Effects of Extracellular Potassium on Ventricular Automaticity and Evidence for a Pacemaker Current in Mammalian Ventricular Myocardium

BERTRAM G. KATZUNG AND JAMES A. MORGENSTERN

SUMMARY Automaticity was induced in isolated guinea pig and cat papillary muscles by application of depolarizing constant current pulses. Increasing extracellular potassium from 1 to 15 mM caused a shift of the pacemaker-like activity to less negative diastolic potentials and a decrease in maximum phase 4 slope. Membrane resistance, estimated from the relation of applied current to maximum diastolic potential, decreased when extracellular potassium was increased. Voltage clamps of cat papillary muscle demonstrated that action potentials activate a time-dependent outward current which has a reversal potential of \(-79.1\) mV (\(\pm 0.99\) SE, \(n = 20\)) at an extracellular potassium concentration of 5 mM. The reversal potential of this current varies with extracellular K\(^+\) with a slope of \(50-60\) mV per 10-fold concentration change. The current is activated by voltage clamps or action potential plateaus in the range of \(-30\) to \(+30\) mV. It has a time constant of deactivation which increases from approximately 100 to over 400 msec as clamp potential is increased from \(-90\) to \(-60\) mV. It is proposed that this current is equivalent to \(I_{\alpha}\) demonstrated in other cardiac tissues and is responsible, in combination with inward currents, for automaticity in ventricular fibers.

It is now clear that under certain conditions ventricular myocardial cells are capable of automatic repetitive depolarization, i.e., pacemaker-like activity. Such conditions include depolarization by direct application of current and by exposure to barium ion. However, little information is available concerning the underlying ionic mechanisms for automaticity in this tissue.

Studies in Purkinje fibers\(^6-7\) and in frog atrial trabeculae\(^8\) have shown that automaticity in these cell types may be ascribed to the decay of time-dependent outward currents in the presence of inward currents of sodium (Na\(^+\)), calcium (Ca\(^{2+}\)), or both. Prior studies with ventricular preparations have provided evidence for the participation of Ca\(^{2+}\) and Na\(^+\) as inward charge carriers during automatic depolarization.\(^2\) Recently, Imanishi and Sura-wiecz reported that depolarization-induced automaticity in guinea pig ventricle is suppressed by increases in extracellular potassium (K\(^+\)). This observation suggests that K\(^+\) permeability is an important factor in ventricular automaticity.

The present study was therefore carried out to document the effects of extracellular K\(^+\) on ventricular automaticity and to study time-dependent currents present during pacemaker depolarization (phase 4).

Methods

Experiments with guinea pig papillary muscles were carried out in a single sucrose gap chamber as described previously. For studies of cat ventricular muscle, right ventricular papillary muscles were removed from cats (2-5 kg) anesthetized with pentobarbital (30 mg/kg, ip). The muscles were mounted and superfused as described for guinea pig tissue.

The solutions used included normal Tyrode's solution.
which contained (mM concentrations): NaCl, 134; MgCl₂, 1.05; CaCl₂, 1.8; NaHCO₃, 11; NaH₂PO₄, 0.42; and glucose, 5.5. KCl was added to final concentrations of 0.5–15 mM, with 5.0 mM taken as the “control” concentration. No correction was made for variation in osmotic pressure resulting from these changes. The solution was saturated with 95% O₂, 5% CO₂. The central (gap) compartment was perfused with isotonic sucrose solution containing (mM) sucrose, 300; glucose, 5.5; CaCl₂, 0.02. This solution was saturated with 100% O₂. The rear (current injection) compartment of the muscle chamber was perfused with normal Tyrode’s solution during the equilibration period and changed to isotonic K⁺ Tyrode’s solution before beginning the experimental protocol. This solution was identical to the normal Tyrode’s except that KCl, 134 mM, replaced NaCl. All experiments were carried out at 35°C.

Instrumentation for current clamps was that previously described. For voltage clamp experiments, the instrumentation was supplemented as follows: (1) A ground clamp of the type described by New and Trautwein helped to reduce the effects of the extracellular resistance. (2) A high speed (150 μsec) electronic switch (Siliconix DG188) permitted switching between current clamp and voltage clamp modes under stimulator control. Total switching time, measured at high sweep speed from end of current clamp to onset of voltage clamp, was less than 20 μsec. (3) An over-voltage protection device, modified from Tsien, was placed between the clamp output and the preparation. This device automatically opened the circuit if the output voltage of the clamp exceeded a preset value (44–88 V) for more than 1 msec.

After mounting, muscles were stimulated at 0.5 Hz with 2- to 3-msec threshold pulses while a stable impalement was obtained. Long depolarizing current pulses then were applied in current clamp mode at 0.05 Hz and the result of depolarization-induced automaticity was recorded. For current clamp studies (guinea pig and cat), the test compartment perfusate then was changed to record the effects of different K⁺ concentrations.

Automaticity was quantified by estimating the average slope of phase 4 depolarization as previously described. This method permits the inclusion of data in which diastolic depolarization does not reach threshold, i.e., does not result in a spike. This is particularly important in defining the negative and positive ends of the curve relating automaticity to maximum diastolic potential.

For voltage clamp studies cat papillary muscles were used exclusively because it was never possible to achieve adequate control, using the criteria of New and Trautwein, with guinea pig preparations. The protocol involved an initial survey of depolarizing current clamps as described above. The duration of the current clamp then was reduced and the electronic switch was programmed to provide voltage clamps at preset potentials following the automatically-inducing current clamp, resulting in a sequential current and voltage clamp pair. Several diastolic clamp potentials then were surveyed to estimate the reversal potential of the recorded current. In some experiments, continuous, multistep voltage clamps were used to measure the activation characteristics of the time-dependent current.

The problem of adequate spatial clamp control in ventricular muscle has been documented and discussed. Therefore, in several separate experiments, homogeneity of membrane potential in the portion of the muscle in the test compartment was evaluated by recording from dual simultaneous impalements during automaticity elicited by long current clamps.

The possibility of inadvertent impalement of Purkinje fibers was ruled out by selection of fibers with a flat diastolic potential, dv/dt less than 400 V/sec, and plateau potential greater than 15 mV at normal resting potentials. Furthermore, addition of epinephrine, 10⁻¹⁰ to 10⁻⁹ M, never induced automaticity in these preparations at normal resting potentials, suggesting that Purkinje fibers are probably absent from the terminal 1.0 mm of most papillary muscles in these species.

Results

CONSTANT CURRENT STUDIES (CURRENT CLAMP)

Figure 1 shows depolarization-induced automaticity under control conditions in typical guinea pig and cat preparations. Note that diastolic depolarization is slower at more negative maximum diastolic potentials. Simultaneous recording from two cells permitted evaluation of the homogeneity of the preparations. There appears to be adequate uniformity of these cell pairs following phase 0 of the action potentials, especially during diastole, since the small differences between cells can be reasonably ascribed to electrotonic effects. Similar results were obtained in six preparations.

The effects of extracellular K⁺ on conventional depolarization-induced automaticity at two different levels of maximum diastolic potential are shown in Figure 2. Start-
VOLTAGE CLAMP EXPERIMENTS

A typical sequential clamp experiment is shown in Figure 4. A survey of current clamps showed that 2 µA induced sufficient depolarization in this muscle to result in significant phase 4 depolarization leading to an "automatic" action potential following the initial elicited action potential (panel 1). The current clamp then was interrupted by a voltage clamp just after the maximum diastolic potential was reached (panel 2). This voltage clamp permitted the recording of the net current flowing during phase 4 (panels 2-4). The voltage-dependence of this

![Figure 4](http://circres.ahajournals.org/)

**Figure 4** Representative current and voltage clamp experiment in cat papillary muscle. Upper trace, current; lower trace, transmembrane potential. The voltage calibrations are at the left, current calibrations at the right, and the time calibration (panel 1) apply to all panels. In each panel the initial segment of the current trace defines the transmembrane potential zero as well as the current zero level. Panel 1 shows typical automaticity induced by a long current clamp. In panels 2-4 the current clamp (I) was interrupted by a 900-msec voltage clamp (V) at the clamp potential shown. Single impalement, K⁺ = 5 mM.
current was evaluated by varying the clamp potential. In the preparation shown in Figure 4, the net current was outward and decayed with time between -80 mV and -30 mV. At clamp potentials negative to -88 mV, a decreasing inward current was seen (panel 4), defining a reversal potential of approximately -88 mV (panel 3). At potentials positive to -50 mV, the time-dependent component began to diminish, and positive to approximately -30 mV an increasing outward current was recorded, suggesting further activation of the same outward current conductance, or perhaps a second reversal or “turnover” potential similar to that described by McGuigan. Similar currents were recorded in 13 cat papillary muscles. An experiment carried out in another papillary muscle in Tyrode’s solution containing 2 mM K⁺ is shown in Figure 5. As can be seen from panels 1 and 2, the reversal potential at this K⁺ concentration lay between -100 and -90 mV (the reversal potential measured in 5 mM K⁺ was -78 mV in this preparation).

Maximum time-dependent current was obtained with a voltage clamp potential of -71 mV (panel 3), slightly negative to the maximum diastolic potential. As the clamp potential was made more positive, the time-dependent component of the outward current diminished and at -42 mV (panel 5), the outward current increased with time, as shown in panel 6.

The “turnover” potential (panel 5, Fig. 5) at which the decreasing outward current becomes an increasing outward current could be interpreted as the point at which time-dependent K⁺ accumulation in the extracellular space and the resulting increase in membrane K⁺ permeability begins to dominate time-dependent conductance changes as described by McGuigan. However, it may more probably result from the fact that the ordinary action potential plateau duration, used here to activate the current, is not sufficient to fully activate the conductance (see section on activation characteristics, below). Thus, voltage clamping to potentials in the activation range (see below) results in increasing activation rather than deactivation as seen at the more negative clamp potentials.

The reversal potential of the time-dependent decreasing outward current was measured in a total of 20 cells in 14 papillary muscles at extracellular K⁺ concentrations of 2–15 mM. These data are plotted in Figure 6. Note that the average slope for all preparations is close to that of a K⁺ electrode (60 mV per 10-fold concentration change). The reversal potential in 5 mM K⁺ was -79.1 mV ± 0.99 (mean ± se, n = 20).

**ACTIVATION OF THE TIME-DEPENDENT CURRENT**

The time and voltage requirements for activation of the diastolic current were studied using continuous voltage clamp. The activation time for the outward current was measured in four cat papillary muscles by varying the duration of an initial (V1) clamp step to the plateau range and noting the magnitude of the time-dependent current flowing during a subsequent fixed (V2) clamp. As shown in Figure 7A, essentially full activation was achieved by V1 steps of 400 msec or longer.

Deactivation or decay time constants for the time-dependent outward current were estimated from semilogarithmic plots of currents recorded during V2 clamp steps to potentials from -90 mV to -70 mV. Straight lines were obtained with time constants of 100 msec (-90 mV) to 400 msec (-70 mV).

Since “steady state” activation of the time-dependent
K+ AND VENTRICULAR PACEMAKER CURRENT

FIGURE 7 Activation characteristics of the time-dependent outward current; tracing of currents (below) recorded during double step voltage clamps (above) from a holding potential of −70 mV; single impalement, K+ = 5 mM, cat papillary muscle. A (left): activation time measured by varying the duration of the activating (V1) clamp to +5 mV. Note the effect on the current flowing during the subsequent (V2) step to −51 mV. B (right): activation voltage measured by varying the amplitude of the V1 clamp of 600-msec duration. V1 and V2 current traces from bottom up correspond to V1 clamp potentials from bottom up. Current traces for V1 clamps to +26 and +36 mV are superimposed for most of their time course during the V2 clamp.

Plotting the net peak time-dependent outward current obtained at two extracellular K+ concentrations as a function of activating clamp potential provided the steady state activation curves shown in Figure 8. The average potential for 50% activation in the three muscles studied at 5 mM K+ was −9 mV. Additional experiments will be required to determine whether a change in extracellular K+ concentration shifts the curve on the voltage axis.

FIGURE 8 Steady state activation curves for the time-dependent outward current at two K+ concentrations. Left panel: net time-dependent outward current (ordinate) was measured from voltage clamp records like those shown in Figure 7B at extracellular K+ concentrations of 2 and 5 mM and plotted against the V1 clamp potential (abscissa). Holding potential was −70 mV, V2 clamp potential was −51 mV. Note that the fully activated current was greater at 2 mM K+ than at 5 mM. Right panel: normalized activation curves derived from the data of the left panel. The dimensionless activation variable X (ordinate) was calculated by dividing each current value from the data plotted on the left side by the maximum current for that K+ concentration and plotted against V1 clamp potential (abscissa).
after the action potential (Figs. 4, 5, and 9). The reversal potential is shifted by changes in extracellular K+ with a slope of 50-60 mV per 10-fold change in concentration. Therefore, it appears probable that this current is carried largely by K+ ions. Finally, the activation potential curve (Fig. 8) lies in the plateau range and is relatively distant from the normal resting potential range. These properties and the time constant of several hundred milliseconds classify this current as belonging to the X, type (Table 1) as described by Noble and Tsien. A similar but quantitatively smaller current can be detected in dog ventricular trabecula (Beefer and Reuter;15 Katzung, unpublished). In calf and sheep ventricle this current is much less well developed (McGuigan;16 Reuter, personal communication).

On the basis of this time-dependent current plus previously described inward currents13, 15 it now is possible to explain the absence of automaticity in ventricle at normal resting potentials and its presence at depolarized potentials. The normal action potential plateau activates the X, conductance. Because of its long time constant, this conductance deactivates during an appreciable fraction of diastole. If the membrane potential repolarizes promptly to the normal resting potential, no significant outward current will flow through the X, conductance, since the resting potential lies very close or negative to the reversal potential and the driving force is therefore negligible or inward. However, if the membrane potential is held at a more positive level by depolarizing current, an appreciable driving force will be present and a significant outward I will flow until deactivation is complete. The initial effect of this outward I_X will be to hyperpolarize the membrane relative to the “stable” diastolic potential at which total steady state depolarizing current is balanced by steady state outward current. As X, deactivates, this hyperpolarizing effect will be lost and progressive phase 4 depolarization will be recorded. If either the sodium system or the calcium system has been reactivated since the preceding action potential, phase 4 depolarization may reach threshold and result in a spike. The resulting plateau can then renew the cycle. This process is analogous to that described for Purkinje fibers which oscillate at depolarized resting potentials2 and for frog atrial fibers.8, 9 Neither ventricular nor frog atrial fibers show diastolic depolarization.

**Table 1** Time-Dependent Outward Currents Activated at Plateau Potentials in Several Cardiac Preparations

<table>
<thead>
<tr>
<th></th>
<th>Purkinje fiber (Ref. 6, I_X)</th>
<th>Frog atrium (Ref. 8, 9, I_X)</th>
<th>Papillary muscle (this study)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Reversal potential</strong></td>
<td>-85 mV ( K^+ = 4) mm</td>
<td>-70 to -40 mV ( K^+ = 4) mm</td>
<td>-79 mV ( K^+ = 5) mm</td>
</tr>
<tr>
<td><strong>Time constant</strong></td>
<td>300 msec (-70) mV</td>
<td>450 msec (-90) mV</td>
<td>300 to 400 msec (-70) mV</td>
</tr>
<tr>
<td><strong>Activation voltage</strong></td>
<td>-50 to +20 mV</td>
<td>-65 to +10 mV</td>
<td>-35 to +30 mV</td>
</tr>
</tbody>
</table>
tation at normal resting potentials, since they appear to lack $I_{Ks}$, a current present in Purkinje fibers$^{21}$ which has a reversal potential significantly more negative than the normal maximum diastolic potential for that tissue.

**EFFECTS OF EXTRACELLULAR K**

Imanishi and Surawicz$^{2}$ first reported that elevated extracellular K$^+$ reduces or abolishes depolarization-induced automaticity in papillary muscle. The results reported above show that this effect involves both a shift to more depolarized diastolic potentials and a reduction in maximum pacemaker slope. The shift to less negative potentials can be partly ascribed to the effect of increased K$^+$ on the reversal potential for $I_{Ks}$. Thus, greater depolarization would be required to achieve the same outward current at the start of phase 4. The reduction in maximum phase 4 slope undoubtedly involves several factors, including the greater total membrane conductance observed as K$^+$ is increased.$^{22}$ The decrease of the time-dependent component resulting from the shift in reversal potential (Fig. 8), and the reduction of deactivation of $I_{Ks}$ as the activation range of the conductance variable (Fig. 8) is reached.

**IMPLICATIONS FOR CARDIAC ARRHYTHMIAS**

The demonstration of a time-dependent current of the $I_{Ks}$ type in ventricular muscle establishes an ionic basis for previously reported pacemaker activity in this tissue. The presence or absence of automaticity in this tissue, as in Purkinje fibers and frog atrial fibers, is thus a function of the diastolic membrane potential and the reversal potential of the pacemaker current. Therefore, it can be predicted that interventions or pathology which reduce diastolic potential without similarly shifting the $I_{Ks}$ reversal potential should result in diastolic depolarization and increase the probability of arrhythmias of the increased automaticity type.$^{23}$ Experimental interventions of this type include direct application of depolarizing current as in this study, induction of currents of injury,$^{24,25}$ and reduction of K$^+$ permeability as caused by Ba$^{2+}$ exposure.$^{26}$ Depolarized Purkinje fibers in an Na$^+$-free, tetraethylammonium (TEA)-containing medium show the same characteristic property, i.e., automaticity which is modulated by hyperpolarizing and depolarizing current and can be abolished by hyperpolarizing to the normal resting potential.$^{27}$ Such fibers have been shown to have a reduced K$^+$ permeability$^{28}$ like that caused by reduced extracellular K$^+$ or Ba$^{2+}$. It is tempting to speculate that a common mechanism of increased automaticity may underlie this type of arrhythmia in atrial, Purkinje, and ventricular cells.

In contrast to the above, interventions which simultaneously depolarize and shift the pacemaker current reversal potential or increase time-independent outward currents should reduce or prevent the occurrence of automaticity but should also increase the probability of arrhythmias of the reentry type, especially those involving slow responses.$^{28}$ Increased extracellular K$^+$ belongs in this category.

**Acknowledgments**

We thank Drs. H. Reuter and L. Hondeghem for useful discussion, C. Cotner for technical assistance, and D. Noack for secretarial assistance.

**References**

3. Imanishi S, Surawicz B: Effect of potassium on slow channel-dependent automaticity in guinea pig ventricular myocardium (abstr). Physiologie 172: 253, 1974
5. Antoni H: Electrophysiological mechanisms underlying pharmacological models of cardiac fibrillation. Naunyn Schmiedebergs Arch Pharmacol 269: 177-199, 1971
24. Katzung BG, Hondeghem LM, Grant AO: Cardiac ventricular automaticity induced by current of injury. Pfluegers Arch 360: 193-197, 1975
25. Deleze J: The recovery of resting potential and input resistance in sheep heart injured by knife or laser. J Physiol (Lond) 208: 547-562, 1970
27. Aronson RS, Cranefield PF: The effect of resting potential on the electrical activity of canine cardiac Purkinje fibers exposed to Na-free solution or to ouabain. Pfluegers Arch 347: 101-116, 1974
Effects of extracellular potassium on ventricular automaticity and evidence for a pacemaker current in mammalian ventricular myocardium.

B G Katzung and J A Morgenstern

_Circ Res._ 1977;40:105-111
doi: 10.1161/01.RES.40.1.105

_Circulation Research_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Copyright © 1977 American Heart Association, Inc. All rights reserved.

Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:

http://circres.ahajournals.org/content/40/1/105