The Electrical Activity of Canine Cardiac Purkinje Fibers in Sodium-Free, Calcium-Rich Solutions

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ABSTRACT Propagated action potentials can be obtained in canine cardiac Purkinje fibers exposed to Na-free solutions containing no inorganic cation other than Ca and K. Essentially similar action potentials are obtained if Na is replaced by tetraethylammonium (TEA), tetramethylammonium (TMA), or choline. In a solution containing 128 mM TEA and 16.2 mM Ca the characteristics of these electrical responses were: maximum diastolic potential, -59 ± 3.3 mV; overshoot, 20 ± 6.8 mV; maximum upstroke velocity, 3.7 ± 2.3 V/s; conduction velocity, 0.1 m/s; and action potential duration, 360 ± 45 ms. The magnitude of the overshoot varied with log Ca_o with a slope of about 30 mV/10-fold concentration change. The upstroke velocity was an approximately linear function of Ca_o. The active response was greatly diminished or abolished by Mn and D-600 but was unaffected by tetrodotoxin. These Ca-dependent responses appeared in a region of transmembrane potential (about -50 mV) at which the rapid Na-dependent upstroke is abolished even when Na is present.

INTRODUCTION

The rapid upstroke of the transmembrane action potential of cardiac Purkinje fibers is generally believed to result in large part from a selective increase in Na conductance. The voltage-dependent characteristics of this Na conductance are similar to those of nerve and skeletal muscle, but cardiac cells show a slow component of inward current not seen in nerve or skeletal muscle. At values of transmembrane potential at which the rapid Na conductance is thought to be completely inactivated, a slow inward current has been observed in cardiac Purkinje fibers and in ventricular and atrial muscle. The slow inward current has been ascribed to Ca and to a combination of Na and Ca. The literature pertaining to these findings has been reviewed recently (see Rougier et al., 1969; Tarr, 1971; Cranefield, Wit, and Hoffman, 1972; De Mello, 1972; Shigenobu and Speralakis, 1972; Reuter, 1973).

Various workers have found propagated action potentials in cardiac fibers depolarized sufficiently to inactivate the rapid Na-dependent upstroke or in

cardiac fibers treated with tetrodotoxin (Engstfeld, Antoni, and Fleckenstein, 1961; Carmeliet and Vereecke, 1969; Pappano, 1970; Mascher, 1970; Cranefield et al., 1972; Shigenobu and Speralakis, 1972). These slow electrical responses are unaffected by tetrodotoxin and are blocked by Mn. Vereecke and Carmeliet (1971 *a*) have shown that cardiac Purkinje fibers can develop propagated action potentials when exposed to solutions containing no inorganic cation other than K, 10 mM Sr, and 0.5 mM Mg. There has, however, been no systematic study of propagated electrical activity in cardiac fibers exposed to no inorganic cation other than Ca and the present article reports the characteristics of such activity.

METHODS

Mongrel dogs were anesthestized with 30 mg/kg of sodium pentobarbital given intravenously. The heart was excised and immersed in a sodium-free solution (see below). Bundles of Purkinje fibers (false tendons) were removed from the right and left ventricles and placed in a beaker containing a sodium-free solution at room temperature (25°–27°C). Bundles to be studied were placed in a tissue bath perfused with solution maintained at 36°–38°C.

Electrical recording and current injection were achieved by means of glass microelectrodes filled with 3 M KCl. Intracellular current was applied by means of a high voltage field effect transistor circuit and current was measured by an operational amplifier which maintained the bath at virtual ground. The timing of the pulses was achieved by means of a digital parallel timing system (Silverman and Eisenberg, 1971). External stimuli were delivered to the tissue through unipolar sintered Ag/AgCl electrodes (Annex Research, Santa Ana, Calif.) or through bipolar teflon coated silver wires.

The following chemicals and drugs were used: NaCl, Merck reagent grade (Merck Chemical Div., Merck & Co., Inc., Rahway, N. J.); KCl, Mallinckrodt analytical reagent; anhydrous $CaCl_2$, Mallinckrodt analytical reagent; $MgCl_2 \cdot 6H_2O$, Mallinckrodt analytical reagent (Mallinckrodt Chemical Works, St. Louis, Mo.); anhydrous dextrose, Baker reagent (J. T. Baker Chemical Co., Phillipsburg, N. J.); Tris as Trizma base, Sigma reagent grade; anhydrous choline chloride, crystalline, Sigma (Sigma Chemical Co., St. Louis, Mo.); tetramethylammonium (TMA) chloride, 99%, Matheson, Coleman, and Bell; tetraethylammonium (TEA) chloride, Eastman (Eastman Organic Chemicals Div., Eastman Kodak Co., Rochester, N. Y.); tetraethylammonium chloride, monohydrate, 99%, Matheson, Coleman and Bell (East Rutherford, N. J.); D-600 hydrochloride, Knoll A. G., Ludwigshafen-Rhein; atropine sulphate U.S.P., Amend; HCl, 37%, Mallinckrodt analytical reagent; $MnCl_2 \cdot 4H_2O$, Baker reagent; tetrodotoxin, Sigma.

Table I gives the composition in millimoles per liter of the solutions used in these experiments. Glass condensed redistilled water was used. Tyrode solution buffered with bicarbonate was bubbled with 95% O_2 and 5% CO_2. All other solutions were buffered with 5 mM Tris brought to pH 7.2–7.6 with HCl. These solutions were bubbled with 100% O_2. Atropine (1.5×10^{-5} M) was added. Tetrodotoxin was dis-

TABLE I

COMPOSITIONS OF SOLUTIONS IN MILLIMOLES PER LITER

Na	Choline	TEA	TMA	Ca	K	Mg	Cl	HPO_4/HCO_3	Glucose	Tris	Total milli-osmoles	Solution no.
150.8	—	—	—	2.7	2.7	0.5	147.4	1.8/12	5.5	—	323.4	1
—	—	146.3	—	4.0	2.7	0.5	158.0	—	5.5	5	322.0	2
—	—	140.1	—	8.1	2.7	0.5	160.0	—	5.5	5	322.0	3
—	—	128	—	16.2	2.7	0.5	164.1	—	5.5	5	322.0	4
—	—	103.7	—	32.4	2.7	0.5	172.2	—	5.5	5	322.0	5
—	128	—	—	16.2	2.7	0.5	164.1	—	5.5	5	322.0	6
—	—	—	128	16.2	2.7	0.5	164.1	—	5.5	5	322.0	7

solved by adding 2 cm^3 of distilled water to the vial; the resulting solution was immediately added to the perfusate. Because of the cholinergic activity of choline and of TEA, atropine was usually added, but in some experiments solution 4 was used without atropine without significant change in the findings.

Some fibers were assayed for Na at the completion of an experiment; the fiber was blotted for 5 s on filter paper (Whatman No. 42) and a wet weight was then obtained. The fiber was dried for several hours under a heating lamp after which a dry weight was obtained. The dried fiber was placed in 2 ml of 0.1 N HNO_3 for 12–48 h. The Na content was determined by means of a quartz spectrophotometer (Beckman Instruments, Inc., Fullerton, Calif.).

The total Na content of eight bundles bathed for 4–6 h with solution 4 was 3.76 ± 1.24 mmoles/kg wet weight. Six of the eight fibers were used in experiments in which typical electrical responses were recorded. The remaining two preparations were immersed in TEA-Ca (solution 4) for a period of 4–6 h, but were not impaled with recording electrodes. The extremely low value for total Na indicates that a large amount of intracellular Na as well as extracellular Na is eliminated by immersion in Na-free solution. Solution 4 (128 mM TEA, 16.2 mM Ca) contained only 0.36 mM Na. These results indicate that the concentration of Na present cannot account for the electrical responses described below.

In the text that follows, concentrations in millimoles/liter are designated simply by the chemical symbol and a subscript to indicate whether intracellular or extracellular concentration is meant. For example, Ca_o is used instead of $[Ca^{++}]_o$.

RESULTS

Propagated action potentials were obtained in fibers exposed to Na-free, Ca-rich solutions in which the NaCl was replaced by choline chloride, by tetramethylammonium (TMA) chloride, or by tetraethylammonium (TEA) chloride. Propagated activity was obtained most readily and reproducibly when Na was replaced by TEA and we will describe the characteristics of such activity in some detail and then briefly will describe the results obtained by substituting choline or TMA for Na.

Electrical Activity in TEA and High Ca

SPONTANEOUS ACTIVITY AND DIASTOLIC POTENTIAL Purkinje fibers perfused with 16.2 mM Ca and 128 mM TEA (solution 4) generally beat spontaneously at a mean rate of 30/min with a range of 17–57/min (20 measurements). Fig. 1 is a record of two typical action potentials showing spontaneous diastolic depolarization. The electrical activity was usually accompanied by visible contractions. The maximum diastolic potential was -59 ± 3.3 mV (20 determinations in eight fibers) with a range of -53 to -65 mV; this comparatively low maximum diastolic potential is discussed separately below.

OVERSHOOT In the typical action potentials shown in Fig. 1, recorded from a fiber in TEA and 16.2 mM Ca, the overshoot is 28 mV, the membrane potential at which the upstroke is initiated is -44 mV, and the maximum diastolic potential is -58 mV. The mean overshoot in 20 determinations in eight fibers was 20 ± 6.8 mV with a range of 14–34 mV. The overshoot is less than that recorded from canine Purkinje fibers in normal Tyrode solution (31 ± 5 mV; Weidmann, 1956), but is comparable to that recorded for Sr action potentials (18 mV at 10 mM Sr; Veerecke and Carmeliet, 1971 *a*).

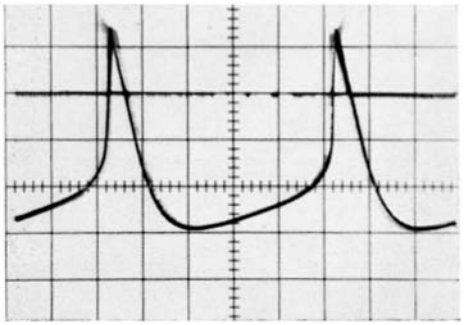

FIGURE 1. Two spontaneous action potentials recorded from a fiber in solution 4 (128 mM TEA, 16.2 mM Ca). Calibration grid: vertical, 20 mV; horizontal, 200 ms. The horizontal line indicates zero transmembrane potential.

Fig. 2 shows the lack of a systematic predictable relationship between the magnitude of the overshoot and the level of membrane potential at which the upstroke is initiated. This behavior is in contrast to that observed in sheep and calf Purkinje fibers in normal Tyrode solution in which the overshoot decreases from an average maximum of 33 mV at -90 mV to zero at about -60 mV (Weidmann, 1956). The mean membrane potential at

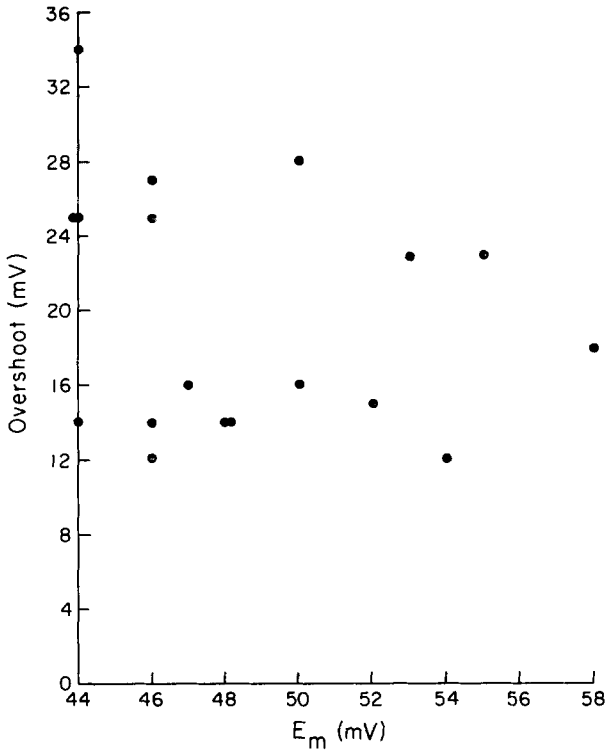

FIGURE 2. Magnitude of action potential overshoot as a function of transmembrane potential at which upstroke was initiated. Ordinate: overshoot in millivolts; abscissa transmembrane potential in millivolts.

which the upstroke was initiated in 128 mM TEA and 16.2 mM Ca was 48 ± 4.0 mV (20 determinations; range of 44–58 mV). The presence of an overshoot at such transmembrane potentials shows that the current-carrying mechanism has a voltage dependency that differs from that of the rapid Na-dependent upstroke.

MAXIMUM RATE OF RISE Fig. 3 is a record of the upstroke of an action potential obtained from a fiber in 16.2 mM Ca and 128 mM TEA (solution 4). The upstroke has a sigmoid configuration and a slow rate of rise. In 20 determinations the mean maximum upstroke velocity was 3.7 ± 2.3 V/s with a range of 1.0–10.0 V/s. This rate is much lower than the maximum upstroke velocity of canine Purkinje fibers in normal Tyrode solution, which often exceeds 500 V/s, but it is comparable to that recorded in TEA-treated crayfish muscle (6.5–28 V/s; Fatt and Ginsborg, 1958) and crab muscle bathed in TEA Ringer solution (36 V/s; Fatt and Katz, 1953). The upstroke velocity is similar to that of the Ca-dependent component of the upstroke of frog heart strips as recorded by Niedergerke and Orkand (1966), namely

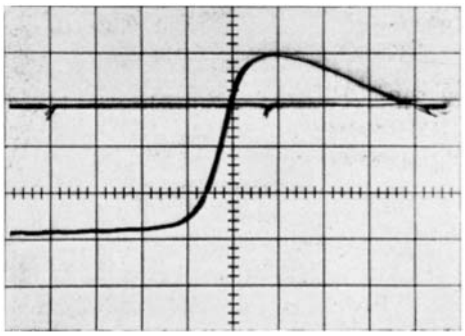

FIGURE 3. Record of action potential upstroke taken at a rapid sweep. Calibration grid: vertical, 20 mV; horizontal, 20 ms.

5.5 V/s at 0.2 mM Ca and 8.5 V/s at 3 mM Ca and to that of Sr action potentials in sheep cardiac Purkinje fibers (5–7 V/s; Vereecke and Carmeliet, 1971 *a*).

THRESHOLD POTENTIAL Fig. 4 shows the initiation of an electrical response by an intracellular stimulus. Application of a current pulse of 50 ms duration initiated a regenerative response 50% of the time (Fig. 4 A). Increasing the duration of the pulse to 70 ms initiated an active response 100% of the time. In this fiber the threshold potential was about −30 mV. When the stimulus was 60 ms long (Fig. 4 B) the latency between stimulus and response varied markedly and the stimuli with the longest utilization time produced responses preceded by what amount to local responses or long steps of depolarization. The first five action potentials are virtually identical in amplitude, overshoot, and upstroke velocity although the "step" of depolarization preceding the action potential varies from about 60 to about 160 ms. The sixth and seventh responses are somewhat reduced in amplitude and upstroke velocity, but arise at a time when the "step" is declining towards the resting potential. The upstroke velocity and amplitude of the action potential thus appear to be uninfluenced by the duration of the prior "step" of depolarization.

CONDUCTION VELOCITY Fig. 5 is a record, taken at a rapid sweep speed, of the upstrokes of two spontaneous action potentials recorded from electrodes 4 mm apart. The conduction time was 40 ms giving an apparent conduction velocity of 0.1 m/s. This conduction velocity is comparable to that recorded for Sr action potentials (0.15 m/s; Veerecke and Carmeliet, 1971 *a*), but is much lower than the values of 1–3 m/s seen in canine cardiac Purkinje fibers *in situ* or in normal Tyrode solution (Hoffman and Cranefield, 1960). The slow conduction velocity is probably related to the slow rate of rise of the action potential upstrokes, but alterations in other determinants of conduction velocity (e.g. core-conductor properties) are also possible.

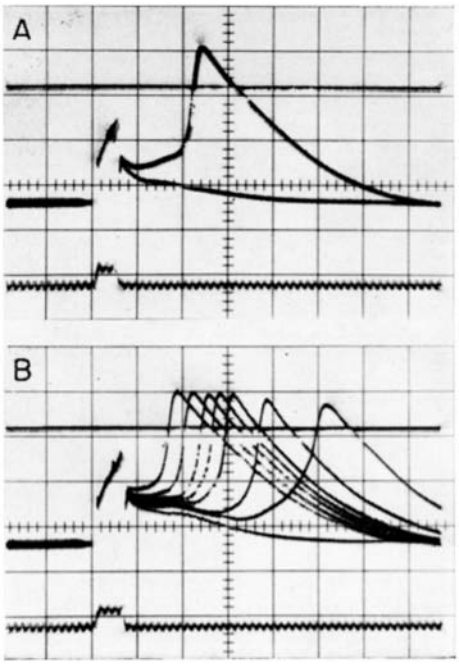

FIGURE 4. Initiation of action potential by consecutive intracellular current pulses (3.8×10^{-7} A). The lower trace in (A) and (B) shows the stimulus. (A), Pulse duration, 50 ms, initiating active response 50% of the time. (B), Pulse duration, 60 ms. Note that amplitude of overshoot and upstroke velocity for the first five responses is identical and independent of the preceding constant depolarization "step." The last two active responses show a decrease in overshoot amplitude and upstroke velocity; no active response occurs after the last depolarization. Calibration grid: vertical, 20 mV; horizontal 100 ms.

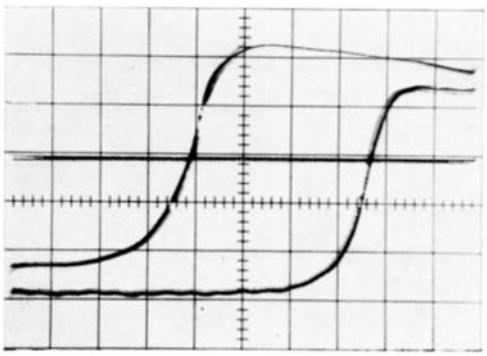

FIGURE 5. Records taken from two electrodes separated by 4 mm. Apparent conduction velocity $\simeq$0.1 m/s. Calibration grid: vertical, 20 mV; horizontal, 10 ms.

ACTION POTENTIAL DURATION Figs. 6 A and B show the configuration of two action potentials of different duration. The duration was measured as the time taken for repolarization to return the membrane to the level of potential at which the upstroke was initiated. The duration of the action

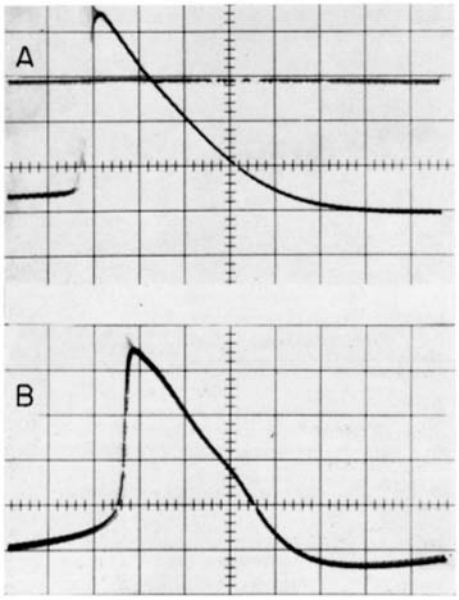

FIGURE 6. Two action potentials of different configurations. (A), "Concave" repolarization phase; (B), "Convex" repolarization phase. Calibration grid: vertical, 20 mV; horizontal, 100 ms.

potential in Fig. 6 A is 430 ms and the repolarization phase has a concave configuration. The action potential duration in Fig. 6 B is 310 ms and the repolarization phase has a more convex configuration. The mean action potential duration in 20 determinations was 360 ± 45 ms. This may be compared to the value of 300–500 ms in canine Purkinje fibers in normal Tyrode solution (Hoffman and Cranefield, 1960). The configuration of the repolarization phase of action potentials recorded in TEA and high Ca was more "triangular" than that recorded in normal Tyrode solution. There was generally no plateau phase but occasionally long plateaus occurred (see Fig. 13, below).

EFFECT OF VARYING CA_o Fig. 7 shows the effect of Ca_o on the electrical response. The records in Figs. 7 A, B, C, and D were taken at Ca_o levels of 32.4 mM, 16.2 mM, 8.1 mM, and 4.0 mM (solutions 5, 4, 3, and 2), respectively. At each concentration a steady state was reached within 30 min. There was a decrease in both overshoot and maximum rate of rise as Ca_o was decreased from 32.4 to 4.0 mM. There was also a decrease in spontaneous rate so that in the lowest concentration external stimulation was necessary.

Fig. 8 is a plot of overshoot as a function of the logarithm of Ca_o. The points can be fitted by a line with a slope of approximately 30 mV for a 10-fold change in concentration, with some deviation at both high and low extremes of concentration. The relatively close agreement between the

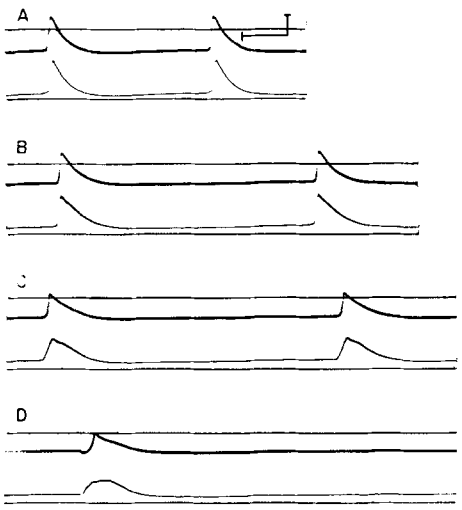

FIGURE 7. Effect of varying Ca_o on action potential. (A), Ca_o = 32.4 mM; (B), Ca_o = 16.2 mM; (C), Ca_o = 8.1 mM; (D), Ca_o = 4.0 mM. Note decrease in magnitude of overshoot and upstroke velocity as Ca_o is decreased; the spontaneous rate decreases as Ca_o is decreased so that external stimulation was necessary in (D). Calibration: vertical bar, 50 mV; horizontal bar, 500 ms.

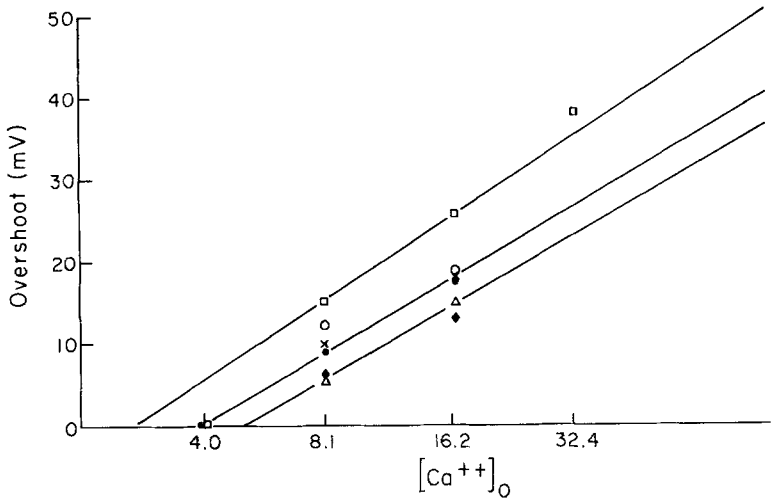

FIGURE 8. Magnitude of overshoot in millivolts as a function of Ca_o. Ca_o is plotted on a logarithmic scale. Each symbol represents a different cell, six cells in four different fibers being represented. The straight lines were fitted by eye; the upper line has a slope of 33 mV/decade; the two lower lines have a slope of 29 mV/decade.

experimental points and the theoretical slope for a Ca electrode (30.5 mV/decade at 37°C) is consistent with the view that Ca conductance is large during the peak of the overshoot. There are so many reasons why the theoretical slope might not be obtained that our observation is somewhat unexpected.

It implies, among other things, that Ca_i remains fairly constant even when Ca_o is varied.

Fig. 9 shows the relative rate of rise of the action potential as a function of Ca_o. The rate of rise and the Ca concentration (16.2 mM) of solution 4 are taken as 1.0. The solutions applied were numbers 5 (2×), 3 (0.5×), and 2 (0.25×). The rate of rise is proportional to Ca concentration as it

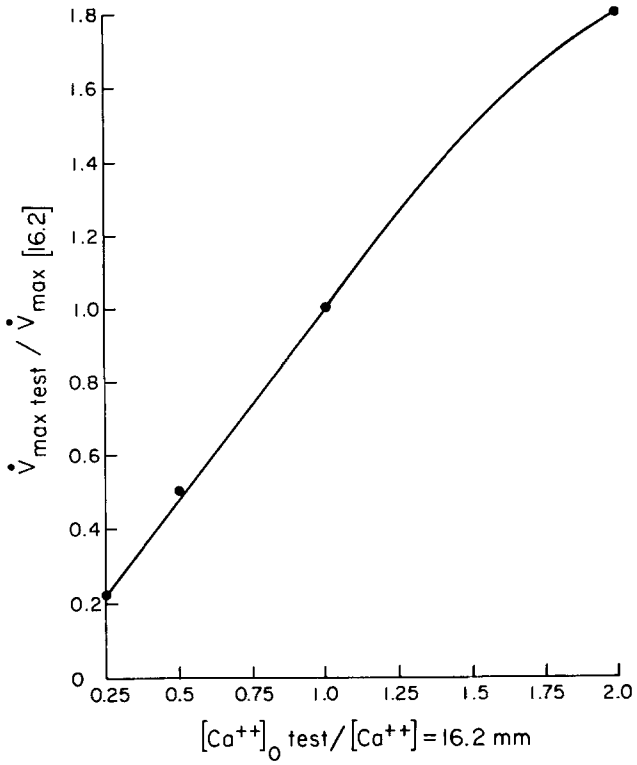

FIGURE 9. Relative maximum upstroke velocity ($\dot{V}_{max}$) as a function of Ca_o, maximum upstroke velocity at Ca_o = 16.2 mM being taken as 1.0. Note that the maximum upstroke velocity is an approximately linear function of Ca_o.

varies from 4 to 16.2 mM. At the highest concentration (32 mM) the rate of rise falls below the line, suggesting a saturation similar to that found for Sr potentials (Vereecke and Carmeliet, 1971 *a*, *b*). It has been found (Beeler and Reuter, 1970) that high Ca_o increases outward current, an effect that could explain the "saturation." The proportionality between Ca_o and rate of rise of the upstroke is further evidence that Ca carries the ionic current responsible for the upstroke.

EFFECT OF BLOCKING AGENTS Among the widely accepted criteria for regarding electrical responses as being Ca-dependent are their being blocked by Mn and being relatively resistant to tetrodotoxin. Fig. 10 shows the effect

of 5 mM Mn on a fiber in solution 7 (128 mM TMA and 16.2 mM Ca) + 10 mM TEA. The spontaneous electrical response was rapidly and progressively diminished. The lowest record shows recovery of electrical activity after Mn had been discontinued for about 20 min. Similar effects were obtained on action potentials of fibers exposed to 128 mM TEA and 16.2 mM Ca.

The effect of D-600, a reputedly specific Ca blocking agent (Kohlhardt et al., 1972) is shown in Fig. 11. The fiber was bathed in 16.2 mM Ca and 128 mM TEA (solution 4) and was driven by external stimuli. The drug caused a progressive decrease in overshoot and rate of rise and the preparation became inexcitable within 20 min. Partial recovery occurred about 20 min after D-600 was discontinued (Fig. 11 F).

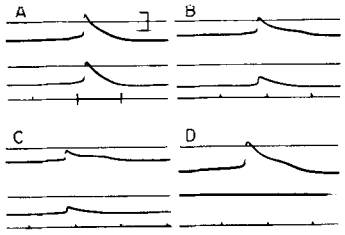

FIGURE 10. Effect of 5 mM Mn on the action potential of a fiber in solution 7 to which 10 mM TEA was added. (A), Control; (B), after exposure to Mn for 3 min; (C), after exposure to Mn for 5 min; (D), 20 min after discontinuing Mn; lower tracing recorded from an electrode which had become dislodged. Calibrations: vertical bar, 50 mV; horizontal bar, 500 ms.

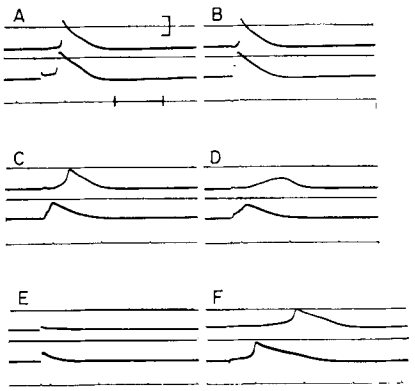

FIGURE 11. Effect of D-600 (1 mg/liter) on the action potentials of fibers in solution 4 (128 mM TEA, 16.2 mM Ca_o). (A), Control; (B), after 7 min exposure to D-600; (C), after 10 min exposure to D-600; (D), after 15 min exposure to D-600; (E), after 20 min exposure to D-600; (F), 20 min after discontinuing D-600. Calibration: vertical, 50 mV; horizontal, 500 ms.

Fig. 12 shows the persistence of electrical activity in a preparation exposed to tetrodotoxin. There is little, if any, difference in the resting potential, amplitude, or maximum upstroke velocity among the three action potentials shown, but the response in Fig. 12 B was obtained after 40 min exposure to 10^{-6} g/ml of tetrodotoxin and that in Fig. 12 C after 20 min exposure to 10^{-5} g/ml of tetrodotoxin. Records of the upstroke taken at a fast sweep speed confirmed the fact that there was no decline in upstroke velocity.

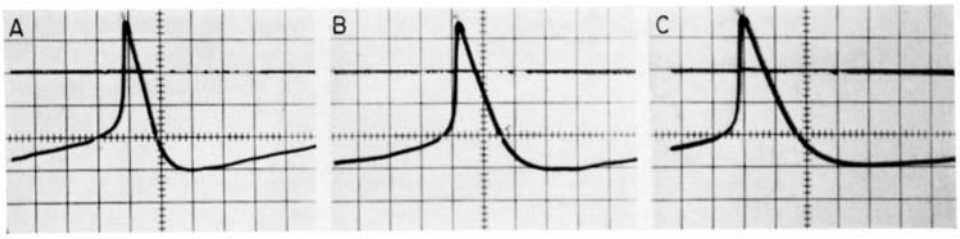

FIGURE 12. Effect of tetrodotoxin on action potential. Records taken from a fiber in solution 4. (A), Control; (B), 40 min after exposure to 10^{-6} g/ml tetetrodotoxin; (C), 20 min after exposure to 10^{-5} g/ml tetrodotoxin. Calibration grid: vertical, 20 mV; horizontal, 100 ms.

ALL-OR-NONE REPOLARIZATION Fibers stored for 3–4 h in 128 mM TEA and 16.2 mM Ca occasionally produced action potentials with plateaus of long duration. An example of an electrical response of this type is shown in Fig. 13 A–F. By applying anodal current pulses during the plateau, active repolarization could be induced (B-F). In general the anodal pulse had to be fairly long (over 50 ms) to evoke all-or-none repolarization. Reexcitation instead of sustained premature repolarization is seen in (G) and (H).

RESTING POTENTIAL The resting potential of fibers exposed to 128 mM TEA is virtually independent of K_o in the range 2–20 mM. Fig. 14 shows the effect of increasing K_o on the resting membrane potential of a quiescent but excitable fiber. The ordinate is resting potential in millivolts and the abscissa is K_o on a logarithmic scale. Solid curve 1 represents experimentally determined values of resting potential at various K concentrations. The interrupted portion of curve 1 is an extrapolation of the linear portion of the experimental curve. Curve 2 represents the theoretical curve calculated from the Nernst relation, $E_K = 61.5$ mV log (K_o/K_i), with the value for K_i assumed to be 173 mM. The slope of the linear portion of the experimental curve is 46 mV for a 10-fold change in K_o, indicating a reasonably high P_K. The marked deviation from linearity at $K_o < 20$ mM strongly suggests a marked reduction in P_K in that range of K_o. A comparable decrease in resting

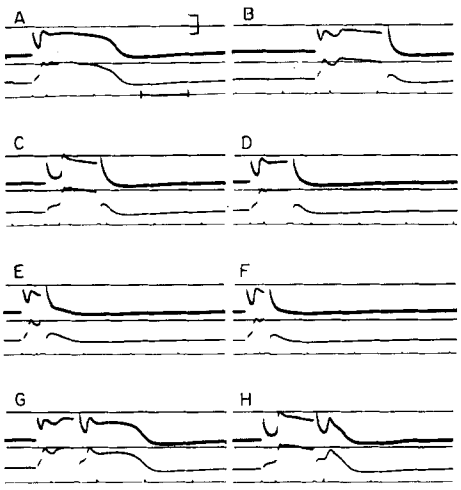

FIG. 13. All-or-nothing repolarization in fibers exposed to solution 4 (128 mM TEA, 16.2 mM Ca). Both the action potential and repolarization were induced by externally applied anodal stimuli. It should be noted that the initial driving stimulus is followed by a marked artifact and a period of variable latency before the appearance of the presumably propagated upstroke. (A) shows a control action potential. Its duration of about 1 s and its definite plateau may be contrasted with the more usual action potentials shown in Fig. 1 and elsewhere. (B–F), active repolarization induced by anodal pulses applied earlier and earlier in the course of the action potential. (E) and (F) are of special interest because it is not easy to "turn off" an action potential in normal Tyrode solution that early in its course. (G) and (H) show reexcitation rather than sustained repolarization. Calibrations: vertical, 50 mV; horizontal, 500 ms.

potential is seen in normal fibers exposed to Tyrode solution when K_o is less than 2.5 mM (Weidmann, 1956; Noble, 1965).

The contribution of Cl to the resting conductance of cardiac Purkinje fibers at normal values of resting potential is relatively small (Carmeliet, 1961; Deck and Trautwein, 1964), but becomes more important at lower levels of membrane potential. Assuming that P_K is decreased to such an extent that the resting potential is largely determined by Cl, the change in membrane potential should approximate $E_m = 61.5$ mV log (Cl_o/Cl_i). At a resting potential of -60 mV and $Cl_o = 160$ mM, $Cl_i = 16$ mM. Using the latter value for Cl_i, the Cl_o necessary to hyperpolarize the membrane by 20 mV is about 320 mM. A quiescent but excitable fiber with a resting potential of -50 mV in solution 4 was exposed to a hyperosmolar solution containing approximately 300 mM TEA chloride. Application of the high Cl solution for 20 min did not cause any change in resting potential. This result suggests that Cl does not primarily determine the resting potential.

Loss of resting potential could result from the presence of impurities, e.g. Ag or other heavy metals, in the TEA. This was considered by Fatt and Katz (1953) as a possible explanation for the depolarization they observed in crab

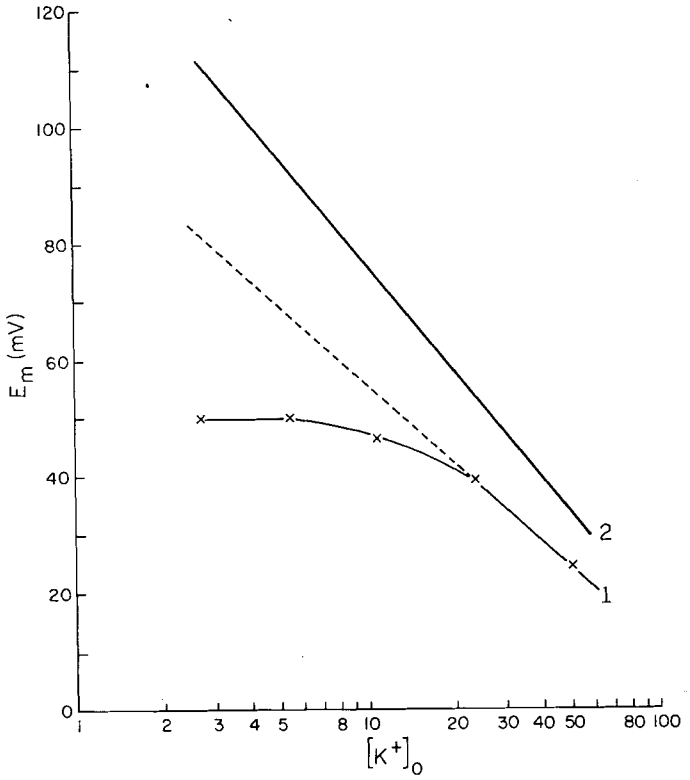

FIGURE 14. Resting potential as a function of K_o in a quiescent fiber exposed to 128 mM TEA and 16.2 mM Ca. Ordinate, resting potential in millivolts; abscissa, K_o plotted on a logarithmic scale. Solid curve 1: experimental points; broken curve 1, extrapolation of the linear portion of solid curve 1 (slope 46 mV/decade). Curve 2: theoretical curve based on $E_m = 61.5$ mV log (K_o/K_i) with K_i taken as 173 mM.

muscle fibers bathed in TEA. We have, however, found the same level of resting potential in solutions made up with TEA obtained from Eastman, from Matheson, Coleman and Bell, and from a specially purified TEA chloride generously provided by Prof. Rafael Lorenté de No.

EFFECTS OF MG-FREE SOLUTIONS Most of our experiments were done in solutions containing TEA chloride and $CaCl_2$ and 0.5 mM Mg. To verify the fact that action potentials can arise when the sole inorganic cation (apart from K) is Ca, one preparation was studied in a solution containing 128 mM TEA, 16.2 mM Ca, and no Mg. The action potentials were essentially the same as those seen when 0.5 mM Mg is present.

Replacement of NaCl by TMA Chloride

Propagated electrical activity, and occasionally spontaneous activity, are seen in fibers in which the NaCl is replaced by TMA chloride (solution 7).

Fig. 10 A shows the spontaneous activity in fibers exposed to 16.2 mM Ca, 128 mM TMA chloride, and 10 mM TEA; the addition of 10 mM TEA often made it easier to obtain activity when TMA was the substituent, but its presence is not essential for such activity. The amplitude of the action potential and of the overshoot are comparable to those seen when TEA chloride is the substituent. Equally important, the resting potential is about the same whether the substituent is TEA or TMA, which suggests that the loss of resting potential is not necessarily caused by a specific action of TEA. The fact that propagated activity is seen in the presence of Ca-rich solutions when the NaCl is replaced by different substituents is further evidence that Ca carries the depolarizing current.

Replacement of NaCl by Choline Chloride

The effects of replacement of NaCl by choline chloride depend on the length of time the fiber is exposed to choline chloride. Immediately after exposure the fiber has a normal resting potential, but with the passage of time the resting potential slowly declines, presumably because choline enters the fibers.

Fibers stored for 2–3 h in solution 6 (128 mM choline chloride, 16.2 mM Ca) show a resting potential of −60 to −40 mV and may or may not be excitable. In fact, about 50% of such fibers do not show spontaneous activity nor can they be excited by applied stimuli. Fibers that can be excited by applied stimuli do show propagated activity, but often only a few responses can be elicited or the fiber can be excited only if it is driven very slowly, for example, once per minute.

If, however, excised fibers are stored in normal Tyrode solution and are perfused with normal Tyrode solution in the tissue bath, upon initiation of perfusion with a solution containing 128 mM choline chloride and 16.2 mM Ca the action potential vanishes almost immediately, but the resting potential remains relatively high. Fig. 15 A shows the activity of a fiber in a solution containing the normal amount of Na; Fig. 15 B shows the absence of activity after perfusion with Na-free solution for less than 1 min. Fig. 15 C shows that after 3 min the resting potential has increased slightly and that the response to extracellular stimulation is a small, low voltage deflection. The obvious interpretation of the results shown in Fig. 15 C is that the Na-dependent upstroke has been abolished by the absence of Na, whereas the resting potential is so high that applied stimuli do not succeed in depolarizing the fiber to its threshold potential. To test this interpretation a drop of 1 M KCl was added to the bath to bring about a transient depolarization (Fig. 15 D). As the resting potential falls the fiber becomes excitable and three propagated action potentials (the first of which shows a double upstroke) are seen in Fig. 15 D. As the KCl washes out, the resting potential gradually

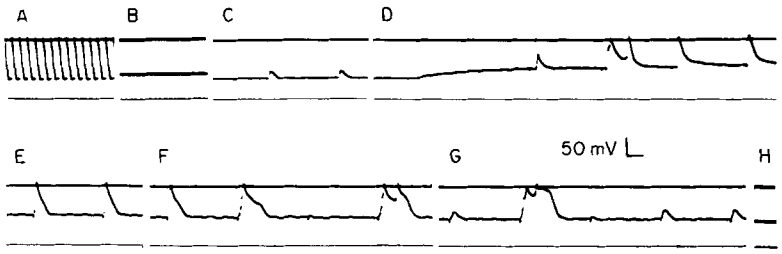

FIGURE 15. The effect of replacing NaCl by choline chloride. (A) shows action potentials in modified Tyrode solution buffered by Tris and bubbled with O_2 (rather than buffered with bicarbonate and 5% CO_2). (B–H), show records obtained in solution 6 (128 mM choline, 16.2 mM Ca). Removal of Na between (A) and (B) leads to immediate loss of excitability. The camera speed is increased between (B) and (C). In (C) the fiber is inexcitable; depolarization by the addition of K makes the fiber excitable in (D). Gradual recovery of resting potential as the K washes away leads to loss of excitability in (G). (H) is a segment of record continuous with that shown in (B) to permit comparison of resting potentials. See text for further details. Calibration: vertical, 50 mV; the horizontal line corresponds to 500 ms in (C)–(G) and to 2 s in (A), (B), and (H).

drifts toward its initial value. Between Fig. 15 D and 15 E a series of some 30 propagated responses was obtained, every stimulus being followed by an action potential. Fig. 15 F shows a further slight increase in resting potential with preservation of excitability. A further slight increase in resting potential in Fig. 15 G is accompanied by a loss of excitability, only one of four stimuli evoking a response. In subsequent records the effect of stimuli was much like that shown in Fig. 15 C. Fig. 15 H is a continuation of the record shown in Fig. 15 B, to permit comparision of the resting potentials.

The records in Fig. 15 have several interesting implications. First, they show that action potentials can be obtained in Na-free solutions in which the substituent is choline; secondly, they show in an almost diagrammatic fashion certain essential features of the normal and the slow response, namely that the normal response arises from resting potentials in the vicinity of −90 mV, but cannot be obtained in the absence of Na, whereas the slow response can be obtained in the absence of Na, but becomes regenerative only in partially depolarized fibers. Finally, this and other records suggest that the duration of the action potential in choline-substituted solutions depends on the level of resting potential, the shorter action potentials being seen at the lower (i.e. less negative) levels of resting potential. Similar shortening of the action potential is, of course, a well known consequence of depolarization induced by elevation of K_o in fibers exposed to normal Tyrode solution.

DISCUSSION

The results presented in this paper show that cardiac Purkinje fibers are excitable in the absence of external Na. This seems to be the first report of

Ca-dependent regenerative electrical responses in cardiac fibers in the absence of both Na and exogenous catecholamines. The evidence that Ca carries the inward ionic current responsible for the upstroke is: (*a*) the slope of the relation between overshoot and Ca_o approximates the expected value of 30.5 mV/10-fold concentration change; (*b*) the rate of rise of the upstroke is an approximately linear function of Ca_o; (*c*) the overshoot and the rate of rise are decreased by agents considered to be blockers of Ca current, namely Mn and D-600; (*d*) propagated electrical activity is seen whether Na was replaced by TEA, by TMA, or by choline.

Other ions which might contribute to the upstroke are Na and Cl. Measurement of the Na content of the tissue and bathing solution shows that both values were too low for this ion to be considered as a possible charge carrier. That outward Cl current contributes to the upstroke is unlikely because it requires ionic current to flow against both an electrical and chemical gradient. Finally, the possibility that TEA itself carries inward current is unlikely since in test solutions in which Ca was increased the overshoot and maximum rate of rise of the upstroke increased in magnitude, yet in those solutions the concentration of TEA was decreased. The most probable interpretation of our findings is that Ca can carry inward current in canine cardiac Purkinje fibers and can sustain all-or-nothing propagated action potentials in such fibers. It is, of course, probable that TEA (and, to a lesser extent, TMA and choline) reduce outward K currents. This would allow action potentials that depend on small inward currents to propagate with a higher safety factor. Whether a reduction of K permeability is necessary to permit propagation of action potentials that depend on inward Ca currents remains to be determined.

Quantity of Inward Current

If propagation occurs at a uniform velocity, when the rate of change of membrane potential is at a maximum, $C_m(dV/dt) = -I$, where I is the net inward current/cm^2, C_m is specific membrane capacity in μF/cm^2, and dV/dt is the maximum rate of rise. If the capacity derived from the foot of the action potential in normal Tyrode solution (2.4 μF/cm^2, Fozzard, 1966) is multiplied by 3.77, a factor relating C_m in normal Tyrode solution to that determined from the foot of the slow upstroke of a Sr action potential (Carmeliet and Willems, 1971), a value of 9 μF/cm^2 is obtained. Taking the mean rate of rise as approximately 4 V/s and C_m as 9 μF/cm^2, the net inward current is about 36 μA/cm^2.[1] This may be compared with Reuter's (1967) finding that in a solution containing no Na and 7.2 mM Ca_o, the net inward current in the voltage range of -50 to $+20$ mV is about 6–40 μA/cm^2.

Our estimate and Reuter's estimate are, as would be expected, far lower

[1] The *maximum* inward current in a propagating action potential occurs later than the moment when dV/dt is maximal, but it exceeds the current at that moment by less than a factor of two.

than the inward current associated with the rapid Na-dependent upstroke. The slow upstroke and the small inward current presumably contribute to the lowering of conduction velocity seen in our study and in Sr potentials (Vereecke and Carmeliet, 1971 *a*) in both of which the conduction velocity is less than 5% of that seen in fibers in which the rapid Na-dependent upstroke is present.

Duration and Shape

The average duration of the action potential in 16.2 mM Ca and 128 mM TEA is 360 ms which is shorter than that of canine cardiac Purkinje fibers in normal Tyrode solution (400 ms). The finding of long action potentials in some of our preparations that were bathed in TEA for 3–4 h before impalement suggests that diffusion of TEA into the cell may be necessary for prolongation to result. Such prolongation has been described in sheep Purkinje fibers exposed to 20 mM TEA (Haldimann, 1963). It presumably depends on inhibition of the delayed outward K current responsible in part for repolarization (Haldimann, 1963; Noble and Tsien, 1972).

The duration of the responses we have described may be determined by decay of the inward current with a time constant far longer than that of the rapid Na current, but shorter than the time constant of activation of the delayed outward current. The decay of the inward current responsible for Sr potentials can, in fact, be fitted by two exponentials with time constants of 170 ms and 1.2 s (Vereecke and Carmeliet, 1971 *b*). The repolarization in the responses we have described might depend on currents with similar characteristics. The possible presence of an inward current with a slow decay is suggested by the fact that responses often cannot be evoked more often than once every 2 or 3 s in fibers exposed to 16.2 mM Ca and 128 mM TEA.

The absence of a "spike" or phase of rapid early repolarization to a distinct plateau may be explained by the frequency dependent membrane capacity of cardiac Purkinje fibers (Fozzard, 1966; Carmeliet and Willems, 1971). In the normal action potential the early rapid repolarization may be caused by the current at the peak of the spike flowing into a capacity in series with a resistance (McAllister, 1968), since about 80% of the membrane capacity is in series with a resistance (Fozzard, 1966). Prior charging by a slow upstroke eliminates the capacitance at the peak of the spike so that no current will be drawn to repolarize the membrane. The same phenomenon has been described for Sr potentials in bovine Purkinje fibers (Carmeliet and Willems, 1971).

Spontaneous Activity

The presence of spontaneous electrical activity in the absence of Na is interesting. Spontaneous diastolic depolarization is generally agreed to be the

result of a time- and voltage-dependent decrease in g_K and a slow inward leak of Na (Noble and Tsien, 1968; Vassalle, 1966; Peper and Trautwein, 1969). The presence of spontaneous diastolic depolarization in the absence of Na means that Ca can carry the current necessary for pacemaker activity. This may be taken as further evidence in favor of a voltage- and time-dependent decrease in g_K being the primary determinant of the kinetics of spontaneous diastolic depolarization.

All-or-Nothing Repolarization

The finding of regenerative repolarization in a fiber with a very long plateau may be taken as evidence for a region of negative slope in the current-voltage relation (Noble and Hall, 1963; Noble and Tsien, 1972). It is interesting that this type of current-voltage relation is found both in normal and Na-free solutions. This regenerative repolarization presumably results from the fact that the anodal polarization brings the membrane potential to a level at which the voltage-dependent permeability responsible for the slow inward current is shut off.

Resting Potential

A low resting potential that is relatively insensitive to K_o in the range 2–20 mM has been reported in cells of the sinoatrial node and in cells of the atrioventricular node (De Mello and Hoffman, 1960) and in embryonic cardiac cells or cardiac cells in tissue culture (Sperelakis, 1972). A TEA-induced decrease in P_K could be the primary cause of the fall in resting potential and there is evidence for such an effect in frog skeletal muscle (Stanfield, 1970). That TEA leaks into the fibers in quantities sufficient to depolarize them seems unlikely because resting potentials were relatively constant in fibers impaled for 2–6 h in the presence of TEA, and because fibers exposed to TEA for 30–60 min before impalement had the same resting potential as fibers soaked in TEA for up to 6 h before the impalement. On the other hand, fibers exposed to choline chloride do show an initially high resting potential that declines over a period of 2–3 h and choline may well diffuse into the fibers, since it is known to do so in feline myocardial fibers (Bosteels, Vleugels, and Carmeliet, 1970).

Although a fall in P_K could be the primary cause of a low resting potential it should be noted that in cardiac fibers P_K may be inversely proportional to the driving force $(E_m - E_K)$, a dependence thought to be mediated by an inwardly rectifying K channel (Noble, 1965). If this relationship between P_K and the driving force obtains in the fibers we have studied then a fall in resting potential from any primary cause would secondarily lead to a fall in P_K and to the changed relationship between resting potential and K_o that we observe. One might, for example, speculate that the primary cause

of the loss of resting potential in Na-free solutions is the loss of Na_i, a loss that would slow the activity of the Na-K pump and eliminate any component of the normal resting potential that depends on electrogenic ion pumping.

The Slow Response

We have suggested that the term "slow response," introduced by Rougier et al. (1969), be used to describe any propagated activity that occurs when the rapid Na-dependent upstroke may be assumed to be absent. This suggestion has been adopted by Shigenobu and Sperelakis (1972). Apart from the Sr potentials reported by Vereecke and Carmeliet (1971 *a*) all previous reports of propagated activity presumably dependent on Ca as a current-carrying ion have been based on preparations in which the rapid Na-dependent upstroke was inactivated by depolarization or blocked by tetrodotoxin; in most such studies excitability was restored or enhanced by the addition of catecholamines. Although the amplitude and upstroke velocity seen in a few of those studies were shown to be related to Ca concentration, questions remain about the effect both of the added catecholamines and of the increased levels of Ca on the movement of Na through the channel responsible for the slow response. Our present findings indicate that propagated activity can be sustained by solutions containing no exogenous catecholamines and no inorganic cation other than Ca and K, and can occur in fibers in which the total Na content is very low. It must, of course, be assumed that the Purkinje fibers and their accompanying terminal sympathetic nerve fibers retain some of their endogenous catecholamines.

It seems probable that the permeability changes that underlie the slow response occur in channels that can allow the passage of either Na or Ca. The striking similarity between the action potentials we have observed and the action potentials of the normal atrioventricular node and the normal sinoatrial node raise the interesting possibility that the normal electrical activity of those cells is a variant of the slow response, especially since those cells normally have a low resting potential that is relatively insensitive to external K. Marshall showed many years ago (1957) that atrial arrest at low temperatures results from reduction of the resting potential and excitability of the atrial fibers at a time when the sinoatrial fibers retain their spontaneous activity. Carpentier and Vassalle (1972) have since shown that activity can be restored to the inexcitable atrial fibers in such a preparation by the addition of norepinephrine. When this is done the atrial fibers remain depolarized and their propagated action potentials, still evoked by the sinoatrial node, have the characteristics of a slow response. The persistence of sinoatrial nodal activity at 20°C might, therefore, be attributed to its depending on a slow response of the sort enhanced in the inactive atrial fibers by the addition of epinephrine.

It is clear that cardiac fibers can function at two distinct levels of resting potential and that a distinct type of action potential corresponds to each of the two levels of resting potential. The mechanism that underlies the normal action potential is a brief and large increase in the permeability to Na; this mechanism operates only at relatively high resting potentials. The mechanism that underlies the slow response operates only in partially depolarized fibers and depends on a greatly prolonged and relatively small increase in the permeability of channels that can admit Ca, Sr, and presumably Na. It is possible that some fibers such as those of the sinoatrial node normally generate only the slow response. Loss of resting potential in a fiber that ordinarily generates the normal response may lead to the appearance of the slow response in those fibers. Such loss of resting potential might arise from elevation of K_o, from reduction of electrogenic ion pumping, or from other causes. Although the inward current responsible for the slow response presumably contributes to the normal action potential, the normal action potential cannot be regarded as being simply a rapid, Na-dependent upstroke followed by a slow response; we have discussed this elsewhere (Cranefield, Wit, and Hoffman, 1972).

ADDENDUM

Since the above article was completed our attention has been drawn to a brief report of the finding that conducted action potentials can be obtained in bovine ventricular myocardium exposed to Na-free solutions containing 1–20 mM Ca and 5 mM caffeine; the NaCl was replaced by sucrose (Verdonck, F., P. Busselen, and E. Carmeliet. 1972. Ca-action potentials and contractions of heart muscle in Na-free solutions. Influence of caffeine. *Arch. Int. Physiol. Biochem.* **80**:167).

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