Cesium Blockade of Delayed Outward Currents and Electrically Induced Pacemaker Activity in Mammalian Ventricular Myocardium

CHARLES F. MEIER, JR., and BERTRAM G. KATZUNG

From the Department of Pharmacology, University of California, San Francisco, California 94143. C. F. Meier's present address is Department of Pharmacology, University of Oklahoma College of Medicine, Oklahoma City, Oklahoma 73190.

ABSTRACT The effects of Cs+, 5–25 mM, were studied in cat and guinea pig papillary muscles using voltage clamp and current clamp techniques. In solutions containing normal K+, the major effects of Cs+ were depolarization of the resting potential and reduction of the delayed outward current (i\textsubscript{\textit{o1}}) between −80 and −20 mV. Both inward and outward portions of the isochronal current-voltage relation (1-s clamps) were reduced by extracellular Cs+. This resulted in a substantial reduction of inward rectification and, by subtraction from the normal I-V relationship, the definition of a Cs+-sensitive component of current. Under current clamp conditions, 5–10 mM Cs+ produced a dose-dependent slowing of repetitive firing induced by depolarization. At higher concentrations (25 mM) the resting potential was depolarized and repetitive activity could not be induced by further depolarization. However, release of hyperpolarizing pulses was followed by prolonged bursts of repetitive action potentials, suggesting partial reversal of blockade or participation of another pacemaker process. The experimental results and a numerical simulation show that under readily attainable conditions, reduction in an outward pacemaker current may slow pacemaker activity.

INTRODUCTION

Diastolic (phase 4) depolarization of cardiac membrane potential has been repeatedly linked to a time- and potential-dependent decay in potassium conductance. In normal sinoatrial nodal cells (Noma and Irisawa, 1976) and in depolarized Purkinje (Hauswirth et al., 1969), atrial (Brown et al., 1972, 1976 \textit{a} and 1976 \textit{b}; Lenfant et al., 1972), and ventricular fibers (Katzung and Morgenstern, 1977) the “pacemaker” current underlying repetitive activity displays kinetic properties similar to the delayed outward potassium current previously identified in Purkinje fibers (Noble and Tsien, 1969 \textit{a} and 1969 \textit{b}) and called \textit{i}_{o1}.
In spite of attempts to characterize the kinetic variables governing the pacemaker potassium currents in these tissues, the relative importance of the magnitude of this current in determining the rate of phase 4 depolarization and thus the rate of repetitive activation remains obscure. For a space-clamped preparation, the rate of change of voltage across the membrane is given by

$$\frac{dV}{dt} = -(i_{in} + i_{out}) \cdot (C_m),$$

where $i_{in}$ includes both $i_{Na}$ and $i_{K}$, and any applied depolarizing current, while $i_{out}$ presumably consists primarily of $K^+$ currents, e.g., $i_{k1}$ and $i_{x}$. Therefore, it might be predicted from a first evaluation of Eq. 1 that interventions which decrease $i_{out}$ should increase the rate of diastolic depolarization.

This prediction can be tested with blockers of channels carrying outward current. Cesium ion ($Cs^+$), which has been shown to block potassium channels in many other tissues (Adelman and Senft, 1968; Hille, 1973; Hagiwara et al., 1976; Gay and Stanfield, 1977), has been reported to block outward current in cardiac cells as well (Isenberg, 1976; Vereecke et al., 1980). However, in spontaneously beating right atrial preparations, $Cs^+$ was reported to have a negative chronotropic effect (Prasad and Midha, 1973). However, neither membrane potential nor current was monitored in that study. In the present study, therefore, repetitive activity induced in cardiac ventricular fibers by application of small depolarizing currents (Katzung, 1975; Imanishi and Surawicz, 1976; Katzung and Morgenstern, 1977) was used as the model of automaticity. The effects of $Cs^+$ on the rate of phase 4 depolarization and on the magnitude of outward currents associated with phase 4 were investigated using current and voltage clamp techniques.

The use of $Cs^+$ was of interest also because blockade of neuronal $K^+$ channels by this ion is strongly unidirectional, i.e., external $Cs^+$ blocks inward $K^+$ current only while internal $Cs^+$ blocks outward $K^+$ current (Adelman and Senft, 1968; Dubois and Bergman, 1975). However, cardiac studies to date suggest that extracellular $Cs^+$ blocks both inward and outward $K^+$ current (Isenberg, 1976; Trautwein and McDonald, 1978; Vereecke et al., 1980). We therefore examined the effects of external $Cs^+$ on both inward and outward components of the 1- to 2-s isochronal current-voltage relation.

The potential dependence and kinetics of the delayed outward current components determined in this study did not differ significantly from those called $I_k$ and $I_x$ in ventricular myocardium by McDonald and Trautwein (1978a and 1978b). Since these currents are also very similar to those previously named $i_{k1}$ and $i_{x2}$ in Purkinje fiber studies, we felt that less confusion would result from using the earlier established Purkinje fiber terminology (see also Vassalle [1977a and 1977b]).

Preliminary reports of some of this research have appeared (Meier and Katzung, 1978a and 1978b).

**Methods**

Experiments were performed on right ventricular papillary muscles using current clamp and voltage clamp techniques.
Tissue Preparation and Perfusion

Cats were anesthetized with 30 mg/kg pentobarbital, intraperitoneal, and guinea pigs were killed by cervical dislocation. Hearts were rapidly excised and dissected in normal physiologic solution at room temperature. Right ventricular papillary muscles or trabeculae of <0.5 mm in diameter were selected for current clamp and voltage clamp procedures.

After a 20- to 30-min recovery period, preparations were mounted in a single sucrose gap chamber (Katzung and Morgenstern, 1977). Prewarmed, oxygenated solutions flowed through the compartments of the chamber at 6 ml/min. Temperature was maintained at 36.5 ± 0.5°C. During the 30-min equilibration period, and during intervals between current clamp and voltage clamp sequences, the preparation was stimulated at 1 Hz with 2-ms current pulses just above threshold.

Electrophysiological Techniques

The membrane potential of the test node was recorded with conventional glass microelectrodes. Whenever possible, a second intracellular microelectrode was used in the test node to evaluate voltage homogeneity. Data from preparations not demonstrating stable characteristics for the duration of the experimental protocol were discarded. The data presented and considered in the analysis of Cs⁺ effects were obtained from single impalements maintained throughout the control and Cs⁺ perfusion periods. The figures show results typical of 5–10 preparations in which that protocol was performed.

Voltage Clamp

Only cat papillary muscles were used. A two-step protocol from the holding potential was usually applied. Because of the difficulty of reaching steady-state outward current during the activating V1 step (probably because of potassium accumulation [McDonald and Trautwein, 1978 a]), we did not attempt to analyze currents during this step but concentrated on the declining outward tail currents flowing during the V2 step. Clamp sequences were separated by at least 20 s to ensure recovery from extracellular potassium accumulation. The extracellular series resistance (Rₑ) was determined using the technique of Goldman and Morad (1977) and only preparations with Rₑ values <200 Ω were used. This value would introduce a maximum potential error of only 1 mV. Therefore, no correction was made for possible artifacts introduced by Rₑ (Attwell and Cohen, 1977).

Current Clamp

Most of the complete current clamp experiments were carried out on guinea pig papillary muscles. Short current clamp procedures were performed on cat papillary muscles to verify all Cs⁺ effects on induced pacemaker activity. Current clamp steps of 1.5 s and varying amplitude were repeated at 20-s intervals.

Data Acquisition and Analysis

Voltage Clamp

Membrane currents and potentials were monitored on a Tektronix, Inc. (Beaverton, Oreg.) 565 oscilloscope and recorded on a Gould Inc. (Cleveland, Ohio) model 2400 chart recorder. Since only slow currents were measured, the frequency response was adequate to accurately reproduce the current components of interest. Outward current tails were measured from these chart records by hand or by a tablet digitizer (Bit Pad-One, Summagraphics Corp., Fairfield, Conn.). Identification of the individual current components comprising the outward current tails was accomplished by graphical analysis (McDonald and Trautwein, 1978 a and 1978 b). Fitting of “least square error” lines to semilogarithmic plots of the data was carried out with the aid of an interactive graphic display and a PDP 11/34 computer system.
(Digital Equipment Corp., Maynard, Mass.). Analysis of selected records by hand gave excellent agreement with the computer assisted analysis. In most cases two exponential components satisfactorily fitted the data. The slower component corresponded to \( i_{\alpha} \) (Noble and Tsien, 1969 a) or \( I_x \) (McDonald and Trautwein, 1978 a). For reasons indicated in the Discussion, no attempt was made to determine whether \( i_{\alpha} \) represents a true ionic current or the effect of \( K^+ \) accumulation in the extracellular space. For convenience, it is referred to below as though it is mediated by a channel. Isochronal current-voltage curves were obtained by applying 1- or 2-s clamps from the holding potential at a rate of 0.05 Hz.

CURRENT CLAMP Current clamp responses were measured as described by Katzung (1975). All comparisons of membrane events were made for pulses yielding equivalent maximal diastolic potential (MDPs). The extracellular series resistance of guinea pig papillary muscles often exceeded 300 \( \Omega \). Therefore, all data obtained from guinea pig tissue were corrected for the potential drop introduced by the applied current across the extracellular series resistance. For the purposes of this study the rate of phase 4 depolarization was defined as the slope of the chord drawn from the MDP to the subsequent point 5 mV positive to the MDP. This slope gives a useful and convenient description of the rate of phase 4 depolarization during the early period of outward current deactivation.

Solutions
Normal physiologic solution consisted of (mM): NaCl, 149; KCl, 4; CaCl\(_2\), 2.5; MgCl, 1.1; dextrose, 5.5; and tris(hydroxymethyl)aminomethane (Tris), 5. The pH was adjusted to 7.3 at 37\(^\circ\)C with concentrated HCl. Isotonic potassium solution for perfusion of the current injection chamber consisted for (mM): KCl, 154; CaCl\(_2\), 2.5; dextrose, 5.5; and Tris, 5. The pH was adjusted to 7.3. The isotonic sucrose solution consisted of (mM): sucrose, 275; CaCl\(_2\), 0.01, and dextrose, 5.5. All solutions were continuously bubbled with 100% \( O_2 \). Unless otherwise noted, all additions to the normal physiologic solutions were made without correction for changes in osmolarity. CsCl was obtained from K & K Laboratories, Inc. (Plainview, N. Y.).

RESULTS
Delayed Outward Currents
Addition of 5–10 mM CsCl to the test node perfusate markedly reduced the magnitude of the time-dependent outward current. Fig. 1 illustrates the effects of 10 mM Cs\(^+\) on the currents recorded during typical two-step voltage clamps. The primary features include development or unmasking of a small inward current at the holding potential (seen before the \( V_1 \) step) and a reduction of \( \sim 40\% \) in the magnitude of the outward tail current during the \( V_2 \) clamp step which was maximal within 80 s of the onset of Cs\(^+\) effects. There was no significant change in the outward current during the \( V_1 \) step into the positive potential range, and no significant change in the kinetics of decay of the outward current tail during \( V_2 \). However, the zero-time intercepts of both the faster tail component (tau 200–400 ms) and the slower one (tau 1.4–2.0 s) were reduced equally. The rapidity of onset of action was comparable to that of a change in perfusate potassium concentration and suggests an extracellular action. The degree of block was concentration dependent,
with almost complete elimination of the outward tail occurring at 20–25 mM Cs$^+$. The time-dependent outward currents may be described by the relationship

$$i_x = \bar{i}_x \cdot \bar{x},$$

(2)

where $i_x$ is the instantaneous current, $\bar{i}_x$ is the value of the fully activated
current at the voltage studied (which may be a nonlinear function of potential), and \( x \) is the instantaneous value of the activation variable. Thus, the observed reduction in outward current magnitude could result from depression of the maximally activated current parameters \( i_{x1} \) and \( i_{x2} \) or from a shift in the \( x_1 \) and \( x_2 \) activation potential range, decreasing \( x_1 \) and \( x_2 \) at the potential studied. However, as shown in Fig. 2, there was no significant shift in the activation range for total time-dependent outward current, despite a marked reduction in the outward tail amplitude at \(-44 \text{ mV}\). This suggests that Cs\(^+\) alters \( i_{x1} \) and \( i_{x2} \), the fully activated current-voltage relations.

**Figure 2. Global activation curves for the time-dependent outward current in cat papillary muscle before and after full development of Cs\(^+\) blockade.** Using a clamp protocol like that of Fig. 1, we extrapolated peak tail currents measured during \( V_2 \) (\(-44 \text{ mV}\)) to the start of \( V_2 \) and plotted them against the series of \( V_1 \) clamp potentials used to activate. Potentials for half-activation: control, \(-21.0 \text{ mV}\); Cs\(^+\), \(-21.5 \text{ mV}\). ◦, Control; ○, Cs\(^+\) (10 mM).

**Isochronal Current-Voltage Relationships**

Because of the problem of potassium accumulation, no attempt was made to obtain steady-state current-voltage curves. A typical isochronal \( I-V \) relation obtained with 1-s clamp steps is shown in Fig. 3. As reported by McDonald and Trautwein (1978 b), inward rectification is present in cat papillary muscle negative to \(-20 \text{ mV}\), but there is no negative slope region. 10 mM Cs\(^+\) clearly reduced both inward and outward currents and virtually linearized the inward rectifier region but had much smaller and inconsistent effects positive to \(-10 \text{ mV}\). The difference between the two curves thus defines the Cs\(^+\)-sensitive component of current flowing at 1 s. As expected, this component crosses the zero-current line very close to the calculated potassium equilibrium potential (crossing at \(-87 \text{ mV}\), \( E_k \) calculated as \(-92 \text{ mV}\)). The Cs\(^+\)-sensitive curve is similar to that reported by Trautwein and McDonald (1978) for bull ventricular muscle. In a few preparations a small increase in outward current was
induced by Cs⁺ positive to −20 mV, suggesting the possibility of stimulation of a Na-K electrogenic pump as reported by Isenberg (1976) or the diminution of potassium accumulation during the pulse. However, in other muscles, no change or a small decrease was observed in the same potential range.

Current Clamp Experiments

Current-MDP relations The rate of phase 4 depolarization depends partly on the decay of the pacemaker current $i_{41}$; thus, the initial rate of phase 4 depolarization ($dV/dt$) is a function of the difference between the instantaneous and steady-state magnitudes of the total current at the time of the MDP (Noble [1975] and Discussion). One method for estimating this current is to plot the net transgap current in current clamp mode against the potential at the MDP. This type of current-voltage relationship provides a quasiinstantaneous measure of the total current flowing at the MDP.

Fig. 4 illustrates the effect of 10 mM Cs⁺ on this current-MDP relationship. The total current is markedly decreased at potentials negative to −20 mV. Similar effects on the current-MDP relationship were observed in guinea pig preparations (Meier and Katzung, 1978 b). The similarity of the Cs⁺-sensitive difference component to that defined by voltage clamp (Fig. 3) is obvious.
DEPOLARIZATION-INDUCED PACEMAKER ACTIVITY As shown in Fig. 5, 5 mM Cs⁺ produced a modest prolongation of action potential and a moderate to marked slowing of repetitive activity during long current pulses. In about half the guinea pig preparations, cessation of repetitive activity occurred first in the more negative range of MDP as shown. The remainder showed greater sensitivity in the less negative range of MDP. Fig. 5 suggests that the primary contributor to the slowing of repetitive firing is a decrease in phase 4 depolarization slope, especially at more negative MDPs. That Cs⁺ does indeed have marked dose-dependent effects on initial phase 4 depolarization rate is shown in Fig. 6. In most muscles a further increase in Cs⁺ concentration above 10 mM resulted in more marked resting depolarization and complete abolition of repetitive activity. Cat preparations were less sensitive than those from guinea pig but also showed depolarization and suppression of automaticity.

Because complete suppression of automaticity was always accompanied by resting potential depolarization, it was important to determine whether hyperpolarization back to the normal resting range could reverse the Cs⁺ suppression of automaticity. However, hyperpolarizing currents did not restore normal responsiveness of Cs⁺-depolarized preparations. Brief current pulses (1-5 s) sufficient to return the resting potential to control levels (-80 to -90 mV) did not reestablish repetitive firing in response to a subsequent depolarizing pulse. Thus, abolition of depolarization-induced rhythmic activity cannot be ascribed to resting depolarization per se.

However, when hyperpolarizing currents were applied for 10-60 s, repetitive firing occurred after release of the current. As shown in Fig. 7, this activity followed a pattern characterized by rapid acceleration to peak frequency followed by gradual slowing and termination in subthreshold oscillations. This
suggests that hyperpolarization of sufficient duration can either reverse Ca\(^+\) suppression of automaticity or unmask some other pacemakerlike process.

**DISCUSSION**

*Current-Voltage Relationship*

Previous work with a variety of membrane preparations has shown that externally applied Cs\(^+\) blocks inward current, whereas internal Cs\(^+\) is selective for outward current. Such preparations have included squid axon (Chandler and Meves, 1965; Adelman and Senft, 1968; Bezanilla and Armstrong, 1972; and Adelman and French, 1978), frog myelinated nerve (Dubois and Bergman, 1975), frog skeletal muscle (Gay and Stanfield, 1977), starfish egg (Hagiwara et al., 1976), and isolated ion channels of sarcoplasmic reticulum (Coronado and Miller, 1979). It is therefore important to note that cardiac ventricular muscle (present results) and Purkinje fibers (Isenberg, 1976) manifest a bidirectional impairment of K\(^+\) currents when Cs\(^+\) is added to the perfusate. Carmeliet (1980) has reported similar bidirectional inhibition of \(^{42}\)K fluxes by Cs\(^+\) in Purkinje fibers. Possible reasons for this bidirectional block by extracellular Cs\(^+\) are considered below.

The pattern of blockade observed in our study consisted of a reduction of isochronal (1-s) currents at negative potentials with strong suppression of the

![Diagram of membrane events in a guinea pig papillary muscle](image-url)

**Figure 5.** Effects of Cs\(^+\), 5 mM, on membrane events in a guinea pig papillary muscle (from oscilloscope photographs). *Upper row*, control; *lower row*, after full development of Cs\(^+\) effects. Activity in the remaining panels was elicited by 2-ms pulses. Action potentials in panels a and d were elicited by 2-ms pulses. Activity in the remaining panels was elicited by current clamp pulses of 1.4 s. Vertical calibrations for all panels are at the right side; the time calibration (under panel e) represents 100 ms for panels a and d, 400 ms for the remainder. Panels e and f were selected for maximum diastolic potentials equal to those in panels b and c (corrected for series resistance). Note that considerably less current was required in the presence of Cs\(^+\).
inward rectifier component, resulting in an almost linear current-voltage relation between $-100$ and $-20$ mV. In addition, there was a dose-dependent decrease in resting potential consistent with a decline in resting $K^+$ permeability. This was responsible for the small inward current seen at the holding potential in the presence of Cs$^+$ (Fig. 1). In a study by Trautwein and McDonald (1978), 10 mM Cs$^+$ was found to have a significant effect on the outward current of bull ventricular trabeculae. Although it did not eliminate the marked negative resistance characteristic of this tissue, it reduced outward current markedly in the anomalous rectifier potential range. The Cs$^+$-sensitive current thus defined was very similar to that obtained in the present study (Figs. 3 and 4). In contrast, these authors did not note a very selective effect on the $I$-$V$ relation in cat papillary muscles, although a diminution of outward current was shown.

**Identity of the Cs$^+$-sensitive Current**

The Cs$^+$-sensitive current components in cardiac Purkinje fibers were shown by Isenberg (1976) to include $i_K$, the time-independent current, and $i_{Ki}$, the "normal" pacemaker current, in these cells. The more recent study of Vereecke et al. (1980) showed that Cs$^+$ "divided" $i_{K1}$ of Purkinje fibers into two components: a Cs$^+$-sensitive, inwardly rectifying portion and a Cs$^+$-insensitive, outwardly rectifying component. In the present study we did not examine $i_{K1}$ over the entire potential range. However, since it is the primary $K^+$ current flowing at potentials negative to $-50$ mV, the reduction in isochronal currents...
negative to this level as well as the decrease in resting potential (Fig. 4) and the appearance of a small inward current (Fig. 1) strongly suggest inhibition of this current.

Since ventricular tissue does not manifest $i_{K_{1}}$, the Cs$^{+}$-sensitive time-dependent component we detected is probably $i_{a1}$. This conclusion is strongly supported by the evidence showing that the Cs$^{+}$-sensitive current has the same time constants and activation range as $i_{a1}$ as previously defined (Katzung and Morgenstern, 1977; McDonald and Trautwein, 1978 b). In the study of Purkinje fibers cited above (Isenberg, 1976), it was reported that $i_{a1}$ in that tissue was nearly insensitive to Cs$^{+}$. However, the time constant of activation of the current referred to was considerably longer (1.4 s) than those reported by Noble and Tsien (1968) and McDonald and Trautwein (1978) (0.15–0.5 s). Furthermore, the clamps used (steps from −96 mV to −40 mV) probably...

![Figure 7](image-url)
FIGURE 8. Reconstruction of the effects of $\bar{r}_{x1}$ reduction using the Beeler and Reuter (1977) model. The figure shows superimposed computer plots of membrane potential (upper), $i_{x1}$ (middle), and total current (lower) for the initial 1.5 s of a long current clamp. $i_{\text{appl}}$ refers to the externally imposed transgap current.

To achieve the same maximum diastolic potential (solid and dashed curves) at 100% and 50% of control $r_{x1}$, the applied current had to be reduced from 2.3 to 1.6 $\mu$A. (Application of 2.3 $\mu$A at the lower $r_{x1}$ resulted in maintained depolarization at $-10$ to $-20$ mV.) The dotted curve shows the effects of an intermediate current intensity at the lower $r_{x1}$. As expected, the action potential duration is greatly prolonged by the reduction in $i_{x1}$, but, in addition, the slope of phase 4 after the maximum diastolic potential is reduced by the change. The reason for the reduction is shown in the lower part of the figure. The total inward current at the lower level of $r_{x1}$ (dashed curve) is smaller than that for the control level (solid curve) at all times during the diastolic interval if imposed current is adjusted for equal MDP.
activated very little $i_{x1}$. It would be useful to restudy this point in Purkinje fibers over the full $i_{x1}$ activation range.

The slower time-dependent component detected in these preparations (tau greater than 1 s for all potentials) corresponds to the current called $i_{x2}$ by Noble and Tsien (1969 a) and $I_x$ by McDonald and Trautwein (1978 b). The difficulty in distinguishing between a distinct membrane channel and $K^+$ accumulation as the underlying cause of this current has been well described by the latter authors. The reduction caused by Cs+ in the present study is consistent with either mechanism. Since it appears to play no role in producing the pacemaker process within a single cycle, no further attempt was made to analyze this slow component.

**Effects of Cs+ on Depolarization-induced Repetitive Activity**

The present results show that Cs+ exposure slows phase 4 depolarization rate if the comparison is made at equal MDPs. It was not possible to compare phase 4 slopes at equal imposed currents because the currents required to induce phase 4 depolarization in the control state caused depolarization to the plateau level and prevented repolarization in the presence of Cs+. This is to be expected from the marked increase in membrane resistance induced by Cs+. In fact, interventions which modify outward current would be predicted to change the MDP (e.g., Brown et al. [1975]) unless this variable is controlled. A change in MDP, in turn, would modify the driving forces of currents flowing at that time and the degree of activation of voltage-sensitive currents, especially (in the present case) $i_{x1}$. A second complicating factor in the case of agents that modify $i_{x1}$ is the change in action potential duration, since a decrease in outward plateau current typically increases the duration of the action potential. Thus, if automaticity is gauged by the change in repetition rate, automaticity will be decreased by this factor.

To study the relative contributions of applied current, MDP, $i_{x1}$, and action potential duration to repetitive activity under more controlled conditions, we modeled depolarization-induced repetitive activity in ventricular muscle using the reconstruction of the action potential by Beeler and Reuter (1977). Using the equations from this model, we computed the effects of an agent that decreases time-dependent outward current. Some results are shown in Fig. 8. For simplicity, a potential-independent reduction in $i_{x1}$ was assumed. As in the biological preparations (Figs. 4 and 5), considerably less applied current was required to achieve the same MDP when $i_{x1}$ was reduced. If applied current was not reduced sufficiently, the MDP was more positive and diastolic interval was decreased relative to that obtained with the same $i_{x1}$ at a more negative MDP.

The analysis above is limited by incomplete information about the effects of Cs+ on other currents as well as uncertainty regarding the interaction of applied current, MDP, and the activation of ionic currents during phase 4 depolarization. It is thus of interest that in two spontaneously active preparations, the sinoatrial node (Prasad and Midha, 1973) and the in vivo dog.
Effects of Hyperpolarization during Cs⁺ Exposure

The hypothesis that the reduction or suppression of repetitive activity by Cs⁺ was the result of resting depolarization was tested by application of hyperpolarizing prepulses before the conventional depolarizing current clamps. Short (<1-s) hyperpolarizations to the control resting potential never restored the pre-Cs⁺ level of repetitive responses to depolarizing pulses. However, as shown in Fig. 7, longer prepulses were followed by repetitive activity, even without application of an additional depolarizing current clamp.

Repetitive activity following release of hyperpolarizing current to the region of the normal resting potential has not, to our knowledge, been reported as an effect of Cs⁺. Although the mechanism was not studied in detail, the following observations can be made. In the absence of a hyperpolarizing prepulse, 25 mM Cs⁺ essentially abolished i_{in} and i_{so}. The findings of others in skeletal muscle (Gay and Stanfield, 1977) and Purkinje fibers (Carmeliet, 1979a) suggest that hyperpolarization to −80 to −90 mV is not sufficient to reduce the degree of K⁺-channel blockade significantly, but rather increases it. Furthermore, the progressive hyperpolarization recorded during the hyperpolarizing current clamps suggests a progressive decrease in membrane K⁺ permeability, due to increasing Cs⁺ blockade of K⁺ channels, extracellular K⁺ depletion, or both. Therefore, it appears unlikely that the automaticity seen under these conditions involves a reversal of i_{in} blockade by Cs⁺. However, in the presence of marked reduction of resting K⁺ permeability, diastolic potential will be much more sensitive to small depolarizing perturbations. Thus, a small oscillation in slow inward current or a minimal decrease in electrogenic pump activity could readily initiate a phase 4 depolarizing sequence.

Reasons for Bidirectional Block by Extracellular Cs⁺ in Cardiac Muscle

Possible explanations for the bidirectional nature of the blockade by Cs⁺ in cardiac Purkinje fibers and ventricular muscle as contrasted with the unidirectional block in nerve include (a) rapid accumulation inside the cardiac cell resulting in “dual unidirectional” blocks, and (b) different properties of the cardiac K⁺ channel that make it susceptible to bidirectional block at an extracellular site.

It is known that Cs⁺ is transported into erythrocytes (Hobbs and Dunham, 1978), skeletal muscle (Sjodin and Beauge, 1967), and heart (Geurin and Wallon, 1979; Carmeliet, 1980). However, the rate of uptake into these tissues was rather slow. In contrast, the half-time for steady-state blockade of outward i_{in} in this study (Fig. 1) was <2 min. Therefore, blockade of this outward K⁺ current from an intracellular site seems unlikely.

The second explanation is more difficult to evaluate. A plausible mechanism can be postulated on the basis of a single-file channel with an external binding site exhibiting a very low dissociation constant for Cs⁺. Considerable evidence has been reported for the single-file, multiple-association-site concept for neuronal K⁺ channels (Hodgkin and Keynes, 1955; Hille, 1973, Begenisich
and De Weer, 1977). Dubois and Bergman (1977) postulate a binding site located in the external half of the node of Ranvier membrane channel that must bind K⁺ for normal gating to occur. A similar binding site in heart is, of course, consistent with the well-known fact that unidirectional efflux of K⁺ from cardiac fibers is strongly dependent on extracellular K⁺ concentration (Carmeliet, 1961). Furthermore, in a K⁺-free solution, Cs⁺ can increase nodal gK (Dubois and Bergman, 1977), showing that Cs⁺ may indeed bind to this gating-permissive site even though it does not impede outward K⁺ current in this preparation. Ciani et al. (1980) have shown that Cs⁺-block data from starfish egg cells are consistent with a two-site model of the K⁺ channel in which the Cs⁺ ion usually occupies the innermost of the two sites but accesses it from the extracellular side. If a similar Cs⁺ binding site is present in the cardiac ix1 channel and if it has sufficiently high affinity for Cs⁺, then the bidirectional block of K⁺ current would be predicted. That such a binding site is not likely to be highly specific for K⁺ ions is suggested by the observation of Carmeliet (1979 b) that in K⁺-free, Na⁺-free superfused Purkinje fibers, addition of small concentrations of Na⁺ has a Cs⁺-like effect, i.e., the current-voltage curve is shifted in the depolarizing direction, inward rectification is abolished, and outward as well as inward current is reduced.

We thank Mr. C. Cotner for his technical assistance and Ms. D. Noack for her secretarial services.

This research was supported by U. S. Public Health Service grant HL-17452.

Please address requests for reprints to B. G. Katzung, Department of Pharmacology, University of California, San Francisco, California 94143.

Received for publication 13 March 1980.

REFERENCES

ADELMAN, W. J., JR., and R. J. FRENCH. 1978. Blocking of the squid axon potassium channel by external caesium ions. J. Physiol. (Lond.). 276:13-25.

ADELMAN, W. J., JR., and J. P. SENFT. 1968. Dynamic asymmetries in the squid axon membrane. J. Gen. Physiol. 51: 1025-114s.

ATTWELL, D., and D. COHEN. 1977. The voltage clamp of multicellular preparations. Prog. Biophys. Mol. Biol. 31:201-245.

BEELEr, G. W., and H. REUTER. 1977. Reconstruction of the action potential of ventricular myocardial fibres. J. Physiol. (Lond.). 268:177-210.

BEGENISICH, T., and P. DE WEER. 1977. Ionic interactions in the potassium channel of squid giant axons. Nature (Lond.). 269:710-711.

BEZANILLA, F., and C. M. ARMSTRONG. 1972. Negative conductance caused by entry of sodium and cesium ions into the potassium channels of squid axons. J. Gen. Physiol. 60:588-608.

BRACHELL, J., C. F. MEIER, B. J. SCHERLAG, G. KABELL, L. HARRISON, and R. LAZZARA. 1980. Differential effects of cesium chloride on sinus node and His Purkinje automaticity in the normal dog heart. Circulation. 62(Suppl. III):138. (Abstr.).

BROWN, H. F., A. CLARK, and S. J. NOBLE. 1972. Pacemaker current in frog atrium. Nat. New Biol. 235:30-31.

BROWN, H. F., A. CLARK, and S. J. NOBLE. 1976 a. Identification of the pace-maker current in frog atrium. J. Physiol. (Lond.). 258:521-545.
Brown, H. F., A. Clark, and S. J. Noble. 1976. Analysis of pace-maker and repolarization currents in frog atrial muscle. *J. Physiol. (Lond.)* 258:547–577.

Brown, H. F., P. A. McNaughton, D. Noble, and S. J. Noble. 1975. Adrenergic control of cardiac pacemaker currents. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 270:522–537.

Carmeliet, E. 1961. Chloride and Potassium Permeability in Cardiac Purkinje Fibres. Presses Académiques Européanes, Brussels.

Carmeliet, E. 1979a. Voltage dependent block of inward going rectification in cardiac Purkinje fibers by external Cs ions. *Arch. Int. Pharmacodyn. Ther.* 242:294–295.

Carmeliet, E. 1979b. Induction of inward going rectification in sheep cardiac Purkinje fibers. *Arch. Int. Pharmacodyn. Ther.* 242:296–297.

Carmeliet, E. 1980. Decrease of K efflux and influx by external Cs ions in cardiac Purkinje and muscle cells. *Pfluegers Arch. Eur. J. Physiol.* 383:143–150.

Chandler, W. K., and H. Meves. 1965. Voltage clamp experiments on internally perfused giant axons. *J. Physiol. (Lond.)* 180:788–820.

Ciani, S., S. Krause, and S. Hagiwara. 1980. A model for the effects of potential and external K⁺ concentration on the Cs⁺ blocking of inward rectification. *Biophys. J.* 30:199–204.

Coronado, R., and C. Miller. 1979. Voltage-dependent caesium blockade of a cation channel from fragmented sarcoplasmic reticulum. *Nature (Lond.)* 280:807–810.

Dubois, J. M., and C. Bergman. 1975. Cesium induced rectification in frog myelinated fibres. *Pfluegers Arch. Eur. J. Physiol.* 355:361–364.

Dubois, J. M., and C. Bergman. 1977. The steady-state potassium conductance of the Ranvier node at various external K-concentrations. *Pfluegers Arch. Eur. J. Physiol.* 370:183–194.

Gay, L. A., and P. R. Stanfield. 1977. Cs⁺ causes a voltage dependent block of inward K⁺ current in resting skeletal muscle fibres. *Nature (Lond.)* 267:169–170.

Goldman, Y., and M. Morad. 1977. Measurement of transmembrane potential and current in cardiac muscle: a new voltage clamp method. *J. Physiol. (Lond.)* 268:613–654.

Guérin, M., and G. Wallon. 1979. The reversible replacement of internal potassium by caesium in isolated turtle heart. *J. Physiol. (Lond.)* 293:525–537.

Hagiwara, S., S. Miyazaki, and N. P. Rosenthal. 1976. Potassium current and the effect of cesium on the current during anomalous rectification of the egg cell membrane of a starfish. *J. Gen. Physiol.* 67:621–638.

Hauswirth, O., D. Noble, and R. W. Tsien. 1969. The mechanism of oscillatory activity at low membrane potentials in cardiac Purkinje fibres. *J. Physiol. (Lond.)* 200:253–265.

Hille, B. 1973. Potassium channels in myelinated nerve. Selective permeability to small cations. *J. Gen. Physiol.* 61:669–686.

Hobbs, A. S., and P. B. Dunham. 1978. Interaction of external alkali metal ions with the Na-K pump of human erythrocytes. A comparison of their effects on activation of the pump and on the rate of ouabain binding. *J. Gen. Physiol.* 72:381–402.

Hodgkin, A. L., and R. D. Keynes. 1955. The potassium permeability of a giant nerve fibre. *J. Physiol. (Lond.)* 128:61–88.

Imanishi, S., and B. Surawicz. 1976. Automatic activity in depolarized guinea pig ventricular myocardin. Characteristics and mechanism. *Circ. Res.* 39:752–759.

Isenberg, G. 1976. Cardiac Purkinje fibers: cesium as a tool to block inward rectifying potassium currents. *Pfluegers Arch. Eur. J. Physiol.* 365:99–106.

Katzung, B. G. 1975. Effects of extracellular calcium and sodium on depolarization-induced automaticity in guinea pig papillary muscle. *Circ. Res.* 37:118–127.

Katzung, B. G., and J. A. Morgenstern. 1977. Effect of extracellular potassium on ventricular
automativity and evidence for a pacemaker current in mammalian ventricular myocardium. 

Lenfant, J., J. Mironneau, and J. K. Aka. 1972. Activité répetitive de la fibre sinoauriculaire
de grenoville: analyse des courants membranaires responsables de l'automatisme cardiaque.
J. Physiol. (Paris). 64:5-18.

McDonald, T. F., and W. Trautwein. 1978 a. Membrane currents in cat myocardium:
separation of inward and outward components. J. Physiol. (Lond.). 274:193-216.

McDonald, T. F., and W. Trautwein. 1978 b. The potassium current underlying delayed
rectification in cat ventricular muscle. J. Physiol. (Lond.). 274:217-246.

Meier, C. F., Jr., and B. G. Katzung. 1978 a. Effects of cesium and barium on depolarization
induced automaticity in ventricular myocardium. Proc. West. Pharmacol. Soc. 21:71-75.

Meier, C. F., Jr., and B. G. Katzung. 1978 b. Effects of Ba++ and Ca++ on depolarization
induced automaticity and membrane currents in mammalian ventricular muscle. Fed. Proc.
37:574.

Noble, D. 1975. The Initiation of the Heartbeat. Clarendon Press, Oxford.

Noble, D., and R. W. Tsien. 1969 a. Outward membrane currents activated in the plateau
range of potentials in cardiac Purkinje fibres. J. Physiol. (Lond.). 200:205-231.

Noble, D., and R. W. Tsien. 1969 b. Reconstruction of the repolarization process in cardiac
Purkinje fibres based on voltage clamp measurements of membrane current. J. Physiol.
(Lond.). 200:233-254.

Noma, A., and H. Irisawa. 1976. A time- and voltage-dependent potassium current in the
rabbit sinoatrial node cell. Pfluegers Arch. Eur. J. Physiol. 366:251-258.

Prasad, K., and K. K. Midha. 1973. Effect of cesium on the properties of cardiac muscle. Jpn.
Heart J. 14:454-466.

Sjodin, R. A., and L. A. Beauge. 1967. Strophanthin-sensitive transport of cesium and
sodium in muscle cells. Science (Wash. D. C.). 156:1248-1250.

Trautwein, W., and T. F. McDonald. 1978. Current-voltage relations in ventricular muscle
preparations from different species. Pfluegers Arch. Eur. J. Physiol. 374:79-89.

Vassalle, M., 1977 a. Cardiac automaticity and its control. Am. J. Physiol. 233:H625-H634.

Vassalle, M. 1977 b. Generation and conduction of impulses in the heart under physiological
and pathological conditions. Pharmacol. Ther. Part B Gen. Syst. Pharmacol. 3:1-39.

Vereecke, J., G. Isenberg, and E. Carmeliet. 1980. K efflux through inward rectifying K
channels in voltage clamped Purkinje fibers. Pfluegers Arch. Eur. J. Physiol. 384:207-217.