

EXTRACELLULAR $[K^+]$ FLUCTUATIONS IN VOLTAGE-CLAMPED CANINE CARDIAC PURKINJE FIBERS

R. P. KLINE

Department of Pharmacology, Columbia College of Physicians and Surgeons, New York, New York

I. S. COHEN

*Department of Physiology and Biophysics, Health Sciences Center, State University of New York,
Stony Brook, New York 11794*

ABSTRACT Membrane currents and extracellular $[K^+]$ were measured in canine Purkinje strands during voltage-clamp steps to plateau or diastolic potentials. Extracellular $[K^+]$ increased during step depolarizations and decreased during step hyperpolarizations. On hyperpolarization, the largest fraction of the K^+ depletion occurred during the initial 500 ms of the voltage-clamp step and was correlated with a potassium depletion current, the i_d . A slower component of the depletion also occurred on hyperpolarization and had a time constant consistent with cylindrical diffusion of potassium within the Purkinje strands. On depolarization, there is an accumulation of K^+ that is correlated with the plateau current i_x . On termination of depolarizing test pulses, the K^+ accumulation decays with a time course similar to the i_x tail current. Surprisingly, no accumulation of K^+ occurred during the arrhythmogenic transient inward current, TI , suggesting that the selectivity of this current should be reevaluated.

The cardiac Purkinje fiber is a functional syncytium in which most of the membrane surface faces clefts between 100 Å and 1 μm in diameter (Mobley and Page, 1972; Hellam and Studt, 1974; Eisenberg and Cohen, 1983). The presence of these narrow clefts lead us to expect significant changes in extracellular $[K^+]$ when the magnitude of the transmembrane K^+ current changes (Attwell et al., 1979; Cohen and Kline, 1982). Direct measurements with K^+ selective electrodes in the extracellular space during the action potential have demonstrated significant increases in cleft $[K^+]$ during the action potential plateau. This K^+ accumulation decays during diastolic depolarization (Kline et al., 1980; Kline and Kupersmith, 1982). To extend these observations, in the present study we employ the voltage clamp and extracellular K^+ selective microelectrodes. Our purpose is to examine the relationship of diastolic and plateau membrane currents to changes in extracellular $[K^+]$. Some background on the relevant currents (i_d , i_x , and the TI) is provided below.

The dominant background current at diastolic potentials in the Purkinje fiber current-voltage relationship is the inwardly rectifying K^+ current iK_1 (Vereecke et al., 1980). On hyperpolarizing voltage steps from plateau potentials to diastolic potentials in the inwardly rectifying potential range, there is a dramatic decrease in the background K^+ efflux. This decreased K^+ efflux (or at potentials negative

to the K^+ equilibrium potential, net K^+ influx) through iK_1 has been postulated to lead to net K^+ depletion from the extracellular spaces is reflected in the K^+ depletion current, i_d (Baumgarten and Isenberg, 1977). More recently, single channel studies of the inward rectifier in isolated guinea pig myocytes have suggested that inactivation of the inward rectifier in the absence of any concomitant $[K^+]$ change could account for part or all of the postulated depletion current (Sakmann and Trube, 1984).

On depolarization at plateau potentials, there are slow time dependent changes in membrane current that have been called i_{x1} and i_{x2} (Noble and Tsien, 1969). These gated currents are thought to be partially, but not entirely K^+ selective. Clearly the initial analysis (Noble and Tsien, 1969) did not consider the presence of the slow inward Ca^{2+} current that is known to exist in the same potential range. Also, more recent experiments have questioned the entire formalism for i_x as initially presented (Jaeger and Gibbons, 1981).

When cardiac Purkinje fibers are exposed to a toxic concentration of cardiac glycosides or an elevated bathing $[Ca^{2+}]$, a transient inward current, TI , may be induced (Lederer and Tsien, 1976; Kass et al., 1978). The TI is most frequently observed following a period of rapid stimulation or on repolarization following a depolarizing voltage-clamp pulse of several seconds in duration. This

oscillatory current is believed to underlie a variety of triggered arrhythmias and so its properties are of substantial clinical interest (Rosen et al., 1980). Present evidence suggests that the *TI* is mediated by a rise in cytosolic $[Ca^{2+}]$ and is carried through a channel that is about equally selective to Na^+ and K^+ (Lederer and Tsien, 1976; Colquhoun et al., 1981). The oscillatory nature of the *TI* has made it difficult to unambiguously characterize its selectivity since the reversal potential has been assessed by changes in the phase of the oscillation rather than by a simple change in the direction of current flow.

We have employed the two microelectrode voltage-clamp technique with extracellular double-barreled K^+ -selective microelectrodes to examine the K^+ dependence of the depletion current, i_d , the plateau current system, i_x , and the arrhythmogenic *TI*. We find that the i_d is accompanied by a rapid phase of extracellular K^+ depletion, which supports previous conclusions based on indirect measures. During depolarizations in the plateau range of potentials, there are slow progressive increases in cleft K^+ ; these K^+ accumulations decay with a time course similar to i_x tail currents, which suggest at least a partial K^+ selectivity for these slow time-dependent plateau membrane currents and/or the role of the K^+ accumulation itself in generating the time dependence. However, no extracellular K^+ accumulation accompanies the *TI*. The absence of extracellular K^+ accumulation with the *TI* raises questions concerning the selectivity of this current.

We used canine cardiac Purkinje fibers of narrow radius (<0.2 mm) cut short (<2 mm) to employ the two microelectrode voltage-clamp technique (Deck et al., 1964; Cohen et al., 1983). Solutions contained in millimoles per liter: $NaCl$ 140, KCl 4 or 8, $CaCl_2$ 4, $MgCl_2$ 2, $NaHCO_3$ 12, NaH_2PO_4 0.4, and 1.5 g/l glucose. The elevated concentration of Ca^{2+} increased the probability of seeing the *TI* current after step depolarizations (Cohen et al., 1983). We continuously bubbled solutions with a 95% O_2 , 5% CO_2 gas mixture. The pH of the solution was 7.12 in the bottle and between 7.3 and 7.4 in the open tissue bath. The temperature was maintained between 32° and 37°C, and constant to within 0.5°C in a given experiment. Double-barreled K^+ -selective microelectrodes were fabricated and calibrated as previously described (Kline et al., 1980; Kline and Kupersmith, 1982; Kline and Morad, 1978), with the additional provision that smaller diameter tips (<1 μm) were required to minimize tissue damage when the electrodes were inserted in the extracellular space. The selectivity and time constant (~ 30 ms) of the K^+ electrode were evaluated before and after the experimental runs. For each experiment, V_K (the K-ISE output) was converted into $[K^+]_0$. From the values of $[K^+]_0$, the time course of the changes in E_K could be calculated. For the bath K^+ levels and K-ISE selectivity encountered during the experiments in Figs. 1 and 2, V_K can be converted to ΔE_K directly (with an error of $< 7\%$) by multiplying the V_K values by a constant equal to $(K_0 + S$

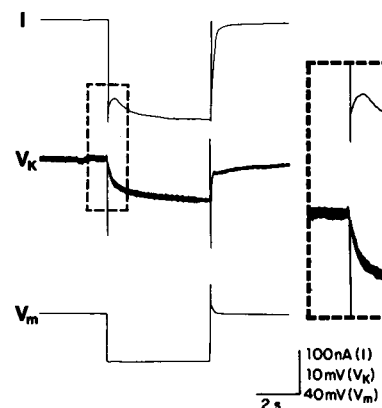


FIGURE 1 From a holding potential of -40 mV, the membrane is hyperpolarized for 5 s to -86 mV. Following the increasingly outward depletion current, the pacemaker current decays inward for the duration of the clamp step. The 10 mV negative deflection on the V_K trace (K-ISE output) indicates a depletion of $[K^+]_0$ levels from 8.0 to 4.7 mM (total depletion of 3.3 mmol/l cleft space). The insert (right panel) shows on expanded scale the first second of the clamp step indicating close correlation between development of the I_d and the rapid decay of $[K^+]_0$. About two-thirds of the total decay of $[K^+]_0$ seen during the clamp step occurs during the first 500 ms. Note that the V_K trace is not a linear indicator of $[K^+]_0$ but is logarithmic; e.g., a 5 mV negative V_K deflection indicates a 1.8-mM depletion for this electrode and bath K^+ value (this is more than one-half of the 3.3-mM depletion indicated by a 10-mV change). The holding current was -12 nA at -40 mV. The time course for the change in E_K is obtained directly from the V_K trace, noting only that the vertical calibration bar (when used to determine the magnitude of E_K) indicates 13 mV.

$Na_0)/K_0$. (K_0 and Na_0 are extracellular K^+ and Na^+ concentrations, and S is the K-ISE selectivity for Na^+ vs. K^+). The derived values of the vertical calibration bars appropriate for determining ΔE_K directly from V_K are given in the legends of Figs. 1 and 2. In Fig. 3, the calculated values for the total change in E_K are given in the legend. The deviation of ΔE_K from a linear dependence on V_K for the largest calibration bar is $\sim 20\%$.

Assuming a $[K^+]_i$ value of 130 mM (within the wide range reported for Purkinje fibers, see Isenberg, 1977; Miura et al., 1977), then the E_K values at holding potentials near the 0 current level (where cleft and bulk $[K^+]$ s should be equal) are -88 mV (4 mM bath potassium) and -70 mV (8 mM bath potassium). The maximum changes in the E_K that we measured (~ 15 mV) would then represent changes in E_K of almost 20%. We maintained the holding potential for all experiments near to the 0 current level (although small current drifts do occur during prolonged voltage clamping). This protocol allowed us to assume, to a first approximation, that cleft and bulk $[K^+]$ s were equal at the holding potential. All calculations for the changes in $[K^+]_0$ following steps away from the holding potential were performed on this basis.

Fig. 1 shows the membrane voltage, V , membrane current, I , and extracellular $[K^+]$, V_K , in response to a hyperpolarizing voltage-clamp step of 5 s from -40 to -86 mV. In the current record, the hyperpolarizing volt-

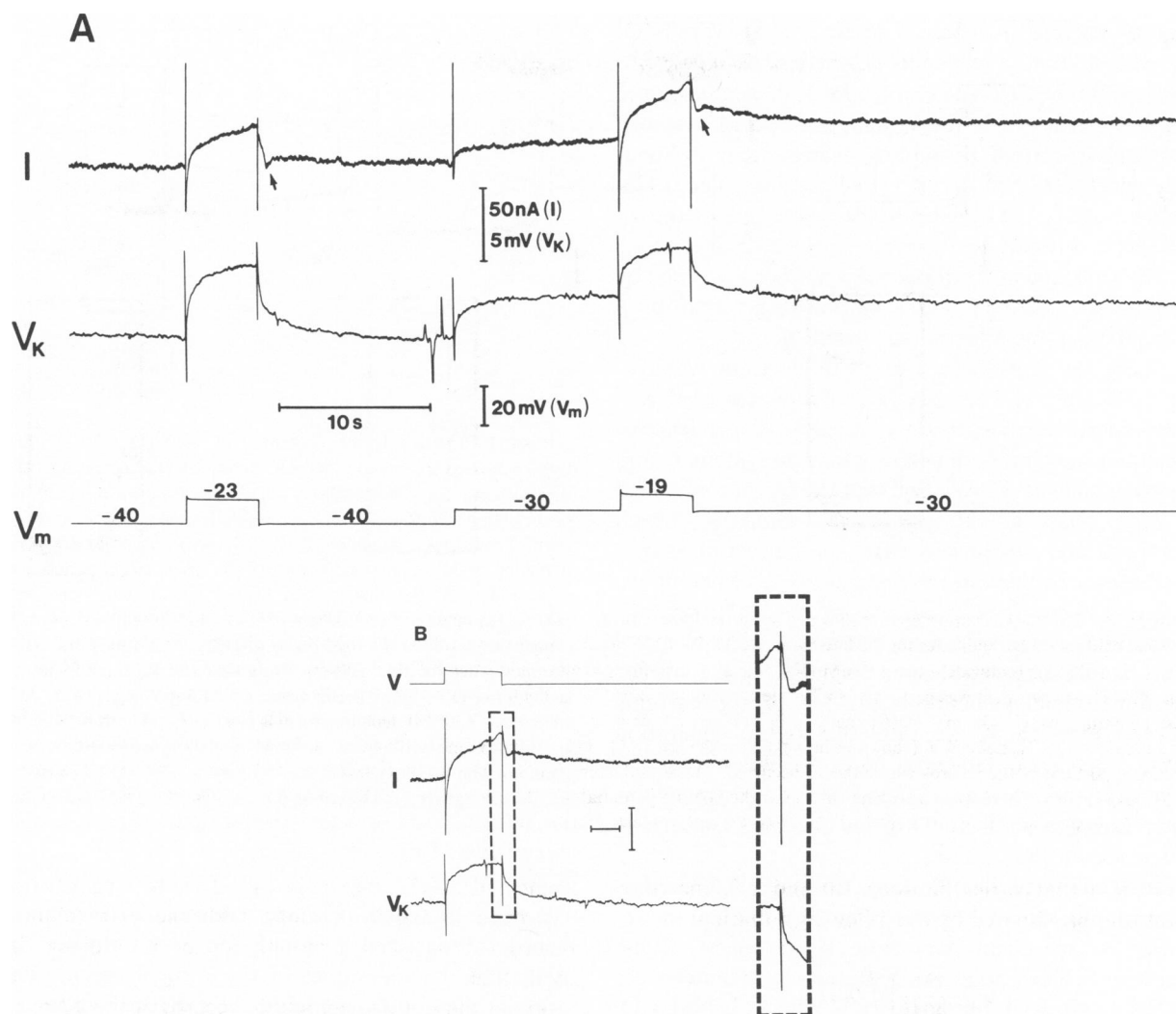


FIGURE 2 (A) The current (I), voltage (V_m), and K-ISE (V_K) traces are shown for a series of voltage-clamp steps in the plateau range of potentials. The $[K^+]$ is 8 mM at -40 mV, and accumulates maximally to 9.2 mM at -30 mV, 10.3 mM at -23 mV, and 10.9 mM at -19 mV. The time course for the change in E_K can be obtained directly from the V_K trace, noting only that the vertical calibration bars (when used to determine the magnitude of E_K) indicate 6.5 mV. The arrows indicate transient inward currents (TIs). The holding current was -6 nA at -40 mV. The MDP was -58 mV. (B) The current (I) and K-ISE (V_K) traces are shown along with membrane voltage (V) during and following the 5-s clamp shown in A from -30 to -19 mV. On return to holding potential, the TI oscillation appears but no positive $[K^+]_o$ deflection is indicated. This is shown more clearly on an expanded scale in the right insert. Note also that the $[K^+]_o$ accumulation and the I_x tail decay slowly toward baseline. The vertical calibration bar is 40 nA (I), 4 mV (V_K), and 32 mV (V). The horizontal bar is 2.5 s. The V_K trace can be used to calculate the change in E_K noting only that the vertical calibration bar represents a 5.2 mV change in E_K .

age step includes a large instantaneous inward jump in current (change in iK_1) followed by a decreasing inward current, the i_d . Later, ~ 500 ms after the hyperpolarizing clamp step begins, the time-dependent current changes direction becoming increasingly inward as the normal pacemaker current ensues. During the initial 500-ms period in which the i_d is seen, there is a rapid decrease in the extracellular $[K^+]$, declining from an initial value of 8 to ~ 5.8 mM. This relationship between the rapid phase of extracellular K^+ depletion and the i_d is further emphasized in the inset to Fig. 1 in which an enlargement of the first second of the current and K^+ decays are shown. This result confirms the supposition based on indirect estimates that

the depletion current is associated with significant extracellular depletion of K^+ (Baumgarten and Isenberg, 1977). In the final 4 s of the current record, there is a slow depletion of K^+ that accompanies the pacemaker decay. On return to the holding potential, there is an escape of the potential from control of the voltage clamp due to activation of fast inward Na^+ current, which is not controllable at normal external $[Na^+]$ at 37°C . During the voltage escape, there is rapid reaccumulation of extracellular K^+ .

The diffusion time constant for a cylinder of radius (r) can be estimated from the time constant of the first and dominant term in the Bessel expansion. Assuming a diffusion coefficient for K^+ (D_K) of $2 \times 10^{-3} \text{ mm}^2/\text{s}$ and a radial

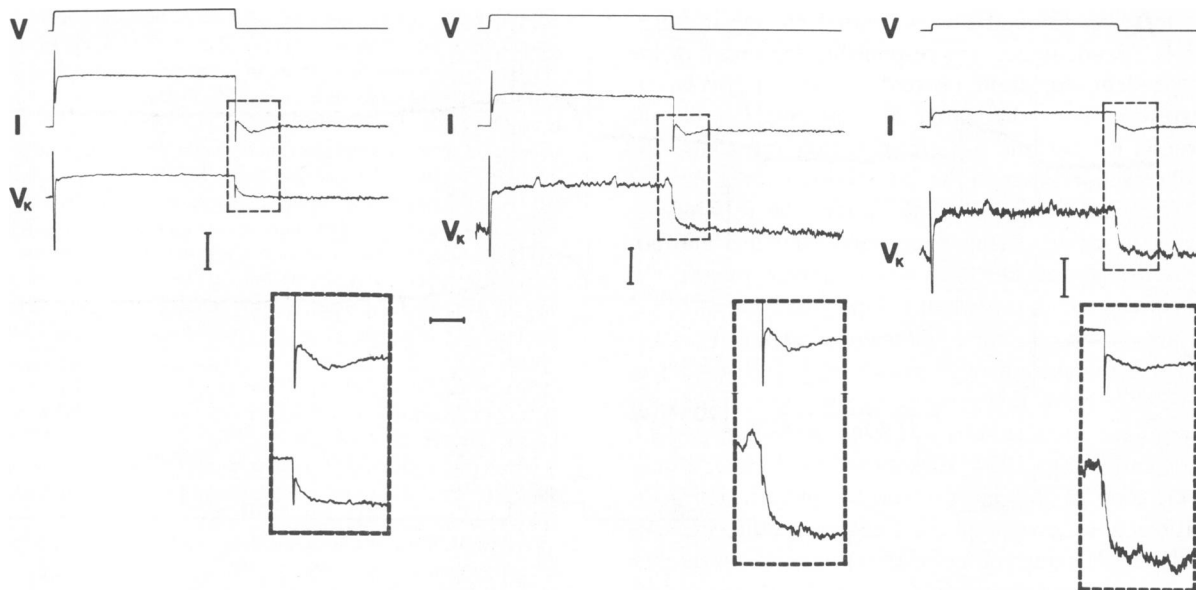


FIGURE 3 Three 10-s clamp steps are shown. The vertical bar indicates 100 nA (I) and 40 mV (V). The horizontal calibration bar is 2 s. The vertical calibration bar indicates for the left-hand panel a 10-mV V_K deflection (equivalent to a 2.3 mM $[K^+]_o$ change), for the center panel a 4 mV V_K deflection (equivalent to a 1.0 mM $[K^+]_o$ change), and for the right-hand panel a 2 mV V_K deflection (equivalent to a 0.5 mM $[K^+]_o$ change). This experiment was performed in Tyrode containing 4 mM $[K^+]_o$. For all three clamp steps, the holding potential was -34 mV. The test potentials were -55 mV (left panel), -49 mV (center panel), and -41 mV (right panel). In each case a T_I was seen with no accompanying $[K^+]_o$ deflection (shown in inserts) even though $[K^+]_o$ levels are only slowly changing at the peak of the T_I . The insets show enlarged portions of the records where the T_I occurred. The actual changes in $[K^+]_o$ were for left, center, and right panels 1.8, 1.5, and 0.7 mM, respectively. There was no holding current at the holding potential of -34 mV, where the $[K^+]_o$ was 4 mM. The changes in E_K for the three clamp steps (left to right) were 15.1, 11.8 and 4.8 mV, respectively.

tortuosity (λ) that varies between 1.0 and 1.5, the time constant is approximated by the following equation:

$$\tau \approx \left(\frac{r\lambda}{2.4048} \right)^2 / D_K.$$

For a preparation of radius 0.15 mm, τ is between 2.0 and 4.5 s. Thus, the slowing of the rate of K^+ depletion during the final 4 s probably represents cylindrical diffusion of K^+ into this preparation of 0.15-mm radius. In fact, during both depolarizing and hyperpolarizing clamp steps, the $[K^+]_o$ changes could be fit as two exponentials, where the slower time constant approximates the cylindrical diffusion time constant of the preparation (see also Kline and Kupersmith, 1982).

Fig. 2 *a* shows a series of voltage-clamp pulses in the plateau range of potentials. Following each depolarization (-40 to -23 , -40 to -30 , -30 to -19), there is a slow change in membrane current that is correlated with a slow increase in extracellular $[K^+]$. On repolarization (-23 to -40 , and -19 to -30), there is a slowly decaying i_x tail current, correlated with a decay of the accumulated extracellular $[K^+]$. To emphasize this correlation between membrane current and changes in extracellular $[K^+]$, the step from -30 to -19 mV is shown in Fig. 2 *b* at expanded gain. Also present in the current record in Fig. 2 *b* immediately following repolarization is the oscillatory T_I current

(indicated by the arrows in Fig. 2 *a*). It is shown further expanded in the inset along with the extracellular K^+ record. There is no accumulation of K^+ during the T_I oscillation.

Fig. 3 shows three additional records of the T_I recorded in another preparation following 10-s depolarizing pulses from potentials of -41 , -49 , and -55 mV to a potential of -34 mV. Again the oscillatory T_I is not accompanied by an accumulation of K^+ . In this preparation a total of nine such clamp steps yielded similar T_I oscillations with no associated K^+ accumulation.

The changes in extracellular $[K^+]$ we report here have been predicted by substantial previous computer modeling and theoretical investigation (Cohen et al., 1982; Attwell et al., 1979; Levis et al., 1983). The extracellular $[K^+]$ is determined by a balance of transmembrane K^+ flux through membrane channels, active transport by the Na/K exchange pump, and diffusion between the bulk solution and the narrow extracellular spaces. When the membrane potential is altered by the voltage clamp, a change in this balance occurs resulting in a net change in the extracellular $[K^+]$. Time-independent K^+ currents can be made time dependent by changes in both the electrochemical driving force for K^+ and the effects of changes in extracellular K^+ on K^+ conductance. When the membrane voltage is hyperpolarized, the K^+ efflux through i_{K1} is reduced or reversed resulting in extracellular K^+ depletion. This change in

driving force on K^+ and the associated change in background K^+ conductance are responsible for creating the time-dependent depletion current, i_d . Alternatively, on depolarization both the gated K^+ current, i_g , and the background K^+ current, i_{K1} , are increased and contribute to the observed increase in the extracellular concentration of K^+ . This accumulation of K^+ alters the relationship between the gating of the membrane channels and the observed membrane current, which makes meaningful analysis of channel gating almost impossible.

The present results were obtained in canine Purkinje fibers where the extracellular spaces, although restricted, are somewhat more favorable than the more commonly used ungulate preparations (Mobley and Page, 1972; Hellman and Studt, 1974; Eisenberg and Cohen, 1983). However, even in this more favorable preparation large fluctuations in extracellular $[K^+]$ accompany hyperpolarizing and depolarizing voltage-clamp pulses. We were able to substantiate previous arguments correlating the i_d with an early phase of cleft K^+ depletion. We have also found that some slow time-dependent plateau currents are accompanied by changes in cleft $[K^+]$. Finally we were not able to record K^+ accumulation during the TI oscillation. This finding is surprising considering the presumed contribution of K^+ to the TI current. The TI oscillations reported here are smaller in magnitude than those recorded during exposures to K^+ -free Tyrode (Eisner and Lederer, 1979) or prolonged exposures to toxic doses of cardiotonic steroids (Lederer and Tsien, 1976). It was necessary to keep the after-contraction associated with the TI current small so as not to dislodge our K^+ electrode from its extracellular position. Nevertheless, given the size of the oscillatory current, its assumed equal Na^+ and K^+ selectivity, the potential at which it was recorded, and the ability of the K-ISE to detect K^+ changes during activation of other currents, we calculate that K^+ accumulation should have been observed if the TI occurred uniformly throughout the preparation (Lederer and Tsien, 1976). Our results, along with other recent studies on the TI current reversal (Clusin et al., 1983; Karagueuzian and Katzung, 1982), suggest that further experiments investigating the selectivity of the TI current are necessary.

We would like to acknowledge helpful discussion and criticism of the manuscript by Dr. Nicholas Datyner, Dr. Gary Gintant, and Nancy Mulrine.

This work was supported by National Institutes of Health grants HL20558 and HL28958 to Dr. I. Cohen and grants HL25798 and HL30557 to Dr. R. Kline. Dr. R. Kline was the recipient of an American Heart Association and an Irma Hirschl trust career scientist award.

Received for publication 14 February 1984 and in final form 29 May 1984.

REFERENCES

- Attwell, D., D. A. Eisner, and I. Cohen. 1979. Voltage clamp and tracer flux data. Effects of a restricted extracellular space. *Q. Rev. Biophys.* 12:213-261.
- Baumgarten, C., and G. Isenberg. 1977. Depletion and accumulation of potassium in the extracellular clefts of cardiac Purkinje fibers during voltage-clamp hyperpolarization and depolarization. *Pfluegers Arch. Gesamte Physiol. Menschen Tiere.* 368:19-31.
- Clusin, W. T., R. Fischmeister, and R. L. DeHaan. 1983. Caffeine-induced current in embryonic heart cells: time course and voltage dependence. *Am. J. Physiol.* 245:H528-H532.
- Cohen, I., R. Falk, and R. Kline. 1982. Pacemaker activity in cardiac Purkinje fibers. A voltage clamp analysis. In *Normal and Abnormal Conduction in the Heart*. A. De Carvalho, M. Lieberman, and B. Hoffman, editors. Futura Publishing Co., Inc., Mount Kisco, NY.
- Cohen, I., R. Falk, and R. Kline. 1983. Voltage-clamp studies on the canine Purkinje strand. *Proc. Roy. Soc. Lond. B Biol. Sci.* 217:215-236.
- Cohen, I., and R. Kline. 1982. K^+ fluctuations in the extracellular spaces of cardiac muscle. Evidence from the voltage clamp and K^+ -selective microelectrodes. *Circ. Res.* 50:1-16.
- Colquhoun, D., E. Neher, H. Reuter, and C. F. Stevens. 1981. Inward current channels activated by intracellular Ca in cultured cardiac cells. *Nature (Lond.)* 294:752-754.
- Deck, K. A., R. Kern, and W. Trautwein. 1964. Voltage-clamp technique in mammalian cardiac fibres. *Pfluegers Arch. Gesamte Physiol. Menschen Tiere.* 280:50-62.
- Eisenberg, B., I. Cohen. 1983. The ultrastructure of the canine Purkinje strand: a morphometric analysis. *Proc. Roy. Soc. Lond. B Biol. Sci.* 217:191-213.
- Eisner, D. A., and J. Lederer. 1979. Inotropic and arrhythmogenic effects of potassium depleted solutions on mammalian cardiac muscle. *J. Physiol. (Lond.)* 294:255-277.
- Hellam, D. C., and J. W. Studt. 1974. A core-conductor model of the cardiac Purkinje fibre based on structural analysis. *J. Physiol. (Lond.)* 243:637-660.
- Isenberg, G. 1977. Sheep Purkinje fiber: Intracellular potassium activity by ion sensitive electrodes in comparison with potassium reversal potential. *Pfluegers Arch. Euro J. Physiol.* 368(Suppl):R3.
- Jaeger, J. M., and W. R. Gibbons. 1981. The effect of 4-aminopyridine on the late outward plateau current in cardiac Purkinje fibers. *Biophys. J.* 33(2, Pt. 2):72a. (Abstr.)
- Karagueuzian, H. S., and B. G. Katzung. 1982. Voltage-clamp studies of transient inward current and mechanical oscillations induced by ouabain in ferret papillary muscle. *J. Physiol. (Lond.)* 327:255-271.
- Kass, R. S., R. W. Tsien, R. Weingart. 1978. Ionic basis of transient inward current induced by strophanthidin in cardiac Purkinje fibres. *J. Physiol. (Lond.)* 281:209-226.
- Kline, R., I. Cohen, R. Falk, and J. Kupersmith. 1980. Activity dependent extracellular K^+ fluctuations in canine Purkinje fibers. *Nature (Lond.)* 286:68-71.
- Kline, R., and J. Kupersmith. 1982. Effects of extracellular potassium accumulation and sodium pump activation on automatic canine Purkinje fibres. *J. Physiol. (Lond.)* 324:507-533.
- Kline, R., and M. Morad. 1978. Potassium efflux in heart muscle during activity: extracellular accumulation and its implications. *J. Physiol. (Lond.)* 280: 537-558.
- Lederer, J., and R. W. Tsien. 1976. Transient inward current underlying arrhythmogenic effects of cardiotonic steroids in Purkinje fibers. *J. Physiol. (Lond.)* 263:73-100.
- Levis, R. A., R. T. Mathias, and R. S. Eisenberg. 1983. Electrical properties of sheep Purkinje strands. Electrical and chemical potentials in the clefts. *Biophys. J.* 44:225-248.
- Miura, D. S., B. Hoffman, and M. R. Rosen. 1977. The effect of extracellular potassium on the intracellular K^+ activity and transmembrane potentials of beating canine cardiac Purkinje fibers. *J. Gen. Physiol.* 69:463-473.
- Mobley, B., and E. Page. 1972. The surface area of sheep cardiac Purkinje fibres. *J. Physiol. (Lond.)* 220:547-563.

- Noble, D., and R. W. Tsien. 1969. Outward membrane currents activated in the plateau range of potentials in cardiac Purkinje fibres. *J. Physiol. (Lond.)*. 220:205–231.
- Rosen, M. R., C. Fisch, B. I. Hoffman, P. Danilo, Jr., D. I. Lovelace, and S. B. Knoebel. 1980. Can accelerated atrioventricular junctional escape rhythms be explained by delayed after depolarization? *Am. J. Cardiol.* 45:1272–1284.
- Sakmann, B., and G. Trube. 1984. Voltage-dependent inactivation of inward-rectifying single-channel currents in the guinea-pig heart cell membrane. *J. Physiol. (Lond.)*. 347:659–683.
- Vereecke, J., G. Isenberg, and E. Carmeliet. 1980. Inward and outward rectifying components of the K efflux in voltage-clamped Purkinje fibers. *Pfluegers Arch. Eur. J. Physiol.* 384:207–217.