

ELECTROPHYSIOLOGICAL STUDIES ON THE HEART OF THE BIVALVE MOLLUSC, *MODIOLUS DEMISSUS*

II. IONIC BASIS OF THE ACTION POTENTIAL

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(Received 26 June 1971)

INTRODUCTION

Investigations concerning the ionic basis of the action potential in nerve and muscle have revealed a variety of mechanisms ranging from total dependence on sodium, first described quantitatively in the squid axon (Hodgkin & Huxley, 1952), to total dependence on calcium in crustacean muscles (Fatt & Ginsborg, 1958; Abbott & Parnas, 1965; Hagiwara & Naka, 1964; Hagiwara & Nakajima, 1966). Between these extremes, various combinations of sodium and calcium conductances comprise spike-generating mechanisms.

For example, calcium and other divalent cations produce spike currents in the absence of sodium, although in normal bathing solutions sodium is the principle source of current (Tasaki, Watanabe & Singer, 1966; Gerasimov, Kostyuk & Maiskii, 1965; Koketsu & Nishi, 1969). In some instances, however, action potentials occurring for several hours in Na-free solutions appear to depend on stores of sodium ions trapped at the surface of the membrane (Chamberlain & Kerkut, 1967; Moreton, 1968; Carlson & Treherne, 1969).

A variety of intermediate examples have been described where both sodium and calcium carry the spike currents (Geduldig & Junge, 1968), where the proportions of the two ions involved vary with the membrane potential (Irisawa, Irisawa & Shigeto, 1969), and where one or more ions are associated with specific components of the action potential. The latter category shows a rich diversity among vertebrate hearts. Sodium has been associated principally with rise time and overshoot of the action potential (Draper & Weidmann, 1951; Brady & Woodbury, 1960; Weidmann, 1955), although calcium also contributes to the spike in some instances (Niedergerke & Orkand, 1966; Matsubara & Matsuda, 1969). Lack of, or interference by, manganese or calcium also blocks the spike plateau (Hagiwara & Nakajima, 1966; Garnier *et al.* 1969; Yanaga & Holland, 1969), although Garnier *et al.* (1969) demonstrated that under certain conditions the plateau amplitude was dependent on external sodium.

Although the contributions of sodium cannot be entirely discounted, calcium appears to be the major current source in visceral smooth muscle (Holman, 1958; Bülbring & Kuriyama, 1963; Nonomura, Hotta & Ohashi, 1966; Brading, Bülbring & Tomita, 1969).

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The experiments communicated in this paper describe qualitatively the multi-ionic currents which contribute to the action potential in the heart of *Modiolus demissus*. The results of these experiments show that *Modiolus* has predominately a calcium spike, as do visceral smooth muscles, and that ionically separable spike and plateau components exist with similarities to those in vertebrate myocardia. The ionic basis of the action potential in *Modiolus* heart is also discussed in comparison to ionic mechanisms previously described in other bivalve hearts (Irisawa, Shigeto & Otani, 1967; Irisawa *et al.* 1969) and in relation to the control of membrane permeability by calcium.

MATERIALS AND METHODS

Simultaneous electrical and mechanical recordings of the heart-beat of *Modiolus demissus* were made by the sucrose-gap technique. A description of the methods, and the formulae for the ionic composition of the test solutions, has been given in the preceding paper (Wilkins, 1971). Intracellular microelectrode recordings were also made simultaneously with the extracellular records, in one set of experiments, by lowering a glass pipette onto the heart in the test chamber of the sucrose gap; the apparatus was modified to this end by extending the opening for the transducer until the heart was exposed.

RESULTS

In the preceding paper (Wilkins, 1971) the heart of *M. demissus* was characterized, in part, by a membrane potential of considerable variability. In addition, the action potential varies considerably in both size and shape. Because of the emphasis placed



Fig. 1. Action potentials recorded simultaneously by sucrose-gap and microelectrode (arrows) techniques. Calibration bars represent 20 mV (vertical); 1 sec (horizontal).

on the shape of the action potential, experiments were conducted specifically to determine the effect, if any, of the extracellular recording method on the spike configuration. Sharp, monophasic spikes as well as action potentials followed by pronounced plateaus have been recorded individually by both intra- and extracellular techniques. Conclusive evidence was gained, however, in simultaneous recordings by both techniques (Fig. 1). Although slightly smaller in amplitude, spike morphology is faithfully reproduced in sucrose-gap recordings.

Effects of sodium-free media

After a brief pause in spontaneous activity, action potentials retaining their spike-like configuration suffered a reduction in both amplitude and duration of the plateau

in Na-free SW (Fig. 6; Wilkens, 1971). In addition, the maximum rate of rise of the spike was reduced 25–50%, depending on the preparation. Long-term dependence on sodium was observed, however, in control experiments of several hours duration. After 2 h in Na-free SW, the amplitude of the spike was unchanged while systolic tension dropped to less than 10% of the control level. Thereafter, spike amplitude declined gradually, without change in the membrane potential, until spontaneous activity disappeared irreversibly after 3 h. Although *Modiolus* heart is Na-sensitive in the sense of long-term viability of the preparation, these results suggest that ions other than, or in addition to, sodium contribute to the spike-generating currents.

A reduction of the plateau, similar to the effect of Na-free SW, results from the substitution of lithium for sodium (Fig. 8A, Wilkens, 1971). It appears, therefore, that lithium fails to substitute for sodium in the maintenance of the spike plateau. Experiments were not performed to determine long-term viability in Li-SW. Differences in the actions of sodium and lithium were noted previously with respect to the membrane potential and sodium pump (Wilkens, 1971).

Effects of tetrodotoxin

Modiolus hearts are nearly insensitive to tetrodotoxin (TTX). In one experiment, TTX had no effect in concentrations between 10^{-7} and 10^{-5} M. In a second experiment (Fig. 2A) only at the largest dose did a slight reduction in spike amplitude, duration, and frequency occur, associated with the formation of a notch near the peak of the potential (see inset, Fig. 2A). A slight increase in diastolic and systolic tone was also observed following TTX treatment. The effect of TTX was also minimal in the absence of calcium (Fig. 2B), a transient interruption in the rhythmical depolarizations being the only observable response. Tetrodotoxin did enhance the contractions of the heart for a few cycles due presumably to a release of residual bound calcium.

Electrical activity in Ca-free SW

Hearts were perfused in Ca-free SW to test the dependence of the spike on extracellular calcium. The possible existence of a calcium spike in the hearts of *Mytilus* and *Crassostrea* has been reported (Irisawa *et al.* 1967; Irisawa, Noma & Ueda, 1968). The action potentials in *Modiolus* hearts underwent a characteristic sequence of modifications in Ca-free solutions (Fig. 3A, B). Hearts were hyper-excited initially; both spike amplitude and rate of rise increased for 1–2 min. Subsequently, membrane depolarization and a decrease in spike amplitude and maximum rate of rise were observed.

The duration and amplitude of the plateau phase changed remarkably in Ca-free SW. The amplitude of the spike plateau increased – actually exceeding the amplitude of the spike. Membrane repolarization was also delayed. Records of action potentials, taken at intervals after the removal of calcium (Fig. 10), illustrate the gradual increase in the plateau. Note the complete loss of the initial spike after 14 min. Thus, in Ca-free SW, spontaneous action potentials undergo a dual transformation – a simultaneous spike reduction and an enhancement of the plateau. This dual change is plotted in Fig. 4 for a typical Ca-free experiment. Although unstable during the first 25 min of the experiment, the spike amplitude subsequently stabilized for 30 min at a level

6 mV greater than the initial peak potential in sea water, then began a gradual decrease for 35 min and then suddenly disappeared. On the other hand, the plateau remained high, and showed little sign of extinction after 2.5 h. The same pattern of activity was observed in each heart subjected to Ca-free SW, although the time required for loss of the spike phase was frequently less (around 15–20 min) and could

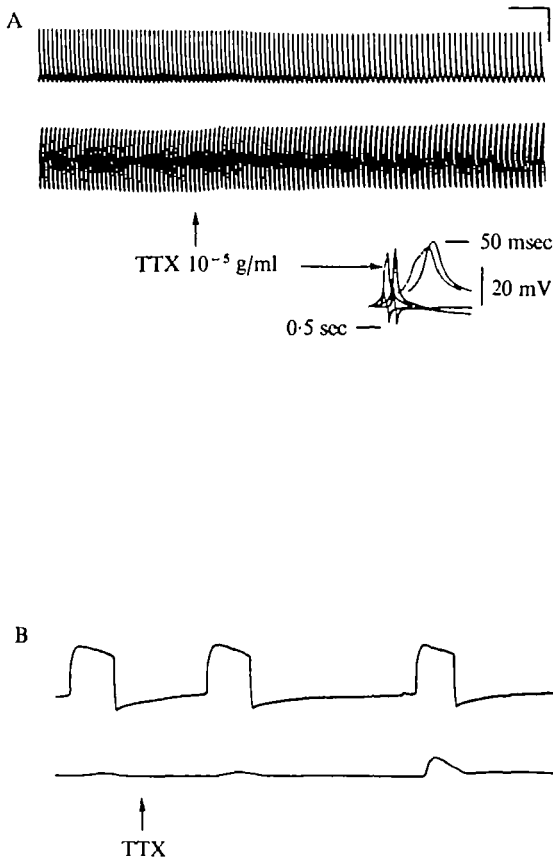


Fig. 2. Effects of tetrodotoxin. Electrical and mechanical activity in the heart of *Modiolus* in response to TTX 10^{-5} M is illustrated in the upper and lower traces, respectively, of each pair. In (A) TTX resulted in a slight decrease in amplitude. The inset in (A) photographically compares spikes recorded simultaneously from the oscilloscope at different sweep speeds. The lower traces are differentiated spikes. In (B) TTX was added after perfusion for 2.2 h in Ca-free SW. Vertical bars equal 20 mV. Horizontal bars equal 30 sec in continuous records, 1 sec in the inset (B).

be shortened by the addition of chelating agents. The time course for complete loss of systolic tension in these hearts was nearly equal to the time required for loss of the spike. In Fig. 4 the spike and contraction disappear within about 3 min of each other.

Records, from the experiment graphed in Fig. 4, show a complete separation of the spike and plateau phases of the action potential (Fig. 3 C, D). An expanded record from the long-lasting depolarizing potential in Fig. 3 C reveals a rapidly rising spike preceding the slowly rising plateau phase. After another 20 min (Fig. 3 D) a short burst of spikes, decreasing in amplitude, preceded each long-lasting potential and then died

out. In the subsequent depolarization (see expanded portion, Fig. 3D) no initial spike occurred.

In Na-free SW spontaneous activity disappeared completely after several hours, although hearts in normal sea water continued to beat for 1–2 days. However, in Ca-free SW only the rapid spike-like phase of the action potential disappeared while the enlarged plateau phase continued to reappear spontaneously for up to 6 h in the absence of all mechanical activity. Fig. 3E illustrates the rhythmical nature of these long depolarizations, each lasting about 40 sec.

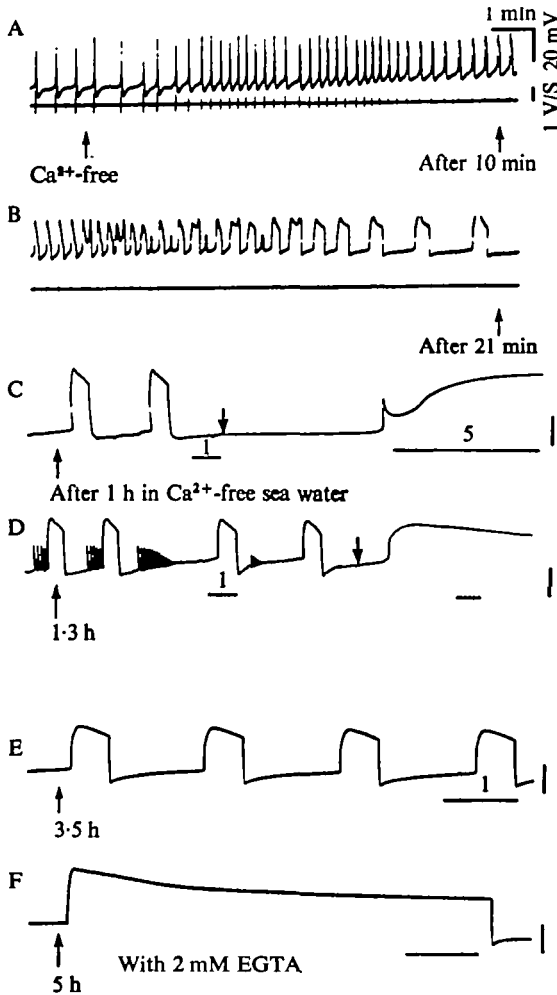


Fig. 3. Effects of calcium-free sea water. A and B are oscilloscope records of the spontaneous action potentials and their differentiated signals, upper and lower trace in each pair, following removal of calcium. Time in Ca-free SW is given at the right-hand arrows. C, D, E and F show spontaneous activity after several hours in Ca-free SW. The rectangular-shaped depolarizations are preceded by a spike (C) after 1 h in Ca-free SW. Spikes occur in bursts (D) and then disappear completely. Downward-pointing arrows indicate the change in recording speed. The two lower traces illustrate the regularity of the pulses after 3.5 h (E) and after 5 h in Ca-free SW, but with 2 mM-EGTA (F). Vertical bars equal 20 mV. Horizontal bars equal 1 min in each trace with the exception of the expanded portions in C and D, which equal 5 sec.

Two millimolar ethylene bis (oxyethylenitrilo)-tetracetic acid (EGTA), added after perfusion in Ca-free SW for 5 h, lengthened the depolarizations to 5–6 min in duration (Fig. 3 F). In one instance prolonged potential shifts of 30 min occurred regularly in Ca-free SW with EGTA. The time necessary for loss of spike and for plateau enhancement was halved when 2 mM-EGTA was added to Ca-free SW prior to the exposure of the heart to the test solution. This procedure often led to a sustained membrane depolarization, equal in amplitude to the depolarizations occurring spontaneously. This potential level, although greater in amplitude than the normal action potential, did not reach zero potential.

A relationship between calcium and the plateau duration was obtained by a gradual

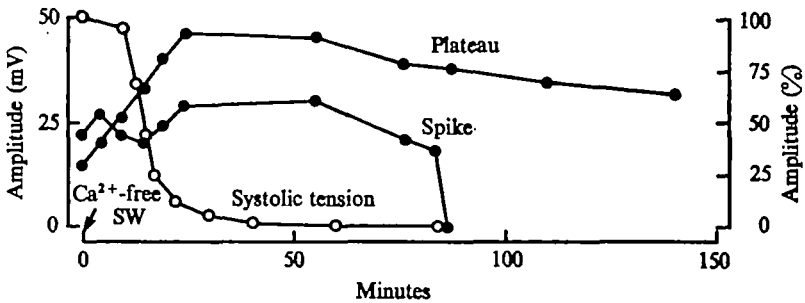


Fig. 4. Comparison of spike and plateau amplitude and systolic tension in Ca-free sea water. Spike and plateau amplitude (left ordinate) versus time after Ca-free sea water (abscissa). Systolic tension (right ordinate) is given as the percentage of the contraction amplitude just prior to the removal of calcium.

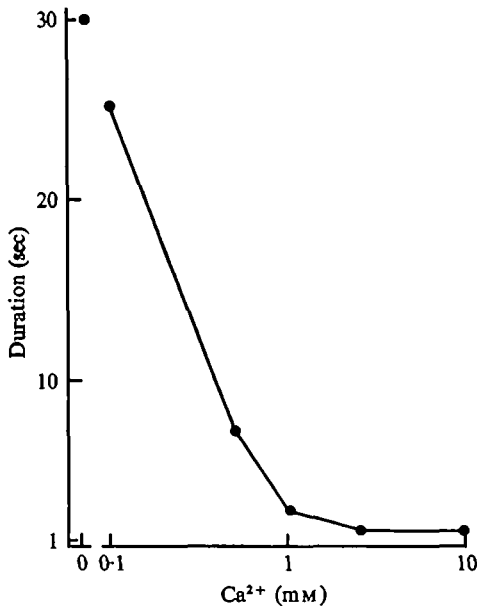


Fig. 5. Dependence of plateau duration on calcium concentration. Duration of the plateau (ordinate) versus the concentration of calcium in sea water (abscissa). Only a slight increase in duration occurs after calcium is removed completely. The data are means from three experiments.

elimination of calcium (Fig. 5); sufficient time was allowed for the response to stabilize at each concentration. Little effect was observed until calcium was lowered to near 1 mM. Below 1 mM the plateau became increasingly sensitive to low calcium.

Effects of manganese

Manganese produced changes in the action potential similar to those seen in Ca-free SW. The Ca-antagonizing effects of manganese have been used previously to identify the ionic components of the spike (Hagiwara & Nakajima, 1966; Nonomura *et al.* 1966; Irisawa *et al.* 1968). Fig. 6 illustrates the action of manganese on *Modiolus* hearts.

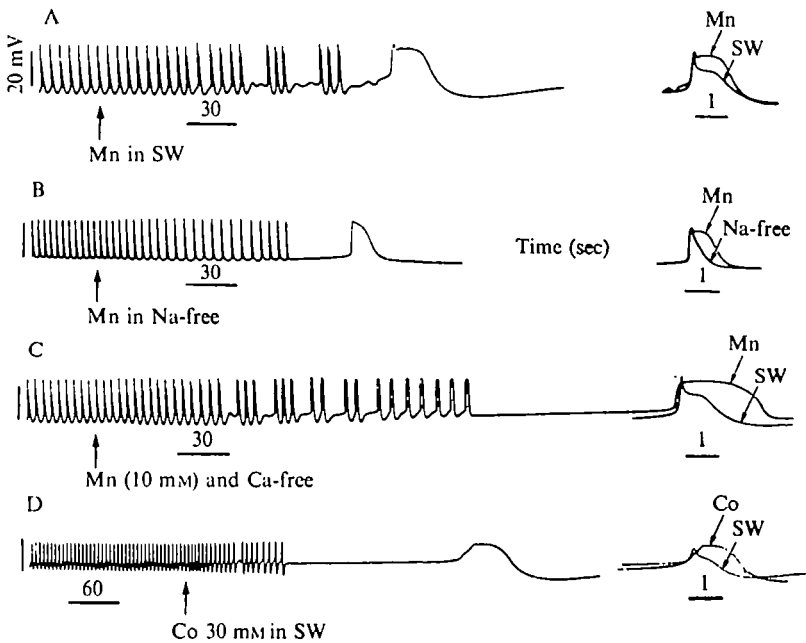


Fig. 6. Effects of manganese and cobalt ions on the action potential. Manganese, 10 mM, added at the arrow in sea water (A), Na-free SW (B), and Ca-free SW (C); cobalt, 30 mM, added in sea water (D). Expanded records at right in each trace are photographically superimposed action potentials recorded before and after the test substance. All vertical bars equal 20 mV.

Manganese rapidly produced an increase in the amplitude and duration of the plateau (Fig. 6A). A similar response to manganese occurred in Na-free SW (Fig. 6B). Note, however, that the rising phase of the action potential still retained its spike-like configuration. If the heart was subjected to manganese and Ca-free SW simultaneously, the spike disappeared rapidly (Fig. 6C). Fig. 6D illustrates the loss of the spike, coupled with an increase in the plateau, following perfusion with 30 mM cobalt. Cobalt and other divalent metal ions also interfere with calcium currents in *Aplysia* membranes (Geduldig & Junge, 1968).

Selectivity of spike and plateau phases

Experimental procedures have been described for separating the components of the action potential. Na-free SW reduces the plateau leaving only the spike, while a

reduction of extracellular calcium exaggerates the plateau phase and, eventually, results in loss of the spike.

When both sodium and calcium were omitted from the perfusing medium, the heart became quiescent and retained a nearly normal level of membrane polarization. If sodium was then selectively readmitted to the perfusate, spontaneous activity resumed in the characteristic form of the Ca-free plateaus (Fig. 7, upper trace). If, on the other hand, calcium was returned to the medium following perfusion with Na- and Ca-free SW, rhythmical spike-like activity reappeared (lower trace). It was, therefore, possible

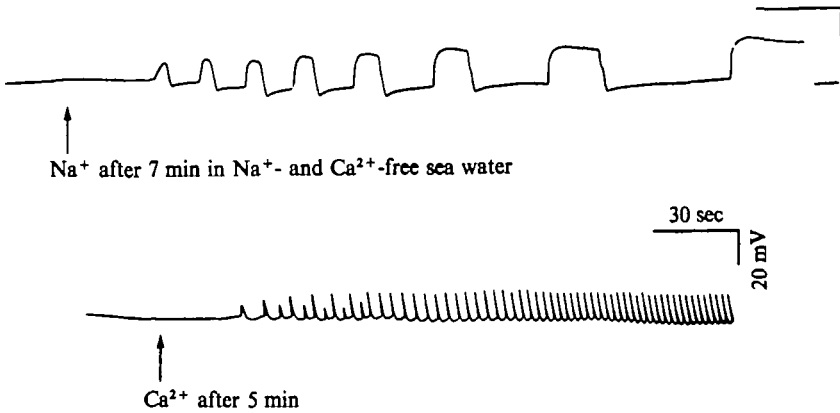


Fig. 7. Selective return of the spike or plateau component. The heart was made quiescent in Na- and Ca-free SW 7 min prior to the addition of sodium (upper trace) and 5 min prior to the addition of calcium (lower trace). Calibration marks are the same for both traces.

to selectively initiate spontaneous activity in either the form of a spike or a plateau. The effects of Na-free, Ca-free, and Na- and Ca-free solutions were fully reversible, and the responses were consistent, regardless of the testing sequence, for periods of 2–3 h. Although some deterioration in the condition of the heart was observed after long exposures to these solutions, the heart appeared to recover fully when each of the three compartments was returned to normal sea water perfusion for several hours. A striking feature of this muscle is, therefore, its ability to withstand prolonged exposures to extreme modifications of its ionic environment.

Calcium spike and Na-free solutions

The effects of calcium deficiency on the duration of the plateau were minimal between 1–10 mM calcium (Fig. 5). Changes in the spike were also minor in this concentration range whereas excess calcium depressed spontaneous activity. Elevated levels of calcium have previously been known to inhibit pacemaker activity in both vertebrate and molluscan hearts (Weidmann, 1955; Krijgsman & Divaris, 1955).

Changing the calcium concentration in the absence of sodium produced similar results. Low calcium decreased spike amplitude and increased its duration (Fig. 8). The increased duration in low calcium and Na-free SW was similar to the effect of manganese in Na-free SW (Fig. 6B). Although not measured directly from a differentiated spike, the rate of rise of the spike (see inset, Fig. 8) was visibly reduced following a decrease in calcium. The sensitivity to reduced calcium, however, was greater in

Na-free SW than was previously observed in sea water with sodium (cf. Figs. 5, 8). The effects of increased concentrations of calcium on the spike were bimodal. At 20 mM- Ca_0 , spike amplitude had increased while the duration was less, as compared with the corresponding parameters of the spike at 10 mM- Ca_0 ; further increases in calcium concentration reversed these effects (Fig. 9). The rate of rise was also measured in this experiment and found to parallel changes in amplitude of the spike. The optimum calcium concentration in Na-free SW varies from preparation to preparation. It appears that calcium is directly involved in the spike current. There is, however, a limit to the ability of calcium to increase the amplitude and rate of rise of the spike.

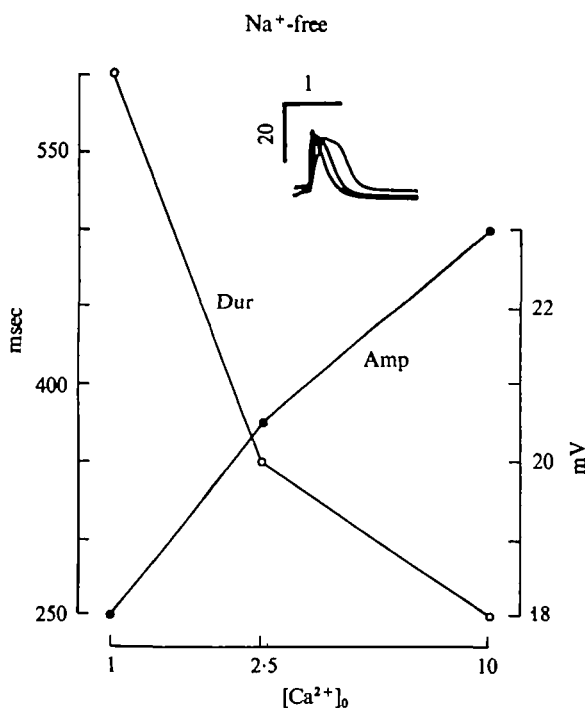


Fig. 8. Effects of reduced calcium in Na-free sea water. Half-amplitude duration (left ordinate) and spike amplitude (right ordinate) versus calcium concentration (abscissa) on a log scale. Action potentials recorded at 1, 2.5 and 10 mM- Ca_0 are photographically superimposed in the inset. Calibration marks are in millivolts and seconds.

Experiments in Ca-free and Mg-free SW

Effects of divalent-free solutions

The absence of magnesium had little effect on the action potential in short-term experiments. When both divalent ions (calcium and magnesium) were omitted, the heart being bathed essentially in isosmotic NaCl (see Table 1, Wilkens, 1971), the heart became depolarized and stopped beating. The depolarization was equivalent in magnitude to the level of the plateaus in Ca-free SW. The additional removal of magnesium, following perfusion in Ca-free SW, again produced a sustained depolarization of the membrane (Fig. 10). This electrical behaviour was similar to the sustained depolarizations observed when EGTA was added to Ca-free SW.

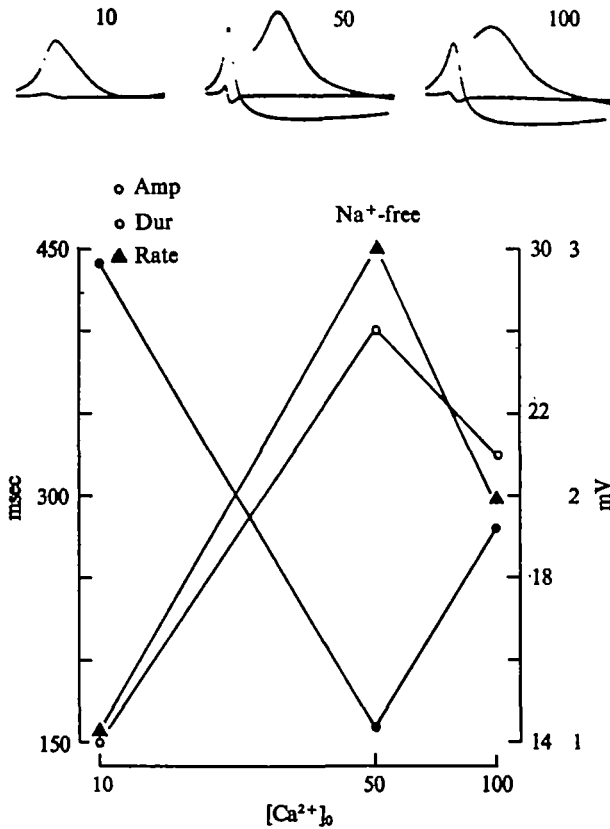


Fig. 9. Bimodal effects of elevated calcium in Na-free sea water. Half-amplitude duration (left ordinate), spike amplitude (right ordinate, mV), and rate of rise (right ordinate v/s) versus Ca_0 (abscissa) on a log scale. Individually recorded action potentials at 10, 50 and 100 mM- Ca_0 illustrate the data. Spikes were recorded simultaneously at different sweep speeds and with a differentiated trace.

The combined effect of eliminating both divalent ions, therefore, is a sustained membrane depolarization close to zero potential. To explain this effect one must assume that the selective permeability of the membrane, which normally maintains the inside-negative cellular potential, has been reorganized. Therefore, in the absence of calcium and magnesium, the permeability regime for sodium and potassium reverses and the membrane becomes more permeable to sodium than to potassium. Alternatively, the membrane may have become completely passive to potassium, thereby reducing the concentration differences of this ion across the cell boundary.

The effects of sodium in Ca- and Mg-free SW

The additional deletion of sodium, following perfusion with Ca- and Mg-free SW, resulted in a repolarization of the membrane, usually to a level greater than the original membrane potential (Fig. 10B-D). By eliminating sodium, the solution perfusing the heart contained only TrisCl (see Table 1, Wilkens, 1971), and in some experiments, Tris plus 10 mM potassium. By readmitting sodium, the membrane could again be depolarized. This cycle was repeated several times with essentially the same results

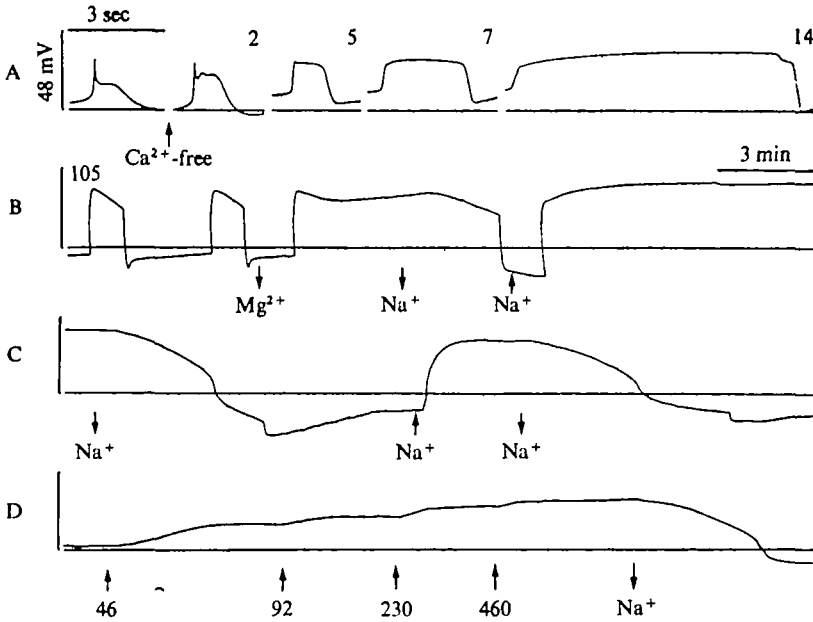


Fig. 10. Effects of changing Na_0 in Ca- and Mg-free sea water. Individual action potentials in (A) were recorded 2, 5, 7 and 14 min after removal of calcium. In (B) after 105 min in Ca-free SW, magnesium was also removed resulting in a stable depolarization of the membrane. A subsequent removal of sodium reversibly repolarized the membrane. This procedure was repeated in (C); sodium was readmitted in steps of 46, 92, 230 and 460 mM in (D). Vertical bars equal 48 mV in each trace; horizontal time mark is seconds in (A), 3 min in (B), (C) and (D). Records are continuous in (B) and (C) and were interrupted 4 min between (C) and (D).

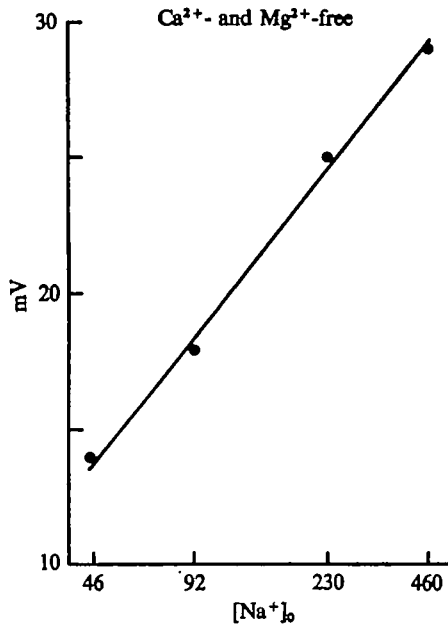


Fig. 11. Membrane potential as a function of sodium in Ca- and Mg-free sea water. Membrane potential (ordinate) versus sodium concentration (abscissa) on a log scale. The data are taken from Fig. 25D.

(Fig. 10C). Stepwise increases in the external sodium concentration were accompanied by partial membrane depolarizations (Fig. 10D). The relationship between the membrane potential and the outside sodium concentration (Na_0) is linear on a logarithmic scale (Fig. 11). The slope of 16 mV per tenfold increase in Na_0 is low, however, indicating that the potential is not a pure sodium effect. Still, a partial reversal of the normal membrane permeability ratio, in Ca- and Mg-free SW, is suggested by the ability of sodium to depolarize the membrane.

Potassium depolarization in Ca- and Mg-free SW

The ability of potassium to depolarize the membrane in the divalent-free condition appears to be determined by the sodium content of the perfusate. A membrane, polarized by a lack of sodium, calcium, and magnesium, was depolarized 13 mV by 5 mM potassium. However, if the membrane was depolarized by readmitting sodium, 100 mM potassium further depolarized the membrane by only 1–2 mV.

Apparently, the membrane does not become less permeable to potassium in Ca- and Mg-free SW. In fact, potassium permeability may have been little affected by the absence of the divalent cations, since depolarization by 50 mM potassium in Na-free, Ca-free and Mg-free SW was 2 mV greater than depolarization by 50 mM potassium in normal sea water (Fig. 4, Wilkens, 1971).

Ca-free experiments in Li-SW

Hearts, beating spontaneously in solutions in which sodium had been replaced by lithium, did not continue to beat when in addition calcium was deleted. Although spikes began to develop long plateaus after 2–3 min in Ca-free Li-SW, the membrane subsequently became depolarized by 15–20 mV and rhythmical activity ceased. That lithium appears to be unable to support prolonged activity in Ca-free solutions is consistent with the fact that lithium failed to support the plateau in normal sea water (Wilkens, 1971).

DISCUSSION

In the heart of *Modiolus demissus* action potentials can be separated into spike and plateau phases by changing the ionic composition of the sea water perfusate.

Ionic dependence of the spike

After short recovery periods *Modiolus* hearts beat for 2–3 h in Na-free SW. Similar responses have been observed in ventricles of *Mytilus* (Irisawa, Shigeto & Otani, 1967) and *Crassostrea* (Irisawa, Noma & Ueda, 1968) and in the taenia coli of the guinea-pig (Bülbring & Kuriyama, 1963). Although spike discharges in the taenia coli persisted for 45–60 min in Na-free solutions, the possibility was not ruled out in these and subsequent experiments (Brading, Bülbring & Tomita, 1969) that sodium contributes directly, if not substantially, to spike currents. Similarly, while the hearts of *Modiolus*, *Mytilus* and *Crassostrea* all beat for 3–6 h in Na-free SW, this evidence is insufficient to exclude the possibility that sodium currents participate in spike generation.

The fact remains, however, that low sodium concentrations had little effect in reducing spike amplitude. Furthermore, in the absence of calcium and magnesium,

sodium was related instead to the slow depolarization seemingly equivalent to the plateau. As expected, tetrodotoxin, in high concentrations, did not significantly alter the spontaneous activity in these hearts. Nor was any effect observed when tetrodotoxin was tested in Ca-free or Na-free solutions.

In Ca-free SW, however, the spike component of the action potential disappeared after 15–90 min. Systolic tension also disappeared in Ca-free SW and the appearance of a close temporal relationship between loss of spike and tension may signal final depletion of bound calcium stores.

The dependence of the spike on calcium was observed directly in the absence of sodium. Spike amplitude and rate of rise were decreased in low calcium and increased in more concentrated calcium although this effect became self-limiting at high concentrations. Clearly, the effects of calcium, over a concentration range of two or more log units, are complex and cannot be explained simply by a linear relation between spike amplitude and the logarithm of the outside calcium concentration. Although the spike is calcium-dependent, calcium nevertheless reduces conductance at high concentrations, apparently by binding rigidly to the membrane. This hypothesis is discussed by Shanes (1958), in terms of the pore-solvent model of the membrane.

Ion dependence of the plateau

Modiolus hearts are characterized by an action potential of variable plateau. Presumably the plateau results from slowly rising inward sodium currents, which have been shown to stimulate an electrogenic sodium pump resulting in a positive after-potential (Wilkins, 1971).

This phase of the action potential, when present, was always reduced in Na-free solutions, whether sodium was replaced by a large impermeant cation (Tris) or by lithium, an ion which supports spike currents in other systems. *Modiolus* hearts and cells of cultured chick hearts (Pappano & Sperelakis, 1969) are exceptions in that lithium fails to replace sodium adequately.

Furthermore, the large, rhythmical rectangular pulses, which continue spontaneously for several hours in Ca-free SW, are believed to develop directly from the plateau. The sodium dependence of these potentials has been established by the experiments in Ca-free and Mg-free SW, in which the potential across the membrane was determined by the concentration of sodium in the medium. In this situation the membrane behaves as a sodium electrode. An almost identical plateau increase was described in the rat myocardium (Garnier *et al.* 1969). In Ca-free and Mg-free solutions containing ethylenediamine tetra-acetic acid (EDTA), as sodium was replaced by saccharose, this membrane also behaved like a sodium electrode with an estimated slope of 59 mV.

It therefore seems reasonable to conclude that, under normal circumstances, the plateau in *Modiolus* heart results from a transient increase in sodium current which is regulated by calcium. The observed variability of this phase of the action potential may be due to differences in calcium metabolism.

The role of calcium in excitability

The membrane-stabilizing functions of calcium have been known prior to the evidence accumulating in many preparations for calcium spikes (Frankenhaeuser &

Hodgkin, 1957). By lowering calcium directly, or by adding Ca-binding agents, resting membranes are depolarized and made to become spontaneously active.

Calcium has similar properties in the *Modiolus* heart, in addition to its role in the spike. Calcium-free SW depolarized the membrane by 10–15 mV, and in three instances a brief membrane activity consisting of small spikes was observed, similar to the effect of citrate on peripheral nerve.

In nerve fibres of the squid and the frog the depolarizing effects of low calcium were reversed or blocked by replacing sodium with choline (Stämpfli & Nishi, 1956). The depolarizing effect of media lacking divalent cations was also reversed in *Modiolus* by removing sodium. Rhythmical membrane depolarizations in *Modiolus* heart nearly reach zero potential in Ca-free solutions, and by eliminating magnesium as well, membrane depolarization is sustained. Similar results have been observed in the heart of the rat (Garnier *et al.* 1969). Thus, membrane permeability to sodium is substantially increased by the removal of the divalent ions.

That potassium failed to depolarize the heart in Ca-free and Mg-free SW was at first surprising. One possibility is that the cation-free effects are due to a marked change in the relative 'resting' membrane permeabilities to sodium and potassium. The change is, apparently, not in P_K ; rather, a large selective increase in P_{Na} occurs, exceeding P_K . The slope of the sodium potential was low in *Modiolus* (16 mV per tenfold increase in Na_0); however, potential shifts resulting from adding or removing sodium in Ca-free and Mg-free SW were as high as 58 mV and, in Ca-free experiments, rhythmical potential shifts of up to 51 mV were observed.

Crayfish muscle fibres respond to low calcium in an almost identical manner to that described here for the heart of *Modiolus* (Reuben *et al.* 1967). Membrane depolarization and increased sodium conductance followed removal of calcium. In addition, when chloride was absent the membrane produced a series of fluctuations whose frequency depended on the concentration of EDTA. The sodium-dependent depolarizations in the crayfish, however, were blocked by TTX whereas those in *Modiolus* were not. Therefore, although the response to low calcium is similar in both muscles, the mechanism of sodium conductance is different.

The role of sodium in excitability

Several lines of evidence have been cited, suggesting that the initial spike component of the action potential in the myocardium of *Modiolus* is, either wholly or in part, due to the influx of calcium across the membrane. However, the reduced rate of rise and the eventual loss of spontaneous activity in Na-free SW still support the possibility that sodium contributes directly to the ionic currents. Koketsu & Nishi (1969) have proposed that the spike in the sympathetic ganglion cells of the bullfrog is normally carried by sodium and that only in low sodium or high calcium does the divalent ion carry the spike. Alternatively, it has been demonstrated that high calcium reduces sodium permeability in crayfish muscle fibres (Reuben, Girardier & Grundfest, 1964); in 13.5 mM calcium, these fibres are impermeable to sodium. In sea water containing 10 mM calcium, sodium permeability in *Modiolus* hearts may also be somewhat limited.

The most probable explanation for spike electrogenesis in *Modiolus* heart is that sodium and calcium contribute partial currents. This contention is supported by the reduced rate of rise in Na-free SW and the slight effects produced by TTX. It is not

known whether sodium and calcium compete for the same channels or carriers (Matsubara & Matsuda, 1969; Neidergerke & Orkand, 1966), and there is no evidence to support a dual-channel hypothesis in this preparation (Geduldig & Junge, 1968).

The lack of clear evidence needed to resolve the precise role of sodium in the spike may be the result of two factors. First is the complexity of the characteristic variability in the membrane potential, spike, and plateau which suggests that the contribution of sodium and calcium to the spike is subject to large individual variations. Secondly, an experimental limitation has been the necessity for maintaining spontaneous activity. In order to determine more quantitatively the ionic mechanisms of the spike, future experiments will be conducted by the technique of double sucrose-gap recording where electrical stimulation is required to initiate the spike.

Comparison of action potentials in bivalve hearts

Studies on the ionic mechanisms of membrane and action potentials by the electrophysiological technique of intracellular recording, or by an equivalent external method, have been reported for three bivalve hearts. The ventricles of *Mytilus edulis* and the Japanese oyster, *Crassostrea gigas*, have been characterized as having a predominantly calcium spike by Irisawa and co-workers. The present study has proposed a somewhat similar ionic mechanism in the heart of *Modiolus*.

Hearts from each of the three species beat spontaneously for several hours in Na-free SW and each was insensitive to tetrodotoxin. These hearts differed somewhat, however, in their responses to Ca-free SW. While the hearts of *Modiolus* and *Crassostrea* produced slow spontaneous depolarizations for several hours in Ca-free SW, activity in *Mytilus* hearts stopped after 20–40 min, with a subsequent decrease in membrane potential (Irisawa, Shigeto & Otani, 1967). There are also inherent differences between these species in the shape of the action potential. Spikes with or without a plateau occur in *Modiolus* hearts whereas in the oysters *Crassostrea virginica* (personal observation) and *Crassostrea gigas* (Ebara, 1969; Irisawa, Kobayshi & Matsubayashi, 1969) the plateau is a constant feature. Although the rate of fall of the spike potential in *Mytilus* is approximately 50% less than the rate of rise, *Mytilus* hearts do not have the plateau (personal observation from sucrose-gap recordings). These observations are in agreement with previous studies on *Mytilus* hearts, using suction electrodes and glass microelectrodes (Irisawa *et al.* 1967; Greenberg, 1970). It is possible that the inability of *Mytilus* hearts to beat in Ca-free SW is related to the lack of a plateau. It is the plateau phase which persists after the loss of the spike in *Modiolus* in Ca-free SW. Calcium sensitivity in *Mytilus* hearts is also relatively high compared with *Modiolus* hearts, since the former failed to beat after exposures to calcium concentrations reduced to 2.3 mM or less in otherwise normal artificial sea water.

The differences in calcium sensitivity and the occurrence of the plateau may also be related to differences in the natural environments of the organisms. *Modiolus demissus* and *Crassostrea virginica* are both found in habitats where salinity fluctuates widely. Thus, the two species which face greater environmental stresses have a plateau and both are electrically active in Ca-free SW, whereas *Mytilus*, which inhabits the more constant subtidal regions, has a greater calcium sensitivity and lacks a plateau.

SUMMARY

1. Electrical activity from the heart of *Modiolus demissus* was recorded simultaneously by sucrose-gap and microelectrode techniques, confirming the validity of the extracellular method of assessing the shape of the action potential.

2. Hearts beat in Na-free SW (Tris) but with loss of the plateau and a reduced rise time. Mechanical contractions did not persist, however, for more than 2 h in the absence of sodium, and the viability of the preparation declined rapidly after 3 h.

3. Lithium substitution (for sodium) also failed to support the plateau and tetrodotoxin had little effect on the spike.

4. Hearts also beat in Ca-free SW. Here, the spike component and contractile activity were lost at nearly the same time although slowly rising plateau-like potentials persisted for over 6 h.

5. EGTA potentiated the effects of Ca-free SW, and manganese had calcium-antagonizing effects producing longer plateaus.

6. Spikes and plateau phases could be selectively initiated from hearts made quiescent in Na-free and Ca-free SW by readmitting either ion individually.

7. In Na-free SW spike amplitude and rate of rise were increased by raising calcium concentration. This effect was self-limiting at high levels of calcium, however, and the spike was reduced.

8. In divalent-free solutions (Ca- and Mg-free SW) the membrane was depolarized to near zero potential. In this situation the membrane permeability to sodium exceeded that to potassium, and the membrane behaved as a sodium electrode.

9. It is concluded that the action potential in *Modiolus* heart is dependent primarily on calcium for the spike component and on sodium for the amplitude of the plateau.

10. Calcium is also important in the control of plateau duration and membrane permeability by reason of its stabilizing properties.

11. A possible correlation exists between the occurrence of the plateau, calcium sensitivity and habitat of three bivalve species. The hearts of *Modiolus* and of the oyster have plateaus and will beat in Ca-free SW, and these bivalves live in estuarine habitats, whereas *Mytilus* lives in a subtidal environment and its heart is sensitive to low calcium and has no plateau component.

The author wishes to acknowledge the direction and support of Dr M. J. Greenberg. Thanks are also due to Dr H. Irisawa for discussions of the results.

Contribution No. 9 from the Tallahassee, Sopchoppy and Gulf Coast Marine Biological Association.

Supported, in part, by U.S.P.H.S. Fellowship No. GM-43039 (General Medical Sciences) and by U.S.P.H.S. Grant No. HE-09283 (National Heart Institute).

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