Delayed Afterdepolarizations and Triggered Activity in Ventricular Muscle from Rats with Streptozotocin-Induced Diabetes

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SUMMARY. Previous studies have shown that myocardium of the diabetic rat has impaired myoplasmic calcium metabolism. Delayed afterdepolarizations and triggered activity are potentiated by conditions believed to increase intracellular calcium concentration therefore, we performed this study to investigate the possibility that myocardium of the diabetic rat is more susceptible than normal tissue to develop afterdepolarizations and triggered activity. We used standard microelectrode techniques to record the electrical activity of papillary muscles from hearts of control rats and rats made diabetic with streptozotocin. We compared the response of control and diabetic preparations to conditions presumed to create progressively more severe degrees of myoplasmic calcium loading, viz. perfusion with solutions containing ouabain (5 X 10^-5 M) and increasing concentrations of calcium (2.4, 4.8, 7.2, and 9.6 mM). Our results showed the following. (1) Ventricular muscle from diabetic rats was more prone than normal myocardium to develop delayed afterdepolarizations and triggered activity under conditions believed to cause myoplasmic calcium overload. (2) The external calcium concentration correlated with the incidence but not the magnitude or coupling interval of the delayed afterdepolarizations in fibers of diabetic rats. (3) The action potentials in fibers of diabetic rats decreased markedly in duration after exposure to ouabain, whereas normal action potentials were not affected significantly; as external calcium was increased with ouabain still present, the action potential duration in diabetic fibers decreased slightly more, whereas the action potential duration in normal fibers did not change significantly. These results suggest that normal rat myocardium is resistant to developing myoplasmic calcium overload, whereas myocardium from the diabetic rat is susceptible to calcium loading, at least as measured by development of afterdepolarizations. (Circ Res 57: 28-34, 1985)

DELAYED afterdepolarizations and triggered activity occur in normal canine Purkinje fibers exposed to toxic concentrations of ouabain (Rosen et al., 1973; Ferrier et al., 1973; Ferrier and Moe, 1973; Aronson and Cranefield, 1974; Cranefield and Aronson, 1974), as well as in tissues from other areas of the heart (Hashimoto and Moe, 1973; Wit and Cranefield, 1976; Wit and Cranefield, 1977; Mary-Rabine et al., 1980). Similar activity has been observed in canine ventricular muscle intoxicated with acetylstrophanthidin (Ferrier, 1976), in hypertrophied ventricular muscle from rats with renal hypertension (Aronson, 1981), and in hypertrophied ventricular muscle from rats loaded with deoxycorticosterone acetate (DOCA) (Heller and Stauffer, 1979).

Previous studies (Fein et al., 1980, 1981, 1983; Penpargkul et al., 1981) have characterized the mechanical properties of cardiac tissue from another pathophysiological model: rats made diabetic by injection of streptozotocin (Schein et al., 1971; Reaven and Reaven, 1974). Several lines of evidence suggest that severe hyperglycemia lasting for several weeks may cause alterations in the handling of myoplasmic calcium by the ventricular muscle tissue. For example, when exposed to solutions containing high calcium or ouabain, diabetic papillary muscles develop a higher resting tension than normal muscles (Fein et al., 1983). Diabetic muscles also have a longer relaxation time than normal muscles (Fein et al., 1980) and sarcoplasmic reticulum (SR) isolated from ventricular muscle from diabetic rats takes up calcium more slowly than normal SR (Penpargkul et al., 1981). Other studies suggest that Na,K-ATPase activity may be depressed in diabetic cardiac tissue (Imanaga et al., 1981). Finally, our previous studies on papillary muscles showed that the action potential duration in diabetic fibers was more sensitive than in normal fibers to the shortening effects of either ouabain or increased levels of external calcium (Fein et al., 1983).

Delayed afterdepolarizations and triggered activity are potentiated by conditions believed to increase the concentration of intracellular calcium, such as the presence of digitalis or high calcium in the external solution (Rosen et al., 1973; Hashimoto and Moe, 1973; Ferrier and Moe, 1973; Allen et al., 1984). Those observations, taken together with evidence of impaired myoplasmic calcium metabolism in myocardium of diabetic rats suggested the possi-
bility that ventricular tissue from diabetic rats might be more susceptible than normal tissue to develop afterdepolarizations and triggered activity. We undertook this study to investigate that possibility.

Methods

Female Wistar rats weighing 200 g were made diabetic with a single intravenous injection of streptozotocin (60 mg/kg) as described previously (Fein et al., 1980). The diabetic state was confirmed by measurement of nonfasting glucose 3–4 weeks after streptozotocin injection. Plasma glucose of control and diabetic rats was measured again when the animals were killed for experiments. Control rats from the same stock were age-matched with diabetic rats.

After rats were anesthetized with ether, hearts were removed and papillary muscles excised from the left ventricles. Diabetic and control muscles were mounted in a tissue chamber and perfused at 37°C with Tyrode's solution of the following composition in mmol/liter: Na+, 151; Ca**, 2.4; Mg**, 0.5; K+, 4.0; Cl−, 147; H2PO4−, 1.8; HCO3−, 12.0; and glucose, 5.5. The solution was gassed with 95% O2/5% CO2.

The study was divided into two series of experiments. In the first series, each diabetic muscle was mounted with a paired control in the tissue chamber, and the two preparations were perfused simultaneously. The muscles were equilibrated in control Tyrode's solution for about 60 minutes before control records were obtained. The muscles then were perfused sequentially with the following experimental solutions: Tyrode's solution containing ouabain (5 × 10−5 M) and Tyrode's solution containing ouabain (5 × 10−5 M) with calcium increased to 4.8 mM. The papillary muscles were perfused with each experimental solution for 15 minutes before recordings were obtained. Seven pairs of muscles from diabetic and control rats were studied in this first series of experiments. In this part of the study, we used the following stimulation protocol during perfusion with each solution: (1) stimulation at a cycle length of 2000 msec for 20 seconds; (2) 10 stimuli at cycle lengths that were decreased from 1000 to 200 msec in decrements of 100 msec; (3) in five pairs of muscles, an additional stimulation protocol was completed successfully: stimulation at cycle lengths of 200, 150, and 100 msec in trains of 10, 20, 30, and 40 stimuli followed by a rest period of 10 seconds after each protocol.

In the second part of the study, we investigated seven individual diabetic muscles and five control muscles. After equilibration in control Tyrode's solution (Ca** = 2.4 mM) for about 60 minutes, each muscle was exposed to Tyrode's solution containing ouabain (5 × 10−4 M) and then sequentially to Tyrode's solution containing both ouabain and increased concentrations of Ca**: 4.8, 7.2, and 9.6 mM. For this series of experiments, we used the following stimulation protocol during perfusion with each solution: (1) stimulation at a cycle length of 2000 msec for 20 seconds; (2) stimulation at cycle lengths of 450, 300, and 150 msec with trains of 10, 20, and 30 stimuli at each cycle length; (3) a quiescent period of 10–15 seconds after each stimulation sequence.

Transmembrane action potentials were recorded by standard microelectrode techniques. Continuous recordings were obtained on a six-channel strip chart recorder (Gould Brush model 260). Photographic recordings of action potentials also were obtained from the oscilloscope with a Polaroid camera.

External stimulating pulses lasting 0.2–0.8 msec were delivered to the tissue through bipolar Teflon-coated silver wires. Patterns of stimulation were selected by a programmable digital timing system interfaced with a pulse generator connected to a stimulus isolation unit.

The amplitude of afterdepolarizations was measured as the maximum peak-to-peak value. The coupling interval was measured as the time from the onset of the upstroke of the last driven action potential to the peak of the following afterdepolarization.

Data Analysis

The analysis of our data is complicated by the fact that afterdepolarizations are all-or-none events. Therefore, it is not physiologically meaningful to treat cases in which afterdepolarizations did not occur as being equivalent to cases in which the afterdepolarizations had an amplitude or coupling interval of zero. Furthermore, the number of fibers that developed afterdepolarizations or triggered activity became larger as the external Ca** concentration (Ca**), was increased and as the rate and duration of preceding stimulation were increased. Therefore, the number of fibers with afterdepolarizations varied widely according to conditions. Accordingly we used two nonparametric statistical tests to analyze our results. The data in Table 2 from our first series of experiments were analyzed by the Wilcoxon rank sum test to establish that the tendency of muscles from diabetic rats to develop afterdepolarizations and triggered activity was statistically significant. The data in Table 3 from our second series of experiments were analyzed by the Cochran Q-test to establish that increasing [Ca**] had a significant influence on the development of afterdepolarizations and triggered activity in papillary muscles from diabetic rats. The data in Table 4 were analyzed by two-way analysis of variance. A P value of <0.05 was considered significant for all statistical tests.

Results

The characteristics of the experimental animals are given in Table 1. All diabetic rats were severely hyperglycemic (range 624–888 mg/dl) and had significantly lower body weights than control rats.

<table>
<thead>
<tr>
<th>Characteristics of Experimental Animals</th>
<th>Series I</th>
<th>Series II</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BW (g)</strong></td>
<td>152 ± 28</td>
<td>259 ± 47</td>
</tr>
<tr>
<td><strong>Glu (mg/dl)</strong></td>
<td>785 ± 10</td>
<td>562 ± 59</td>
</tr>
<tr>
<td><strong>n</strong></td>
<td>7</td>
<td>7</td>
</tr>
</tbody>
</table>

Data are given as mean ± so; BW = body weight; Glu = serum glucose. Statistical significance determined by Student's t-test for unpaired data.
These differences have been noted previously (Fein et al., 1980).

The first series of experiments was designed to determine whether papillary muscles from diabetic hearts were more susceptible than those from control hearts to develop delayed afterdepolarizations and triggered activity. Therefore, in these experiments we recorded simultaneously from diabetic and control papillary muscles. Statistical significance was determined by the Wilcoxon rank sum test.

Table 3 summarizes the incidence of delayed afterdepolarizations and triggered activity in the seven diabetic papillary muscles under all experimental conditions. In five control papillary muscles subjected to the same experimental interventions, a small (1.8-mV) afterdepolarization developed in only one preparation and only under the most extreme conditions (9.6 mM [Ca++]o, ouabain 5 X 10^{-5} M, 30 preceding action potentials at a cycle length of 150 msec). In contrast, a progressively larger number of diabetic preparations developed delayed afterdepolarizations and triggered activity under conditions of higher [Ca++]o, higher stimulation density. Statistical analysis of the data in Table 3 showed that increasing [Ca++]o significantly influenced the incidence of afterdepolarizations at cycle lengths of stimulation of 150 and 300 msec but not at a cycle length of 450 msec.

Figure 1B shows representative records from diabetic and control preparations under conditions of increasing [Ca++]o and ouabain. Under control conditions, the diabetic fiber developed a delayed afterdepolarization with a slow time course after the train of rapid stimulation. After exposure to 4.8 mM [Ca++]o and ouabain, a delayed afterdepolarization similar to that recorded in 2.4 mM [Ca++]o and ouabain occurred in the diabetic fiber. In 9.6 mM [Ca++]o and ouabain, the diabetic fiber developed a single triggered response that was followed, in turn, by a delayed afterdepolarization with a faster time course than that of the afterdepolarizations seen in lower [Ca++]o. The control preparation did not develop afterdepolarizations or triggered activity when subjected to the same experimental conditions.

Figure 2 shows the effects of stimulation parameters and [Ca++]o on the amplitude of delayed afterdepolarizations. Contrary to what we expected, these data show that addition of ouabain and increasing [Ca++]o to 4.8 mM inhibited development of afterdepolarizations. When [Ca++]o was increased to 7.2 and 9.6 mM (ouabain still present), the amplitude of the afterdepolarization increased again to values similar to those recorded in control solution.

Figure 3 shows that the coupling interval of delayed afterdepolarizations in diabetic preparations
Figure 1. Panel A: effect of the number of preceding driven action potentials on diastolic electrical activity in papillary muscles from hearts of control and diabetic rats. The number above each pair of records indicates the number of driven action potentials evoked at a cycle length of 150 msec. The external solution contained 9.6 mM [Ca++] and ouabain, 5 X 10^-5 M. The first two action potentials in each record were the last of 10 action potentials elicited at the control cycle length of 2000 msec. The number at the lower left of each record is the resting membrane potential in mV. The diabetic preparation developed a small delayed afterdepolarization after 10 driven action potentials. The delayed afterdepolarization was larger and had a more rapid time course after 20 driven action potentials; after 30 driven action potentials, a triggered response was evoked, and was followed by a delayed afterdepolarization. The control preparation failed to develop either afterdepolarizations or triggered activity under the same experimental conditions. Panel B: effects of [Ca++] and ouabain on diastolic electrical activity in papillary muscles from diabetic and control hearts. In each record, the period of rapid stimulation consisted of 30 action potentials evoked at a cycle length of 150 msec. The number at the lower left of each record indicates the resting membrane potential in mV. The diabetic preparation developed small delayed afterdepolarizations without ouabain and 2.4 mM [Ca++], and with ouabain and 4.8 mM [Ca++]. In the presence of ouabain and 9.6 mM [Ca++], a single triggered action potential developed, and was followed by delayed afterdepolarization. The control preparation failed to develop either delayed afterdepolarizations or triggered activity under the same experimental conditions.

Table 4 summarizes the effects of ouabain alone and increasing [Ca++] in the presence of ouabain on action potential parameters of diabetic and control preparations. As suggested by the records in Figure 4, the most obvious effect of exposure to ouabain was marked shortening of the action potential duration in diabetic fibers. Increasing [Ca++] in the presence of ouabain caused additional shortening of action potential duration in diabetic fibers, but the relative degree of shortening became smaller and smaller as [Ca++] increased from 4.8 to 9.6 mM. Statistical analysis of the data in Table 4 by two-way analysis of variance showed that: (1) the resting potential was significantly less negative and the amplitude significantly less in diabetic than control fibers, (2) the action potential was significantly longer in diabetic than control fibers, (3) treatment with ouabain and ouabain with increasing [Ca++].
TABLE 3
Incidence of Delayed Afterdepolarizations and Triggered Activity in Papillary Muscles of Diabetic Rats under Conditions Presumed to Cause Progressive Degrees of Calcium Overload

<table>
<thead>
<tr>
<th>CL/No.</th>
<th>2.4 mM [Ca(^{++})](_{o})</th>
<th>4.8 mM [Ca(^{++})](_{o})</th>
<th>7.2 mM [Ca(^{++})](_{o})</th>
<th>9.6 mM [Ca(^{++})](_{o})</th>
<th>No. developing delayed afterdepolarizations*</th>
</tr>
</thead>
<tbody>
<tr>
<td>450/10</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>450/20</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>450/30</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>300/10</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>300/20</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>300/30</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>150/10</td>
<td>1</td>
<td>1</td>
<td>4</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td>150/20</td>
<td>2</td>
<td>4</td>
<td>3</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>150/30</td>
<td>3</td>
<td>3</td>
<td>5</td>
<td>6</td>
<td>7</td>
</tr>
</tbody>
</table>

* Each number is the fraction of seven diabetic preparations that developed delayed afterdepolarizations under the designated experimental conditions.
† Indicates both afterdepolarizations and triggered activity. CL, cycle length of stimulation; No., number of stimuli at the designated cycle length. P values were obtained by applying the Cochran Q-test to the data for each CL/No. vs. the sequential alterations in [Ca\(^{++}\)]\(_{o}\) and ouabain given across the table.

caus ed a significant increase in amplitude and decrease in duration of action potentials but did not affect resting potential significantly, and (4) the only significant interaction was that treatment caused the duration of action potentials in diabetic rats to shorten but did not affect the duration of control action potentials.

Discussion

Our results show that ventricular myocardium from diabetic rats is more prone than normal myocardium to develop delayed afterdepolarizations under conditions believed to cause myoplasmic Ca\(^{++}\) overload. Previous studies have reported that normal (Ferrier, 1976; Hiroaka et al., 1979, 1981) and hypertrophied (Aronson, 1981) ventricular muscle can develop afterdepolarizations under conditions presumed to produce Ca\(^{++}\) overload. However, our results describe certain features of afterdepolarizations not previously reported: (1) This is the first...
description of delayed afterdepolarizations in cardiac muscle from diabetic rats. (2) The level of [Ca^{++}]_o appeared to correlate with the incidence but not the magnitude of delayed afterdepolarizations in diabetic fibers. (3) The coupling interval of the delayed afterdepolarization was not affected predictably by [Ca^{++}]_o.

We also found that action potentials of diabetic fibers responded differently than action potentials of normal fibers to treatment with ouabain and ouabain with increasing [Ca^{++}]_o. Whereas action potentials in diabetic fibers decreased markedly in duration after exposure to ouabain, normal action potentials were not affected significantly. As [Ca^{++}]_o was increased with ouabain still present, the action potential duration in diabetic fibers decreased slightly more, whereas the action potential duration in normal fibers did not change significantly.

We assume that treatment with ouabain and ouabain with increased [Ca^{++}]_o produces graded degrees of myoplasmic Ca^{++} loading. This assumption seems reasonable, since a recent study by Allen et al. (1984) showed that increasing [Ca^{++}]_o from 2–8 mM in the presence of strophanthidin caused an increase in [Ca^{++}]_i as measured by aequorin luminescence. If this assumption is correct, then our results have some interesting pathophysiological implications with respect to both normal and diabetic myocardium. First, they suggest that the normal rat myocardium is very resistant to developing myoplasmic Ca^{++} overload, at least as measured by development of afterdepolarizations and alterations in action potential duration. Second, diabetic fibers show increased susceptibility to Ca^{++} loading, as reflected by development of afterdepolarizations. On the other hand, diabetic fibers do not show the expected response if one assumes that the magnitude of afterdepolarizations is proportional to the degree of Ca^{++} overload. This finding suggests that, although Ca^{++} loading may be a prerequisite for afterdepolarizations to develop, the dynamic behavior of this phenomenon may depend on other factors such as the amount of Ca^{++} that enters the myoplasm via the slow inward current. Although we have not measured slow inward current directly, the marked shortening of the action potential in diabetic fibers as [Ca^{++}]_o was increased is consistent with more rapid inactivation of the slow inward current under

<table>
<thead>
<tr>
<th>[Ca^{++}]_o (mM)/ouabain (M)</th>
<th>RMP (mV)</th>
<th>AMP (mV)</th>
<th>APD_{90} (msec)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Diabetic</td>
<td>Control</td>
<td>Diabetic</td>
</tr>
<tr>
<td>2.4/0</td>
<td>64 ± 6</td>
<td>76 ± 4</td>
<td>80 ± 10</td>
</tr>
<tr>
<td>2.4/5 × 10^{-5}</td>
<td>71 ± 6</td>
<td>72 ± 8</td>
<td>93 ± 5</td>
</tr>
<tr>
<td>4.8/5 × 10^{-5}</td>
<td>72 ± 5</td>
<td>76 ± 7</td>
<td>98 ± 8</td>
</tr>
<tr>
<td>7.2/5 × 10^{-5}</td>
<td>75 ± 6</td>
<td>78 ± 6</td>
<td>104 ± 5</td>
</tr>
<tr>
<td>9.6/5 × 10^{-5}</td>
<td>77 ± 9</td>
<td>78 ± 9</td>
<td>104 ± 5</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± sd. Group refers to the difference between diabetic and control independent of treatment. Treatment refers to differences produced by the treatment (ouabain and increasing [Ca^{++}]_o) independent of whether the preparation was from a diabetic or control heart. Interaction refers to an effect of treatment that is significantly different in diabetic or control preparations. P = probability value for diabetic vs. control based on the F test. Degrees of freedom = 50. RMP = resting membrane potential; AMP = amplitude of action potential; APD_{90} = duration of action potential to 50% of complete repolarization.
these conditions. Support for this view is provided by a study in fibers of the canine coronary sinus rats that showed that acceleration of repolarization decreased the amplitude of delayed afterdepolarizations (Henning and Wit, 1984). Another possibility is that increasing [Ca++]o, even in the presence of ouabain, does not produce a monotonic increase in the level of myoplasmic Ca++ in diabetic fibers.

Regardless of the precise mechanism involved, our results show that myocardium of diabetic rats has a propensity to develop Ca++ overload as reflected by the appearance of delayed afterdepolarizations. Under certain conditions, these afterdepolarizations might reach the threshold for triggered activity and thereby lead to arrhythmias in diabetic myocardium.

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INDEX TERMS: Diabetes • Afterpotentials • Triggered activity • Calcium overload

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