Effects of Components of Ischemia and Metabolic Inhibition on Delayed Afterdepolarizations in Guinea Pig Papillary Muscle

W.A. Coetzee and L.H. Opie

Delayed afterdepolarizations (DADs) may develop into triggered automaticity and ventricular arrhythmias. However, the potential role of DADs in the genesis of ischemic arrhythmias is not clear. We studied the effects of different components of severe ischemia (acidosis, hypoxia, lactate, increased potassium, and the absence of glucose) on DADs. DADs were evoked using trains of 30–60 externally applied pulses at a rate of 4–5 Hz in the presence of isoproterenol (10⁻⁷ M) or dibutyryl cyclic 3', 5' adenosine monophosphate (dB-cAMP, 10⁻³ M). Acidosis, caused by the addition of protons (pH = 6.8), increased the amplitude of DADs from 3.2 ± 0.4 to 5.9 ± 0.5 mV (n = 8, p < 0.001). DADs were abolished by hypoxia (pO₂ < 35 mm Hg, n = 7, p < 0.001) from control values of 3.4 ± 0.3 mV. DADs were also abolished by neutral lactate (20 mM, n = 7, p < 0.001) in the absence of glucose. Acidotic lactate (20 mM, pH = 6.8), however, was unable to abolish DADs. Increasing the extracellular potassium concentration to 16.2 mM decreased DAD amplitude from 3.6 ± 0.27 mV to 1.3 ± 0.1 mV (n = 5, p < 0.002) with an associated reduction of membrane potential from −86.2 ± 0.9 to −58.6 ± 0.9 mV. The overall effect of simulated ischemia (all components tested together) was to abolish DADs (n = 8, p < 0.001), with hypoxia as the most important factor. Neither the glycolytic inhibitors iodoacetate (0.1 mM) and 2-deoxyglucose (10 mM with 10 mM pyruvate) nor the absence of glucose changed the amplitude of DADs. 2-Deoxyglucose (10 mM) in the absence of pyruvate, cyanide (0.5–2.0 mM), and dinitrophenol (0.1–1.0 mM) each abolished DADs. We interpret these findings to mean that DADs are unlikely to occur in severe myocardial ischemia. (Circulation Research 1987;61:157–165)

The mechanism of ventricular arrhythmias during early ischemia and reperfusion is not well understood. 1,3 Reentrant excitation 4 appears to play a dominant role during early ischemia, 1,4 although other factors, including abnormal automaticity, 7 cannot be excluded. Delayed afterdepolarizations (DADs) can cause arrhythmias, 6 especially during digitalis poisoning. 5 Because of the dependence of DADs on Ca²⁺ ions, 10 their development should be favored if the cytosolic Ca²⁺ increases in ischemic tissue as suggested by an increase in the intracellular Ca²⁺ concentration during hypoxia (Snowdowne et al 11 but see Allen and Orchard12) and during metabolic inhibition. 13,14 Furthermore, the tissue content of cAMP increases during myocardial ischemia, 15,16 and cAMP promotes the formation of DADs when injected intracellularly. 17 Therefore, it may be expected that conditions for the generation of DADs exist in myocardial ischemia. However, there are several major changes in cellular metabolism during myocardial ischemia other than those in intracellular Ca²⁺. The myocardium becomes severely hypoxic, the extracellular potassium concentration rises, the intracellular pH falls, and because of the reduced blood supply, glucose uptake into the cell is reduced and tissue lactate accumulates. These factors have an unknown effect on the generation of DADs. Because of the lack of a suitable in vitro model of ischemia, components of ischemia (substrate omission, lactate, acidosis, high potassium, and hypoxia) were mimicked in the superfusion bath of a guinea pig papillary muscle to study the effect of ischemia on DADs.

Materials and Methods

Preparation

The experiments were performed on guinea pig papillary muscle obtained from the right ventricle of guinea pigs (120–350 g) that were killed by cervical dislocation. Hearts were removed within 1 minute and were arrested in cold (4°C) modified Tyrode's solution. Papillary muscles (<1.0 × 0.5 mm) were dissected from the right ventricle and tied to a cotton string to facilitate mounting in the tissue bath. Recovery for 45 minutes was allowed before any recording was commenced.

Superfusate

The Tyrode’s solution used had the following composition (in mM): NaCl 127.0, KCl 5.4, CaCl₂ 2.5, MgCl₂ 0.5, NaHCO₃ 23.0, and glucose 5.0. The temperature was 37°C (±0.5°C) and pH 7.4 (95% O₂–5% CO₂). Ionic composition was changed in the superfusate to achieve the desired conditions.
certain conditions without adjustment for osmolarity. In some experiments, the pH was decreased by the addition of hydrogen chloride. When lactic acid was added, the pH was corrected with NaOH. Hypoxia (pO₂ = 25–35 mm Hg in a sample from the perfusion bath) was obtained by equilibration with 95% N₂-5% CO₂ (pH 7.4 at 37°C). To diminish the diffusion of oxygen into the solution, glass tubing was used as far as possible, while a small hood, through which nitrogen was introduced, was placed directly over the preparation.

**Intracellular Recordings**

Action potentials were recorded from the surface cells of the preparations using intracellular glass microelectrodes with a tip resistance of 15–25 MΩ when filled with 3 M KCl. Action potentials were elicited by point stimulation using rectangular pulses with a duration of 0.5–1.0 msec and an amplitude of 150% threshold. The pulses were applied through a thin (0.1 mm) platinum wire that was electrically isolated except for the tip. The indifferent Ag-AgCl electrode was placed close to the preparation.

**Protocol for Development of Delayed Afterdepolarizations**

Unless otherwise stated, DADs were provoked throughout this study using methods of rapid stimulation (Figure 3 of Vassalle and Carpentier,9 Ferrier9) by a train of externally applied stimuli that consisted of 30–90 pulses delivered at a frequency of 5 Hz. DADs were always observed in the presence of isoproterenol (10⁻⁷ to 10⁻⁴ M) following the last action potential in the train (Figure 1B). The amplitude of the DAD was a function of the duration of the train of stimuli, but this aspect was not studied in detail. The train duration and frequency were kept constant for any particular experiment. DADs were sometimes elicited by dB-cAMP instead of isoproterenol; DADs appeared after 10 minutes of dB-cAMP (2.5 x 10⁻⁶ M) and gradually increased in size over a period of 30–45 minutes (data not shown). DADs were also sometimes evoked by the addition of ouabain and isoproterenol. Action potentials and DADs were photographed from the oscilloscope screen. Because of the small size of DADs, they were measured at a high sensitivity (2–5 mV/division).

**Drugs**

The following drugs were added: isoproterenol (Winthrop Laboratories, Durban, South Africa), ouabain (Sigma Chemical Co., St. Louis, Mo.), dibutyryl cyclic adenosine 3',5' monophosphate (dB-cAMP, Sigma), L(+)-lactic acid (Sigma), pyruvate (Boehringer Mannheim Corp., Pinelands, South Africa), 2-deoxyglucose (Sigma), NaCN (Merck Pharmaceuticals, Johannesburg, South Africa), and dinitrophenol (BDH Chemicals, Poole, England).

**Definitions**

We defined delayed afterdepolarizations as positive deflections of the membrane potential following an action potential.8 A DAD-mediated extrasystole was defined as an action potential developing from a DAD rather than from an external stimulus.

**Analysis**

All results are expressed as mean ± SEM. Statistical analysis was done using the Fisher exact test or the paired Student's t test at a 1% level of significance.

**Figure 1.** Effect of increased extracellular potassium concentration (5.4 mM, 13.5 mM, and 16.2 mM, not given in this sequence) on DADs. Train of 30 pulses were given at rate of 5 Hz. Isoproterenol (10⁻⁷ M) led to the formation of DAD following the last action potential in the train. Note shortening of time to peak of DAD. Only lower portions of action potentials are displayed. Membrane potential before onset of stimulation was −84 mV, −85 mV, −61 mV, and −57 mV in the respective panels (from top to bottom).
Results

Effects of Potassium on Delayed Afterdepolarizations

Increasing the extracellular potassium concentration from 5.4 to 16.2 mM decreased DAD amplitude from 3.6 ± 0.3 to 1.3 ± 0.1 mV (n = 5, p < 0.002) and the membrane potential from -86.2 ± 0.9 to -58.6 ± 0.9 mV. A potassium concentration of 13.5 mM decreased the amplitude of DADs to 1.5 ± 0.4 mV (n = 4, p < 0.005). In the example shown in Figure 1, the extracellular potassium concentration was increased to 13.5 or 16.2 mM (Figures 1C and 1D), and the amplitude of the DAD was respectively decreased from a control value of 3.2 mV to 2.4 or 1.3 mV. The rate of change of membrane depolarization during the train of stimuli was increased by isoproterenol (Figure 1B) but decreased by an increased [K], in the presence of isoproterenol (Figures 1C and 1D). This phenomenon is not well understood. Explanations might include an accumulation of potassium in the extracellular spaces or a stimulation of a Ca2+-dependent outward current.

Effects of Decreased pH on Delayed Afterdepolarizations

When the extracellular pH was decreased from 7.4 to 6.8, the DAD amplitude increased from 3.2 ± 0.4 to 5.9 ± 0.5 mV (n = 8, p < 0.001). In the example shown in Figure 2, the amplitude of the DAD increased from 3.0 to 7.5 mV within 5 minutes. Note the decrease in time to peak potential and the general steepening of the DAD. The DAD amplitude continued to increase (11 mV at 10 minutes) until it reached threshold and gave rise to an extrasystolic action potential (arrow) at 15 minutes. At return to normal pH, the DAD again decreased in size. DAD-mediated extrasystoles were seen in 3 preparations on lowering the pH.

Effects of Substrate Manipulation on Delayed Afterdepolarizations

The omission of glucose from the superfusate for a period of 30 minutes left the amplitude of DADs unchanged at 2.2 ± 1.0 mV, compared with the control amplitude of 2.6 ± 0.7 mV (n = 5, p = 0.29). Neutralized lactate (20 mM, pH = 7.4) in the absence of glucose reversibly abolished DADs from control amplitudes of 3.5 ± 0.7 mV (n = 7, p < 0.001, Table 1), while the action potential duration at 90% repolarization was shortened from 223 ± 8 to 160 ± 23 msec (n = 5, p < 0.01). The decrease of DADs by lactate was not caused by the high osmolarity since substituting lactate by 30 mM mannitol in the presence of glucose did not abolish DADs (results not shown). When the pH was adjusted to 6.8 instead of 7.4 (see “Materials and Methods”), lactate (20 mM) had variable effects. In some preparations, the amplitude of DADs was increased, while in others, it decreased. The mean DAD amplitude was not changed from a control value of 3.0 ± 0.4 mV to 5.4 ± 1.3 mV (n = 10, p = 0.09).

Effect of Hypoxia on Delayed Afterdepolarizations

Using the standard train method of stimulation in the presence of isoproterenol, hypoxia led to the disappearance of DADs within 20 minutes from a control amplitude of 3.4 ± 0.3 mV in all the preparations studied (n = 7, p < 0.001). In an attempt to produce DADs that might depend less on Ca2+ entry through the calcium channels (see “Discussion”), an alternative method was employed of decreasing the extracellular potassium concentration to 2.7 mM while the preparation was stimulated at 1 Hz in the presence of isoproterenol (3 × 10⁻⁷ M) and ouabain (7 × 10⁻⁷ M). In the preparation shown in Figure 3A, DADs developed within 30 minutes following each action potential. Hypoxia led to the complete abolition of DADs within 10 minutes (n = 5, p < 0.005). Accompanying
the inhibition of DADs, the action potential duration shortened from 181 ± 8 msec to 111 ± 4 msec (n = 5, p < 0.005), and the resting membrane potential showed a small depolarization of 2–4 mV. Reoxygenation after a period of hypoxia increased the amplitude of DADs. Within 1 minute of reoxygenation after a 10-minute period of hypoxia, a small burst of DAD-mediated triggered activity was observed (not shown). After cessation of triggered activity, the DAD reappeared with an increased amplitude and, at 6.5 minutes of reoxygenation, reached a peak potential of 22 mV from a maximum diastolic potential of −94 mV. At the arrow, the DAD amplitude reached threshold to cause an extrasystolic action potential to appear. Similar results were found in three other preparations, where DADs increased in amplitude, and DAD-mediated extrasystoles and triggered activity occurred within the first minute on reoxygenation after a period of hypoxia. Hypoxia also led to the abolition of DADs elicited using dB-cAMP (results not shown).

**Effects of Ischemic Superfusate on Delayed Afterdepolarizations**

To simulate ischemia in the perfusion bath, different interventions were combined: the extracellular potassium concentration was increased to 16.2 mM; a β-adrenoceptor agonist, isoproterenol (10⁻⁶ M), was first added after which the extracellular pH was decreased to 6.8; 20 mM neutral lactate was added; glucose was omitted to simulate considerably decreased glucose availability, and the superfusate was subjected to severe hypoxia. Hypoxia was elicited at the end of 60 pulses and was not inhibited by a 10-minute exposure to high potassium, low pH (6.83), 20 mM lactate, and the absence of glucose. When the preparation was made hypoxic during these conditions, however, DADs disappeared after 10 minutes and reappeared within the first minute on reoxygenation after a 20-minute period of hypoxia. DADs were selectively abolished by hypoxia (n = 8, p < 0.001) after they had been evoked (DAD amplitude, 1.8 ± 0.3 mV) in the combined presence of other components of ischemia (isoproterenol, high potassium, low pH, lactate, and absence of glucose).

**Effects of Metabolic Inhibitors on DADs**

The removal of glucose from the superfusate, the addition of 0.1 mM iodoacetate in the presence of

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**Table 1. Effects of Components of Simulated Ischemia on DAD Amplitudes**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Detail</th>
<th>Control (mV)</th>
<th>Intervention (mV)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Acidosis</td>
<td>pH = 6.8</td>
<td>3.2 ± 0.4</td>
<td>5.9 ± 0.5*</td>
<td>8</td>
</tr>
<tr>
<td>2. Hypoxia</td>
<td>pO₂ &lt; 35 mm Hg</td>
<td>3.4 ± 0.3</td>
<td>0*</td>
<td>7</td>
</tr>
<tr>
<td>3. Lactate</td>
<td>20 mM, pH = 7.4</td>
<td>3.5 ± 0.7</td>
<td>0*</td>
<td>7</td>
</tr>
<tr>
<td>4. Lactate</td>
<td>20 mM, pH = 6.8</td>
<td>3.0 ± 0.4</td>
<td>5.4 ± 1.3</td>
<td>10</td>
</tr>
<tr>
<td>5. High K⁺</td>
<td>13.5 mM</td>
<td>2.4 ± 0.4</td>
<td>1.5 ± 0.4*</td>
<td>4</td>
</tr>
<tr>
<td>6. High K⁺</td>
<td>16.2 mM</td>
<td>3.6 ± 0.3</td>
<td>1.3 ± 0.1*</td>
<td>5</td>
</tr>
<tr>
<td>7. Simulated ischemia</td>
<td></td>
<td>1.8 ± 0.3†</td>
<td>0* ‡</td>
<td>8</td>
</tr>
</tbody>
</table>

*p < 0.005 for comparison of two columns.
†Conditions 4 and 6.
‡Conditions 2, 4, and 6.
glucose, or the addition of 2-deoxyglucose (10 mM) in the presence of the alternative substrate, pyruvate. (10 mM), did not decrease the amplitude of DADs (Table 2). When glycolysis was inhibited using 2-deoxyglucose (10 mM) in the absence of pyruvate or glucose, the action potential duration was not significantly

\( p = 0.06 \)

changed (from 158±6.9 mV to 143±12.3 mV, \( n = 5 \)), but the DAD amplitudes were decreased from 2.4±0.4 to 0.6±0.3 (\( n = 6, p < 0.01 \)). The inhibition of mitochondrial metabolism by cyanide (0.5-2.0 mM) or by the uncoupling agent dinitrophenol (0.1-1.0 mM) led to a rapid shortening of the action potential with a small (1-3 mV) associated hyperpolarization of the membrane potential. Both drugs also caused DADs to disappear in less than 10 minutes.

**Note added in proof:** Results similar to ours were reported by Lucas and Ferrier (J Mol Cell Cardiol 1986;18:1143-1156). They showed that simulated ischemia suppressed digitalis-induced DADs, whereas reperfusion potentiated them.

**Discussion**

We found that delayed afterdepolarizations (DADs) evoked by rapid pacing and isoproterenol in a guinea pig papillary muscle could be abolished by hypoxia and neutral lactate. The amplitude of DADs was decreased by raising the potassium concentration and was increased by acidosis. The overall effect of simulated ischemia (hypoxia, high potassium, glucose omission, low pH, and high lactate) led to a rapid shortening of the action potential with a small (1-3 mV) associated hyperpolarization of the membrane potential. Both drugs also caused DADs to disappear in less than 10 minutes.

**Delayed Afterdepolarizations and Triggered Activity**

DADs are caused by the transient inward current (\( i_{\text{in}} \)), which is described for both Purkinje fibers and isolated myocardial cells. The nature of this current is not known. Both an electrogenic sodium–calcium exchange and a novel membrane current passing mainly Na+ ions have been proposed as the charge carrier. Whatever the mechanism, it is generally accepted that intracellular calcium plays an important role in the genesis of DADs—an idea that was first stressed by Kass et al. The involvement of intracellular Ca\(^{2+} \) in the development of DADs is underlined by their enhancement by \( \beta \)-adrenoceptor agonists and proven by the direct injection of Ca\(^{2+} \). A direct effect of cAMP on the provocation of DADs was demonstrated by the intracellular injection of cAMP or a cAMP-dependent protein kinase, which caused DADs to appear. DADs are also caused by inhibition of the Na-K pump with digitalis leading to an increased cytosolic sodium concentration and thus to an increased cytosolic Ca\(^{2+} \).

DADs occur more readily in Purkinje fibers than in ventricular muscle fibers. DADs can reach threshold potential, thereby inducing spontaneous discharge in the form of triggered or repetitive activity in both Purkinje fibers and in ventricular muscle in the appropriate conditions.

**Components of Ischemia and Delayed Afterdepolarizations**

Ventricular arrhythmias of the heart often occur during early myocardial ischemia. The study of the genesis of early ischemic arrhythmias in vitro, however, is hampered by the lack of a suitable model of myocardial ischemia. Perfusion of pig hearts with hypoxic, hyperkalemic, and acidotic perfusate closely mimicked the electrical changes caused by ischemia and also led to early (first 10 minutes) arrhythmias, while electrical changes during ischemia in the dog heart were reproduced by hypoxia in the presence of potassium infusion. We chose to mimic ischemia by a combination of hyperkalemia, acidosis, lactate, hypoxia, and the absence of glucose.

The extracellular potassium concentration increases in the ischemic zone to values ranging between 12–16 mM. We used 13.5 and 16.2 mM in our experiments. The amplitude of the DADs was decreased by increasing extracellular potassium, which is consistent with previous findings. There is uncertainty about the
direct effect of extracellular potassium on the transient inward current. Decreased levels of \([K]\) leads to the appearance of the transient inward current,
possibly through an inhibitory effect on the Na-K pump. For an increased \([K]_o\), both an inhibitory effect or no effect is described. The transient inward current is also a direct function of the membrane potential, and depolarization will lead to a reduced \(i_{\text{Na}}\), beyond voltages of about \(-40\) mV. In the range of potentials relevant to this study (\(-85\) to \(-55\) mV), depolarization will actually increase \(i_{\text{Na}}\), which can, therefore, not explain the decreased DAD amplitude by increased \([K]_o\). The most likely explanation for the inhibitory effect of high \([K]_o\) on DADs is the increased potassium conductance associated with high \([K]_o\), opposing any depolarizing action of an inward current.

During myocardial ischemia, both the intracellular and extracellular pH decreases. The intracellular pH can fall from 7.25 to 6.8 in globally ischemic rat hearts. In regional ischemia caused by coronary artery ligation in the dog, tissue pH falls from 7.05 to 6.47 in 60 minutes. The intracellular pH also decreases on exposure to acidicotic Tyrode’s solution. The decrease of extracellular potassium and ouabain, that led to Ca overload during the initial phase of the action potential. Most of the Ca entry occurs within 2-5 msec and has an inactivation time constant of 10-20 msec. Most of the Ca entry continues during the initial phase of the action potential. Second, even though the calcium current decreases during metabolic inhibition, the intracellular Ca concentration may increase, which should favor the development of DADs. Third, when DADs were evoked using methods, such as low extracellular potassium and ouabain, that led to Ca overload through inhibition of the Na-K pump and, secondary, via the sodium–calcium exchange and thus decreased the dependence on Ca entry via the action potential, hypoxia still abolished DADs. Abolition of hypoxia by DADs evoked by digitalis is accompanied by an increase in resting tension, showing that the disappearance of DADs is not due to a decrease of cellular Ca in these conditions. Recent preliminary experiments indicate that the transient inward current underlying DADs is abolished by DNP before any changes in other membrane currents, which rules out configuration changes of the action potential as a major cause for the abolition of DADs (W.A. Coetzee and L.H. Opie, unpublished observations). Still, it is difficult to exclude the effects of a changed intracellular environment during hypoxia on Ca uptake/release by the sarcoplasmic reticulum.

Table 2. Effect of Different Inhibitors of Glycolysis and Mitochondrial Metabolism on DAD Amplitude

<table>
<thead>
<tr>
<th>Condition</th>
<th>(n)</th>
<th>Concentration</th>
<th>Duration (min)</th>
<th>DAD amplitude (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No glucose</td>
<td>4</td>
<td>...</td>
<td>30</td>
<td>2.6 ± 0.7</td>
</tr>
<tr>
<td>Iodoacetate</td>
<td>4</td>
<td>0.1 mM</td>
<td>20</td>
<td>2.7 ± 0.6</td>
</tr>
<tr>
<td>2-Deoxyglucose*</td>
<td>6</td>
<td>10 mM</td>
<td>10</td>
<td>2.4 ± 0.4</td>
</tr>
<tr>
<td>2-Deoxyglucose plus pyruvate</td>
<td>6</td>
<td>10 mM</td>
<td>30</td>
<td>2.7 ± 0.4</td>
</tr>
<tr>
<td>Cyanide*</td>
<td>5</td>
<td>0.5-2.0 mM</td>
<td>&lt;10</td>
<td>2.6 ± 0.4</td>
</tr>
<tr>
<td>Dinitrophenol*</td>
<td>10</td>
<td>0.1-1.0 mM</td>
<td>&lt;10</td>
<td>2.8 ± 0.4</td>
</tr>
</tbody>
</table>

*p < 0.01 for comparison of two columns.

**Role of Action Potential Shortening in Abolition of DADs During Metabolic Inhibition**

The reason DADs were abolished during conditions of hypoxia and metabolic inhibition is probably not because of changes in the resting membrane potential (\(E_m\)). With hypoxia, \(E_m\) decreased by a few millivolts, while a small increase in \(E_m\) was found during application of DNP. Both had the same inhibitory effect on DADs. A logical mechanism to explain the inhibitory effect of hypoxia and metabolic inhibition on DADs is by the associated shortening of the action potential. The protocol for the development of DADs relies heavily on the involvement of the calcium channels (high stimulation rate and \(\beta\)-adrenoceptor agonist stimulation) to increase the intracellular Ca concentration. Therefore, a decrease in action potential duration and, thus, the slow inward current during hypoxia should lead to less Ca entry and, therefore, to a reduced DAD amplitude. However, a number of factors argue against this proposal. First, although DAD amplitudes were completely abolished by hypoxia, action potential durations were not (Figure 3A), so there should still have been some time for Ca entry during the action potential. Second, even though the calcium current decreases during metabolic inhibition, the intracellular Ca concentration may increase, which should favor the development of DADs. Third, when DADs were evoked using methods, such as low extracellular potassium and ouabain, that led to Ca overload through inhibition of the Na-K pump and, secondary, via the sodium–calcium exchange and thus decreased the dependence on Ca entry via the action potential, hypoxia still abolished DADs. Abolition by hypoxia of DADs evoked by digitalis is accompanied by an increase in resting tension, showing that the disappearance of DADs is not due to a decrease of cellular Ca in these conditions. Recent preliminary experiments indicate that the transient inward current underlying DADs is abolished by DNP before any changes in other membrane currents, which rules out configuration changes of the action potential as a major cause for the abolition of DADs (W.A. Coetzee and L.H. Opie, unpublished observations). Still, it is difficult to exclude the effects of a changed intracellular environment during hypoxia on Ca uptake/release by the sarcoplasmic reticulum.

**DADs: Possible Dependence on Energy Metabolism**

An alternative mechanism that may be considered is that DADs are abolished (at least, in part) by a decreased availability of energy in the form of ATP. Several arguments can be raised to favor this hypothesis. First, the Ca uptake mechanism by the sarcoplasmic reticulum is dependent on the hydrolysis of ATP, while DADs are thought to be caused by a cyclic release/uptake of Ca by the membranes of the sarcoplasmic reticulum. Second, DAD amplitude and action potential duration decreased simultaneously.
in this study. The decreased action potential duration can be ascribed to an increase in a background potassium current,\textsuperscript{53,54} which is caused by a marked decrease of intracellular ATP concentration.\textsuperscript{58-60} The increase in potassium conductance during metabolic inhibition is also reversed by an intracellular injection of ATP.\textsuperscript{61} This observation cannot be used as conclusive evidence for the role of energy depletion, since injection of ATP also lengthens the action potential,\textsuperscript{61} allowing more time for Ca\textsuperscript{2+} entry and, thus, an increased likelihood for DADs. Third, in the absence of external substrates, 2-deoxyglucose abolished DADs without appreciable shortening of the action potential. Deoxyglucose is an inhibitor of glycolysis and, therefore, causes a critically decreased influx of pyruvate from glycolysis into the citrate cycle. Lactate abolished the DADs in a normal extracellular pH, which was not due to a decrease in the rate of production of glycolytic ATP since pyruvate or acetate did not mimic the effect of lactate (W.A. Coetzee and L.H. Opie, unpublished observations). Some direct effect of lactate ion on the cell membrane, perhaps by interfering with the mechanism responsible for the transient inward current or by the associated shortening of the action potential,\textsuperscript{62} cannot be excluded.

Thus, the true mechanism of abolition of DADs by metabolic inhibition is open to debate, and further work with voltage clamping to keep the membrane potential constant during metabolic inhibition is needed before any definite conclusions can be drawn.

Possible Role of DADs During Myocardial Ischemia and Reperfusion

Reperfusion after myocardial ischemia is associated with an increase of Ca\textsuperscript{2+} influx during reperfusion\textsuperscript{63} leading to an increased concentration of free intracellular Ca\textsuperscript{2+}, as measured with aequorin,\textsuperscript{14} and also to a restitution of energy production. In this study, DADs were increased in amplitude during reoxygenation, often reaching threshold to cause repetitive firing of action potentials in otherwise quiescent, if not stimulated, preparations. DAD-mediated arrhythmias were also seen in reperfused dog Purkinje fibers, but not in ventricular muscle, that had been subjected to a prior period of simulated ischemia.\textsuperscript{64} A major difference between our study and that of Ferrier et al\textsuperscript{64} is that we found DAD-mediated triggered activity to occur in ventricular muscle rather than in Purkinje fibers. The apparent discrepancy might possibly be explained by species differences, by the different tissues studied, or perhaps by the severity of hypoxia during the period of simulated ischemia. Nevertheless, our findings support those of Ferrier et al\textsuperscript{64} in that DADs were only observed in the reoxygenation period and not during simulated ischemia. It may be supposed that DADs are a possible source of ischemic arrhythmias because of the combination of increased circulating catecholamines,\textsuperscript{48-50} rise of tissue cAMP,\textsuperscript{12} and tachycardia found in the early phases of acute myocardial ischemia in vivo. However, hypoxia alone (Figure 3) or in combination with other factors occurring during myocardial ischemia (Figure 4) is able to abolish DADs provoked by isoproterenol and rapid pacing. However, the provocation of DADs required special conditions including a high extracellular Ca\textsuperscript{2+} concentration, which complicated a simple comparison to the in vivo situation. In the partial ischemic zone (e.g., the transition between the ischemic and normoxic area in regional ischemia), the contribution of DADs to ventricular arrhythmias cannot be ruled out. In zones of mild ischemia, where tissue hypoxia and potassium loss may be more moderate,\textsuperscript{67} DADs may not be inhibited as in the severely ischemic myocardium. It should be noted that the earliest activity during single premature beats, as well as the initial beats of a ventricular tachycardia, was recorded from the non-ischemic zone close to the ischemic border.\textsuperscript{7} Further work is now required to assess the role of DADs in such arrhythmias.

Acknowledgments
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KEY WORDS • arrhythmias • ischemia • automaticity • energy metabolism
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W A Coetzee and L H Opie

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