An \( \alpha_1 \)-Adrenergic Receptor Subtype Is Responsible for Delayed Afterdepolarizations and Triggered Activity During Simulated Ischemia and Reperfusion of Isolated Canine Purkinje Fibers

Victor Molina-Viamonte, MD; Evgeny P. Anyukhovsky, PhD; and Michael R. Rosen, MD

**Background.** We used standard microelectrode techniques to study delayed afterdepolarizations and triggered activity induced by \( \alpha_1 \)-adrenergic stimulation in canine Purkinje fibers in the setting of simulated ischemia and reperfusion.

**Methods and Results.** The ischemic environment included 10.8 mM \([Ca^{2+}]_0\), 10 mM \([K^+]_0\), 40–50 mm Hg \(Po_2\), 20 mM lactate (pH 6.7), and \(1 \times 10^{-7}\) M phenylephrine. During ischemia, there was the variable occurrence of abnormal automaticity, early afterdepolarizations, and delayed afterdepolarizations. During reperfusion, 100% of preparations manifested delayed afterdepolarizations and 40% manifested triggered activity. Decreasing \(Po_2\) to less than 20 mm Hg markedly reduced the incidence of delayed afterdepolarizations and triggered activity, as did increasing \(Po_2\) to values of more than 90 mm Hg. WB 4101, an \( \alpha_1 \)-subtype–selective competitive blocker that antagonizes the effects of \( \alpha_1 \)-agonists to induce phosphoinositide metabolism and increase \([Ca^{2+}]_0\), significantly reduced the incidence of delayed afterdepolarizations and triggered activity. In contrast, the \( \alpha_2 \)-subtype–selective blocker chloroethylclonidine, an alkylating agent, had no effect on afterdepolarizations or triggered activity.

**Conclusions.** Our results indicate that a specific \( \alpha_1 \)-adrenergic pathway is involved in the induction of triggered activity in the setting of ischemia and reperfusion and suggest that interventions used to block this specific pathway have the potential to be antiarrhythmic. They also emphasize the importance of the magnitude of hypoxia in the expression of the arrhythmias. (*Circulation* 1991;84:1732–1740)

Delayed afterdepolarizations are oscillations in membrane potential that generate a subset of cardiac arrhythmias referred to as “triggered.”1 Although initial studies of delayed afterdepolarizations and triggered activity concentrated on those induced by digitalis,2–5 subsequent studies have suggested that a variety of interventions can generate them, including cardiac hypertrophy, myocardial ischemia and reperfusion, myocardial infarction, elevated \([Ca^{2+}]_0\), and \( \alpha_1 \)– and \( \beta \)-adrenergic catecholamines (see References 1 and 6 for review). Common to all of these causal factors is an increase in free intracellular calcium and/or an adjustment in the operation of the Na–Ca exchanger, which induces oscillations in membrane potential via induction of a transient inward current.1,6

The purpose of the present study was to determine the role of \( \alpha_1 \)-adrenergic stimulation in the genesis of delayed afterdepolarizations and triggered activity in an isolated tissue model of ischemia and reperfusion. We previously used this model to identify the role of \( \alpha_2 \)-receptor stimulation in the genesis of automatic arrhythmias during simulated ischemia and reperfusion.1,6 In the course of these studies, we noted that delayed afterdepolarizations and triggered activity did not occur. This was of particular concern to us because other investigators have demonstrated delayed afterdepolarizations and triggered activity in a similar (although not identical) setting.9
Assuming delayed afterdepolarizations did occur, the other goal was to identify whether α₁-adrenergic-subtype specificity was demonstrable. In performing these studies, we took advantage of the fact that the use of selective antagonists can identify the contribution of subtypes of α₁-adrenergic receptors to both normal and abnormal automatic rhythms. For example, chloroethylclonidine is an alkylating agent that irreversibly blocks an α₁-receptor subtype whose effector coupling induces a decrease in normal automaticity. This receptor-effector pathway involves stimulation of the Na-K pump via a 41-kd GTP regulatory protein that is a pertussis toxin substrate. In contrast, WB 4101 is a competitive antagonist that blocks α₁-adrenergic stimulation of phosphoinositide metabolism and resultant increases in [Ca²⁺] in cardiac myocytes. We previously demonstrated that WB 4101 blocks α₁-adrenergic-induced increases in automaticity that occur both in normally polarized Purkinje fibers and at low membrane potentials in fibers superfused with simulated ischemic solutions. We viewed the current studies of α₁-adrenergic–subtype specificity and contributions to triggered activity as important because if there were subtype specificity, it would have important pathogenetic and therapeutic implications.

Methods

We anesthetized adult mongrel dogs weighing 12–18 kg with sodium pentobarbital (30 mg/kg i.v.). The hearts were removed through a left or right thoracotomy and placed into ice-cold Tyrode’s solution equilibrated with 95% O₂-5% CO₂ and containing (mM) NaCl 137, NaHCO₃ 18, CaCl₂ 2.7, MgCl₂ 0.5, Na₂HPO₄ 1.8, dextrose 5.5, and KCl 4 (pH 7.4). We dissected Purkinje fiber bundles from left and right ventricles and mounted them in a Lucite chamber perfused with Tyrode’s solution warmed to 37±0.5°C. The solutions were pumped through the chamber at a rate of 15–18 ml/min.

We stimulated the Purkinje fiber bundles using standard techniques to deliver square-wave pulses 1.5 msec in duration and two times threshold via bipolar Teflon-coated silver wire electrodes. The fibers were impaled with 3 M KCl–filled glass capillary microelectrodes having tip resistances of 8–25 MΩ. The microelectrodes were coupled via an Ag-AgCl junction to a preamplifier with high-input impedance and input capacity neutralization. The tissue bath was grounded through a 3 M KCl-Ag-AgCl junction. Transmembrane action potentials were displayed on an oscilloscope, photographed with Polaroid film, and recorded on a strip-chart recorder for further analysis.

In all experiments, we measured maximum diastolic potential, action potential amplitude, and action potential duration to 100% repolarization (APD₁₀₀). We also determined the incidence of abnormal rhythms and the occurrence of afterdepolarizations and triggered activity during simulated ischemia and during reperfusion. The amplitude of delayed afterdepolarizations was measured as described previously.

Protocols

The basic experimental design incorporated a control solution, simulated ischemia, and reperfusion. In all experiments, control involved 60 minutes of equilibration, 40 minutes of simulated ischemia, and 20 minutes of reperfusion. The protocol was based on previous reports from our laboratory and from others. The preparations were paced at cycle lengths of 500, 400, 350, 300, and 250 msec at frequent intervals during control and reperfusion. This permitted us to observe transmembrane potential characteristics and the occurrence of delayed afterdepolarizations. At the end of the control period, drive was discontinued, and the preparation was permitted to beat spontaneously throughout the period of simulated ischemia. At 30 minutes of ischemia, the pacing protocol was repeated. Immediately after return to the control superfusate, the pacing protocol was reinitiated. Then, the pacing protocol was repeated, permitting 30-second pauses between drive trains, throughout reperfusion until the control membrane potential was reattained.

When the effects of WB 4101 (1×10⁻⁷ M) or chloroethylclonidine (1×10⁻⁷ M) were studied, each

<table>
<thead>
<tr>
<th>TABLE 1. Test Solutions in Preliminary Experiments and Occurrence of Delayed Afterdepolarizations</th>
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<tr>
<td><strong>Test solution</strong></td>
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<tr>
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<tr>
<td></td>
</tr>
<tr>
<td>1 (n=3)</td>
</tr>
<tr>
<td>2 (n=3)</td>
</tr>
<tr>
<td>3 (n=1)</td>
</tr>
<tr>
<td>4 (n=2)</td>
</tr>
<tr>
<td>5 (n=1)</td>
</tr>
<tr>
<td>Phenylephrine</td>
</tr>
<tr>
<td>6 (n=10)</td>
</tr>
<tr>
<td>Phenylephrine</td>
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</tbody>
</table>

In all solutions: pH, 7.4 during control and reperfusion and 6.7 during ischemia; PO₂, 200 mm Hg or more during control and reperfusion and 40–50 mm Hg during ischemia; sodium lactate, 20 mM during ischemia.
TABLE 2. Effects of Changing Po2 on Occurrence and Amplitude of Delayed Afterdepolarizations

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Ischemia</th>
<th>Reperfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Po2 (mm Hg)</td>
<td>MDP (mV)</td>
<td>AB AUTO (%)</td>
</tr>
<tr>
<td>Group</td>
<td></td>
<td>MDP (mV)</td>
<td></td>
</tr>
<tr>
<td>1 (n=6)</td>
<td>≤20</td>
<td>70±2*</td>
<td>50</td>
</tr>
<tr>
<td>2 (n=10)</td>
<td>40–50</td>
<td>70±1*</td>
<td>30</td>
</tr>
<tr>
<td>3 (n=6)</td>
<td>90–110</td>
<td>69±1*</td>
<td>0</td>
</tr>
<tr>
<td>4 (n=5)</td>
<td>350–500</td>
<td>69±0.5*</td>
<td>20</td>
</tr>
</tbody>
</table>

MDP, maximum diastolic potential; AB AUTO, abnormal automaticity; DAD, delayed afterdepolarizations.

In all groups, 10.8 mM Ca\(^{2+}\) and 1 x 10\(^{-7}\) M phenylephrine were used throughout and 20 mM lactate was used during ischemia. Measurements are made at a basic cycle length of 250 msec.

*p<0.05 compared with control.

†p<0.05 compared with group 2.

Results

Induction of Delayed Afterdepolarizations and Triggered Rhythms

To test the effects of various interventions on the development of delayed afterdepolarizations, we had to obtain a reliable model of afterdepolarizations and triggered activity. Our intent was to use a model in which delayed afterdepolarizations occurred as closely as possible to 100% of the time during reperfusion. The following is a sequential description of preliminary experiments that permitted the selection of solutions needed to consistently obtain delayed afterdepolarizations.

We commenced by using solutions (during control, ischemia, and reperfusion) similar to those previously described by Ferrier et al (Table 1). All simulated ischemic solutions contained (mM) lactate 20; NaHCO\(_3\) 5; KCl 4 or 10; CaCl\(_2\) 2.7, 5.4, 7.8, or 10.8; MgCl\(_2\) 0.5; NaH\(_2\)PO\(_4\) 1.8; and dextrose 5.5. NaCl was adjusted according to the changes in NaHCO\(_3\), CaCl\(_2\), and KCl. In the setting of simulated ischemia, Po2 was 40–50 mm Hg (pH 6.7). Using the combinations of [Ca\(^{2+}\)]\(_0\) [K\(^{+}\)]\(_0\) and phenylephrine shown in Table 1 (groups 1–5), we did not observe delayed afterdepolarizations during reperfusion. Only by increasing CaCl\(_2\) to 10.8 mM in the presence of 1 x 10\(^{-7}\) M

TABLE 3. Effects of Interventions on Maximum Diastolic Potential, Abnormal Automaticity, and Delayed Afterdepolarizations

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Ischemia</th>
<th>Reperfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MDP (mV)</td>
<td>MDP (mV)</td>
<td>AB AUTO (%)</td>
</tr>
<tr>
<td>Group</td>
<td></td>
<td>MDP (mV)</td>
<td></td>
</tr>
<tr>
<td>1 (n=10)</td>
<td>1 x 10(^{-7}) M</td>
<td>96±1</td>
<td>70±1*</td>
</tr>
<tr>
<td>2 (n=9)</td>
<td>1 x 10(^{-7}) M plus 1 x 10(^{-7}) M</td>
<td>97±1</td>
<td>72±1*</td>
</tr>
<tr>
<td>3 (n=9)</td>
<td>1 x 10(^{-7}) M plus 1 x 10(^{-7}) M</td>
<td>95±1</td>
<td>68±1*†</td>
</tr>
</tbody>
</table>

MDP, maximum diastolic potential; AB AUTO, abnormal automaticity; DAD, delayed afterdepolarizations.

MDP and DAD were measured at a basic cycle length of 250 msec.

In all groups, 10.8 mM [Ca\(^{2+}\)], was used throughout and Po2 of 40–50 mm Hg and 20 mM lactate were used during ischemia.

*p<0.05 compared with control.

†p<0.05 compared with group 2.

‡p<0.05 compared with group 1.
phenylephrine did we observe delayed afterdepolarizations consistently (Table 1, group 6). As a result of these experiments, in all subsequent studies we used control and reperfusion solutions that contained (mM) NaCl 131, KCl 4, CaCl₂ 10.8, NaHCO₃ 18, MgCl₂ 0.5, NaH₂PO₄ 1.8, and dextrose 5.5 (pH 7.4 and PO₂ of 350–500 mm Hg). The ischemic solution was (mM) NaCl 118, KCl 10, CaCl₂ 10.8, NaHCO₃ 5, and sodium lactate 20. MgCl₂, NaH₂PO₄, and dextrose were as above (pH 6.8 and PO₂ was 40–50 mm Hg. Phenylephrine (1×10⁻⁷ M) was included during control, ischemia, and reperfusion.

In one series of experiments, we varied oxygen tensions during conditions of simulated ischemia to determine the effects of this intervention on the development of afterdepolarizations and abnormal rhythms during ischemia and reperfusion. In individual experiments, PO₂ was 20, 40–50, 90–110, and 350–500 mm Hg. Methods for varying PO₂ have been described by us previously.⁷

Effects of Varying PO₂ on Development of Abnormal Rhythms and Afterdepolarizations During Ischemia and Reperfusion

Table 2 shows the effects of increasing PO₂ from less than 20 to more than 350 mm Hg during ischemia on abnormal rhythms and afterdepolarizations induced by 1×10⁻⁷ M phenylephrine and 10.8 mM [Ca²⁺]₀ during simulated ischemia and reperfusion.

**FIGURE 1.** Recordings of effects of varying oxygen tensions during ischemia on amplitudes of delayed afterdepolarizations during reperfusion; panels A, B, C, and D represent different fibers. Panel A: PO₂ of 20 mm Hg during simulated ischemia. After 5 minutes of reperfusion, a small delayed afterdepolarization is observed after a train of overdrive stimulation. Panel B: PO₂ of 40 mm Hg during simulated ischemia. Note a prominent delayed afterdepolarization after 5 minutes of reperfusion. Panel C: PO₂ of 100 mm Hg during simulated ischemia. After 5 minutes of reperfusion, a small delayed afterdepolarization is observed. Panel D: PO₂ of 350 mm Hg during simulated ischemia. A small delayed afterdepolarization is seen.

**FIGURE 2.** Plot of effects of varying PO₂ during simulated ischemia on delayed afterdepolarization amplitude (DAD AMP) during reperfusion. Amplitude at PO₂ of 40–50 mm Hg significantly differed from that at PO₂ of 20 mm Hg or less and from PO₂ of 90–110 and 350–500 mm Hg (p<0.05).
During ischemia at $P_O_2$ of 20 mm Hg or less, membrane potential depolarized from $-97\pm2$ mV to $-70\pm1$ mV ($p<0.05$). Abnormal automaticity was present in 50% of fibers, and no delayed afterdepolarizations were observed. In another group of fibers (shown also in Table 3), maintaining $P_O_2$ at 40–50 mm Hg during ischemia resulted in reduction of abnormal automaticity to 30% ($p>0.05$). In addition, delayed afterdepolarizations with a mean amplitude of $7\pm1$ mV developed in 40%, and 10% presented triggered activity. At $P_O_2$ of 90–110 mm Hg, there was no abnormal automaticity, but the incidence of delayed afterdepolarizations decreased to 17% with no triggered activity. Finally, when $P_O_2$ was increased to 350–500 mm Hg, abnormal automaticity and early afterdepolarizations occurred in 20% of fibers, and there were no delayed afterdepolarizations. In all of the abovementioned groups, action potential amplitude and $APD_{100}$ decreased significantly during ischemia. However, no significant differences were observed among groups (data not shown).

Reperfusion restored maximum diastolic potential (Table 2), action potential amplitude, and $APD_{100}$ to control values in all groups (data not shown for action potential amplitude and $APD_{100}$). When $P_O_2$ had been maintained at 20 mm Hg or less during ischemia, abnormal automaticity and early afterdepolarizations did not occur during reperfusion. In this case, 83% of fibers had small delayed afterdepolarizations (Figures 1A and 2) but no triggered activity. On reperfusion after maintaining $P_O_2$ at 40–50 mm Hg during ischemia, 100% of fibers had delayed afterdepolarizations with an amplitude of $9\pm1$ mV ($p<0.05$) (Figures 1B and 2), and 40% had triggered activity (data from Table 3). Increasing $P_O_2$ to 90–110 mm Hg during simulated ischemia resulted in a reduction to 83% in the incidence of delayed afterdepolarizations during reperfusion as well as a significant reduction in delayed afterdepolarization amplitude to $3\pm0.3$ mV ($p<0.05$) (Figures 1C and 2). In addition, no triggered activity was observed (Table 2). Oxygen tensions of 350–500 mm Hg during simulated ischemia reduced the incidence of delayed afterdepolarizations during reperfusion to 80% (Figures 1D and 2); their amplitude was $4\pm1$ mV.

**Effects of Chloroethylclonidine and WB 4101 on Delayed Afterdepolarizations and Triggered Rhythms**

In the control setting, maximum diastolic potential was $-96\pm1$ mV (Table 3, group 1). No significant changes in membrane potential were seen with WB 4101 or chloroethylclonidine (Table 3 and Figure 3). Control automatic rate is not presented here (or in any of the tables) because the experimental protocol, which involved frequent periods of pacing at various drive cycle lengths, prevented most of the preparations from developing stable automatic rates; only three of the 10 preparations in Table 3 (group 1) developed stability (rate, 11±4 beats/min). By way of comparison, automaticity was stable in three of the nine fibers superfused with phenylephrine plus WB 4101 (Table 3, group 2: rate, 3±0.3 beats/min) and in three of nine fibers superfused with phenylephrine plus chloroethylclonidine (Table 3, group 3: rate, 14±6 beats/min).

During simulated ischemia, membrane potential depolarized significantly, and action potential amplitude and $APD_{100}$ decreased significantly (Table 3 and Figure 3). However, in the presence of WB 4101, maximum diastolic potential was greater than with chloroethylclonidine ($p<0.05$), and $APD_{100}$ was less than in the control or chloroethylclonidine groups (Figure 3). Normal automaticity ceased during simulated ischemia, and abnormal automaticity was observed in 30% of fibers superfused with phenyleph-
rime. The incidence of abnormal automaticity was not significantly altered by WB 4101 or chloroethylclonidine. Early afterdepolarizations were seen (Figure 4), but their incidence was inconsistent across groups of fibers, and as a result no data analysis is presented. Delayed afterdepolarizations with a mean amplitude of 7±1.0 mV at a basic cycle length of 250 msec occurred in 40% of fibers during simulated ischemia, and triggered activity occurred in 10% (Figure 5). These were not modified significantly by chloroethylclonidine or WB 4101 (data not shown).

On reperfusion, maximum diastolic potential was restored to control values (Table 3 and Figure 3) within 6–8 minutes and abnormal automaticity ceased in all three groups. For the fibers superfused with phenylephrine alone (Table 3, group 1), delayed afterdepolarizations with an amplitude of 9±1.0 mV occurred in 100% at a basic cycle length of 250 msec and triggered activity occurred in 40% (Figures 4D and 5C). Mean maximum diastolic potential at which delayed afterdepolarizations were seen was −90±2 mV. Delayed afterdepolarizations only persisted for a brief interval and did not occur after 8–10 minutes of reperfusion.

In the presence of WB 4101, a reduction in the incidence of delayed afterdepolarizations from 100% to 33% (p<0.05) was observed (Figure 6), and the amplitude for the three preparations with delayed afterdepolarizations was 1±0.5 mV (p<0.05) (Table 3, group 2). There was no triggered activity. In contrast, in the presence of chloroethylclonidine (Table 3, group 3), delayed afterdepolarizations were observed in 100% of fibers and had a mean amplitude of 8±0.5 mV (Figure 6). Twenty-two percent manifested triggered activity.

Discussion

The findings in the present study relate to three aspects of delayed afterdepolarizations and triggered activity during ischemia and reperfusion: the conditions needed to maximize the occurrence of delayed afterdepolarizations, the relation of arrhythmias induced by delayed afterdepolarizations to those induced by automaticity and early afterdepolarizations and the role of α1-adrenergic stimulation.

It is clear from our preliminary studies (summarized in Table 1) that the conditions needed to maximize the occurrence of delayed afterdepolarizations and triggered activity were a solution containing high [Ca2+]o and an α-agonist throughout the experiment. This is not meant to imply that other experimental conditions are not associated with these events; in the study by Ferrier et al,9 the minimal conditions required were quite different, including 4 or 10 mM [K+]o, 2.5 mM [Ca2+]o, 20 mM lactate, and no adrenergic amine. Although we tested similar solutions initially (in three experiments with 4 mM [K+]o and three with 10 mM [K+]o), we did not see delayed afterdepolarizations. This is not surprising, as Ferrier et al did not observe them routinely during reperfusion (A. Lukas, personal communication). It is possible that had we used a greater number of

![Figure 4. Tracings from a representative experiment showing control, simulated ischemia, and reperfusion. Panel A: At the end of the control period, delayed afterdepolarizations were not observed after pacing (Pacing cycle length, 250 msec). Panel B: After pacing was terminated, fiber was allowed to beat spontaneously. Note that superfusion with "ischemic" Tyrode's solution induced membrane depolarization and loss of normal automatic activity. Panel C: After 30 minutes of ischemia, fiber showed spontaneous activity and early afterdepolarizations. Panel D: At 5 minutes of reperfusion, restoration of membrane potential was evident. Note a prominent delayed afterdepolarization immediately after a pacing train was terminated.](http://circ.ahajournals.org/Download/fig/s1fig4.jpg)
preparations, our result would have been consistent with theirs. However, our goal in the present study was to identify the conditions under which delayed afterdepolarizations were maximized during reperfusion; hence, we modified the superfusate.

In so doing, we took advantage of the fact that elevation of free intracellular Ca²⁺ is a key factor in the induction of delayed afterdepolarizations (see References 1 and 6 for review). The current responsible for these oscillations is thought to be either a transient inward current carried by Na⁺, whose operation is contingent on high levels of [Ca²⁺]o, or a current dependent on the Na⁺–Ca²⁺ exchanger. We considered two means for elevating [Ca²⁺]o in these experiments. One was the use of an α-adrenergic amine (phenylephrine); the other was the elevation of [Ca²⁺]o. These interventions have been shown to induce delayed afterdepolarizations.¹⁷ From Table 1 it is clear that the combination of 10.8 mM [Ca²⁺]o plus phenylephrine was invariably associated with delayed afterdepolarizations as an end point. For this reason, we incorporated these components into all solutions in all experiments.

Coetzee and Opie¹⁸ demonstrated the effect of changes in energy supply in the induction of delayed afterdepolarizations and have shown that with severe ischemia, including Po₂ of less than 30 mm Hg, the afterdepolarizations are minimized. Our studies with different Po₂ values are consistent with this result (Table 2). At Po₂ of 20 mm Hg or less, delayed afterdepolarizations were seen 83% of the time during reperfusion, but they were extremely small and not associated with triggered activity. Furthermore, no delayed afterdepolarizations were seen during simulated ischemia. At Po₂ of 40–50 mm Hg, the occurrence and amplitude of delayed afterdepolarizations were maximal during both ischemia and reperfusion, as was the occurrence of triggered activity. As Po₂ was further elevated, the afterdepolarizations diminished and triggered activity was not seen. Hence, given a constant [Ca²⁺]o and the presence of an α-adrenergic agonist, the occurrence of delayed afterdepolarizations and incidence of triggering during ischemia and reperfusion were rigorously dependent on maintenance of mild hypoxia. These observations also explain why we did not observe delayed afterdepolarizations and triggered activity in earlier studies of ischemia-induced automatic rhythms.⁷,⁸ In these experiments, we maintained Po₂ at 20 mm Hg or less, which minimizes delayed afterdepolarizations. Furthermore, in many of these experiments, we used 2.7 mM [Ca²⁺]o instead of 10.8 mM. Therefore, it appears that whether a rhythm is triggered or automatic in the presence of ischemia and reperfusion is strongly influenced by the extent of hypoxia as well as the elevation that occurs in [Ca²⁺].

Our previous research also demonstrated early afterdepolarizations during ischemia, but under the experimental conditions used, their occurrence was inconsistent.⁷,⁸ A similar result was seen here; that is, early afterdepolarizations appeared in all settings of ischemia and somewhat inconsistently during reperfusion.
Consideration of \( \alpha_1 \)-adrenergic actions on the mechanisms studied is facilitated by referring to Table 3. In interpreting data in this table, we considered the following information about \( \alpha_1 \)-adrenergic–subtype blockers. In cardiac tissues, WB 4101 blocks an \( \alpha_1 \)-receptor that is linked to the stimulation of phosphoinositide metabolism and an increase in free \([Ca^{2+}]_i\).\(^\text{11}\) In addition, WB 4101 competitively antagonizes \( \alpha_1 \)-adrenergic–induced increases in automaticity of normal Purkinje fibers. In contrast, chloroethylclonidine antagonizes the decrease in normal automaticity induced by \( \alpha \)-agonists.\(^\text{11}\) The mechanism is thought to be noncompetitive block of the stimulation of Na–K pumping induced by \( \alpha \)-agonists. In ischemic Purkinje fibers with \( \alpha \)-adrenergic–induced automatic rhythms, WB 4101 suppressed these rhythms and chloroethylclonidine enhanced them.\(^\text{8}\) In contrast to our previous research, no effect on abnormal automatic rhythms was exerted by either blocker in the present studies. This might reflect several procedural differences from the previous study. First, both \([Ca^{2+}]_i\) and \( Po_2 \) were higher in the present study to enhance the occurrence of delayed afterdepolarizations. Second, in this setting, the frequency of abnormal automaticity was only 30% (compared with 50% when \( Po_2 \) was 20 mm Hg or less and 2.7 mM \([Ca^{2+}]_i\]). Given these important differences in experimental conditions, it was not altogether surprising that a different effect on the automatic arrhythmias was seen.

One might ask if—given the different experimental conditions—both chloroethylclonidine and WB 4101 were binding to their respective receptors and blocking them as expected. That this was the case is suggested by the results seen during ischemia (Table 3). In the presence of WB 4101, the membrane was significantly more hyperpolarized than in the presence of chloroethylclonidine. This is consistent with our earlier results\(^\text{8}\) and is expected based on the action of chloroethylclonidine to block the Na–K pump—stimulating effect of \( \alpha \)-agonists while leaving the phosphoinositide metabolic effects unopposed and the action of WB 4101 to do the opposite.\(^\text{11}\)

Given the differing effects of WB 4101 and chloroethylclonidine on \( \alpha_1 \)-adrenergic receptor–effector coupling pathways, one might predict the blocker of phosphoinositide metabolism and resultant \([Ca^{2+}]_i\) accumulation to suppress delayed afterdepolarizations and triggered activity. This result was seen during reperfusion such that WB 4101 reduced the
incidence and amplitude of delayed afterdepolarizations significantly and abolished triggered activity. In contrast, chloroethylclonidine had no effect on the frequency of occurrence of delayed afterdepolarizations, their amplitude, or the incidence of triggered activity during reperfusion.

Previous studies in intact animals have emphasized the role of α-adrenergic mechanisms in the genesis of reperfusion-induced arrhythmias. These studies used prazosin as the antagonist, thereby implicating the receptor as being α1- rather than β-adrenergic (propranolol was ineffective). Although our earlier studies used prazosin and then WB 4101 and chloroethylclonidine to identify the role of α1-subtypes in automatic rhythms associated with ischemia, the present study differs importantly in that it concentrates on reperfusion arrhythmias that are triggered. Therefore, our results emphasize the α1-adrenergic–subtype specificity of triggered rhythms during reperfusion and suggest that selective block of the α1-pathway involved in the accumulation of free [Ca2+], might be an important antiarrhythmic intervention for prevention and treatment of reperfusion arrhythmias.

Acknowledgments

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References


Key Words • early afterdepolarizations • chloroethylclonidine • α1-adrenergic agonists • hypoxia
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