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THE IONIC DEPENDENCE ON THE CARDIAC ACTION POTENTIAL IN BIVALVE MOLLUSCS: SYSTEMATIC DISTRIBUTION*

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Abstract—1. Isolated ventricles from bivalve species in the subclasses Heterodonta and Paleoheterodonta stop beating in Na-free media but not in Ca^{2+}-free medium.

2. Ventricles from species in the subclass Pteriomorphia stop beating in Ca^{2+}-free medium but not in Na-free media.

3. The cardiac action potentials of pteriomorphs have a Ca-dependent spike and may or may not have a Na-dependent plateau. Paleoheterodont cardiac action potentials lack a spike; those of heterodonts always have a plateau and may or may not have a spike.

INTRODUCTION

The ionic dependence of the action potentials of different excitable tissues is highly variable (Reuter, 1973; Taylor, 1974; Luttgau, 1977; Almers, 1978). The currents underlying action potentials result from the movement of ions through selectively permeable protein channels in the lipid bilayer membrane (Hille, 1978); thus the tissue specific variation in the ionic requirements for excitability probably reflects qualitative differences in membrane composition and structure.

The ionic basis of cardiac excitability in bivalve molluscs has been examined in detail in only three species: the oyster *Crassostrea gigas* (Irisawa *et al.*, 1968); and two mussels, *Mytilus edulis* (Irisawa *et al.*, 1967) and *Geukensia demissa* (=*Modiolus demissus*) (Wilkens, 1972b). Isolated ventricles from *M. edulis* beat in Na-free seawater, but in Ca-free seawater all electrical and mechanical activity ceases (Irisawa *et al.*, 1967). Ventricles of *C. gigas* and *G. demissa* also beat in Na-free seawater; in Ca-free seawater, mechanical activity stops, although modified action potentials can continue (Irisawa *et al.*, 1968; Wilkens, 1972b).

The shape of the cardiac action potential of *G. demissa* is variable, but two components are usually discernible: a fast spike followed by a slower plateau (Wilkens, 1972b). Deletion of either Ca or Na from the bathing medium results in the loss of the spike or plateau component, respectively (Wilkens, 1972b), suggesting that the myocardial spike in *Geukensia* is dependent largely on Ca, while the plateau depends primarily on Na. A plateau is always present in the action potentials of *C. gigas* hearts, but is not observed in the cardiac action potential of *M. edulis* (Irisawa *et al.*, 1967, 1968). Wilkens (1972b) related the inability of *M. edulis* ventricles to beat in Ca-free seawater to this lack of a Na-dependent plateau component.

The oysters and mussels, including *C. gigas, M. edulis* and *G. demissa*, belong to the bivalve subclass Pteriomorphia (Keen, 1971). Thus, the organisms whose cardiac physiology has been intensively investigated are relatively closely related. Recently, however, Greenberg & Roop (1977) found that isolated ventricles of the clams *Macrocallista nimbosa, Mercenaria mercenaria*, and *Spisula solidissima* would beat in Ca-free seawater but not in Na-free seawater. Moreover, these three species are in the bivalve subclass Heterodonta. These observations, though certainly not representative of all species in the class, still suggested that the sensitivity of bivalve cardiac excitability to deletion of either Ca or Na from the bathing medium might be distributed by taxon.

To test the generality of the proposed distribution, we have surveyed 25 bivalve species to determine whether cardiac excitability in the subclass Pteriomorphia is insensitive to deletion of Na and sensitive to deletion of Ca, while that of species in the subclasses Heterodonta and Paleoheterodonta is insensitive to deletion of Ca but sensitive to Na deletion. The results substantiate the suggested systematic correlation.

We have also attempted, with less success, to relate the shape of the cardiac action potential of these animals to the ionic sensitivity of excitability exhibited by their hearts.

MATERIALS AND METHODS

Animals

Some of the animals used in these experiments were collected from various sites in northern Florida; Alligator Harbor, Franklin County (*Cyrtopleura costata, Chione cancellata, Dinocardium robustum, Trachycardium egmontianum, Cardita floridana, Noetia ponderosa, Modiolus squamosus, Atrina rigida, Aequipecten irradians, Mercenaria campechiensis, Geukensia demissa*); Ochlockonee Bay, Wakulla County (*Rangia cuneata, Polymesoda caroliniana*); Lake Talquin, Leon County, (*Corbicula manilensis, Villosa*

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villosa, Elliptio icterina, Anodonta peggyae); the Ochlockonee River, Gadsden County (*Lampsilis teres, Lampsilis claibornensis, Elliptoidias sloatianus*); and St Joe Bay, Gulf County (*Macrocallista nimbosa*). Additional species were obtained from the Supply Department of the Marine Biological Laboratory, Woods Hole, Massachussetts (*Anadara ovalis, Mytilus edulis, Modiolus modiolus, Spisula solidissima, Ensis directus, Tagelus plebius, Mercenaria mercenaria, Mya arenaria*) or other commercial sources (*Crassostrea virginica, Brachiodontes recurvus, Tresus nuttali, Tivela stultorum, Semele decisa, Saxidomus nuttali, Chama pellucida, Lima scabra*). *Ostrea palmula* was collected from the Miraflores Third Locks Lake in the Panama Canal Zone.

The marine species were maintained in aerated seawater (24–28‰); the freshwater species were kept in aerated water dipped from the mighty Sopchoppy River (Wakulla County).

Ion substitution

Ventricles were isolated by the conventional methods of Welsh and Taub (1948) and Greenberg (1965). The preparations were immersed in aerated organ baths (22–24°C), stretched between a stainless steel hook and a force transducer (Grass Model FT. 0.3C). Mechanical activity was recorded with an ink-writing oscillograph (Grass Model 7). The standard bathing medium for ventricles of marine species was natural seawater; hearts from freshwater clams were bathed with seawater diluted to 50 mOsm with distilled water. Nominally Na-free and Ca-free seawaters were prepared by the methods of Wilkens (1970) and adjusted to the proper osmolality with distilled water. In most experiments, Na was replaced with Tris (Sigma Chemical Co.); in nominally Ca-free medium, Ca was replaced with Na.

Electrical recording

An isolated ventricle was placed in a small Petri plate coated on its bottom with a layer of electrical embedding resin (Dow Corning Sylgard 183). One side of the heart, at the auricular–ventricular junction, was pinned to the dish with a dissecting pin; the other side was attached to a force transducer via a bell crank. Mechanical activity was recorded on one channel of an ink-writing oscillograph (Grass Model 7).

Suction electrodes were constructed from 1 cm^3 disposable plastic syringes attached to a short length of glass pipet with a polished tip (aperture *ca.* 0.3 mm). The voltage difference between the suction electrode and a grounded silver/silver chloride indifferent electrode immersed in the bath, was amplified (Grass Model P6 12) and recorded with the oscillograph.

RESULTS

Survey of ion sensitivity

Typical heart responses from a representative species of each of the subclasses to Na-free and Ca-free seawater are shown in Fig. 1, and the results of the survey are summarized in Table 1.

All of the pteriomorph hearts tested, with the exception of *Modiolus modiolus*, beat in Na-free seawater and were arrested in Ca-free seawater. In contrast, the heterodont and paleoheterodont hearts were spontaneously active in Ca-free, but not Na-free, seawater.

Heterodonta. Nearly all heterodont hearts exposed to Na-free seawater were immediately arrested in diastole. Moreover, after 40 min exposure, the arrest was usually irreversible; repeated washing of the preparation with seawater would not restore spontaneous activity, even if 10^{-5} M 5-hydroxytryptamine was applied. Hearts from some heterodont species were less sensitive to Na-free seawater than others. For example, *Mya arenaria* hearts beat for 10 min in

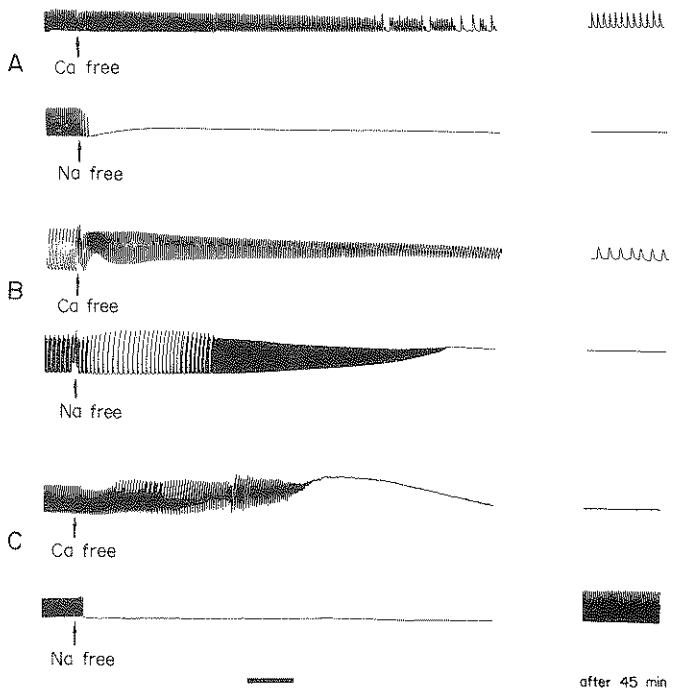

Fig. 1. Typical responses of bivalve ventricles to Na-free seawater and to Ca-free seawater. Record A is *Macrocallista nimbosa* (subclass Heterodonta), B is *Elliptio icterina* (subclass Paleoheterodonta), and C is *Noetia ponderosa* (subclass Pteriomorphia).

Table 1. Response of hearts of bivalve molluscs to Ca^{2+} free or Na^+ free seawater

SUBCLASS Order	Family	Species	Na^+ free	Ca^{2+} free
PTERIOMORPHIA				
ARCOIDA	Arcidae	*Anadara ovalis*	+	0
		Noetia ponderosa	+	0
MYTILOIDA	Mytilidae	*Mytilus edulis*	+	0
		Modiolus modiolus	0	0
		Modiolus squamosus	+	0
		Geukensia demissa	+	0
	Pinnidae	*Atrina rigida*	+	0
HETERODONTA				
VENEROIDA	Carditidae	*Cardita floridana*	0	+
	Carditidae	*Trachycardium egmontianum*	0	+
		Dinocardium robustum	0	+
	Mactridae	*Spisula solidissima*	0	+
		*Rangia cuneata**	0	+
	Solenidae	*Ensis directus*	0	+
	Solecurtidae	*Tagelus plebius*	0	+
	Corbiculidae	*Corbicula manilensis**	0	+
		*Polymesoda caroliniana**	0	+
	Veneridae	*Mercenaria mercenaria*	0	+
		Chione cancellata	0	+
		Macrocallista nimbosa	0	+
MYOIDA	Myidae	*Mya arenaria*	0	+
	Pholadidae	*Cyrtopleura costata*	0	+
PALEOHETERODONTA				
UNIONIDAE	Unionidae	*Lampsilis claibornensis**	0	+
		*Anodonta peggyae**	0	+
		*Elliptio icterina**	0	+
		*Villosa villosa**	0	+

+ = Maintenance of spontaneous contractions. 0 = Cessation of spontaneous contractions. *All seawaters diluted to 50 mOsm for this species. n = 2–10 hearts for each species.

Na-free seawater, and then became quiescent. *Polymesoda caroliinana* and *Corbicula manilensis* hearts beat for 40 and 10 min, respectively, in dilute (50 mOsm) Na-free seawater before stopping.

In Ca-free seawater, heterodont hearts beat for 2 hr or more, but the beat frequency declined slowly during the test period. Amplitude decreased gradually in some preparations, increased in some, and was unchanged in others.

Paleoheterodonta. Paleoheterodont hearts beat at first in dilute Na-free seawater, but the frequency and the amplitude decayed until arrest after 20–30 min. The effects were reversible upon return to dilute seawater.

Paleoheterodont hearts responded to Ca-free seawater with decreased beat frequency and variable changes in amplitude, but arrest did not occur during the test period.

Pteriomorphia. Pteriomorph hearts were transiently arrested by exposure to Na-free seawater, however, following a quiescent period of 10–20 min, spontaneous contractions resumed and continued for 2 hr. In Ca-free seawater, the amplitude gradually decayed until the hearts stopped, usually within 2–20 min. The Ca-free arrest of pteriomorph hearts was reversible upon return to normal seawater.

Sodium substituents

The arrest of heterodont and paleoheterodont hearts in Na-free (Tris) seawater could have been due to some pharmacological effect of the Tris rather than to the deletion of Na. Therefore, hearts from several heterodont, paleoheterodont, and pteriomorph species were exposed to a set of artificial seawaters in which the Na salts were variously replaced by isosmotic concentrations of sucrose, choline chloride, CsCl, LiCl, or RbCl. The results are shown in Table 2.

None of the hearts tested, except those of *Geukensia demissa*, were active in sucrose water. Choline seawater arrested all hearts except those of *G. demissa*, *Modiolus squamosus*, and *Noetia ponderosa*, and hearts of the latter two species beat at a very low frequency. Most, but not all, of the hearts showed some tolerance of Li seawater. *Trachycardium egmontianum*, *N. ponderosa*. *Modiolus modiolus*, *M. squamosus*, and *G. demissa* hearts beat for over an hour in Li seawater; hearts from three other species beat for 20–50 min before stopping. Those hearts which beat temporarily in Li seawater showed a gradual decrease in amplitude until arrest occurred. *Mercenaria mercenaria* and *Chione cancellata*, both heterodonts in the family Veneridae, showed particular sensitivity of cardiac excitability to Li seawater.

Replacement of NaCl by CsCl or RbCl caused immediate systolic arrest in all hearts tested. The contracture was prolonged for 10–20 min, followed by very gradual relaxation.

Ouabain

The effects of 10^{-5} M ouabain on the hearts of a number of bivalve species are shown in Table 3. Ouabain was usually ineffective on paleoheterodont and

Table 2. Responses of bivalve hearts to various sodium substituted seawaters

SUBCLASS	Species	Na substituent	Time to arrest (min)	Length of exposure (min)
HETERODONTA				
	Trachycardium egmontianum	sucrose	0	30
	T. egmontianum	choline	0	60
	T. egmontianum	Li	>60	60
	T. egmontianum	Rb	0	60
	T. egmontianum	Cs	0	60
	Cardita floridana	sucrose	0	30
	Macrocallista nimbosa	sucrose	0	55
	Mercenaria mercenaria	sucrose	0	30
	M. mercenaria	choline	0	60
	M. mercenaria	Li	5	60
	M. mercenaria	Rb	0	60
	M. mercenaria	Cs	0	30
	Chione cancellata	sucrose	0	30
	C. cancellata	choline	0	60
	C. cancellata	Li	0	50
	C. cancellata	Rb	0	60
	C. cancellata	Cs	0	60
	Spisula solidissima	choline	0	60
PALEOHETERODONTA				
	Elliptoidias sloatianus	sucrose	0	60
	Elliptio icterina	sucrose	0	40
	E. icterina	choline	0	40
	E. icterina	Li	2	60
	E. icterina	Rb	0	60
	E. icterina	Cs	0	60
	Lampsilis teres	sucrose	10	60
	L. teres	choline	0	60
	L. teres	Li	30	60
	L. teres	Rb	0	60
	L. teres	Cs	0	60
	Anodonta peggyae	sucrose	0	40
	A. peggyae	choline	5	60
	A. peggyae	Li	20	60
PTERIOMORPHIA				
	Noetia ponderosa	sucrose	0	80
	N. ponderosa	choline	>50	50
	N. ponderosa	Li	>60	60
	N. ponderosa	Rb	0	60
	N. ponderosa	Cs	0	60
	Modiolus squamosus	sucrose	0	50
	M. squamosus	choline	>60	60
	M. squamosus	Li	>60	60
	M. squamosus	Rb	0	60
	M. squamosus	Cs	0	60
	Geukensia demissa	sucrose	>60	60
	G. demissa	choline	>60	60
	G. demissa	Li	>60	60
	G. demissa	Rb	0	60
	G. demissa	Cs	0	60
	Modiolus modiolus	sucrose	>60	60
	M. modiolus	choline	0	60
	M. modiolus	Li	>60	60
	M. modiolus	Cs	0	60
	Mytilus edulis	sucrose	0	40
	M. edulis	Li	>40	40
	Crassostrea virginica	sucrose	15	60
	C. virginica	choline	>90	90
	C. virginica	Li	50	90
	Atrina rigida	sucrose	0	60
	A. rigida	choline	0	60
	A. rigida	Rb	0	60
	A. rigida	Cs	0	60

n = 2–4 hearts for each species.

Table 3. Responses of bivalve hearts to 10^{-5} M Ouabain

SUBCLASS	Species	Effect
HETERODONTA		
	Tresus nuttali	none
	Semele decisa	none
	Tivela stultorum	incr. tone, frequency
	Macrocallista nimbosa	incr. tone, frequency
	Mercenaria campechiensis	incr. tone
	Mercenaria mercenaria	none
	Cardita floridana	none
	Chama pellucida	none
	Saxidomus nuttali	none
	Dinocardium robustum	none
	Spisula solidissima	none
	Mya arenaria	none
PALEOHETERODONTA		
	Lampsilis claibornensis	none
	Elliptio icterina	none
	Anodonta peggyae	none
	Villosa villosa	none
PTERIOMORPHIA		
	Noetia ponderosa	none
	Atrina rigida	incr. tone, then arrest
	Aequipecten irradians	incr. frequency
	Guekensia demissa	incr. tone
	Modiolus squamosus	arrest
	Brachiodontes recurvus	incr. tone, frequency
	Ostrea palmula	incr. tone
	Crassostrea virginica	none
	Lima scabra	none

heterodont hearts (except those of some venerids), but elicited positive tonotropic responses from most pteriomorph hearts.

Electrical activity

Suction electrode recordings of the cardiac action potentials of several species from each of the three subclasses studied are shown in Fig. 2. The action potentials of heterodont and paleoheterodont hearts are slow and the plateau phase is prominent. Although a spike may or may not be present in heterodonts, paleoheterodont cardiac action potentials lack this component entirely. Action potentials of pteriomorph hearts are generally spike-like, and the plateau phase is often, though not always, lacking.

DISCUSSION

The ionic sensitivity of the bivalve myocardium appears to be related to taxon. No heterodont or paleoheterodont hearts were able to beat in seawater with Tris replacing Na, suggesting that Na is required to maintain cardiac excitabiliy in these two subclasses. Pteriomorph hearts, with the exception of those of *Modiolus modiolus*, are spontaneously active in Tris seawater, but not in Ca-free seawater. Cardiac excitability in this subclass appears to be insensitive to lack of Na, but the presence of Ca is required.

Relatively low concentrations of Tris irreversibly reduce the force of contraction of rabbit hearts and attenuate the responses of vascular smooth muscle to vasoactive substances (Gillespie and McKnight, 1976;

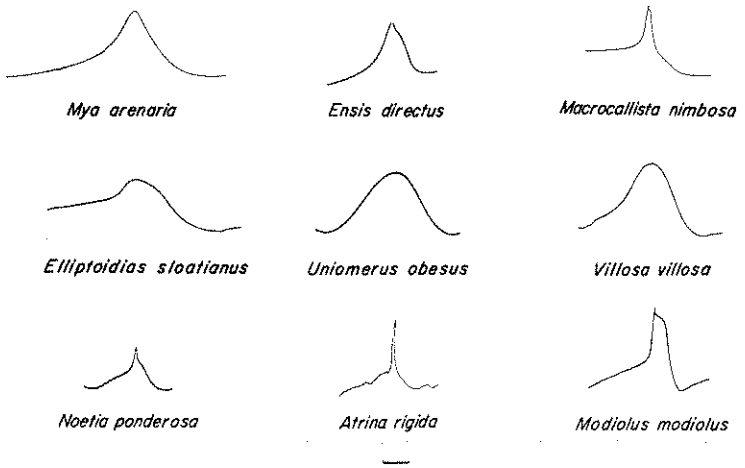

Fig. 2. Cardiac action potentials of selected bivalves. Time mark = 1 sec.

Prasad *et al.*, 1978). In molluscan neurons, 10 mM Tris attenuates acetylcholine (ACh) responses, suggesting that Tris has some action as an ACh antagonist (Wilson *et al.*, 1977). Since many bivalve hearts are quite sensitive to ACh (Welsh and Taub, 1948), the failure of some of them to beat in our Na-free seawater (540 mM Tris) might be due to a depressor effect of the Tris.

The threshold for ACh depression of pteriomorph hearts is generally 1–3 orders of magnitude above that for heterodont hearts, but paleoheterodont hearts, which also do not beat in Tris seawater, are also relatively insensitive to ACh (Greenberg, 1965). In addition, we found no difference in the responses of heterodont hearts to Tris seawater in the presence or absence of 10^{-6} M benzoquinonium, a potent ACh antagonist in bivalve hearts (Ladd & Thorburn, 1955).

The effectiveness of Na substituents other than Tris is more equivocal, but the results do not contradict the suggested correlation. As expected, none of the heterodont or paleoheterodont hearts beat in sucrose seawater. Among the pteriomorph hearts tested, only that of *Geukensia demissa* beat in sucrose seawater, despite the presence of normal Ca concentrations in this medium.

Hearts from four pteriomorph species were able to beat in choline seawater, again suggesting Na independence of cardiac excitability in this subclass.

Lithium ion has been shown to substitute effectively for sodium in several invertebrate excitable tissues (Hodgkin, 1951; Gardner & Kerkut, 1968; Sattelle, 1972). Wilkins (1972a) found that relatively brief exposure to Li seawater arrested *G. demissa* hearts; we have been unable to repeat this result. Presumably Li is unable to substitute for Na in the sodium pump (Gardner & Kerkut, 1968), and arrest may ensue in hearts when disruption of the Na pump depolarizes the membrane. Wilkins (1972a) found that ouabain depolarized *G. demissa* hearts by 2–3 mV. It is, however, difficult to relate ouabain sensitivity and Li sensitivity. *Mercenaria mercenaria* hearts are insensitive to ouabain but stop beating immediately in Li seawater. *G. demissa* hearts beat well in Li seawater, but ouabain produces a pronounced positive tonotropic effect. The sodium channels in *M. mercenaria* hearts (and those of other venerid species) may be much less permeable to Li than the Na channels of other bivalve hearts.

Cesium and rubidium seem to act like K, depolarizing the tissue and causing prolonged contracture of the hearts.

Recently, Plumb (1979) found that intracellular Ca is regulated partly by exchange diffusion of external Na and internal Ca at the sarcolemma in the ventricle of the heterodont clam *Tivela stultorium* (family Veneridae). Intracellular myocardial Ca in the pteriomorph *G. demissa*, in contrast, shows no sensitivity to external Na. This mechanism, if general to heterodonts, may account for the sensitivity of their hearts to Na-free media. However, if Li is unable to substitute for Na in the exchange diffusion process, Li seawater should not maintain the contractility of heterodont hearts. Venerid hearts are quite sensitive to Li seawater, but *Trachycardium egmontianum* (family Cardiidae) hearts are not.

It is tempting to associate the shape of the cardiac action potential of a bivalve species with the ionic requirements for excitability of the heart: spike-only action potentials, such as those of the pteriomorphs *Mytilus edulis* and *Atrina rigida* are characteristic of Ca-sensitive hearts, while plateau-only action potentials, such as those of paleoheterodonts and some heterodonts (e.g., *Spisula solidissima*) are associated with Na sensitivity. A complication is presented by cardiac action potentials in both the subclasses Heterodonta and Pteriomorphia with both spike and plateau, but differing sensitivities to deletion of ions from the bathing medium. *Noetia ponderosa* hearts are insensitive to Na deletion, but the cardiac action potential has some evidence of a plateau. The cardiac action potential of *Macrocallista nimbosa* has a small spike, but the heart is insensitive to Ca deletion.

Studies of bivalve phylogeny show a closer relationship between the subclasses Heterodonta and Paleoheterodonta than between either of these two and the subclass Pteriomorphia (Cox, 1960; Scarlato & Starobogatov, 1978). The results of this survey provide considerable physiological evidence in support of this view.

Further surveys of large numbers of species are necessary to substantiate our suggested correlation. Certainly our results show that there can be considerable variability in the expression of excitability of homologous tissues within a class. Also, generalization of pharmacological and physiological results from hearts of a few species of Bivalvia to be representative of the entire class is probably unwarranted.

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