Automatic Activity in Depolarized Guinea Pig Ventricular Myocardium

Characteristics and Mechanisms

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SUMMARY Membrane potential was changed uniformly in segments, 0.7-1.0 mm long, of guinea pig papillary muscles excised from the right ventricle by using extracellular polarizing current pulses applied across two electrically insulated compartments. Rhythmic automatic depolarizations (RAD) occurred in 90% of preparations superfused with Tyrode's solution at maximum diastolic membrane potentials ranging from -35.2 ± 7.5 (threshold) to +4.0 ± 9.2 mV. The average maximum dV/dt of RAD ranged from 17.1 to 18.0 V/sec within a membrane potential range of -40 to +20 mV. Raising extracellular Ca²⁺ concentration [Ca²⁺]₀ from 1.8 to 6.8 mM, or application of isoproterenol (10⁻⁶ g/ml) enhanced the rate of RAD, but lowering [Ca²⁺]₀ to 0.4 mM or exposure to MnCl₂ (6 mM) abolished RAD. RAD were enhanced by lowering extracellular K⁺ concentration [K⁺]₀ from 5.4 to 1.5 mM. RAD were suppressed in 40% of fibers by raising [K⁺]₀ to 15.4 mM, and in all fibers by raising [K⁺]₀ to 40.4 mM. This suppression was due to increased [K⁺]₀, and not to K⁺-induced depolarization because it persisted when membrane potential was held by means of a conditioning hyperpolarizing pulse at the level of control resting potential. The slope resistance increased gradually after maximum repolarization. These observations suggest that the development of RAD in depolarized myocardium is associated with a time-dependent decrease in outward current (probably K current) and with increase in the background inward current, presumably flowing through the slow channel carrying Ca or Na ions, or both.

AUTOMATICITY of ventricular myocardial fibers has been observed after superfusion with K⁺-free and Ca²⁺-free solution, exposure to Ba²⁺ salts, excessive mechanical stretch, and several other interventions (for references, see Antoni and Cranefield, p. 213). Common to all these interventions is the depolarization to levels of membrane potential at which the rapid inward sodium current is partly or completely inactivated. Automatic activity of ventricular myocardial also has been induced by depolarizing direct current pulses of several seconds' duration applied across a single sucrose gap in sodium-free and regular Tyrode's solution. Recently Katzung showed that Ca and Na ions influence automaticity of papillary muscles from guinea pigs and cats and dog trabeculae after depolarizations to membrane potentials less negative than -60 mV.

Imanishi used the partition chamber method of Kamiyama and Matsuda to study the effect of depolarizing currents on quiescent dog Purkinje fibers. In this preparation depolarization induced Ca²⁺-sensitive oscillations which were attributed to ionic current passing through the slow channel. Subsequently, we have shown that the same procedure induces spontaneous rhythmic automatic depolarizations of guinea pig ventricular papillary muscles. We have confirmed and extended the observations of Katzung, who studied the same phenomenon but used the single sucrose gap method. Our studies not only supplement the evidence that rhythmic depolarizations depend on Ca²⁺ but also show that these depolarizations strongly depend on K⁺. Our results indicate that both outward and inward currents play a role in the genesis of spontaneous rhythmic depolarizations in depolarized ventricular myocardium.

The purpose of this paper is to report (1) the detailed characteristics of rhythmic automatic depolarizations (RAD) in homogeneously depolarized myocardium within a wide range of membrane potentials, (2) the effects of Ca²⁺, Mn²⁺, and isoproterenol on RAD, (3) the effects of changes in extracellular K⁺, and (4) the measurements of slope resistance during diastolic depolarization of rhythmic responses.

Methods

Guinea pigs weighing 250-300 g were killed by a blow on the neck. The hearts were rapidly removed and papillary muscles, 3-5 mm long and 0.5-1.0 mm in diameter, were dissected from the right ventricle. The schematic diagram of the experimental setup is shown in Figure 1.
The tendinous end of the preparation was fastened with a thread to a small rubber block (G) glued to the floor of a chamber. The latter consisted of two compartments: A (1.5 ml) and B (0.6 ml). The longer segment of the muscle occupied compartment A, and the shorter segment with the tendinous end (0.7 -1.0 mm long), compartment B. After the muscle was mounted the compartments were separated physically and insulated electrically by a Vaseline-coated plexiglass plate (P) (0.2 mm thick) which was fitted tightly into a slot between the compartments. Compartment B was perfused with Tyrode's solution (control) or other test solution at a rate of 2.5 ml/min, and compartment A with Tyrode's solution or isotonic KG solution at a rate of 2.5 ml/min. The latter was used to detect possible leakage between the two compartments. We assumed an absence of leakage when the presence of KCl solution in compartment A did not change the resting membrane potential of cells in compartment B. The bath temperature was maintained at 36-37°C. The preparation was stimulated by a Grass model S4GR stimulator (Si) at a constant rate of 0.5-1.0 cycles/sec through a pair of silver wire (diameter = 0.1 mm) electrodes which were insulated except at their tips (D). The driving stimuli were monophasic pulses 3 msec in duration and twice diastolic threshold strength. A Grass S-88 stimulator (SII) was used to deliver extracellular polarizing square pulses of current of various strengths (5 x 10^-4 to 10^-3 A) and durations (2-9 sec) through a pair of spiral Ag-AgCl electrodes, one in each compartment. A resistor (28 kΩ) in series with the stimulator (SII) maintained the polarizing current nearly constant. The driving stimulus (S1) was turned off before application of polarizing currents. In some experiments, a third Grass stimulator, model S-88 (SIII), was connected in series with SII to alter the membrane potential before or during application of the depolarizing current pulses.

Membrane potentials were measured as potential differences between a pair of intra- and extracellular standard glass microelectrodes, positioned close to each other near the partition plate. The resistance of the electrodes ranged from 10 to 20 MΩ. The measured membrane voltage was led to a differential DC preamplifier (Transidyne, model MPA 6).

Relative membrane resistance was determined by measuring voltage changes induced by small square hyperpolarizing pulses 20-50 msec in duration. These pulses were superimposed on the spontaneous responses occurring during application of long depolarizing current pulses. The electrotonic potentials induced by the hyperpolarizing current pulses were measured in tracings magnified 6x. The amplitude of each deflection was measured as the difference between the membrane potential at the center of the pulse and the potential at the same moment during diastole in the absence of hyperpolarization.

We realize that a 20-msec, or even a 50-msec, current pulse may be not sufficiently long to fully charge the membrane capacitance, but we could not use longer pulses for the evaluation of slope resistance because the total duration of diastolic depolarization was only about 100 msec. Therefore, our results probably did not measure the total membrane input resistance at each locus of current application. However, the measurements were made at intervals longer than 4.4 msec, i.e., the membrane time constant in mammalian ventricular muscle.13

The membrane potential, the maximum dV/dt, and the

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Figure 1 Schematic diagram of the experimental setup. A and B, chamber separated by partition plate (P); M, microelectrodes; S, specimen (guinea pig papillary muscle); D, stimulating electrodes; E, Ag-AgCl output plate electrode of the feedback current for bath clamping system; G, rubber block used as a base for pinning the thread attached to the tendinous end of the papillary muscle; S1, SII, and SIII, stimulators. Each stimulator output was delivered through an individual stimulus isolation unit.
amplitude of depolarizing current were displayed simulta-
neously on the screen of a Tektronix 5103N storage oscil-
loscope, and photographed with a Polaroid camera.

The possible errors in the measurement of the mem-
brane potential stemming from polarization-induced
changes in the extracellular potential were minimized by
the use of an extracellular voltage clamp system. The
extracellular voltage clamp sensed the potential immedi-
ately adjacent to the preparation through the extracellular
electrode, and drives the surrounding bath as required
to hold the extracellular potential at a virtual ground poten-
tial. The bath was driven through an Ag-AgCl plate (4
mm wide) electrode (Fig. 1, E) attached to the walls of
the chamber, and bent to conform approximately to the exter-
ernal contour of the tissue in compartment B (Fig. 1). To
test the effectiveness of this clamp, we measured the
potential between two extracellular microelectrodes
within the bath during application of depolarizing current
pulses. Weaker depolarizing pulses required to depolarize
the membrane to potentials more negative than 0 mV
produced no potential difference between the two microe-
lectrodes, but stronger pulses required to depolarize the
membrane to positive potentials caused potential differ-
ences up to 5 mV. In the absence of the clamp, the
maximum potential difference between these two microe-
lectrodes in six experiments ranged from 6 to 12 mV.

The cycle length, overshoot, and maximum dV/dt of the
automatic responses appearing in depolarized myocar-
dium were determined by averaging the values of all re-
ponses recorded during a single application of the depo-
larizing pulse, excluding the dV/dt and the overshoot of
the first response, and the duration of the first cycle.

The composition of Tyrode’s solution (control) was:
NaCl, 137.0; KCl, 5.4; CaCl2, 1.8; MgCl2, 1.05;
NaHCO3, 11.9; NaH2PO4, 0.3; and glucose, 5.0. Isopro-
terol (ISP) was added directly to the control solution to
produce a concentration of 5 x 10⁻⁷ or 10⁻⁸ g/ml.

Changes in concentration of Ca²⁺ or K⁺ in Tyrode’s solu-
tion were made without adjusting for change in osmolality.
The manganese solution was prepared by adding 4-6
mm MnCl₂ to tromethamine (Tris) buffer-Tyrode’s solu-
tion (Tris = 5.4 ITIM) equilibrated with 100% O₂. Before
use the Tris buffer solutions were titrated with HO to pH
7.4 at a temperature of 36°C. It has been shown previously
that this buffer does not change the physiological prop-
ties of cardiac fibers. Statistical evaluation of results was
performed with Student’s t-test.

Results

EFFECT OF DEPOLARIZING CURRENTS IN TYRODE’S
SOLUTION

The effect of depolarizing currents 3–9 seconds in dura-
tion and of varying strength was studied in 69 fibers. The
resting membrane potential ranged from —78 to —88 mV.

The procedure had no deleterious effects on the prepa-
ration. After each series of depolarizations the resting
membrane potential and the duration of the action poten-
tial returned to control values and repeated series of depo-
larizations produced identical results in several consecu-
tive tests. The most negative maximum diastolic potential
at which RAD appeared averaged —35.2 ± 7.5 mV, and
the membrane potential at which RAD ceased averaged
+4.0 ± 9.2 mV.

Figure 2 shows results of a typical experiment in which
the strength of the depolarizing current was increased in
steps from panels A to F. In panel A, the first three steps
produced a passive electrotonic potential, the fourth step
an action potential with a stable resting membrane poten-
tial, the fifth step an action potential with a slight diastolic
depolarization, and the sixth step induced spontaneous
rhythmic activity. This activity occurred at a more rapid
rate with stronger depolarizing currents (B–E). Panels B–
D show that the maximum diastolic potential became
gradually more negative. We attribute this phenomenon to
the time-dependent decrease in membrane resistance in-
herent in the current-voltage relation that obtains within
this range of membrane potentials. In all three records (B,
C, D), the first response differed in shape and amplitude
from the subsequent responses. This probably was due to
the less negative takeoff potential of the first response.

In all records the duration of the first cycle was longer
than that of the second cycle but the durations of cycles follow-
ing the first cycle were constant. The responses in panel A
began abruptly, which suggests an electrotonic interaction
with neighboring fibers. In other records (B–E) the transi-
tion from phase 2 to phase 0 was smooth and gradual.
Depolarization to about 0 mV elicited oscillations of low
amplitude (E). Such oscillations also occurred in some
experiments after two or more full-sized spontaneous de-
polarizations (e.g., see Fig. 5B2). When the applied currents depolarized the fiber to potentials more positive than +10 mV, spontaneous depolarizations did not occur (F).

Table 1 summarizes the effect of membrane potential on the cycle length, maximum dV/dt, and the overshoot of RAD. The oscillations of amplitude lower than 10 mV induced by depolarization to membrane potentials less negative than –10 mV are not included in the analysis of these results. The average cycle lengths within the ranges of membrane potential from –40 to –10 mV and –10 to +20 mV were similar. The average maximum values for dV/dt within these two membrane potential ranges also were not significantly different from each other. Within the membrane potential range from –50 to –40 mV the average cycle length, maximum dV/dt, and overshoot were significantly different from the corresponding average values at other levels of depolarization.

## SPATIAL HOMOGENEITY OF MEMBRANE POTENTIAL

The voltage homogeneity during application of depolarizing currents of various strengths was tested in seven fibers by comparing the transmembrane potentials recorded simultaneously with two pairs of microelectrodes. The schematic diagram of the method and the results of two experiments in two different ventricular fibers are shown in Figure 3. Two microelectrode pairs (1 and 2) were placed at the opposite end of the preparation (distance, 0.8 mm), and the transmembrane potentials were recorded simultaneously by independent amplifying systems (1 and 2 in C). The transmembrane potentials recorded at both sites were the same at all levels of depolarization, and the shapes, duration, and amplitudes of the responses were nearly identical (Fig. 3A and B).

To determine the dependence of spontaneous automatic activity in depolarized myocardium on the membrane current passing through the slow channel, we studied the effects of changes in extracellular calcium, isoproterenol, and manganese.

### EFFECT OF EXTRACELLULAR CALCIUM

The effect of [Ca\(^{2+}\)]\(_o\) on RAD was studied in six fibers superfused with low [Ca\(^{2+}\)]\(_o\) (0.4–0.6 mM) and in five fibers superfused with high [Ca\(^{2+}\)]\(_o\) (6.8 mM). A decrease in [Ca\(^{2+}\)]\(_o\) from 1.8 to 0.4 mM abolished RAD in four fibers, while a decrease to 0.6 mM decreased the rate, the overshoot, and the maximum dV/dt in two fibers. Figure 4 shows that the increase in [Ca\(^{2+}\)]\(_o\) increased the average overshoot from 59.6 ± 11.5 to 72.2 ± 5.7 mV, increased the average maximum dV/dt from 17.3 ± 5.6 to 23.7 ± 6.8 V/sec, and decreased the average cycle length from 510.0 ± 36.0 to 442.3 ± 32.1 msec. All these changes were statistically significant. From the plot of the change in overshoot against the logarithm of the change in [Ca\(^{2+}\)]\(_o\), we estimated a 22.7-mV change in the overshoot amplitude for a 10-fold change in [Ca\(^{2+}\)]\(_o\).

### EFFECT OF ISOPROTERENOL

In nine nondepolarized fibers ISP (10\(^{-5}\) g/ml) shifted phase 2 to more positive potentials and prolonged the action potential duration without changing the maximum dV/dt (Fig. 5B1). In depolarized fibers ISP increased the rate of RAD, the amplitude of the overshoot, and the maximum dV/dt in four fibers, and induced RAD in four of five fibers in which the depolarizing currents of varying strength had failed to produce spontaneous depolarizations in control solution (Fig. 5A2). These effects are consistent with increased activation of slow inward current by ISP. ISP in a concentration of 5 × 10\(^{-7}\) g/ml had an effect similar to that of ISP at 10\(^{-5}\) g/ml. Figure 5C shows that the ISP effect was almost completely reversible within 40 minutes after washing out the drug.

### EFFECT OF MANGANESE

Several investigators have used Mn\(^{2+}\) to selectively block the slow inward current.\(^{15-17}\) The effect of 5 and 6 mM Mn\(^{2+}\) was studied in three fibers. Both concentrations...
Figure 4 Overshoot, maximum dV/dt, and cycle length of rhythmic automatic depolarizations (RAD) at two different Ca\(^{2+}\) concentrations in five preparations. Circles indicate mean values, and vertical bars standard deviations. *P < 0.001; **P < 0.01. See text.

had the same effect. The typical effect of Mn\(^{2+}\) on RAD is illustrated in Figure 6. This figure shows that RAD were abolished by exposure to 6 mM Mn\(^{2+}\) (panel B) but reappeared after washing the Mn\(^{2+}\) out with control solution (panel C). The same result was observed in two other preparations.

**EFFECT OF EXTRACELLULAR POTASSIUM**

The effect of [K\(^+\)]\(_o\) on RAD was studied in eight fibers superfused with high [K\(^+\)]\(_o\) (15.4 or 40.4 mM) and in three fibers superfused with low [K\(^+\)]\(_o\) (1.5 mM) solution. Typical effects of low and high [K\(^+\)]\(_o\) are shown in Figure 7. It can be seen that the rate of depolarizations increased following change from control (7A1) to low K solution (7A2). Such an increase in rate occurred in all experiments and was due to both more rapid repolarization and more rapid after-depolarization. In this experiment RAD occurred in the control solution after depolarization to —8 mV but not after depolarizations to more negative potentials (7A1). Following a decrease in [K\(^+\)]\(_o\) to 1.5 mM (7A2) RAD occurred after depolarization to —22 mV. However, in two other experiments low [K\(^+\)]\(_o\) had no effect on the threshold potential for rhythmic responses. Similarly low [K\(^+\)]\(_o\) had no effect on the level of membrane potential at which RAD disappeared.

Figure 7B1 shows data from an experiment from another preparation in which the rate of RAD in the control solution was faster than in Figure 7A1. Following the increase of [K\(^+\)]\(_o\) to 15.4 mM (7B2) depolarization to the same membrane potential as in the control solution induced RAD at a slower rate than in Figure 7B1. This effect was observed in three of five fibers superfused with 15.4 mM [K\(^+\)]\(_o\). It was caused by slower repolarization and slower after-depolarization. In the remaining two fibers, an increase in [K\(^+\)]\(_o\) totally abolished the RAD.

Raising [K\(^+\)]\(_o\) to 40.4 mM totally abolished RAD in tests on three fibers. Results of a typical experiment are shown...
**FIGURE 6** Effect of Mn^{2+} on rhythmic automatic depolarizations (RAD) in a depolarized ventricular muscle fiber. At the top is shown the strength of the applied depolarizing current pulses; in the middle, the transmembrane potentials; and at the bottom, maximum dV/dt. A, control: RAD occur after depolarization to −32 mV. B, 20 minutes after application of 6 mM MnCl₂, depolarizations to membrane potentials ranging from −70 to +18 mV do not elicit RAD. C, 30 minutes after return to control solution without Mn^{2+}: RAD occur again after depolarization to −32 mV.

**FIGURE 7** Effect of changes in [K⁺]₀ on rhythmic automatic depolarizations (RAD) in depolarized ventricular muscle fibers in two different experiments (A and B). A, 20 minutes after change from control [K⁺]₀ = 5.4 mM (1) to [K⁺]₀ = 1.5 mM (2). Low [K⁺]₀ hyperpolarizes the resting membrane potential by 7 mV, and increases the rate of RAD (A2). B, 20 minutes after change from control [K⁺]₀ = 5.4 mM (1) to [K⁺]₀ = 15.4 mM (2). High [K⁺]₀ depolarizes the resting membrane potential by 18 mV, and decreases the rate of RAD (B2). In A, membrane potential is shown at the top, dV/dt at the bottom. In B, dV/dt is at the top, relative strength of applied current in the middle, and membrane potential at the bottom. See text.

In Figure 8, in the control solution (K⁺ = 5.4 mM) and resting membrane potential = −80 mV, RAD were elicited by depolarizations to −40 mV (A) and −27 mV (B) but not by depolarizations to membrane potentials more negative than −47 mV (A) or more positive than −4 mV (C). However, when the same fiber was superfused with [K⁺]₀ = 40.4 mM and the resting membrane potential became −48 mV, RAD failed to appear following depolarizations ranging from −38 mV (D) to +20 mV (E). Figure 8F shows that in this instance suppression of RAD was due to increased [K⁺]₀ and not to K-induced depolarization, because it persisted when the membrane potential was held at the level of the control resting potential by means of a conditioning hyperpolarizing pulse of about 4 seconds’ duration. Subsequently, the preparation was superfused with solution containing 15.4 mM [K⁺]₀ and depolarizing currents were applied when the resting membrane potential increased to −62 mV, but no RAD appeared following depolarizations within the −53 to +13 mV range (G). In Figure 8E, F, and G are shown the characteristic slow repolarization and long duration of action potentials elicited by depolarizing currents in high K⁺ solution. The slowing of repolarization increased progressively with increasing levels of depolarization. Figure 8H and I shows that the effects of high [K⁺]₀ were reversi-
The homogeneous and synchronous activity at both ends of the preparation indicates that RAD are due to true ventricular automaticity rather than to circus movement or reentry resulting from inhomogeneous depolarization. This conclusion is supported by the observations that in most records the transition from phase 4 to phase 0 was smooth and gradual, and that the responses were of uniform shape and duration. Most of our records were undistorted by electrotonic interactions with the neighboring fibers. We would have expected such interactions, due to different rates of firing or to different amplitudes of individual responses to depolarization, to occur even in homogeneously depolarized preparations. However, in our experiments the ranges of cycle lengths and overshoots of RAD were narrow, and the level of depolarization had no significant effect on these parameters at potentials less negative than $-40\, \text{mV}$. Within the membrane potential range from $-50$ to $-40\, \text{mV}$ the average cycle length, maximum dV/dt, and overshoot were significantly different from the corresponding average values at other levels of depolarization. This suggests that the rapid Na$^+$-carrying system may not have been completely inactivated and thereby could have contributed to the genesis of RAD within this potential range. The latter finding may explain the predominance of electrotonic interactions during depolarizations to membrane potentials less negative than $-40\, \text{mV}$.

We have considered the possibility that the RAD were induced not by direct depolarization of the ventricular fibers but by electrotonic spread of impulses conducted from depolarized Purkinje fibers contained within the papillary muscles. However, a previous study in which the same method was used showed that pacemaker activity in quiescent Purkinje fibers was initiated by depolarizations to membrane potentials ranging from $-60$ to $-50\, \text{mV}$. Automaticity within this range occurred in several fibers but such fibers were not used in this study.

Our study shows that some preparations were less "responsive" to the depolarizing currents, as manifested by a narrower range of potentials at which responses occurred, slower rates of RAD, or their cessation before the end of the depolarizing current pulse. In about 10% of preparations depolarizing currents elicited only low amplitude oscillations. However, even in these fibers RAD could be produced after application of isoproterenol. The causes of individual differences in the "responsiveness" were not obvious, because the ages and weights of all guinea pigs were similar and all preparations were handled in a similar manner.

The use of a bath clamp minimized the possible errors in the measurement of membrane potentials following application of polarizing currents. The maximum error stemming from potential differences was 5 mV. However, errors of such magnitude were expected only during application of stronger depolarizing currents required to hold the membrane at positive potentials. Therefore, they detract from the accuracy of the values of membrane potentials.
MECHANISMS

Several investigators studied rhythmic automatic, or oscillatory activity in Purkinje fibers and ventricular myocardial fibers. Katzung pointed out that the maintenance of sustained rhythmic activity depends on three processes: slow depolarization which prevents the return to maximum diastolic potential, rapid depolarizing spike, and repolarization to maximum diastolic potential. The precise mechanism for these processes has not been completely clarified but all investigators postulate some type of an interaction between time-dependent variations in outward and inward ionic membrane currents (for references see Cranefield and Katzung). Our study adds evidence for the role of both the outward and the inward current in the genesis of RAD.

We have shown that the slow depolarization can be completely suppressed by Mn++, (Fig. 6), which is believed to block the inward current passing through the slow channel, and by high [K+]o (Fig. 8), which is assumed to increase the outward current by decreasing the membrane resistance. The effect of high [K+]o in our study was independent of the depolarization induced by the K ion and resembled the effect of K+ on in Purkinje fibers. It should be mentioned that high [K+]o does not always suppress spontaneous activity in depolarized cardiac fibers. Thus, Aronson and Cranefield induced spontaneous rhythmic depolarizations in Purkinje fibers superfused with high Ca++, Na+-free solution and depolarized by K+ to about −50 mV. In our laboratory spontaneous depolarizations were never observed in guinea pig papillary muscles depolarized by high [K+]o to various levels of membrane potentials, even after an increase in [Ca++]o and addition of isoproterenol (unpublished observations of W. Poplawska and B. Surawicz). The differences between our results and the results of Aronson and Cranefield may be due to the difference in the fiber types, or to species difference. For instance, it has been reported that high [K+]o and epinephrine transform the oscillatory afterpotentials into sustained rhythmic activity in the bovine but not in the similarly treated canine Purkinje fibers (see Cranefield, p. 211).

An increase in the relative membrane resistance during diastolic depolarization found in this study is in agreement with the results of Katzung, who applied current pulses at 5 Hz (pulse duration not stated) to the same type of preparation. Katzung stated that in the absence of full voltage clamp analysis his results do not reveal whether the change in membrane resistance is the cause or the result of the observed phase 4 depolarization. We face the same problem of interpretation. However, if the current-voltage relation in our fibers is similar to that in Purkinje fibers (for references see Noble or in frog ventricular fibers), then the observed diastolic depolarization is due more likely to a time-dependent increase in membrane resistance and not to the anomalous (inward-going) rectification. We measured the membrane resistance at membrane potentials less negative than −20 mV, i.e., within a potential range in which the membrane displays not an inward-going but a time-dependent delayed (outward-going) rectification. This would be expected to cause a time-dependent decrease rather than an increase in membrane resistance. Such a time-dependent decrease in membrane resistance is also suggested by the gradual decrease (more negative) of the maximum diastolic potentials during application of depolarizing current pulses (Figs. 2B-D, Figs. 3A, 6B, and 7B, and 8B). Our results suggest, therefore, that change in membrane resistance is the cause rather than the result of diastolic depolarization.

The following results of our study support the hypothesis that the slow inward current plays an important role in the genesis of automatic depolarizations: (1) sensitivity to extracellular Ca++ concentration, (2) the effect of isoproterenol, and (3) the effect of manganese. We found that the calculated change in amplitude of the overshoot for a 10-fold change in Ca++ was 22.7 mV, whereas the expected change calculated from the Nernst equation was about 30 mV. Reuter and Scholz found a similar discrepancy between the recorded and expected responses to changes in [Ca++]o, following depolarizations in sheep and calf trabeculae superfused with Na+-free solution. They attributed this discrepancy to the possible influence of membrane currents carried by other ions.

In our experiments the most negative membrane potential at which RAD appeared was about −35.2 mV, and the maximum dV/dt averaged 17.3 V/sec. These results are also consistent with the hypothesis that RAD depend on the membrane current passing through the slow channel. In guinea pig myocardium the threshold potential for the slow inward current is about −40 mV and the current is maximum for depolarizations ranging from −10 to +10 mV. In dog Purkinje fibers the maximum dV/dt of action potentials dependent on the slow inward current (slow responses) ranged from 5−20 V/sec.

It is reasonable to attribute the repolarization of RAD to the time-dependent outward current which has been recorded in ventricular muscle fibers (for references see Trautwein). The current has been analyzed by Ochi and Nishiye, for the guinea pig, Beeler and Reuter for the dog, and McGuigan for sheep and calf fibers. Ochi and Nishiye have suggested that the outward current is carried by K ions. This outward current will be expected to overcome the inward current when the membrane potential approaches the equilibrium potential for the inward current. Table 1 shows that the overshoot of the RAD exceeded the normal overshoot in ventricular muscle fibers by about 10−20 mV. and was close to the calculated reversal potential of calcium current (+60 mV) in dog ventricular muscle in normal Tyrode’s solution. This finding lends further support to the role of Ca++ current in the genesis of RAD.

In our study low [K+]o accelerated the repolarization of RAD. This effect was similar to the effect of low [K+]o on the initial phase of repolarization of the Purkinje and ventricular fibers. We attributed it to an increased driving force due to increased difference between membrane potential and K+ equilibrium potential. A similar mechanism may be postulated in this study. The slowing of repolarization in high [K+]o solution most likely is caused by the stronger current required to depolarize the fibers.
superfused with high $[K^+]_o$ solution. Morad and Trautwein showed that the duration of action potential in sheep ventricular fibers became progressively longer with application of progressively stronger pulses of constant depolarizing current. Similarly, in dog and cat papillary muscles depolarized with Tyrode’s solution containing KCl (13.5 mmol/liter) the duration of action potential increased with increasing strength of depolarizing stimuli.

**RELATION TO CARDIAC ARRHYTHMIAS**

Cranefield suggested at least two ways in which spontaneous depolarization may occur in nonpacemaker fibers in situ, namely, by the injury current that flows between an infarct and the adjacent tissue or by the current spreading electrotonically across the region of block. Solberg et al. observed pacemaker-like activity in depolarized ventricular myocardial fibers from the infarcted dog heart, and from the ischemic human ventricle. Scherlag et al. showed that certain arrhythmias in the ischemic myocardium may arise from the epicardium, and suggested that this could be due to pacemaker-like activity in the ventricular fibers. Our study shows that such activity could occur in depolarized myocardium. However, if the depolarization were caused by potassium liberated from damaged fibers, the high K$^+$ concentration might be expected to oppose the specific effect of depolarization and prevent the appearance of automatic depolarizations.

Both depolarization and increased $[K^+]_o$ may be of importance in the genesis of arrhythmias in the ischemic or damaged myocardium. Our experimental model shows that depolarization and high extracellular K$^+$ concentration produce opposite effects on spontaneous electrical activity in the ventricular myocardium. These observations suggest that an improved understanding of cardiac arrhythmias may require an increased knowledge of various interrelations between the levels of polarization and the ionic milieu in the myocardium.

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