is held responsible for a spectrum of tachyarrhythmias following ischemia and reperfusion in the intact feline heart.5–7 Studies in isolated canine Purkinje fibers have furthered our understanding of possible mechanisms for these arrhythmias. For example, experiments using a modified Tyrode’s solution to mimic an
ischemic environment have shown that phenylephrine induces an increase in abnormal automatic rhythms occurring at low membrane potentials that can be terminated with prazosin but not propranolol.8 The purpose of the present experiments was to test the subtype specificity of a1-adrenergic induction of arrhythmias during simulated cardiac ischemia. Hence, we studied the effects of WB 4101 and of chloroethylnonidine on arrhythmias occurring in "ischemic" and "reperfused" Purkinje fibers.

**Methods**

Mongrel dogs weighing 10–20 kg were anesthetized with 30 mg/kg i.v. sodium pentobarbital. Their hearts were removed 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 5.5, and KCl 2.7. Purkinje fiber bundles were excised from the right and left ventricles and placed in a Lucite chamber perfused with Tyrode’s solution warmed to 37°C. Solutions were pumped through the chamber at a rate of 12 ml/min, changing chamber content three times each minute in both the control and ischemic settings. The chamber temperature was 37±0.3°C, and pH during nonischemic periods was 7.3±0.5. All fiber bundles were impaled with 3 M KCl–filled glass capillary microelectrodes having tip resistances of 10–20 MΩ. The electrodes were coupled by an Ag/AgCl junction to a WPI amplifier (model KS-700, World Precision Instruments, New Haven, Conn.). The transmembrane action potentials were displayed on an oscilloscope (model 5115, Tektronix, Beaverton, Ore.) and a Gould-Brush strip-chart recorder (model 2400, Gould, Cleveland, Ohio). The photographs were made on Polaroid film for subsequent analysis. The tissue chamber was connected to ground through a 3-M KCl/Ag/AgCl junction.

For stimulation of Purkinje fiber bundles, standard techniques were used to deliver square-wave pulses 1.0 msec in duration and 1.5 times threshold via bipolar Teflon-coated silver electrodes.3 In all fibers transmembrane action potential characteristics were measured both during spontaneous activity and during periods of stimulation. To determine pH, PCO2, and PO2 of the superfusates a blood gas analyzer (model 158, Corning Glass Inc., Corning, N.Y.) was used.

**Protocols**

After equilibration in control Tyrode’s solution, superfusion was begun with one of two solutions modified to mimic some of the components of an ischemic environment.9 One ischemic solution (normal-calcium ischemic Tyrode’s) contained (mM) CaCl2 2.7, NaCl 137, NaHCO3 5, NaH2PO4 1.8, KCl 10, MgCl2 0.5, and dextrose 5.5. In the second ischemic solution (high-calcium ischemic Tyrode’s), all components were identical to the first, except that CaCl2 was increased to 10.8 mM and NaCl was reduced to 129 mM. In previous experiments we have shown that this elevation of extracellular Ca2+ concentration, in its own right, has no effect on automaticity in the control and ischemic settings and increases automaticity during reperfusion.8 In both solutions, reduction of [HCO3-] to 5 mM reduced pH to approximately 6.7 in the presence of 95% N2–5% CO2. Gases were delivered in the stock bottles and via bubbling directly into an equilibration chamber immediately proximal to the tissue bath. The superfusate delivery system was isolated completely from room air, and all connecting tubes were made of glass to minimize the gas exchange. In this way, bath PO2 could be maintained at 10–25 mm Hg for the duration of the ischemic period.8

After the automatic rhythm had stabilized, all Purkinje fibers were superfused for 40 minutes in control Tyrode’s solution and then for 40 minutes with ischemic Tyrode’s solution; they then were reperfused for 40 minutes with control Tyrode’s solution. Spontaneous transmembrane action potential characteristics were recorded throughout the experiment, and measurements reported here were recorded at the end of the equilibration and reperfusion periods. After 35 minutes of equilibration, ischemia, and reperfusion, Purkinje fibers were stimulated for 1 minute at cycle lengths of 500 and 300 msec, and the action potential characteristics were measured. We then discontinued stimulation to observe whether delayed afterdepolarizations or triggered activity occurred.

The experiments were performed on eight groups, consisting of 10 fibers each (Table 1). We selected the phenylephrine concentrations (5×10⁻⁸ and 1×10⁻⁷ M) based on our previous studies,1,3,8 which showed that these concentrations have a pronounced α-adrenergic receptor effect on the electrical activity of Purkinje fibers. To exclude the influence of β-adrenergic receptor stimulation, 2×10⁻⁷ M propranolol was added to all solutions. We have previously demonstrated this concentration to have no effect on the transmembrane potential or automaticity in control, ischemic, or reperfused fibers.8 The concentrations selected of chloroethylnonidine and WB 4101 (both 1×10⁻⁷ M) were the highest that we had previously shown to have no effect, alone, on automaticity or on the transmembrane potentials of Tyrode’s superfused Purkinje fibers.1 Moreover, in control experiments (Table 2) we have found that chloroethylnonidine (1×10⁻⁷ M) has no effect on automaticity in the control, ischemic, or reperfused setting. Hence, despite reports of its actions as a partial α1-agonist,1,9 there is no evidence for such an effect in the present study. As shown in Table 1, in each group of fibers all the pharmacological agents investigated were added to the control solutions as well as to the ischemic solutions. This permitted us to investigate their effects both on normal automaticity and on electrical activity of Purkinje fibers during simulated ischemia.

**Pharmacological Agents**

We purchased phenylephrine and propranolol from Sigma Chemical Co., St. Louis, and chloroeth-

Statistical Analysis

Data are expressed as mean±SEM. The statistical technique used was analysis of variance, with Scheffe’s test when the F value (by analysis of variance) permitted this.10 For experiments on the incidence of abnormal automaticity, Fisher’s exact test was used. Significance was determined at p<0.05.

Results

Figure 1 is a representative experiment, illustrating the characteristics of a normal automatic focus under control, ischemic, and reperfused conditions in the absence of an α-agonist. Note the high membrane potential and regular rhythm occurring during control, the depolarization of the membrane and quiescence of the fiber subsequent to the onset of ischemia, the interposition of pacing during ischemia, and the repolarization and gradual reemergence of normal automaticity on reperfusion. A contrasting pattern of behavior is shown in Figure 2, recorded in the presence of 1×10^{-7} M phenylephrine. The initial depolarization and cessation of normal automaticity during ischemia and hyperpolarization and reemergence of normal automaticity on reperfusion demonstrated in Figure 1 are seen here as well. However, the quiescence during ischemia is interrupted by a spontaneous rhythm manifesting both phase 4 depolarization and early afterdepolarizations. Modulation of the type of automatic rhythm demonstrated in Figure 2 was the major focus of this study. The occurrence of early afterdepolarizations was sporadic and, hence, not quantified. As for delayed afterdepolarizations and triggered activity, these were never seen.

The addition of phenylephrine to propranolol, alone and in the presence of WB 4101 or chloroethylclonidine, had important effects not only on abnormal automaticity but also on normal automatic rate and on membrane potential. The dose dependence of the α₁-adrenergic effects on automaticity is seen as follows: in control solutions with propranolol alone, automatic rate was 28±4.5 beats/min. This was reduced to 24±5.5 beats/min with propranolol plus

### Table 1. Pharmacological Interventions Performed During Simulated Ischemia and Reperfusion Protocols

<table>
<thead>
<tr>
<th>Control</th>
<th>Ischemia</th>
<th>Reperfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>CT</td>
<td>NCIT</td>
<td>CT</td>
</tr>
<tr>
<td>CT+10^{-7} M PE</td>
<td>NCIT+10^{-7} M PE</td>
<td>CT+10^{-7} M PE</td>
</tr>
<tr>
<td>CT+10^{-7} M PE+CEC</td>
<td>NCIT+10^{-7} M PE+CEC</td>
<td>CT+10^{-7} M PE+CEC</td>
</tr>
<tr>
<td>CT+10^{-7} M PE+WB</td>
<td>NCIT+10^{-7} M PE+WB</td>
<td>CT+10^{-7} M PE+WB</td>
</tr>
<tr>
<td>CT+CEC</td>
<td>NCIT+CEC</td>
<td>CT+CEC</td>
</tr>
<tr>
<td>CT+5x10^{-8} M PE</td>
<td>NCIT+5x10^{-8} M PE</td>
<td>CT+5x10^{-8} M PE</td>
</tr>
<tr>
<td>CT+5x10^{-8} M PE+CEC</td>
<td>NCIT+5x10^{-8} M PE+CEC</td>
<td>CT+5x10^{-8} M PE+CEC</td>
</tr>
<tr>
<td>CT+5x10^{-8} M PE+CEC</td>
<td>HCIT+5x10^{-8} M PE+CEC</td>
<td>CT+5x10^{-8} M PE+CEC</td>
</tr>
</tbody>
</table>

CT, control Tyrode’s solution; NCIT, normal-calcium ischemic Tyrode’s solution; HCIT, high-calcium ischemic Tyrode’s solution; PE, phenylephrine; CEC, chloroethylclonidine; WB, WB 4101. In all experiments 10^{-7} M CEC and WB 4101 were used. All Tyrode’s solutions contained 2x10^{-7} M propranolol.

### Table 2. Incidences of Normal and Abnormal Automaticity During Ischemia and Reperfusion

<table>
<thead>
<tr>
<th>Automaticity conditions</th>
<th>Control Normal</th>
<th>Control Abnormal</th>
<th>Ischemia Normal</th>
<th>Ischemia Abnormal</th>
<th>Reperfusion Normal</th>
<th>Reperfusion Abnormal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiments using 1×10^{-7} M PE</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>2*</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Prop</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>5†</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Prop+PE</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Prop+PE+WB</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Prop+PE+CEC</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>9†</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Experiments using 5×10^{-8} M PE</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>2‡</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Prop+PE</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>2‡</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Prop+CEC</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>4‡</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Prop+PE+CEC</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>4‡</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Prop+PE+CEC+10.8 mM Ca^{2+}</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>9</td>
<td>10</td>
<td>0</td>
</tr>
</tbody>
</table>

Normal, normal automaticity; Abnormal, abnormal automaticity; PE, phenylephrine; Prop, propranolol; WB, WB 4101; CEC, chloroethylclonidine; n=10 for all conditions.

*p<0.05 vs. corresponding value for Prop+PE+CEC (for 1×10^{-7} M PE); †p<0.05 vs. corresponding value for Prop+PE+WB; ‡p<0.05 vs. corresponding value for Prop+PE+CEC+10.8 mM Ca^{2+}.
5×10⁻⁶ M phenylephrine and to 15±3.8 beats/min with propranolol plus 1×10⁻⁷ M phenylephrine (p<0.05). Figure 3 demonstrates the decrease in control automatic rate induced by propranolol plus 1×10⁻⁷ M phenylephrine. The rate was further reduced to 11±4.7 beats/min with propranolol plus phenylephrine in the presence of WB 4101, and increased to 22±4.9 beats/min with propranolol plus phenylephrine in the presence of chloroethylclonidine (p<0.05).

During control and reperfusion, membrane potential in the presence of chloroethylclonidine was significantly lower than that in the other groups (p<0.05); in the presence of WB 4101, membrane potential was higher (Figure 4). Similarly, during ischemia, the greatest membrane depolarization was seen in the presence of chloroethylclonidine, and the least was seen in the presence of WB 4101.

Table 2 summarizes experiments in which rhythm and arrhythmias in the presence of 1×10⁻⁷ M phenylephrine are quantified. All preparations showed only normal automaticity during control, regardless of whether the superfusate was the control solution containing 2×10⁻⁷ M propranolol alone, propranolol plus phenylephrine, or these two drugs plus either WB 4101 or chloroethylclonidine. The 20% incidence of abnormal automaticity during ischemia (Table 2) is comparable with the 21% reported in our initial study. The incidence of abnormal automaticity during ischemia increased to 50% in the presence of 1×10⁻⁷ M phenylephrine. Abnormal automaticity was completely abolished by WB 4101 and increased to 90% by chloroethylclonidine.

The effects of the various interventions on transmembrane action potentials during pacing at a basic cycle length of 500 msec are reviewed in Figure 5. As stated earlier, the concentrations of antagonists used were those that, alone, have no effect on the action potential. The fibers with lowest maximum diastolic potential during the entire control/ischemia/reperfusion sequence were those in the presence of chloroethylclonidine. No significant differences among the groups occurred for maximum rate of rise of phase 0 of the action potential. Action potential duration at 90% repolarization was shortest in the presence of WB 4101 during ischemia and was longest in the presence of chloroethylclonidine. This is consistent with block of the Na-K pump–stimulating actions of phenylephrine by chloroethylclonidine.

The relation of extracellular calcium concentration to the effects of phenylephrine on automaticity during ischemia and to the increase in abnormal automaticity seen with phenylephrine plus chloroethylclonidine are shown in Table 2. Here, we used concentrations of phenylephrine (5×10⁻⁸ M) and chloroethylclonidine (1×10⁻⁷ M) that, alone, had no effect on abnormal automaticity during ischemia. Controls were not done for abnormal automaticity.
during ischemia in the presence of propranolol plus 10.8 mM Ca^{2+}, because data reported previously by our laboratory^{8} combined with preliminary data (Olga Budanova, personal communication) indicate that 33% of 21 Purkinje fibers showed abnormal automaticity in this setting. This does not differ significantly from the values for propranolol plus phenylephrine, propranolol plus chloroethylclonidine, or propranolol plus phenylephrine plus chloroethylclonidine shown in Table 2. However, in the presence of 10.8 mM Ca^{2+} plus 5 \times 10^{-8} \text{M phenylephrine}, 1 \times 10^{-7} \text{M chloroethylclonidine}, and propranolol, there was a significant increase in automaticity.

**Discussion**

Transient occlusion of coronary vessels has long been known to result in arrhythmias during ischemia and during reperfusion. In a previous publication,^{8} we had demonstrated the contribution of \( \alpha_1 \)-adrenergic
stimulation to the initiation of ischemic, automatic arrhythmias, as well as $\alpha_1$- and $\beta$-adrenergic contributions to reperfusion arrhythmias. In the present study, we concentrated solely on $\alpha_1$-adrenergic mechanisms; hence, we used the $\beta$-blocker propranolol throughout. The reason for focusing on so finite a subject as the $\alpha_1$-adrenergic receptor was the ability, using new antagonists, to attempt the subclassification of $\alpha_1$-adrenergic effects on rhythm. We believed that this would have important implications both from the point of view of understanding the mechanism as well as from the point of view of potential therapy.

With respect to therapy, data published in the 1960s indicated that a nonselective $\alpha_1$- and $\alpha_2$-blocker, phentolamine (which also evidenced quinidine-like actions), suppressed arrhythmias that occurred in patients with myocardial ischemia and infarction. It is unclear why the promising early reports of efficacy here were not followed up. It may be that the concurrent recognition of the antiarrhythmic actions of lidocaine, plus the relative unavailability of phentolamine (the major use of which was in testing for pheochromocytoma), made the former the more promising drug. In addition, the side effects of nonselective $\alpha$-blockade, especially hypotension, would be of concern. The availability of $\alpha_1$-adrenergic subtype–selective blockers suggests the possibility of developing an antiarrhythmic modality having far greater specificity and less toxicity than was previously the case. However, we are not implying that the two agents used here, chloroethylclonidine and WB 4101, are prototypical agents that might be developed for clinical needs. Although the latter is a competitive blocker, the former is an alkylating agent having effects that appear to be irreversible, and it has partial agonistic actions, as well.

In studies of normal ventricle, WB 4101 blocks the $\alpha_1$-adrenergic agonist–induced increase in phosphoinositide metabolism. In so doing, WB 4101 would be anticipated to reduce the increase in free intracellular calcium that results from metabolism of phosphoinositides. WB 4101 also blocks completely the increase in automaticity that $\alpha_2$-agonists induce in normal Purkinje fibers having high levels of membrane potential. This action uses an $\alpha_1$-adrenergic receptor/effector pathway whose signal transduction is independent of the 41-kDa family of GTP regulatory proteins.

In contrast, chloroethylclonidine abolishes the decrease in automaticity that is induced in Purkinje fibers having high levels of membrane potential. This decrease in automaticity has been shown to depend on signal transduction via a 41-kDa GTP regulatory protein that is a pertussis toxin substrate. Moreover, the effector limb of this pathway has been studied in detail and appears to depend on stimulation of an outward current that is carried by the Na-K pump and is associated with a decrease in intracellular sodium activity. The fact that this decrease in free intracellular sodium is seen as a primary event (i.e., is not preceded by an $\alpha_1$-adrenergic–induced increase in free intracellular sodium activity) has led to the suggestion that the decrease in automaticity may not be dependent on a second messenger.

In the face of this body of information, the results of the present study provide both useful confirmatory data about normal automaticity and useful new data about abnormal automaticity in the setting of simulated ischemia. With respect to normal automaticity, it is clear that in the control superfusate, which contained propranolol, phenylephrine induced a concentration-dependent decrease in automatic rate. This decrease was antagonized by chloroethylclonidine and potentiated by WB 4101, as would be anticipated from our previous work. Of note, as well, is that, in the presence of chloroethylclonidine, the fibers were significantly more depolarized than in the presence of WB 4101 (although chloroethylclonidine and WB 4101, when superfused alone, have no significant effect on membrane potential). All these data support the view that chloroethylclonidine antagonizes an Na-K pump-stimulating effect of phenylephrine, whereas WB 4101 leaves the pump-stimulating pathway fully operative.

It is in the setting of simulated ischemia that the most dramatic differences between the effects of the antagonists were seen. In considering ischemic arrhythmias, we were working with abnormal automaticity only. We saw no delayed afterdepolarizations, and early afterdepolarizations were too sporadic in their occurrence to permit systematic analysis. The reason for the lack of delayed afterdepolarizations during ischemia and reperfusion may relate to the Po2 of the superfusate. Other investigators have reported delayed afterdepolarizations during ischemia and reperfusion have used Po2 of 40–50 mm Hg. In preliminary experiments with Po2 of 40–50 mm Hg and in the presence of high levels of extracellular calcium and $\alpha$-agonist, we have been able to reproduce these results. However, it appears that at lower Po2 delayed afterdepolarizations do not occur, an observation that has been made for guinea pig heart as well. We insisted on using a low Po2 because we wished to mimic a setting approaching anoxia rather than modest hypoxia. As in our previous study, the $\alpha$-agonist induced automaticity in 50% of ischemic preparations (previously shown to be blocked by prazosin). The fact that WB 4101 completely suppressed the arrhythmias whereas chloroethylclonidine significantly enhanced them can be explained by the subtype–specific actions of the antagonists. Moreover, the fact that the ischemic fibers were significantly more depolarized in the presence of chloroethylclonidine than WB 4101 also lends itself to understanding the subtype specificity of the antagonists. It is known that abnormal automatic rhythms are influenced importantly by the membrane potential of preparations. Indeed, the higher the membrane potential, the more that fibers move out of the range of the abnormal automatic pacemaker mechanism and into the range where the normal pacemaker current is, $i_n$ acting. Hence, one mechanism whereby the two antagonists may be inducing the changes seen in automatic arrhythmias may be
via the relative hyperpolarization of the membrane seen with agonist and WB 4101 (antiarrhythmic) and the relative depolarization of the membrane seen with agonist and chloroethylclonidine (arrhythmogenic). This argument would suggest as well that greater Na-K pump stimulation occurs in the presence of WB 4101, whereas pump stimulation is blocked more in the presence of hypoxia and chloroethylclonidine than in the presence of hypoxia alone.

In addition to the above, it is possible to relate the ability of α1-adrenergic stimulation to increase automaticity to a pathway that involves phosphoinositide metabolism and, with this, increases in intracellular calcium. Molina-Viamonte et al.20 have previously suggested that the increase in free intracellular calcium might tend to enhance automaticity via a nonspecific cation conductance. To the extent that WB 4101 blocks the phosphoinositide metabolic-stimulating action of α1-agonists, we would expect the related increase in automaticity to be antagonized (as was, indeed, the case: see Table 2). To the extent that chloroethylclonidine, by blocking the Na-K pump-stimulating actions of α-agonist, would free additional agonist to act at the phosphoinositide metabolic stimulating site, we would expect arrhythmogenicity to be enhanced. This, too, was the case (see Table 2). Hence, both the membrane potential–dependent effects of α-agonist and the actions on phosphoinositide metabolism may be important contributors to the arrhythmogenic actions of chloroethylclonidine and the antiarrhythmic actions of WB 4101.

It is important to stress that, even though chloroethylclonidine is a partial agonist, this action did not express itself in the present studies (see Table 2). Hence, the heightened arrhythmogenicity of the α1-agonist in the presence of chloroethylclonidine can be attributed to blockade rather than stimulation of α1-receptors (the latter we have previously shown to decrease automaticity and increase maximum diastolic potential19). Moreover, the important role of calcium in these experiments is also emphasized in Table 2, where a concentration of calcium that, in its own right, has no significant action on automaticity8 induced a marked increase when added to subthreshold concentrations of phenylephrine and chloroethylclonidine.

In conclusion, α1-adrenergic subtype blockers have specific actions on automatic arrhythmias that occur during ischemia. These actions are important both from the point of view of our understanding the mechanisms responsible for the automatic rhythms and from the point of view of considering new means—or reconsideration of old means—for therapy. A number of questions remain unanswered and are to be the subject of further investigation, including possible changes in α1-adrenergic receptor subtypes in the ischemic myocardium. Certainly, previous studies6–7 have suggested that there is an overall increase in α1-receptor number after ischemia and reperfusion in the cat heart in vivo. Whether these derive from one particular subtype remains to be seen.

Acknowledgments
The authors express their gratitude to Dr. Irina Golyakhovsky for her assistance in performing certain of these studies and to Mrs. Eileen Franey for her careful attention to the preparation of the manuscript.

References
13. Han C, Abel PW, Minneman KP: α1-Adrenoceptor subtypes linked to different kinds of mechanisms for increasing intracellular Ca2+ in smooth muscle. Nature (Lond) 1987;329:333

Key Words: ischemia · reperfusion · electrophysiology · chloroethylclonidine · WB 4101 · sympathetic modulation
Abnormal automatic rhythms in ischemic Purkinje fibers are modulated by a specific alpha 1-adrenergic receptor subtype.

E P Anyukhovsky and M R Rosen

Circulation. 1991;83:2076-2082
doi: 10.1161/01.CIR.83.6.2076

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1991 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/83/6/2076

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org//subscriptions/