

# Electrogenic Sodium Extrusion in Cardiac Purkinje Fibers

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**ABSTRACT** Thin canine cardiac Purkinje fibers in a fast flow chamber were exposed to K-free fluid for 15 s to 6 min to initiate "sodium loading," then returned to K-containing fluid to stimulate the sodium pump. The electrophysiological effects of enhanced pump activity may result from extracellular K depletion caused by enhanced cellular uptake of K or from an increase in the current generated as a result of unequal pumped movements of Na and K, or from both. The effects of pump stimulation were therefore studied under three conditions in which lowering the external K concentration ( $[K]_o$ ) causes changes opposite to those expected from an increase in pump current. First, the resting potential of Purkinje fibers may have either a "high" value or a "low" (less negative) value: at the low level of potential, experimental reduction of  $[K]_o$  causes depolarization, whereas an increase in pump current should cause hyperpolarization. Second, in regularly stimulated Purkinje fibers, lowering  $[K]_o$  prolongs the action potential, whereas an increase in outward pump current should shorten it. Finally, lowering  $[K]_o$  enhances spontaneous "pacemaker" activity in Purkinje fibers, whereas an increase in outward pump current should reduce or abolish spontaneous activity. Under all three conditions, we find that the effects of temporary stimulation of the sodium pump are those expected from a transient increase in outward pump current, not those expected from K depletion.

## INTRODUCTION

The hyperpolarization seen in cardiac cells in which the rate of active sodium extrusion is enhanced after a period of "sodium loading" has been taken as evidence that the Na extrusion is electrogenic (Page and Storm, 1965; Glitsch, 1969, 1972; Hiraoka and Hecht, 1973), and the results of a recent study of the potassium dependence of that hyperpolarization are, similarly, most readily interpreted in terms of electrogenic sodium-potassium exchange (Glitsch et al., 1978). Nevertheless, it has proved difficult to rule out an alternative explanation, that the hyperpolarization arises because K uptake by the cells is rapid enough to cause depletion of K ions just outside the cell membrane, thereby shifting the K equilibrium potential ( $E_K$ ) in a negative direction (cf. Page and Storm, 1965; Adrian and Slayman, 1966; Gadsby et al., 1977). In the case of cardiac Purkinje fibers, however, there are three experimental conditions under which a reduction in external K concentration,  $[K]_o$ , is known to produce effects opposite to those expected from an increase in the rate of electrogenic sodium extrusion.

First, in low chloride fluids containing  $\sim 4$  mM K, Purkinje fibers may be

“switched” from their normal resting potential of about  $-90$  mV to a “lower” stable level near  $-40$  mV; at that lower level of membrane potential, a reduction in  $[K]_o$  leads to further depolarization (Gadsby and Cranefield, 1977 *b*). At the lower level of potential, therefore, pump-induced depletion of extracellular K should cause depolarization, whereas an increase in the rate of electrogenic sodium extrusion should always cause hyperpolarization. Second, in Purkinje fibers at the *high level* of membrane potential, both an increase in outward sodium pump current and a moderate reduction in  $[K]_o$  cause hyperpolarization, but in regularly stimulated fibers, an increase in pump current would be expected to shorten the action potential, whereas a decrease in  $[K]_o$  is known to lengthen it (Weidmann, 1956; Noble, 1965; Vassalle, 1965). Finally, if Purkinje fibers are spontaneously active at the high level of membrane potential, an increase in outward pump current should slow or abolish the spontaneous depolarization underlying the rhythmicity whereas a reduction in  $[K]_o$  is known to enhance it (Vassalle, 1965). We report here that under each of the above conditions stimulation of the sodium pump causes changes of the kind expected to result from enhanced electrogenic sodium extrusion rather than those expected to result from K depletion.

In the present study, thin bundles of cardiac Purkinje fibers, suspended in a fast-flow chamber, were exposed to K-free perfusate for brief periods to initiate sodium loading and were then switched back to K-containing solution; a similar method of stimulating the sodium pump was used by Noma and Irisawa (1974, 1975) for small preparations of rabbit sinoatrial nodal cells. With this technique, effects attributable to pump stimulation are seen on readdition of potassium after as little as 1 min of zero  $[K]_o$  perfusion (cf. Noma and Irisawa, 1975). In contrast to the slow development and decay of the hyperpolarization seen when cardiac preparations are rewarmed after prolonged periods of hypothermia (see, e.g., Hiraoka and Hecht, 1973), the hyperpolarization after brief periods of sodium loading reaches a peak within a few seconds of switching back to K-containing from K-free solution and then decays within a few minutes (Noma and Irisawa, 1975). The present method thus allows a single microelectrode impalement to be maintained throughout several cycles of sodium loading and subsequent pump stimulation, thereby facilitating the demonstration of even small effects and permitting effects of pump stimulation to be studied in the same cell under several different experimental conditions.

We find that small changes in pump activity can rapidly alter both low and high levels of resting potential in Purkinje fibers, markedly change the duration of the action potential, and suppress both pacemaker activity arising near the high level of membrane potential and rhythmic activity sustained by afterpotentials arising near the lower level of membrane potential. These results suggest that changes in the level of activity of the sodium pump may have major functional significance not only for cells in intact hearts but also for excitable cells in various other tissues, e.g., other cells exhibiting more than one resting potential, or exhibiting pacemaker or “bursting” activity.

A preliminary report of some of these findings has been published in abstract (Gadsby and Cranefield, 1977 *a*).

## MATERIALS AND METHODS

Small unbranched bundles of Purkinje fibers, 100–300  $\mu\text{m}$  wide and 1–4 mm long, were dissected from the right ventricles of dog hearts immersed in Tyrode's solution at room temperature. The bundles were suspended between fine insect pins in the narrow channel of a modified Hodgkin-Horowitz (1959) fast flow system. A flow rate of 5 ml/min was maintained throughout all experiments except for those of Figs. 2 and 4 *b* in which the flow rate was 20 ml/min. The composition of the solution near the center of the channel could be changed with a half-time of less than 50 ms at the higher rate and of  $\sim 0.5$  s at the lower rate. This perfusion system and the recording systems used with it have been described in detail previously (Gadsby and Cranfield, 1977 *b*). Conventional glass microelectrodes filled with 3 M KCl, with resistances of 15–30 M $\Omega$  and tip potentials initially less negative than  $-3$  mV, were used for potential recording.

The composition of the Cl-containing Tyrode's solution used during dissection and for some experiments, for example, those on electrically driven preparations (see Figs. 5, 6, and 7), was: 137 mM NaCl; 4 mM KCl; 12 mM NaHCO<sub>3</sub>; 1.8 mM NaH<sub>2</sub>PO<sub>4</sub>; 0.5 mM MgCl<sub>2</sub>; 2.7 mM CaCl<sub>2</sub>; 5.5 mM dextrose. In K-free Tyrode's solution the 4 mM KCl was replaced by 4 mM NaCl. These solutions were prewarmed and equilibrated with a 95% O<sub>2</sub>–5% CO<sub>2</sub> mixture. The low Cl solution used in most experiments contained: 146 mM Na-isethionate (Koch-Light, Colnbrook, Buckinghamshire, England); 4 mM K-methylsulphate (Hopkin & Williams, Chadwell Heath, Essex, England); 5 mM Hepes (*N*-2-hydroxyethyl-piperazine-*N'*-2-ethanesulfonic acid; Sigma Chemical Co., St. Louis, Mo.; adjusted to pH 7.3); 0.5 mM MgCl<sub>2</sub>; 2.7 mM Ca-methanesulfonate (made with methanesulfonic acid; Eastman Kodak Corp., Rochester, N.Y.); 5.5 mM dextrose. K-free low Cl solution was made by replacing the 4 mM K-methylsulphate with 4 mM Na-isethionate. Fluids containing intermediate K concentrations were obtained by mixing appropriate quantities of the 4 mM K and zero K solutions. These low Cl solutions, which contained no added bicarbonate, were all prewarmed and equilibrated with pure oxygen. When required, the cardiac steroid, 3-acetylstrophanthidin (kindly provided by Eli Lilly & Co., Indianapolis, Ind.), was added from a refrigerated stock solution of  $5 \times 10^{-3}$  M acetylstrophanthidin in ethanol: control experiments showed that 0.1% by volume ethanol had no effect on the membrane potential of Purkinje cells in a [K]<sub>o</sub> range of 0–4 mM. The temperature of the solutions was continuously monitored close to the preparation with a small thermistor bead and was kept between 35 and 37° C.

## RESULTS

*The Transient Hyperpolarization*

Fig. 1 *a* shows a record from a quiescent fiber with a resting potential of  $-32$  mV in 4 mM K, low Cl fluid: the fiber depolarized abruptly to about  $-20$  mV in K-free solution. After 1 min of exposure to zero [K]<sub>o</sub>, the return to 4 mM [K]<sub>o</sub> was accompanied by a transient hyperpolarization, the membrane potential "undershooting" its original resting level by 14 mV within a few seconds and then more slowly returning to that control level, reaching it after  $\sim 3$  min. In Fig. 1 *b* four records are superimposed for comparison of the effects of 15, 30, 60, and 90 s of exposure to zero [K]<sub>o</sub> on the hyperpolarization seen on returning to 4 mM [K]<sub>o</sub>: the hyperpolarization was clearly increased by prolonging the prior exposure to zero [K]<sub>o</sub>.

In the experiments shown in Fig. 2, fibers at the lower level of resting potential in 4 mM [K]<sub>o</sub> were exposed to zero [K]<sub>o</sub> for prolonged periods which

were interrupted by brief, 5- to 10-s, "test" exposures to 4 mM  $[K]_o$ ; Fig. 1 shows that 10 s is long enough for the transient hyperpolarization to reach its peak yet too short for it to decay appreciably. In this way the development of the hyperpolarization could be followed as the period of K-free perfusion was prolonged. In Fig. 2 *a* the peak hyperpolarizations, measured from the initial potential level in 4 mM  $[K]_o$ , were 6, 10, 21, and 67 mV after exposure to zero  $[K]_o$  for 30, 60, 120, and 180 s, respectively. At the peak of the final hyperpolarization the membrane potential reached  $-94$  mV and then declined only to  $-92$  mV where it subsequently remained: the resting potential of this fiber could thus have either a low value, near  $-30$  mV, or a high value, near  $-90$  mV. A similar transition from the lower to the higher level of resting potential can

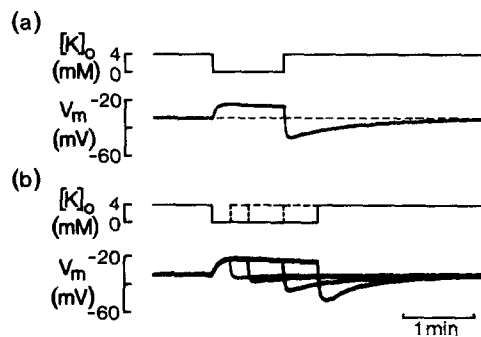


FIGURE 1. Transient hyperpolarization from the low level of resting potential evoked by return to 4 mM  $[K]_o$  after brief periods of exposure to zero  $[K]_o$ . The upper lines indicate the changes in  $[K]_o$  and the lower traces show the resulting changes in membrane potential,  $V_m$ . (a) Dashed line is drawn at the level of the steady resting potential in 4 mM  $[K]_o$ . (b) Superimposed records from four runs made during a maintained impalement. The exposures to zero  $[K]_o$  lasted 60 s in (a) and 15, 30, 60, and 90 s in (b); low Cl solutions throughout.

often be effected by applying a weak, hyperpolarizing current pulse via a second intracellular microelectrode (see Figs. 3 and 4 of Gadsby and Cranfield, 1977 *b*).

Fig. 2 *b* shows results obtained when the exposure to zero  $[K]_o$  was continued even after the membrane potential reached the "high" level during test exposures to 4 mM  $[K]_o$ . The peak hyperpolarization during test flushes with 4 mM  $[K]_o$  continued to increase as the K-free perfusion was prolonged, both before and after the abrupt transition to the higher potential level had first occurred.

These results can be interpreted as follows. In K-free fluid the pump is slowed and Na efflux reduced, but Na influx continues and  $[Na]_i$  therefore rises (Page et al., 1964) to a level determined by the duration of the exposure to zero  $[K]_o$ . Since the pump is stimulated by internal Na ions (Glitsch et al., 1976), on returning to K-containing fluid the rate of Na extrusion is enhanced until  $[Na]_i$  is brought back to its initial steady-state level. If the size of the hyperpolarization is an index of pump activity, the progressive increase in amplitude of the hyperpolarization seen with each successive test exposure to 4 mM  $[K]_o$  in Fig. 2

suggests that  $[Na]_i$ , and hence the subsequent increase in pump current, continue to rise throughout the period of exposure to K-free fluid. This interpretation is supported by the observation that the rate of change of membrane potential during the initial seconds of each test exposure to 4 mM  $[K]_o$  increases as the period of exposure to zero  $[K]_o$  is prolonged; this is seen most clearly in those later test exposures which result in an abrupt transition to the high level of membrane potential (Fig. 2 *b*).

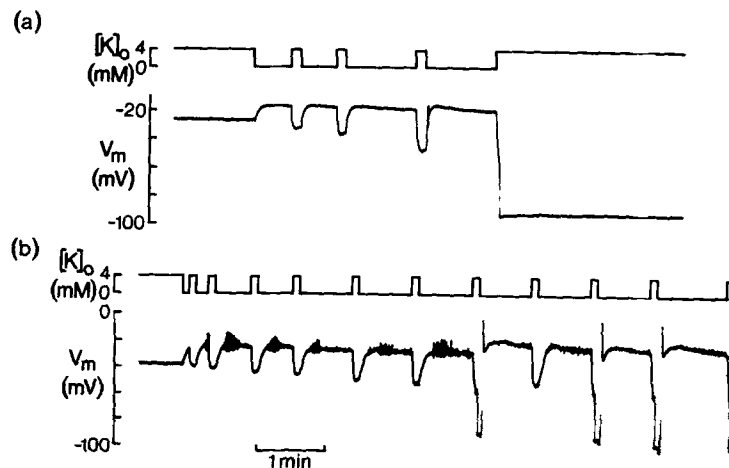


FIGURE 2. Time-course of development of the transient hyperpolarization. Fibers with resting potentials initially at the low level were subjected to prolonged exposure to zero  $[K]_o$  interrupted by 5- to 10-s exposures to 4 mM  $[K]_o$ , as indicated by the upper lines; lower traces are pen recordings of the resulting changes in membrane potential,  $V_m$ . (a) A maintained shift of the membrane potential to the higher resting level followed the return to 4 mM  $[K]_o$  after 3 min in zero  $[K]_o$ . (b) A return to zero  $[K]_o$  when the membrane potential is near the high level in 4 mM  $[K]_o$  initiates an upstroke of an action potential. Small oscillations in membrane potential are occasionally seen in fibers depolarized in K-free fluid. Low Cl solutions throughout.

#### *Effects of $[K]_o$ on the Transient Hyperpolarization*

Since the rate of sodium extrusion in many cells is known to depend strongly on  $[K]_o$  (Glynn, 1968; Baker, et al., 1969; Sjodin, 1971; Glitsch et al., 1976), we studied the effect on the transient hyperpolarization of varying the level of  $[K]_o$  with which the fiber was equilibrated before and after a fixed period of Na-loading in K-free fluid.  $[K]_o$  was varied over the range 0.25–4 mM and a period of at least 3 min was allowed for the resting potential (always at the lower level) to stabilize at each new K concentration. The peak hyperpolarization was then measured on returning to the different levels of  $[K]_o$  after a 1- or 2-min exposure to K-free fluid. A single impalement could often be maintained throughout an entire experiment so that a transient hyperpolarization could be evoked from one to four times (usually twice) at each of five different  $[K]_o$  levels; occasionally, reliable results could be obtained at only two or three  $[K]_o$

values. Some results from a typical experiment are shown in Fig. 3 *a*. In this experiment the steady resting potential ranged from  $-28$  mV at  $0.5$  mM  $[K]_o$  to  $-30$  mV at  $4$  mM  $[K]_o$ . After a 1-min loading period the rate of potential increase and the peak amplitude of the hyperpolarization both increased as the equilibrating  $[K]_o$  was raised from  $0.5$  to  $4$  mM. For each experiment, the average peak hyperpolarization at a given  $[K]_o$  was normalized with respect to the average at  $1$  mM  $[K]_o$  and the means of those ratios from 10 fibers are plotted against  $[K]_o$  in Fig. 3 *b*.

The graph in Fig. 3 *b* does not describe activation of the pump by external K ions, but inasmuch as the low level of resting potential and the slope conductance near that level both appear to change little with  $[K]_o$  over the range  $0.25$ – $4$

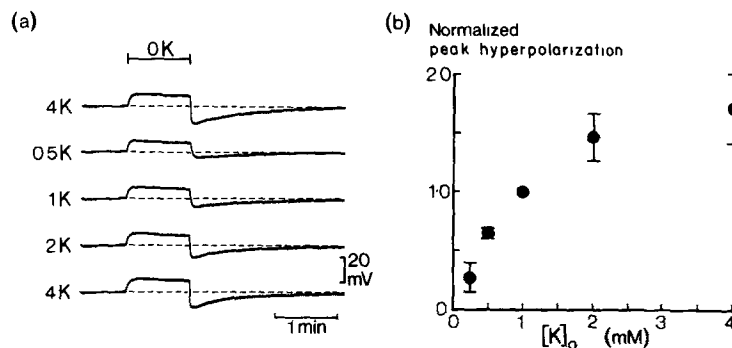


FIGURE 3. Effect of  $[K]_o$  on the transient hyperpolarization at the low level of resting potential. (a) Transient hyperpolarizations recorded during a maintained impalement after equilibrating the fiber to the different K concentrations given (in millimolar) at the left of each record. The 1-min exposure to zero  $[K]_o$  during each run is indicated by the upper bar. Dashed lines are drawn at the levels of the steady resting potentials at each K concentration: the resting potential of this fiber ranged from  $-30$  mV at  $4$  mM  $[K]_o$  to  $-28$  mV at  $0.5$  mM  $[K]_o$ . (b) Average values of the normalized peak hyperpolarizations from 12 experiments on 10 fibers are plotted against  $[K]_o$ ; the vertical bars indicate  $\pm$ SD. Results of eight experiments using 1-min exposures to zero  $[K]_o$  have been pooled with results of four experiments using 2-min exposures to zero  $[K]_o$ . The peak amplitude of the transient hyperpolarization at  $1$  mM  $[K]_o$  averaged  $7$  mV (range  $4$ – $14$  mV) over the 12 experiments. The number of fibers contributing to the average at each value of  $[K]_o$  is:  $0.25$  mM, 6;  $0.5$  mM, 7;  $1$  mM, 10;  $2$  mM, 8;  $4$  mM, 5. The points probably do not describe the  $[K]_o$  dependence of pump activity (see text). Low Cl solutions throughout.

mM (Dudel et al., 1967; Gadsby and Cranefield, 1977 *b*),<sup>1</sup> the points in Fig. 3 *b* may be taken to indicate, approximately, how the peak amplitude of the transient increase in pump current varies with  $[K]_o$  under the conditions of these experiments. (If membrane slope conductance tends to increase with  $[K]_o$ , then the peak amplitude of the pump current should have a steeper dependence on  $[K]_o$  than does the peak of the hyperpolarization plotted in Fig. 3 *b*.) However, one might expect the initial level of  $[Na]_i$  and, consequently, the subsequent degree of pump stimulation, to have been smaller at the higher of

<sup>1</sup> Gadsby, D. C., and P. F. Cranefield. Unpublished observations.

the  $[K]_o$  values used here (Ellis, 1977). Therefore, assuming the Na-K coupling ratio of the pump to be independent of  $[K]_o$ , the  $[K]_o$  dependence of pump activation is expected to be steeper than the  $[K]_o$  dependence of the hyperpolarization shown in Fig. 3 *b*.

#### Cardiac Steroids

Application of acetylstrophanthidin at the low level of resting potential causes depolarization and abolition of the transient hyperpolarization otherwise seen on returning to 4 mM  $[K]_o$  after brief exposures to K-free solution. The upper of the three voltage traces in Fig. 4 *a* shows the usual transient hyperpolarization following a return to 4 mM  $[K]_o$  after a 1-min exposure to K-free fluid. The fiber was then exposed to  $2 \times 10^{-6}$  M acetylstrophanthidin and the membrane potential (second voltage trace) began to decline with a few seconds, reaching a

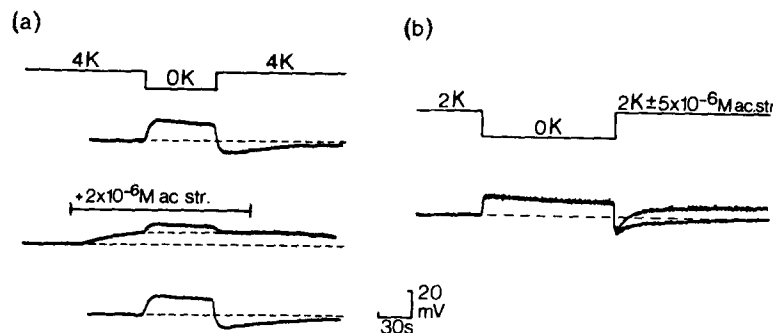


FIGURE 4. Effect of acetylstrophanthidin (ac. str.) on the transient hyperpolarization at the lower level of resting potential. The upper lines in (a) and (b) indicate the  $[K]_o$  changes during each run. (a) Two control runs were made just before, and 10 min after, respectively, the test run during which acetylstrophanthidin was applied for the period indicated by the bar. Dashed lines are drawn at the steady control resting potential in 4 mM  $[K]_o$ ,  $-47$  mV, and at the steady potential level in the presence of acetylstrophanthidin. (b) Two records have been superimposed to illustrate the rapid action of acetylstrophanthidin on the transient hyperpolarization. The dashed line is drawn at  $-41$  mV, the steady resting potential in 2 mM  $[K]_o$ ; the transient hyperpolarization decayed to this level in the control run. In the test run the fiber was switched to 2 mM  $[K]_o$  fluid containing acetylstrophanthidin after the exposure to zero  $[K]_o$ . Low Cl solutions throughout.

new steady level, some 8 mV lower, in about 1 min. This depolarization suggests that inhibition of the sodium pump by acetylstrophanthidin abolishes a steady component of outward membrane current. To test whether the pump was completely inhibited, the fiber was again exposed to K-free solution for 1 min. The further depolarization seen in K-free solution is discussed below; on returning the fiber to 4 mM  $[K]_o$  the membrane potential returned merely to the level seen just before the exposure to K-free fluid, the absence of any transient hyperpolarization demonstrating that the pump was, indeed, completely inhibited. On washing out the acetylstrophanthidin, the membrane potential began to move slowly towards the level seen in 4 mM  $[K]_o$  before addition of the drug. A few minutes later, after the control resting potential

had been reestablished, the fiber was again exposed to K-free fluid, and the usual transient hyperpolarization (third voltage trace) was observed on returning to 4 mM  $[K]_o$ .

Because the level of acetylstrophanthidin used in these experiments appears to inhibit the pump completely, the added depolarization seen on switching to K-free fluid in the presence of acetylstrophanthidin (Fig. 4 *a*) is presumably caused not by further inhibition of the pump, but by a reduction in outward K current (cf. Noble, 1965; Gadsby and Cranefield, 1977 *b*).

Fig. 4 *a* illustrates both the extent and the rapidity of the depolarization that follows application of acetylstrophanthidin at the low level of resting potential. The average size of the rapid depolarization caused by  $2-5 \times 10^{-6}$  M acetylstrophanthidin was 9 mV (SD  $\pm$  3 mV, 11 trials on six preparations). The half-time for that depolarization averaged 26 s (SD  $\pm$  6 s, seven experiments on five fibers). In contrast, the slowness with which the membrane repolarizes on washing out acetylstrophanthidin probably reflects the slow dissociation of the drug from sodium pump sites. This would tend to obscure any transient pump stimulation expected to result from the rise in  $[Na]_i$  that occurs while the pump is inhibited.

The rapidity with which acetylstrophanthidin acts is further illustrated in Fig. 4 *b*. Here, a record showing the usual transient hyperpolarization, elicited in 2 mM K solution, after a 2-min exposure to K-free fluid is superimposed on another record made, during the same impalement, when the fiber was switched from K-free fluid to 2 mM K solution containing  $5 \times 10^{-6}$  M acetylstrophanthidin. In the latter record, the membrane potential increases as usual during the first few seconds after readdition of K but then rapidly declines to a steady lower level, showing that acetylstrophanthidin acts with little delay on the pump-induced hyperpolarization and that its action is essentially complete within about 1 min.

#### *Effects on Action Potentials*

Fig. 5 shows the effects of a 1-min period of sodium loading in zero  $[K]_o$  on action potentials recorded in a Purkinje fiber which was stimulated at a rate of 60/min except while depolarized in K-free fluid. The membrane potential between action potentials (the diastolic potential), initially steady at  $-87$  mV in 4 mM  $[K]_o$ , declined rapidly to about  $-35$  mV in zero  $[K]_o$ . When regular stimulation was resumed 5 s after switching back to 4 mM  $[K]_o$ , the diastolic potential was  $-92$  mV, but it slowly returned to the control level of  $-87$  during the next 2-3 min. The durations of sample action potentials, displayed on an oscilloscope and photographed, were measured from the upstroke to the point where a line drawn at the level of the resting potential intersects the tangent fitted to the final phase of rapid repolarization (Niedergerke and Orkand, 1966). These durations, normalized with respect to the control duration before exposure to K-free fluid, are plotted in the graph below the potential record. Although the first two action potentials obtained on resuming stimulation after the period of sodium-loading were longer than the controls, the action potentials rapidly shortened to  $\sim 80\%$  of the control duration and then slowly lengthened, regaining the control duration after some 3 min of recovery in 4 mM  $[K]_o$ .



Much larger effects after a 6-min exposure to K-free fluid are shown in Fig. 6; the stimulation rate was 75/min in this experiment. The upper curve shows the recovery of the diastolic potential, and the lower curve that of the action potential duration, after the return to 4 mM  $[K]_o$ . The action potentials shown at the bottom were photographed immediately before sodium loading, then 60, 90, 140, and 350 s after the return to 4 mM  $[K]_o$ , and their durations are plotted in the graph as open circles. At the lower left, the initial control action potential is superimposed on that recorded 60 s after the return to 4 mM  $[K]_o$  to illustrate the marked hyperpolarization and shortening of the action potential at that time; at the lower right, the action potential obtained after 60 s is superimposed on those recorded after 90, 140, and 350 s of recovery to illustrate the gradual return of the resting potential and action potential duration to control values.

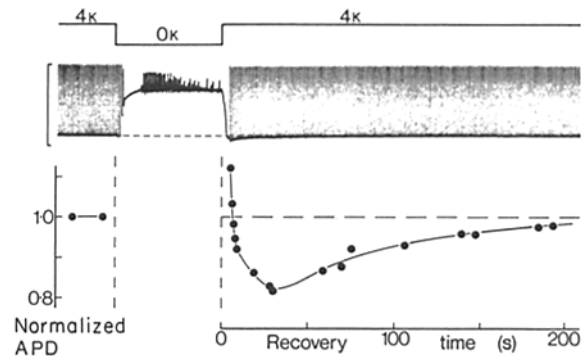


FIGURE 5. Changes in action potential duration (APD) in Cl-containing Tyrode's solution following a 1-min exposure to K-free fluid, indicated by the upper line. The middle pen recording shows the resulting changes in membrane potential. The fiber was stimulated at 60/min except while depolarized in zero  $[K]_o$  (when small oscillations in membrane potential sometimes gave rise to slow response action potentials). The vertical bar next to the pen recording represents 100 mV and its top indicates the zero reference potential; the dashed line shows the control level of the diastolic potential,  $-87$  mV. The filled circles plotted below the pen recording, on the same time scale, give the durations of sample action potentials normalized with respect to the control duration which is indicated by the broken horizontal line.

The maximum shortening of the action potential is not seen until some 20 s after returning the fiber to 4 mM  $[K]_o$  (Figs. 5 & 6), as if processes tending to lengthen the action potential initially interfere with the shortening effect of enhanced pump activity. At least two processes, both of subsidiary interest in the present context, may contribute. In Purkinje fibers, the first action potential on resuming stimulation after a prolonged pause is lengthened, and subsequent action potentials shorten towards the steady-state duration as stimulation is continued (see, e.g., Hoffman and Cranefield, 1960; Miller et al., 1971). Secondly, diffusion of K ions to cells in the interior of the fiber bundle is probably slow enough for the first few action potentials to rise while the K concentration there is still rising. Action potentials in Purkinje cells are known to be prolonged at low external K concentrations and to shorten as  $[K]_o$  is

increased (Weidmann, 1956; Noble, 1965; Vassalle, 1965). Since Purkinje cells are syncytially connected, the time-course of repolarization of action potentials in more superficial cells, where  $[K]_o$  has already equilibrated, would be affected by electrotonic interaction with longer action potentials arising in deeper cells still exposed to a lower  $[K]_o$ . Whatever the causes of those early changes, the action potential is clearly seen to be shortened at a time when the resting potential is increased, and the resting potential and duration of the action

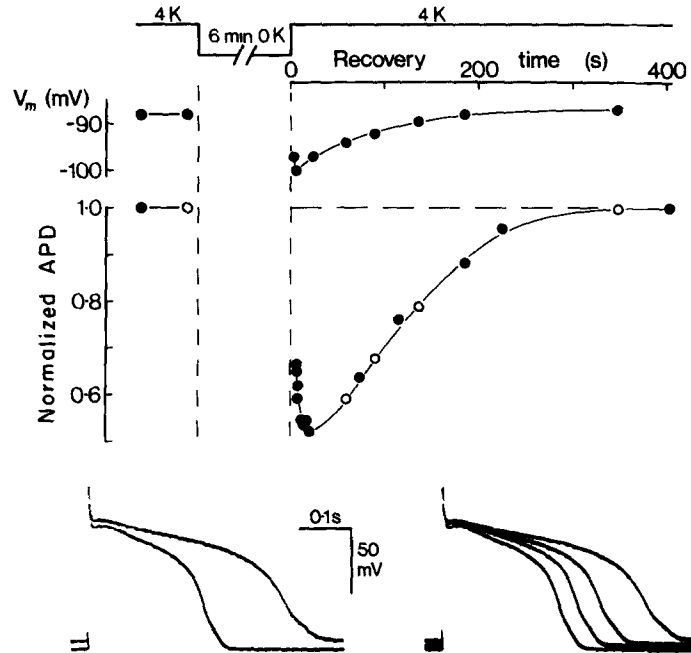


FIGURE 6. Changes in action potential duration (APD) in 4 mM K, Cl-containing, Tyrode's solution during recovery from a 6-min exposure to zero  $[K]_o$  (indicated by the upper line). The fiber was stimulated at 75/min except in K-free fluid. The upper set of points gives values of membrane potential ( $V_m$ ) during diastole at 4 mM  $[K]_o$  before and after exposure to zero  $[K]_o$ ; the lower points give corresponding durations of sample action potentials normalized to the control duration, indicated by the broken horizontal line. The open circles give durations of the representative action potentials illustrated below (see text). The horizontal time calibration for these action potentials also marks the zero reference potential.

potential both return to control levels over a similar period of time. The latter observation suggests that the hyperpolarization and shortening of the action potential have a common origin and thus favors the interpretation that both are caused by a transient increase in outward pump current. A temporary depletion of extracellular K, caused by enhanced pump activity, could explain the increase in resting potential but not the shortening of the action potential.

The results of Figs. 5 and 6 suggest that the shortening of the action potential and the hyperpolarization are both greater after longer periods of exposure to K-free fluid. Fig. 7 summarizes similar results from 11 preparations, each

represented by a different symbol. In Fig. 7 *a*, the ordinate gives the durations of the shortest action potentials (normalized with respect to the control values) recorded in 4 mM  $[K]_o$  after exposures to K-free solution for the period (1–6 min) indicated on the abscissa. The maximum shortening did not occur until 15–25 s after reintroducing 4 mM  $[K]_o$ , possibly as a result of slow equilibration of K in the extracellular spaces (as considered above). Because such an effect might tend to contribute to the hyperpolarization seen in the first few seconds after the return to 4 mM  $[K]_o$ , again by electrotonic interactions between cells in the bundle, the hyperpolarizations plotted in Fig. 7 *b* were measured when the reduction in action potential duration was maximal, i.e., 15–25 s after the return to 4 mM  $[K]_o$ . Fig. 7 demonstrates that both shortening and hyperpolarization on returning to 4 mM  $[K]_o$  are greater after longer exposures to K-free fluid

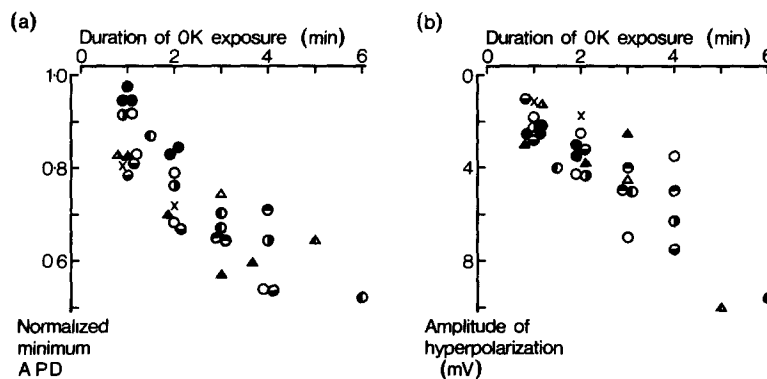


FIGURE 7. The extent of the shortening of the action potential (*a*) and the hyperpolarization of the membrane (*b*) in 4 mM K, Cl-containing Tyrode's solution are increased as the duration of the prior exposure to K-free solution is prolonged. Combined results from 11 fibers, each represented by a different symbol. (*a*) The ordinate gives minimum action potential duration, normalized to the steady control duration recorded before and after full recovery from exposures to K-free fluid of duration indicated on the abscissa. (*b*) Corresponding amplitudes of the transient hyperpolarization, measured when the duration of the action potential was minimal, are plotted.

and provides further support for the hypothesis that the level of  $[Na]_i$  and, hence, the rate of electrogenic sodium extrusion are both higher after longer exposures to zero  $[K]_o$ .

#### *Effects on Automatic Activity*

Vassalle (1970) concluded that the extra sodium influx occurring during periods of "overdrive" (stimulation at rates higher than the intrinsic spontaneous rate) temporarily increases pump activity, and that the temporary quiescence and increase in maximum diastolic potential after such periods (e.g., 1 min at a rate of 120/min) both reflect transiently enhanced electrogenic sodium extrusion: K depletion, secondary to pump stimulation, cannot account for these effects, since a fall in  $[K]_o$  enhances spontaneous activity (Vassalle, 1965). The aftereffects of a brief exposure to K-free fluid on automaticity are shown in Fig.

8 *a*; they are qualitatively similar to the aftereffects of a period of overdrive. This fiber was initially spontaneously active at a rate of about 30/min in 4 mM K, Cl-containing Tyrode's and it depolarized during the 2-min exposure to zero  $[K]_o$  giving rise to spontaneous, "slow response" action potentials. Immediately after the return to 4 mM  $[K]_o$ , the membrane potential increased to a level 6 mV more negative than the initial, steady, maximum diastolic potential, and spontaneous activity was abolished. The membrane potential then slowly declined until spontaneous action potentials reappeared after a 63-s period of quiescence. The rate was initially 6/min but gradually returned to about 30/min after some 4 min of recovery in 4 mM  $[K]_o$ . The hyperpolarization and the

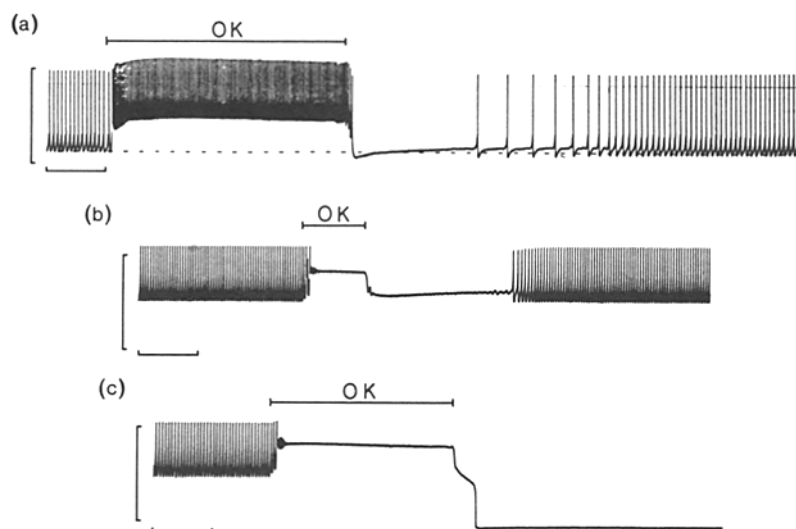


FIGURE 8. Effects of brief exposures to K-free fluid on spontaneous activity arising from either the higher (*a*) or the lower (*b* and *c*) level of membrane potential in Purkinje fibers. The horizontal bars over the records indicate the exposures to K-free solution. The vertical calibration bars represent 100 mV, and their upper ends indicate the zero reference potential; the horizontal calibrations are 30 s. (*a*) Record shows effects on spontaneous activity arising from the high level of potential in 4 mM K, Cl-containing Tyrode's solution. The dashed line indicates the control maximum diastolic potential,  $-87$  mV. (*b*) and (*c*) Records show effects on spontaneous, slow response activity in a different fiber in low Cl solution;  $[K]_o$  was 2 mM in (*b*) and 2.5 mM in (*c*).

period of quiescence were both found to be increased after longer exposures to K-free fluid, as they are after longer periods of overdrive.

Fig. 8 *b* shows that rhythmic, slow response activity arising near the low level of membrane potential (Cranefield, 1975) also may be temporarily abolished after a brief exposure to K-free solution. In this instance reduction of  $[K]_o$  to zero caused the fiber to become quiescent, the membrane potential remaining at  $-18$  mV during the 30-s exposure to K-free fluid. On returning to 2 mM  $[K]_o$ , the membrane potential rapidly increased to  $-43$  mV, and then slowly declined until slow response action potentials arose from small oscillations in

membrane potential after 73 s of quiescence. The spontaneous rate was initially somewhat reduced but returned to the steady control rate within a few seconds; the maximum diastolic potential level remained unaltered.

The effect of a longer period of exposure to K-free fluid (90 s) is shown in Fig. 8 *c*. The current generated by the sodium pump on returning to K-containing solution was transiently large enough to cause a maintained shift of the membrane potential from the lower level to the higher resting level. This maintained shift of potential naturally abolished the rhythmic activity that had been present at the lower level of membrane potential.

#### DISCUSSION

In the present experiments, Purkinje fibers with resting potentials initially at the lower level (ca.  $-30$  to  $-40$  mV) transiently hyperpolarize when they are returned to K-containing solution after brief periods of exposure to K-free fluid (Figs. 1–4). Persuasive evidence that the hyperpolarization is caused by enhanced activity of the sodium-potassium exchange pump is its rapid and reversible abolition by acetylcholinesterase, a specific inhibitor of the sodium pump (Fig. 4). Furthermore, since the pump is known to be stimulated by  $[K]_o$  in a low concentration range (Glitsch et al., 1976, 1978), the increase in amplitude of the hyperpolarization, after a fixed duration of exposure to zero  $[K]_o$ , obtained by increasing  $[K]_o$  over the range 0.25–4 mM (Fig. 3) provides further support for the above hypothesis.

However, three alternative possibilities should be considered. First, an increase in  $[Na]_i$  reduces the electrochemical potential gradient for Na ions, and thereby reduces the “background” inward Na current which is largely responsible for maintaining the lower level of resting potential (Gadsby and Cranefield, 1977 *b*), so that hyperpolarization might result. Second, a rise in  $[Na]_i$  may lead to a rise in intracellular calcium ion concentration,  $[Ca]_i$ , (Glitsch, et al., 1970) which might, in turn, increase the steady-state membrane potassium conductance ( $g_K$ ) (Isenberg, 1977), thereby causing hyperpolarization as long as the membrane potential is less negative than  $E_K$ . Either possibility necessarily presupposes the presence of a K-dependent sodium pump, to account both for the rise in  $[Na]_i$  during the exposure to K-free fluid and for its subsequent fall after the return to 4 mM  $[K]_o$ . Moreover, the rise in  $[Na]_i$  during exposure to zero  $[K]_o$  should not be smaller in the presence of acetylcholinesterase than in its absence, so that the hyperpolarization after exposure to zero  $[K]_o$  should also not be diminished if it were to arise by either of the above mechanisms. However, Fig. 4 *a* shows that the hyperpolarization is completely abolished by acetylcholinesterase, the membrane potential in 4 mM  $[K]_o$  being the same just after the exposure to zero  $[K]_o$  as immediately before it. That hyperpolarization is, therefore, most readily attributed to enhanced pump activity. Finally, a possible contribution made by time- and voltage-dependent conductance changes should be considered because it is known that, in the “plateau” range of voltages, depolarization caused by current injection is followed by a transient hyperpolarization on termination of the current pulse. The depolarization is believed to “turn on” a potassium conductance which is fully activated within seconds in the voltage range of interest and which subsequently decays, also

within seconds, after termination of the depolarization (McAllister and Noble, 1966; Gadsby and Cranefield, 1977 *b*). To explain the present results, however, the time-courses of both "activation" and "inactivation" of that potassium conductance would have to be slowed by at least one order of magnitude because no transient hyperpolarization is seen until after the fiber has been depolarized in K-free fluid for some 15–30 s (Fig. 1 *b*), and the transient hyperpolarization, once elicited, takes minutes, and not seconds, to decay (Fig. 1, 3 and 4). Furthermore, the amplitude of the transient hyperpolarization continues to increase as the period of depolarization in K-free fluid is prolonged up to several minutes (Fig. 2). In addition, the activated K-conductance would have to be acetylcholinesterase-sensitive (Fig. 4). Thus, a time- and voltage-dependent change in conductance would seem to be an unlikely explanation for the transient hyperpolarization observed in the present experiments.

*Extracellular K Depletion or Electrogenic Na Extrusion?*

The observed aftereffects of brief periods of exposure to K-free fluid, namely, transient hyperpolarization from the low level of membrane potential, temporary shortening of the action potential in driven preparations, and temporary quiescence of spontaneously active preparations, can all be accounted for by a temporary increase in the rate of electrogenic Na extrusion but not by a transient depletion of K ions just outside the cell membrane.

Reducing  $[K]_o$  at the lower level of resting potential causes depolarization, not hyperpolarization. This can be seen in Figs. 1 and 2 in which the first reduction in  $[K]_o$  from 4 mM to zero, caused an immediate and monotonic decline in membrane potential without even a small initial transient hyperpolarization. Since the K concentration at the surface of all cells in the bundles is unlikely to have fallen instantaneously to zero, it follows that the low resting potential at any  $[K]_o$  lower than 4 mM is less negative than that at 4 mM. Nor can it be argued that this relationship is changed by brief periods of sodium-loading at zero  $[K]_o$ , since even after some 2 min of sodium-loading, the immediate response to a sudden reduction of  $[K]_o$  is still a decline in membrane potential (Fig. 2). The transient hyperpolarization from the low resting potential seen on returning to 4 mM  $[K]_o$  must therefore be attributed to enhanced electrogenic sodium extrusion. Near the *high* level of resting potential, on the other hand, the switch from 4 mM  $[K]_o$  to zero  $[K]_o$  does give rise to a brief, transient increase in membrane potential which precedes the usual depolarization (see Fig. 2 *b*), but this is consistent with the hyperpolarization normally seen in fibers at the high level of resting potential in response to a moderate reduction in  $[K]_o$  (see Fig. 1 of Gadsby and Cranefield, 1977 *b*).

Two alternative explanations, other than enhanced pump activity, for the shortening of the action potential after brief exposure to K-free solution, are that the resulting increase in  $[Na]_i$  might lead to a rise in  $[Ca]_i$  and thus in  $g_K$  or that the rise in  $[Na]_i$  lowers inward Na current. When  $[Na]_i$  is raised, the inward driving force on Na ions is diminished, thus reducing inward Na current. A sufficiently large reduction in Na current would lower the rate of rise and amplitude of the upstroke of the action potential which might, in turn, cause a reduction in action potential duration. However, the shortening of the action

potential after return to 4 mM  $[K]_o$  after as long as 4 min of exposure to K-free fluid is, in fact, often accompanied by a small, temporary increase in the maximum rate of rise, which probably results from the concomitant, transient increase in resting potential.<sup>2</sup> An increase in  $g_K$  mediated by a rise in  $[Ca]_i$  is more difficult to rule out but is not consistent with the marked prolongation of action potentials observed on application of cardiac steroids (Isenberg and Trautwein, 1974). That prolongation presumably reflects abolition of a steady-state component of outward pump current and lends support to the present finding that small changes in pump activity can markedly affect the duration of the action potential in cardiac Purkinje fibers. That finding might also bear on the changes in action potential duration known to follow alterations in stimulation rate or in  $[K]_o$ . Thus, a maintained shortening of the action potential follows an increase in drive rate and a prolongation follows a reduction in rate (Hoffman and Cranfield, 1960; Miller et al., 1971; Carmeliet, 1977) and these changes are qualitatively consistent with the expected changes in  $[Na]_i$  and hence pump rate. The maintained shortening of the action potential seen on raising  $[K]_o$  and the prolongation seen on lowering  $[K]_o$  (e.g., Weidmann, 1956; Noble, 1965; Vassalle, 1965; Ito and Surawicz, 1977) might also be partly attributable to changes in pump current, since the pump rate is known to increase with  $[K]_o$  over a limited concentration range (e.g., Glitsch et al., 1976, 1978).

We would emphasize that our results do not demonstrate the absence of extracellular K depletion during enhanced pump activity, but instead demonstrate that such depletion cannot account for the effects observed in the present experiments. In fact, there are good reasons for expecting some K depletion to occur, particularly when pump activity is enhanced sufficiently to cause the membrane to hyperpolarize beyond the high level of resting potential (see, e.g., Maughan, 1973).

#### *Steady-State Pump Current*

The rapid depolarization caused by application of  $2 \times 10^{-6}$  M acetylcholine (Fig. 4) strongly suggests that sodium pump activity directly affects the lower level of resting potential in quiescent fibers. Two other changes expected to follow inhibition of the sodium pump, namely, a rise in  $[Na]_i$  and an accumulation of K just outside the membrane secondary to net K efflux, cannot explain this acetylcholine-induced depolarization. A rise in  $[Na]_i$  should reduce the inward Na current and thus tend to hyperpolarize; a rise in  $[K]_o$  is known to cause hyperpolarization of fibers at the low resting potential (Gadsby and Cranfield, 1977 *b*). The efflux of potassium will also cause  $[K]_i$  to fall, but it seems unlikely that a fall in  $[K]_i$  could cause the observed depolarization. Even if the membrane were to behave like a K electrode at the lower resting potential (which it does not), a depolarization of some 9 mV in 1 min would require the cells to lose almost one-third of their total K content in that time. Indeed, it seems possible that a fall in  $[K]_i$  might even cause hyperpolarization just as an increase in  $[K]_o$  is known to do.

<sup>2</sup> Gadsby, D. C., and P. F. Cranfield. Unpublished results.

The average size of the rapid depolarization caused by acetylstrophanthidin was 9 mV. A transient hyperpolarization of similar size is induced following exposure to K-free fluid for ~ 1 min (Fig. 1-4). Assuming the membrane slope conductance to be approximately linear for  $\pm 10$  mV displacements from the low resting potential, and the Na:K coupling ratio of the pump to be unchanged during the stimulation and inhibition of the pump described above, these observations suggest that the amplitude of the steady pump current abolished by acetylstrophanthidin is about equal to the peak increase in pump current resulting from a 1-min period of Na-loading at zero  $[K]_o$ . In other words, the resting rate of electrogenic Na extrusion appears to be approximately doubled after a 1-min exposure to K-free fluid. If the rate of Na extrusion is proportional to  $[Na]_i$  over the concentration range of interest (see, e.g., Thomas, 1972), the present findings would seem to indicate that the intracellular Na concentration "seen" by the sodium pump in canine Purkinje cells may be doubled during a 1-min exposure to K-free fluid. If the resting level of  $[Na]_i$  in canine Purkinje cells is similar to that in sheep Purkinje cells, i.e., ~7 mM (Ellis, 1977), then this result might be taken to imply that  $[Na]_i$  can increase at a rate of about 7 mM/min in K-free fluid. Ellis (1977) reported that, in sheep Purkinje cells,  $[Na]_i$ , measured with Na-sensitive microelectrodes, increases at a rate of ~0.5 mM/min when Na extrusion is abolished by a maximal concentration of cardiac steroid, and Glitsch et al. (1976) found that, in guinea pig atria exposed to cardiac steroids,  $[Na]_i$  rose at a rate of 1.7 mM/min. If the surface-to-volume ratio of the canine Purkinje cells used in the present experiments were to be greater than that of guinea pig atrial cells and sheep Purkinje cells, that might account for some of the apparent discrepancy between the present findings and those of Glitsch et al. (1976) and Ellis (1977). However, an intriguing alternative possibility is that the intracellular Na concentration seen by the pump, presumably the Na concentration at the inside of the cell membrane, is not equivalent to the bulk  $[Na]_i$  determined with Na-sensitive microelectrodes or by chemical analysis (cf. Mullins, 1963). If so, this would clearly have important electrophysiological implications, because fluctuations in net membrane Na fluxes might then have larger and more rapid effects on activity of the sodium pump than would be predicted by measured changes in bulk  $[Na]_i$ .

#### *Possible Antiarrhythmic Effects of Electrogenic Sodium Extrusion*

Among the possible causes of cardiac arrhythmias are reentry secondary to circus movement of excitation, spontaneous activity at the low level of resting potential, and "triggered" rhythmic activity sustained by afterdepolarizations (Cranefield, 1975, 1977). The slow conduction upon which reentry depends seems to be possible only at low levels of membrane potential and could, therefore, be prevented by a sufficient increase in membrane potential. Spontaneous slow-response action potentials at low levels of membrane potential are naturally abolished by membrane potential shifts to the high resting level (present study, Fig. 8 c; see also Gadsby et al., 1978, Fig. 5 c). The delayed afterdepolarizations that sustain rhythmic activity in triggered arrhythmias depend on small inward currents and so might be suppressed by a sufficient increase in outward membrane current. An increase in outward pump current



resulting from enhanced electrogenic sodium extrusion could cause hyperpolarization, reduction in the rate of pacemaker depolarization at any potential level, and reduction in the amplitude of delayed afterdepolarizations, and could thus be antiarrhythmic.

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