The ionic basis of cardiac activity in the bivalve mollusc *Perna perna*

Rosângela Pereira Ferreira, Luiz C. Salomão*

*Department of Physiology, University of São Paulo, São Paulo, Brazil*

Received 25 October 1999; received in revised form 7 December 1999; accepted 24 January 2000

**Abstract**

The ionic basis of cardiac activity and aspects of excitation–contraction (E–C) coupling were investigated in the isolated heart of the bivalve mollusc *Perna perna*, using the sucrose-gap technique. The role of the principal ions was established employing artificial seawater, in which specific ion concentrations were modified, and ion channel blockers. The mean membrane resting potential (MP) and the action potential (AP) were $-33\pm0.7$ mV ($n=89$) and $13\pm0.3$ mV ($n=71$), respectively. The MP potential was primarily dependent on K$^+$ ions. Three types of cardiac APs were identified: fast, slow and spike-plateau potentials. Cardiac activity was maintained in Na$^-$ or Ca$^{2+}$-free salines but ceased when either Cd$^{2+}$ or EDTA was added to these salines. Other Ca$^{2+}$ channel blockers reduced the amplitude and increased duration of the cardiac APs. Tetrodotoxin (TTX) and procaine did not alter the AP. The data showed that the depolarizing phase of the AP was dependent on Ca$^{2+}$ influx while the plateau phase, when present, resulted from Na$^+$ influx that was modulated by Ca$^{2+}$. The mechanical responses were more sensitive to changes in extracellular Ca$^{2+}$ concentration than were the electrical responses. © 2000 Elsevier Science B.V. All rights reserved.

**Keywords:** *Perna perna*; Membrane potentials; Heart; Mollusc; Sucrose-gap technique

1. **Introduction**

The mollusc heart is myogenic in nature and its pacemaker cells are modified muscle fibers. However, efforts to identify the pacemaker have failed because this system is believed to be a diffuse structure (Irisawa, 1978). One of the advantages of studying
myogenic hearts is that their activity can be maintained in vitro for long periods in appropriate physiological salines, free from the interference of neural inputs and cardiomodulatory factors.

In vitro electrophysiological recordings of membrane potential (MP) of isolated mollusc hearts, obtained using either intracellular or suction electrodes, or with the sucrose-gap technique, have shown a variety of cardiac action potentials (APs): fast, slow and spike-plateau (Jones, 1983). The initial phase of depolarization was primarily due to Na$^+$ influx, as seen in an oyster species (Irisawa and Kobayashi, 1964), or to Ca$^{2+}$ influx as was seen in Crassostrea gigas (Irisawa et al., 1968) and Spisula sachalinensis (Filippov et al., 1988). The plateau phase derived from a transient Ca$^{2+}$-modulated Na$^+$ current as demonstrated in Modiolus demissus (Wilkens, 1972b) and Mercenaria mercenaria (Devlin, 1993). Fast APs and the spike of spike-plateau APs were Ca$^{2+}$ dependent while the slow AP was Na$^+$ dependent (Jones, 1983).

The aim of the present study was to establish the ionic basis of cardiac excitability in the marine bivalve mollusc *Perna perna* (Mytilidae) and to examine the role of the identified ions in the process of excitation–contraction (E–C) coupling.

2. Materials and methods

Specimens of the intertidal bivalve *Perna perna* (Linne, 1758) were collected from banks off the coast of Sao Paulo State (23° 49′ S; 45° 27′ W), Brazil, and transported to the Department of Physiology of the University of Sao Paulo. They were maintained in 100-l aerated aquaria in sea water of 34½ salinity.

The sucrose-gap apparatus consisted of two cylindrical lucite hemi-chambers, each 13.5 mm long and 17 mm in external diameter, with a central orifice of 3.3 mm diameter. Each chamber also had three 2-mm diameter orifices arranged radially. In the upper chamber, these orifices allowed the inflow and outflow the test solutions, and the insertion of the test electrode, respectively. In the lower chamber, the orifices allowed perfusion with a depolarizing solution (0.5 M KCl) and insertion of the reference electrode. A rubber disk with the same external and internal diameters of the hemi-chambers, covered with thin latex membranes, interposed between them, formed the sucrose chamber. The sucrose chamber was perfused with a 1.0-M sucrose solution through needles inserted radially into the rubber disk.

The hearts were removed from the mussels by first opening a window on the dorsal side of both valves. After exposing the pericardial chamber, the atrio-ventricular regions were tied off, the ventricles isolated and the rectum removed. The isolated ventricles were then inserted into the sucrose chamber through a small hole in the latex membranes, leaving most of the ventricle in the upper hemi-chamber. To record the mechanical activity, one end of the ventricle was tied to the lower chamber while the other was connected to an F-60 myographic transducer connected to a Narco traces 40 physiograph (Narco Bio-System). The RP and AP were recorded with saline bridges connected to the upper and the lower chambers, and calomel electrodes connected either to a voltmeter (Keithley), an oscilloscope (Tektronix 5103 N) or a physiograph (Narco Bio-System).
Test solutions were obtained by modifying Pantin (1948) artificial seawater (ASW) which contains the following salts per liter solution: NaCl (23.43 g), KCl (0.73 g), CaCl₂ (1.12 g), MgCl₂ (5.01 g), NaHCO₃ (0.21 g), Na₂(SO₄)₃ (3.95 g) and NaBr (0.06 g). The osmolality of each solution was measured with a Fiske osmometer. Where specific ion-free solutions were used, the solution was osmotically adjusted with choline-chloride, potassium aspartate or sodium aspartate. Ca²⁺ and Cl⁻ concentrations were measured by microtitration (Beckman-Spinco Model 150) and Na⁺ and K⁺ concentrations by flame photometry (Zeiss PM Q II). The ionic concentrations of the control saline were (in mM): Na⁺ (460), K⁺ (10), Ca²⁺ (10), Cl⁻ (540).

The following ion channel blockers were added directly to the perfusion solutions: tetrodotoxin (TTX) or procaine for Na⁺ channels; cobalt chloride, manganese chloride, cadmium iodide and verapamil for calcium channels; tetraethylammonium (TEA), 4-aminopyridine (4-AP) and cesium chloride for K⁺ channels.

Although the composition of the control ASW was identical in all experiments, inexplicable variability was present in the cardiac activity of each preparation. For this reason, the respective control values were previously established in each experimental condition. All experiments were performed at room temperature (24±1°C).

Data were given as the mean ± standard error of the mean (S.E.M.). Statistical analysis was performed using ANOVA followed by the Student–Newman–Keuls (SNK) test, or a paired t-test. Minimum significance was set at P = 0.05.

3. Results

3.1. Characterization of membrane potentials

Three types of cardiac APs (fast, spike-plateau and slow) were identified in P. perna ventricles perfused with ASW of 34% saline by using the sucrose-gap (Fig. 1). The mean values obtained for the resting potential (RP) and AP amplitude (measured from the RP to the AP peak) were -33 ± 0.7 (n = 89) and 13 ± 0.3 mV (n = 71), respectively. The depolarizing phase was never positive.

3.2. Effects of extracellular potassium and chloride ions

An increase in extracellular K⁺ concentration from 10 to 100 mM caused significant depolarizations at a rate of 18 mV decade⁻¹ rather than the 58 mV predicted from the Nernst equation for a perfect K⁺ selective electrode (Fig. 2). The amplitude of APs recorded in salines containing 1, 5, 10 and 20 mM KCl was not changed, however, in 50 mM KCl, the amplitude was markedly reduced. Reductions in external KCl from 10 to 5 or 10 to 1 mM caused positive inotropic response. Conversely, an increase from 10 to 20 mM KCl induced negative chronotropic and inotropic responses, however not significant by t-test (Fig. 3). At KCl concentrations of 50 mM or greater cardiac activity was reduced and eventually ceased. However, rhythmicity returned when the hearts were perfused with the control ASW.

The addition of the K⁺ channel blocker, TEA (50 mM), caused depolarization (from
Fig. 1. Different types of cardiac action potential obtained by the sucrose-gap method from the isolated heart of *Perna perna* perfused with artificial seawater of 34\% salinity (control). Fast (A), spike-plateau (B), and slow action potentials (C).

\[ -41 \pm 7 \text{ to } -29 \pm 4 \text{ mV} \] and an increase in AP amplitude (from 13\% to 21\%). 4-AP (20 mM) also caused depolarization, and cardiac activity ceased. Cesium chloride (50 mM) did not alter either RP or AP (Fig. 4).

AP amplitude was initially reduced in Cl\(^-\)-free solutions although after 30 min in Cl\(^-\)-free ASW they returned to the control values. During this time, the RP was

![Fig. 2. Relationship between the resting potential and extracellular potassium concentration in isolated heart of *Perna perna*. The asterisks indicate significant differences compared to the control concentration. Data is a mean from eight to 13 hearts.](image-url)
depolarized and returned to initial values only when the hearts were perfused with control saline.

3.3. Effects of sodium ions

A reduction in external Na⁺ concentration caused hyperpolarization of the RP and increased AP amplitude. In external Na⁺ concentration of 47 mM the RP changed from $-36 \pm 2.5$ to $-41 \pm 2$ mV and the amplitude increased from $10 \pm 0.8$ to $13 \pm 0.6$ mV. Cardiac activity was not abolished in Na⁺-free solutions except after the addition of
Fig. 4. Action potentials recorded from isolated hearts of *Perna perna* perfused initially with artificial seawater of 34% salinity (control saline), then either TEA (50 mM) (A), 4-AP (20 mM) (B) or CsCl (50 mM) (C). 4-AP was added from a stock solution at the time indicated by the arrow.

Cd²⁺ (10 mM) (Fig. 5). The Na⁺ channel blockers TTX or procaine did not alter RP or AP. Na⁺ concentrations lower than that of the control saline and Na⁺-free saline caused a negative chronotropic response coupled to increased systolic force (Fig. 6).

3.4. Effects of calcium ions

In Ca²⁺-free solutions, cardiac activity was maintained for at least 30 min; the AP profile exhibited a prolonged plateau phase. A reduction in Ca²⁺ concentration from 10 (control) to 1 mM or an increase from 10 to 40 mM did not affect either RP or AP amplitude. After a 5-min perfusion with Ca²⁺-free solution plus EDTA (2 mM),

Fig. 5. Action potentials (upper trace) and respective cardiograms (lower trace) recorded from isolated hearts of *Perna perna* when perfused with artificial seawater of 34% salinity (control saline), followed by a Na⁺-free saline and a Na⁺-free saline plus CdI₂ (10 mM). CdI₂ was added from a stock solution at the time indicated by the arrow.
Fig. 6. Frequency (A) and systolic force (B) recorded from the isolated heart of *Perna perna* perfused with different Na\(^+\) concentrations. The data are the mean±S.E.M.; asterisks indicate significant differences between the control saline and salines containing different Na\(^+\) concentrations.
depolarization occurred and rhythmicity was gradually lost; the APs exhibited an atypical profile with a prolonged repolarization period (about 4 min) (Fig. 7A). In all cases, the hearts recovered their normal activity after 40-min perfusion with control saline.

Typical response to Ca$^{2+}$ channel blockers are shown in Fig. 7B–D. Cadmium, cobalt or manganese increased AP duration and finally abolished cardiac activity at concentrations of 10, 30 and 40 mM, respectively. Similar results were obtained with L-type channel blocker verapamil (0.65 mM).

Reductions in Ca$^{2+}$ concentration caused a positive chronotropic effect while conversely Ca$^{2+}$ increases caused a negative chronotropic effect. Systolic force was unaffected by calcium concentrations lower than normal, but at 20 mM Ca$^{2+}$, systolic force increased twofold (Fig. 8).

4. Discussion

The AP types known from different molluscs species, fast, spike-plateau and slow,
Fig. 8. Frequency (A) and systolic force (B) recorded from isolated hearts of *Perna perna*, perfused with salines containing different Ca$^{2+}$ concentrations. The data are the mean±S.E.M.; the asterisks indicate significant differences between the control and test salines.
were observed in \textit{P. perna}, using the sucrose-gap methods. The spike-plateau type was induced by reducing Ca\textsuperscript{2+} concentration, as observed in \textit{M. demissus} (Wilkens, 1972b). The RP or diastolic potential was determined primarily by a K\textsuperscript{+} gradient in the range of 10–100 mM external K\textsuperscript{+} and was a linear function of log[K\textsuperscript{+}]\textsubscript{o} within that range. Selectivity to K\textsuperscript{+} in \textit{P. perna} was low (18 mV decade\textsuperscript{-1}) compared to other molluscs (23 mV decade\textsuperscript{-1} in \textit{Helix pomatia}, Kiss and St.-Rózsa, 1973; 45 mV decade\textsuperscript{-1} in \textit{Mytilus edulis}, Irisawa et al. 1967; 46 mV decade\textsuperscript{-1} in \textit{M. demissus}, Wilkens, 1972a; 50 mV decade\textsuperscript{-1} in \textit{L. stagnalis}, Brezden and Gardner, 1984). Although the diastolic potential was closely related to [K\textsuperscript{+}]\textsubscript{o}, Cl\textsuperscript{−} ions were important since depolarization occurred in a Cl\textsuperscript{−}-free solution, an effect also seen in other mollusc species (Wilkens, 1972a; Brezden and Gardner, 1984; Dorset and Evans, 1989).

AP amplitude was reduced only in Ca\textsuperscript{2+}-free solutions although large reductions in Ca\textsuperscript{2+} were needed; this indicated that one component of the depolarizing phase in \textit{P. perna} ventricles was an inward Ca\textsuperscript{2+} current sensitive to the non-specific Ca\textsuperscript{2+} channel blockers and to the L-type channel blocker verapamil. In Ca\textsuperscript{2+}-free solutions there was a conspicuous occurrence of a plateau phase, the duration of which increased with perfusion time. The same effect was observed when the Ca\textsuperscript{2+}-chelator EDTA was added to the saline.

Although AP amplitude did not change in a Na\textsuperscript{+}-free solution, amplitude was enhanced when the Na\textsuperscript{+} concentration was reduced ten-fold suggesting that Na\textsuperscript{+} ions play little or no role in the depolarizing phase. The Na\textsuperscript{+} channel blockers, TTX or procaine did not change the AP, suggesting that the Na\textsuperscript{+} channel present in the heart of \textit{P. perna} was TTX-insensitive as described in the hearts of other mollusc species (\textit{M. edulis}, Irisawa et al., 1967; an oyster species, Irisawa et al. 1968; \textit{M. demissus}, Wilkens, 1972b; \textit{Spisula sachalinensis}, Filippov et al. 1988). Given these results, we suggest that the depolarising phase was due to an inward Ca\textsuperscript{2+} current, and the plateau to Na\textsuperscript{+} influx. The putative Na\textsuperscript{+} channels were sensitive to Cd\textsuperscript{2+} since cardiac activity that persisted in Na\textsuperscript{+}-free saline was abolished by the addition of Cd\textsuperscript{2+}. The plateau appeared to be Na\textsuperscript{+}-dependent and modulated by Ca\textsuperscript{2+} since the duration of the plateau seen in Ca\textsuperscript{2+}-free solution was reduced by the addition of Ca\textsuperscript{2+}. Such modulation does not necessarily imply the existence of a Na\textsuperscript{+}-specific channel but rather, the presence of a non-selective cation channel unable to discriminate between Na\textsuperscript{+} and Ca\textsuperscript{2+} (Hess and Tsien, 1984). We possibly identified a Ca\textsuperscript{2+} channel that, at low extracellular Ca\textsuperscript{2+} concentrations, was permeant to Na\textsuperscript{+}, as that observed in guinea pig cardiac myocytes (Hess and Tsien, 1984). Wilkens (1972b) and Devlin (1993) have suggested that the extracellular Ca\textsuperscript{2+} concentration modulated the transient Na\textsuperscript{+} current responsible for the AP plateau in \textit{Modiolus} and \textit{Mercenaria}. Wilkens (1972b) suggested that such a plateau was characteristic of species exposed to the reduced and variable salinities found in intertidal and estuarine regions. However, species living in stable environments in which Ca\textsuperscript{2+} concentration was fairly stable, did not show the AP plateau. \textit{Perna perna} is found in intertidal regions and was tolerant to a broad range of salinities.

The contractile mechanism in \textit{P. perna} was more sensitive to a reduction in extracellular Ca\textsuperscript{2+} concentration than was the electrical response. During perfusion with a Ca\textsuperscript{2+}-free saline plus EDTA, mechanical activity ceased before electrical activity. Curiously, an initial increase in systolic force was observed concomitant with extracellular Ca\textsuperscript{2+} reduction.
An increase in systolic force was seen when hearts were perfused with either low Na\textsuperscript{+} salines or a Na\textsuperscript{+}-free solution. This may be related to a rise in cytosolic calcium due to: (i) an increase in calcium influx facilitated by absence of Na\textsuperscript{+}, assuming that the same channel was shared by these two ions; (ii) a reduction in Na\textsuperscript{+}-Ca\textsuperscript{2+} antiporter activity as a consequence of the lack of or low Na\textsuperscript{+} concentrations in the extracellular medium.

Deaton and Greenberg (1980), have proposed that the ionic sensitivity of the bivalve myocardium might be distributed by taxon. Bivalves of the subclass Pteriomorphia are sensitive to Ca\textsuperscript{2+} but not to Na\textsuperscript{+} removal, while the opposite is seen in the Heterodonta and Paleoheterodonta. Of 25 species studied, Deaton and Greenberg (1980) found that hearts from the Heterodonta and Paleoheterodonta did not beat in Na\textsuperscript{+}-free seawater, but that activity was maintained in Ca\textsuperscript{2+}-free medium, suggesting a critical role for Na\textsuperscript{+} in cardiac excitability. Conversely, hearts from pteriomorphia bivalves maintained their spontaneous activity in the absence of Na\textsuperscript{+} but not in Ca\textsuperscript{2+}-free medium. The present results for the pteriomorphia mytilid *P. perna* clearly support this hypothesis, although the Ca\textsuperscript{2+} requirement seems to be very low.

**Acknowledgements**

We thank to Centro de Biologia Marinha–CEBIMAR, for supplying the mussels. This work was partially supported by CNPq–Conselho Nacional de Pesquisa, Brazil. [SS]

**References**


Irisawa, H., Shigeto, N., Otani, M., 1967. Effect of Na\textsuperscript{+} and Ca\textsuperscript{2+} on the excitation of the *Mytilus* (Bivalve) heart muscle. Comp. Biochem. Physiol. 23 (1), 199–212.


