Instantaneous and Delayed Outward Currents of the Bullfrog Atrial Muscle in Ca-free or Na-deficient Conditions

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Abstract Effects of Ca-free or Na-deficient Ringer solution on the membrane currents of the bullfrog atrial muscle were studied using the double sucrose-gap method. Instantaneous outward current (I_{k1}) decreased in Ca-free and increased in Na-deficient conditions. The amplitude of nominal "fully activated delayed outward current" diminished under both sets of conditions. The diminution, however, was greater in Na-deficient medium (10 mM Na) and its activation curve shifted about 13.5 mV toward a more negative potential. In Ca-free Ringer, no such shift was observed, and the activation of the delayed outward current became slower and sigmoidal while the deactivation was faster than in the control. Contrarily, in Na-deficient Ringer, the activation became faster and exponential and the deactivation was slower. The current tail was composed of two exponentially declining components, and the slower, K-accumulation-related component (I_a) was suppressed in Ca-free and the faster component (I_{xs}) diminished in Na-deficient media. These findings may indicate that in the atrial muscle the instantaneous outward current is modified by a Na-Ca exchange mechanism and that the delayed outward current consists of two components, I_{xs} and I_a , which are susceptible to Na and Ca ions, respectively.

Key Words: frog atrial muscle, membrane current, delayed outward current, Na- or Ca-free, K-accumulation.

In a variety of excitable tissues, Ca ions modify the potassium conductance. In squid axons, external Ca ions partially neutralize fixed negative charges on the surface of the membrane and produce a shift of the activation curve of the delayed potassium current (FRANKENHAEUSER and HODGKIN, 1957). Ca ions inside of the cell are also known to regulate some of the potassium channels in neurones of *Helix aspersa*, and a Ca-sensitive component of the delayed outward current has been identified (MEECH, 1972, 1974). A similar Ca-sensitive component was observed in rat uterine smooth muscle (MIRONNEAU and SAVINEAU, 1980). In

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heart muscle, however, the complexity of outward current systems leads to different conclusions, *i.e.*, a direct action of Ca ions on the instantaneous potassium current (KASS and TSIEN, 1976; BASSINGTHWAIGHTE *et al.*, 1976; ISENBERG, 1977a, b, c), a shift of the potential dependency of delayed potassium current, or an increase in potassium accumulation (DiFRANCESCO and McNAUGHTON, 1979).

Effects of Na ions on potassium conductance in the myocardium are far less clear. In sheep Purkinje fibers, DUDEL *et al.* (1967) studied the outward membrane current induced by the absence of Na ions and suggested the possible effects of Na ions on the potassium and non-sodium currents. The Na ions might affect the outward current directly, or indirectly *via* changes in active and passive ion transport systems. There is, however, increasing evidence that Na ions affect the outward current profoundly by altering the internal Ca concentration as the result of a Na-Ca exchange diffusion mechanism (GOTO *et al.*, 1981).

The aim of the present work was to examine the effects of external Ca ions in comparison with those of Na on the bullfrog atrium. We obtained evidence that the Na-Ca exchange regulates the instantaneous outward current. Delayed outward current is also affected by Ca and Na; the slower component of the delayed outward current is sensitive to Ca ions, while the faster component is sensitive to Na ions.

METHODS

Preparations and chamber. The preparations used were thin muscle bundles (0.4–0.5 mm in diameter and 7–8 mm in length) which were obtained from the right atrium of bullfrog, *Rana catesbeiana* (September 1980 to March 1981). After incising the atrium, the atrial muscle bundles which ran in parallel were isolated and the epicardium was removed, under a binocular microscope (*ca.* 120 $\times$). The muscle bundles were mounted in a chamber which consisted of five compartments, for the double sucrose-gap experiments (GOTO *et al.*, 1978; TSUDA, 1979). The central compartment (0.4 mm width) was perfused with normal or test solutions, the left and right intermediate compartments (1.0 mm width) with isotonic sucrose solution, and the terminal compartments (10 $\times$ 5 mm pools) with isotonic KCl solution (127 mM).

Experiments and recordings. The experimental arrangements for measuring the gap potentials and for clamping the membrane potential have been previously described (GOTO *et al.*, 1978). Different pairs of low-resistance Ag/AgCl electrodes were used to inject the current and to measure the potential through the left and right isotonic KCl pools, and in the central test compartment, two Ag/AgCl plate electrodes were placed to avoid possible polarization due to current application. The extracellular resistance in the test node containing normal Ringer solution was negligible and the potential of the node was held virtually at earth. With this apparatus, a gap resting potential of -70 – -80 mV and gap action potentials

exceeding 100 mV were commonly obtained, and this situation continued over several hours. Since the gap action potential is a product of the actual membrane potential and the short circuit factor, $[r_o/(r_o+r_i)]$, where r_o is the outside, and r_i the inside longitudinal resistance in the sucrose-gap, respectively, the factor was nearly 0.9 in the present experiments. The theoretical distribution of the potential in the test node was calculated to be a 13% decay, when the space constant was assumed to be 0.69 mm (BROWN *et al.*, 1976b).

All experiments were done at $17.0 \pm 0.5^\circ\text{C}$, and in each experiment the temperature change did not exceed 0.4°C . The muscle in the test compartment was initially driven at a frequency of 0.1 Hz with a square wave pulse (5–10 msec, about twice the threshold voltage), and was equilibrated in normal Ringer solution for about 1 hr before starting the voltage-clamp experiments. The membrane potential, current, and contractile tension were monitored on a triple beam oscilloscope (Nihon Kohden, VC-9), photographed with a long-recording camera (Nihon Kohden, PC-2B), and simultaneously recorded on a four-channel rectified pen recorder (Nihon Kohden, PMP 3004).

Voltage clamp experiments. The membrane potential was clamped using a voltage clamp feedback amplifier (Nihon Kohden, CEZ 1100). The potential was usually held at the resting gap potential or at a slightly hyperpolarized state up to 10 mV (–80 to –90 mV) except where indicated in the text. In order to determine the voltage-current relationship, long hyperpolarizing or depolarizing pulses of 2.0–7.0 sec and different voltages were applied at 1.0–2.0 min intervals. Usually, three series of examination were repeated for the control, test solution and after washout with control solution, respectively. The mean values of pre- and post-controls were plotted in comparison with those in the test solution. It was possible to obtain 10–15 series of voltage-current relationships with good reproducibility from one preparation.

Measurement of activation and its voltage dependency of the delayed outward current. The extent of activation of the delayed outward current was determined on the current tail. Usually, two-step clamp pulses were used to determine the time dependency of activation. The first step was a conditioning depolarization of 110 mV and of different duration (0.1–5.0 sec) from the holding potential, and the second step was a constant depolarization of 30–40 mV and 10 sec duration (Figs. 5 and 6). The conditioning depolarization of 110 mV was large enough to activate sufficiently the delayed outward current (see Fig. 4), while the second step of 30–40 mV depolarization was well below the threshold of the delayed outward current but well above the reversal potential (–70––75 mV) in the normal condition. Thus, the amplitude of the tail current which shows the grade of activation, was measured from the current level for the second step which was accompanied by the first step equal to the second step in voltage (see Fig. 3).

Similarly, to determine the voltage dependency of the steady state activation of the delayed outward current, the two-step clamp method was used. In this

case, the first step was a depolarizing pulse of constant duration (2.0–5.0 sec) but of different amplitudes (40–140 mV) and the second step was the same as above. Even the 5.0 sec duration of the first step was not long enough but did activate nearly 90% of the outward current, and hence the maximum amplitude of the tail was taken as a measure of the steady state activation. However, longer pulses tended to damage the preparation when the pulse voltage became large.

Analysis of rate of decay of the delayed outward current. The tail current as a function of time after the end of the first pulse was plotted on logarithmic scales. The amplitude of the current was always taken as the difference in currents with or without the first step pulse being larger than the second step. This procedure was important especially when a creep of current occurred for the sole application of the second pulse, as in the case of sodium deficiency, where potassium accumulation was enhanced. Two or three exponential components of the tail current were analysed, as has been done for the frog atrium (BROWN *et al.*, 1977, 1980).

Solutions. The composition of normal Ringer solution was as follows (mM): NaCl 110.0, KCl 2.5, CaCl_2 1.0, Na_2HPO_4 2.14, NaH_2PO_4 0.85 and glucose 20.0, and pH was 7.4 ± 0.1 . Ca-free solution was prepared by eliminating CaCl_2 from the normal solution. Na-deficient Ringer (5–30 mM Na) was made by substituting NaCl with isotonic Tris-Cl (Tris (hydroxymethyl) aminomethane-HCl) or sucrose. Long (more than 20 min) and total elimination of Na ions often produced irreversible damage of the preparation after reintroduction of the normal solution. In either case of Tris-Cl or sucrose substitution, Na-deficiency produced effects which were similar in nature, but the effects of sucrose substitution were more conspicuous than those of Tris-Cl. An analysis of the effects, however, was done only in the latter case because of the possible influence of Cl ions in the former.

About one-third of the experiments (44 cases) were done in the presence of 4.0 mM Mn ions. Mn was used to show up the instantaneous outward current more clearly by eliminating the slow inward current. In these cases, Tris-Cl buffer was used instead of the phosphate buffer. The results of these two series of experiments with and without Mn appeared to be comparable. However, in the presence of Mn ions, the slower component of delayed outward current was generally depressed (see Fig. 9).

RESULTS

1. Current-voltage relationships in Ca-free and Na-lacking conditions

General effects of Na-lacking or Ca-free solutions on the membrane currents were first examined for the current-voltage relationships. Figure 1 shows records of the effects of Na-deficiency in which most of the NaCl (100 mM) was replaced with Tris-Cl. Mn ions (4 mM) were present throughout. As can be seen, the basal current level at the holding potential (–90 mV) shifted slightly outward in the case of Na-deficiency, possibly contributing to hyperpolarization of the mem-

Fig. 1. Voltage dependency of the membrane currents of the bullfrog atrial muscle. Mn ions (4 mM) were present throughout. A: control responses in Ringer solution. B: responses in Tris-Ringer containing 10 mM NaCl. Depolarizing pulses of 7.0 sec duration were applied with 90 sec intervals and 10 mV steps, and the membrane voltage (V) and current (I) were recorded simultaneously. Note a change in instantaneous and delayed outward currents. Further explanations are given in the text. Vertical calibrations from top to bottom are 100 mV, 1.0 μA , and 50 mg. Horizontal calibration marks 5.0 sec.

brane in unclamped conditions (GOTO *et al.*, 1972). Instantaneous outward current (mainly I_{K1}) for low voltages of depolarization was augmented and anomalous rectification became less prominent in Na-deficient solution. For larger depolarizing pulses, the time-dependent delayed outward current (mainly I_x) developed, and after reduction of Na concentration this current was enhanced in the early phase but was rather depressed in the late phase of current development.

General effects of Ca-free solution were also examined in the constant presence of Mn ions (4 mM), although the records are not shown. In this case the basal current level shifted inward under Ca-free conditions, and the instantaneous as well as delayed outward currents were depressed.

The current-voltage relationships in control and in Ca-free solutions are illustrated in Fig. 2A. In these experiments, however, Mn was absent. The basal current level shifted slightly inward as before, corresponding to the depolarization in unclamped conditions (MILLER and MÖRCHEN, 1978). On the terminal current at the end of a 2 sec pulse, inward background current for hyperpolarization, instantaneous outward current for depolarization of less than 50 mV, and net instantaneous and delayed outward current for stronger depolarizations were all inhibited in the Ca-free conditions.

Figure 2B shows the effects of Na-free Ringer in which the external NaCl was completely replaced by Tris-Cl. In contrast to the effects seen in the Ca-free solution, the basal current level shifted outward, and the instantaneous outward current for low voltage of depolarizations was enhanced, while the net outward current for larger depolarizing pulses (more than 100 mV) was depressed. The time-dependent delayed outward current, therefore, appeared to be inhibited in

Fig. 2. Current-voltage relationships of the atrial muscle membrane. A: comparison of the relationships in normal Ringer with that in Ca-free Ringer. B: similar comparison with the relationships in Na-free Tris-Ringer. In these cases, depolarizing and hyperpolarizing pulses of 2.0 sec duration were applied, and the holding potential was -80 mV throughout. Mn ions were not used. In each figure squares denote the terminal current level at the end of pulses, and circles, the peak value of inwardly directed current. The inward current was plotted for showing the condition of the muscle under voltage clamp. Under control conditions, the current crosses the abscissa at a voltage more than 100 mV which corresponds to the amplitude of the action potential. The amplitude decreases in Ca-free, and is markedly diminished in Na-deficient media. Tris-Cl tends to depress the slow inward current. Further explanations are given in the text.

this case, since the net outward current decreased despite an enhancement of the instantaneous outward current.

2. Voltage dependency of the delayed outward current activation in Na-lacking and Ca-free conditions

To determine the "nominally" steady state activation curve for the delayed outward current, two-step clamp pulses were used (see METHODS). Figure 3 shows the records obtained from experiments performed on the same preparation throughout, in the control (A), Na-deficient (B), and Ca-free conditions (C). In these cases, the first step was a rather short depolarization of 2.0 sec with different amplitudes (40 – 140 mV) from the holding potential of -90 mV, and the second step was a sustained depolarization of 10.0 sec and 40 mV. As can be seen in the figures, the tail current increased with increasing amplitude of the first step pulse and gradually became saturated with the stronger pulses. The maximal amplitude attained was largest in control Ringer, next largest in Ca-free, and smallest in

Fig. 3. Voltage dependency of the activation of delayed outward current. A: control records in normal Ringer solution, B: records in Na-deficient Tris Ringer (10 mM NaCl), and C: those in Ca-free Ringer. A two-step clamp method was used (see text), and membrane voltage (V), current (I), and tension responses (T) were recorded simultaneously. The tension responses were markedly depressed in Na-deficiency and almost disappeared in Ca-free conditions. Relationships between the tension and membrane currents will be analysed in later reports now in preparation. Vertical calibrations are 100 mV, 1.0 μA , and 50 mg from top to bottom. Horizontal calibration marks 3.0 sec.

Fig. 4. Relationships between the membrane voltage and the activation of delayed outward current. A: comparison of voltage dependency of the activation in normal, Na-deficient Tris, and Ca-free Ringer solutions. Means of four series of experiments on activation are plotted superposing on the shift of the basal current level for the second step pulse (at the end of the first step) which is shown as horizontal thin lines. In these cases, the first step was of depolarizing pulses of 2–5 sec and 30–140 mV, and the second step, of 10 sec and 30 mV depolarization. B: the normalized steady state activation curves. The basal current shifts are omitted.

Na-deficient solutions. For weaker pulses near threshold levels, increase in the current tail was gradual in normal and Ca-free media, while in Na-deficient conditions the increase was rather sudden, suggesting a lowering in threshold of the current below the second pulse of 40 mV.

The basal current level for the holding potential shifted outwardly in Na-deficient and inwardly in Ca-free solution. Background current for 40 mV depolarization (second step level) decreased slightly in Ca-free conditions, while in Na-deficient conditions it increased markedly inducing an outward creep of the current.

Figure 4A shows the shifts of basal current level and the relationships between the magnitude of the outward current tail and the membrane potential of the first step pulse, which were obtained from three series of similar experiments with longer first step pulses and the data in Fig. 3. Again it can be seen that the tail current increased with increasing depolarization and the maximal amplitude appeared slightly smaller in Ca-free and much smaller in Na-deficient solutions. Figure 4B shows the steady state activation curves where the data were normalized by taking the largest amplitude in each condition as unity. The membrane potential with half-maximal activation (V_h) was +1 mV for the control, +2 mV for the Ca-free, and -12.5 mV for the Na-deficient condition. Thus, Na-deficiency produced a shift in the voltage dependency of activation of the outward current by 13.5 mV toward a negative potential.

3. Effects of Ca- and Na-deficiency on the time course of activation of the delayed outward current

The time courses of activation were analysed for the current tail. Figure 5 shows a series of records of the current tails on repolarization to 40 mV, after conditioning pulses of 110 mV of different durations (0.1–2.5 sec). It can be seen that the peak of the tail current approached a maximum with the lengthening of the initial conditioning pulse in each case of the control (A), Na-deficient (B), and Ca-free conditions (C). The rate of activation (increase of the peak), however, appeared faster in Na-deficient and slower in Ca-free media than in the control solution, while the rate of deactivation (decay rate of the tail) was slower in Na-deficient and faster in Ca-free solution.

Basal current level for the holding potential (−80 mV) again shifted outwardly

Fig. 5. Time dependency of the activation of delayed outward current. A: control records in normal Ringer, B: records in Na-deficient Tris Ringer (10 mM NaCl), and C: those in Ca-free Ringer. Two-step pulses were used where the duration of the first step was varied (see text), and membrane voltage (V) and current (I) were recorded. Vertical calibrations from top to bottom are 100 mV, 1.0 μ A, and 50 mg. Horizontal calibration marks 3.0 sec.

Fig. 6. Time course of the activation of delayed outward current. A: comparison of the time course of activation in normal, Na-deficient Tris, and Ca-free Ringer solution. B: the normalized time courses. Means of four series of experiments are plotted. Further explanations are given in the text.

in Na-deficient and inwardly in Ca-free Ringer solution. Background outward current for 40 mV depolarization (second step level) also showed a marked outward creep in Na-deficient conditions while in Ca-free solution, the current was less than with the control.

Mean time courses of activation of the delayed outward current in control, Na-deficient, and Ca-free conditions ($n=4$) are plotted in Fig. 6A. In Na-deficient solution, the activation was fast and exponential, but the maximal amplitude attained was less than half that of the control (45%). In the Ca-free condition, there was a latency of activation of about 0.3 sec, and then the outward current developed sigmoidally and attained 80% of the control level. For test pulses longer than 3.0 sec, however, the amplitude of the tail tended to decline, suggesting an accumulation of potassium in the immediate exterior of the cells. But the real nature of the decline is unknown at present. Thus, taking the amplitude at 3.0 sec as 100%, the grade of activation was normalized and compared, as shown in Fig. 6B. It became clearer that the activation of delayed outward current was faster in Na-lacking and slower in Ca-free conditions, showing a half activation time of 1.15:0.5:1.28 sec and one-fourth activation time of 0.66:0.22:0.95 sec for the normal, Na-lacking, and Ca-free media, respectively.

4. Effects of Na-deficient and Ca-free solutions on the decay of the outward current tail

In Fig. 7, tail current changes as a function of time at the second step pulse of 40 mV were plotted on logarithmic scales. Different symbols indicate decay of currents following the first conditioning depolarization of various magnitudes (70–120 mV) and 2 sec duration. The plots did not fit a straight line with the normal Ringer solution for any conditioning depolarization, suggesting that the current consisted of more than one component. When the later points were hand

Fig. 7. Two components of the outward current tails in bullfrog atrial muscle. Data which are similar to those of Fig. 3A are analysed in semi-logarithmic plots. Early and late components of the current tail can be distinguished, and are tentatively termed I_{xs} and I_a , respectively (see text). Likewise, the time constant of decay of each component is shown as τ_s and τ_a .

fitted with a straight line, they decayed with a time constant of 11.3–19.5 sec. The line was then subtracted from the measured point to give a remaining current change, which fits well to a line with a time constant of 1.1–1.3 sec.

In all of our preparations there apparently existed these early and late current components. The time constants of the early component roughly correspond to those of the slow component ($i_{x,s}$) reported by BROWN *et al.* (1976a, 1980) and NOBLE (1976), and the time constants of late component, to those of the accumulation and pump component (i_a). Therefore, they were tentatively named early (I_{xs}) and late (I_a) components of the outward current, respectively. In some preparations which showed pacemaker activity, a much faster component was also observed. This may correspond to the fast component ($i_{x,f}$) in the sinoatrial node of the bullfrog (BROWN *et al.*, 1977, 1980), although most of our preparations were quiescent.

As seen in Fig. 7, conditioning first step pulses of 90–120 mV depolarization decayed with almost the same time constant in each I_{xs} and I_a . Therefore, effects of Na-deficient and Ca-free solutions on the decay tail were examined using a step pulse of 100 mV depolarization. The results are shown in Fig. 8.

When Ca-free solution was introduced, the slower I_a of the two components was markedly suppressed, while the faster I_{xs} was little changed (Fig. 8A). Similar effects of Ca-deficiency were also observed in the presence of Mn ions, 4 mM (Fig.

Fig. 8. Two components of the outward current tail and the effects of external Na and Ca ions. A: the effects of Ca-free Ringer, and B: those of Na-deficient Tris Ringer (10 mM NaCl) on current components. Experimental procedures are explained in the text. Note that Ca-removal depressed the late component while Na-deficiency inhibited both the initial and late components.

Fig. 9. Selective depression of the late component of the outward current tail by Mn ions (4 mM), and further, by Ca-removal.

9). In this case, Mn alone depressed the I_a without affecting I_{xs} , and depletion of Ca ions further depressed the I_a . Since Mn ions in a low concentration (2–5 mM) are known to eliminate Ca inward current, our data strongly suggest that the late component, I_a , of outward current was Ca-sensitive.

On the other hand, in Na-deficient Tris-Ringer, both the early and late components were depressed (Fig. 8B). The depression was more conspicuous in the early component, and on some occasions, the component could not be analysed. In the presence of Mn and absence of Ca ions, where the late component was already suppressed, depletion of Na ions immediately abolished the early component, I_{xs} . Similar abolition of I_{xs} by Na depletion was also observed in the presence of Ba ions (0.1 mM, not shown) which depressed potassium accumulation and hence I_a .

DISCUSSION

We have obtained evidence of the existence in the bullfrog atrial muscle of outward currents which are sensitive to Ca and Na ions, as previously described in the cases of other tissues. The instantaneous outward current (mainly I_{k1}) decreased in Ca-free solution, while it increased under Na-deficient conditions. The activation of delayed outward current was slow and sigmoidal and the deactivation was fast in Ca-free solution. Contrarily, in Na-deficient Ringer, the activation of outward current was fast and exponential and the deactivation was rather slow.

In amphibian and mammalian hearts the removal of Ca ions from the bathing medium produces a prolongation of action potential, while reduction of Na concentration causes a shortening. GARNIER *et al.* (1969) suggested, regarding the effects of Ca-removal on frog atrial muscle, that the slow inward sodium current is responsible for the prolongation. However, MILLER and MÖRCHEN (1978) demonstrated that in the frog ventricle $[Ca]_o$ has a direct effect upon potassium permeability and that a possible reduction of I_{k1} due to decrease in $[Ca]_o$ is responsible for the prolongation of the action potential. KASS and TSIEN (1975, 1976) arrived at a similar conclusion with regard to Purkinje fibers under voltage clamp. These latter observations are in good accord with our present results. However, we have further clarified the nature of a pronounced delay of activation of the delayed outward current after removal of the Ca ions. Therefore, it may be concluded that both the depression of I_{k1} and the apparent delay of activation of the outward current are responsible for the delayed repolarization of action potential.

Contrarily, a shortening of action potential in reduced Na Ringer is mainly considered to be due to a marked increase of instantaneous outward current, I_{k1} . The increase may be induced by an increase of $[Ca]_i$ (BASSINGTHWAIGHTE *et al.*, 1976; ISENBERG, 1977a, b) as the result of Na-Ca exchange diffusion after reduction of $[Na]_o$ (REUTER and SEITZ, 1968). In the bullfrog atrium, however, the delayed outward current was activated earlier and at a lower voltage of depolarization

in Na-deficient conditions (Figs. 4 and 6), and thus the enhanced outward current may also contribute to the fast repolarization of the action potential, although the net outward current for more than 100 mV depolarization diminished (Fig. 2).

The cause of the delay or reduction of activation of the delayed outward current after removal of Ca ions was not fully clarified, but the presence of a Ca-sensitive component, I_a , was identified. It is known that Ca-removal inhibits the potassium permeability, reducing I_{k1} directly from the outside, as noted, or indirectly from the inside of the membrane *via* a decrease in $[\text{Ca}]_i$ (MEECH, 1974; MEECH and STANDEN, 1975; ISENBERG, 1977a, b), and this may cause a delay or reduction in the I_{k1} -related potassium current or accumulation to the immediate exterior of the membrane (BROWN *et al.*, 1976a, 1980). Actually, a considerable depression of the late component (I_a) of delayed outward current in Ca-free or Mn-containing media (Figs. 8 and 9) was observed in the present experiments. A concomitant decrease and depressed creep of the instantaneous outward current (Figs. 3 and 5) also support this view. In *Helix aspersa* neurones, the slower component of the delayed outward current was suppressed in Ca-free conditions while the faster one was unchanged (MEECH and STANDEN, 1975), and similar results were also demonstrated in rat uterine smooth muscle (MIRONNEAU and SAVINEAU, 1980). All these data taken together suggest that voltage-dependent and Ca-mediated activations of the outward current are two functionally distinct systems, even in the myocardium.

The effects of reduced $[\text{Na}]_o$ on the outward current were rather complicated. The reduction of $[\text{Na}]_o$ not only produced (1) an increase of instantaneous outward current, I_{k1} , and (2) a decrease of both early and late components of the delayed outward current, but also (3) an acceleration of the onset and (4) a shift in voltage dependency of the activation. The increase of I_{k1} could be interpreted by an increase in $[\text{Ca}]_i$ due to Na-Ca exchange. The decrease of I_{xs} and I_a , on the other hand, seemed to be accounted for by a marked potassium accumulation due to the increased I_{k1} during depolarization, since these outward current tails are depressed in high potassium media (BROWN *et al.*, 1980; DiFRANCESCO and NOBLE, 1980).

The depression of the early component (I_{xs}) of delayed outward current, however, should receive attention, since this component was specifically inhibited by reduction of Na ions. Neither removal of Ca ions nor application of Mn and Ba ions (less than 4 mM) as well as tetrodotoxin (unpublished observation) ever affected I_{xs} . In these conditions where the late component (I_a) was already suppressed, depletion of Na ions immediately eliminated I_{xs} . Thus, I_{xs} could be distinguished as the Na-sensitive early component from the Ca-sensitive late component, I_a , of the delayed outward current.

The acceleration of the onset and the shift in voltage dependency of the outward current induced by the reduction of Na ions are not explicable at present. The shift to a more negative potential may contribute to faster activation, but

the details have yet to be determined. DiFRANCESCO and NOBLE (1980) analysed the time course of potassium current following potassium accumulation in the frog atrium, and their analyses predicted that the leftward shift of activation curves by the accumulation does occur but is fairly small. As for the nature of the shift, a surface charge effect of Ca ions should also be considered (FRANKENHAUSER and HODGKIN, 1957). In Na-deficient conditions, $[Ca]_i$ may possibly increase greatly, and this neutralizing of the internal negative surface charges would cause a shift of voltage-dependency to a more negative potential (Fig. 4). However, such was ruled out, since when $[Ca]_o$ was reduced or increased (unpublished observation) no appreciable shift of activation occurred. In Purkinje fibers, ISENBERG (1977c) failed to detect a shift of voltage-dependency of i_{K_2} even when Ca ions were injected intracellularly, and KASS and TSIEN (1976) also failed to observe the shift when $[Ca]_o$ was altered, although DiFRANCESCO and McNAUGHTON (1979) did report considerable shifts. Thus, the complexity of outward current systems in heart muscle leads to different conclusions with regard to the occurrence of a shift in potential dependency. Moreover, as discussed by these authors, alteration of external Na or Ca concentrations would cause not only a change in surface charges, but also changes in the Na-K and Na-Ca exchange pump and in the action of endogenous catecholamine. These studies are ongoing in our laboratory.

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