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Effect of thermal and salinity stress on *Perna viridis* heart (L.)

Shaila Bhat & P V Desai*

Department of Zoology, Goa University, Taleigao Plateau, Goa 403 205, India

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Cardiac electrophysiology of *Perna viridis* mussels showed fast action potentials under stress. The time course of fast action potentials under salinity stress was 4.0-4.6 msec and an amplitude range of 2.71 mV-5.81 mV. The mussels exhibited a time course of 1-5.25 msec and an amplitude of 2.5 mV-5.5 mV under thermal stress.

Most of the marine life is confined to a much narrower biokinetic zone extending from about 27°-37°C. Hence, a slight change in temperature can affect the physiological processes, mobilisations and energy consumptions. Water is used as coolants to various industries and release of such waters previously used as coolants can warm the receiving water bodies, thereby subjecting the aquatic forms present therein, to thermal stress. There is hardly any report on the effect of thermal and salinity stresses on cardiac electrophysiology of mussels. Therefore, an attempt has been made to study the electrical properties of the *Perna viridis* heart under thermal and salinity stress.

Materials and Methods

Perna viridis were collected from their local habitat (Mandovi estuary, 73° .52′ .30″ E long, 15° .33′ N lat) and maintained in well aerated tanks containing sea water collected from their natural habitats. The animals were allowed to acclimate to the following laboratory conditions: housed in tanks (30l) containing sand at the bottom and sea water (salinity=30 ppt) maintained at 26°-27°C and fed on dried, powdered marine algae and diatoms for 15 days. Then, they were subjected to salinity stress by exposing them to diluted sea water (1, 5, 10, 20 and 30% dilution, which corresponded to salinity of 29.7, 28.5, 27, 24 and 21 ppt respectively) for 48 hr, and then used for electrophysiological studies. The sea water was diluted with double glass distilled water and then the salinity was estimated using salinometer (Systronics, India; accuracy ±0.1 ppt). The thermal stress was induced by subjecting the exposed hearts to the natural sea water maintained 0°, 5°, 10°, 20°, 30°, 40°, 50°, 60°C for 15 min. For each temperature regimen, separate controls and experimental sets were used. The electrical properties of the hearts were recorded by placing platinum electrodes (0.35 mm diam. and resistance 0.1Ω) on the ventricular surfaces and connecting them to the computerised Unkelscope (MIT, USA; version 1984). Source for vertical trace used was analog 0, with a span of 50 mV full scale. Span on horizontal trace used was 200 sec full scale and the sample rate was 0.2s at 5 Hz. In all, 256 samples were recorded per animal per set. For each experiment 5 sets of 5 animals each were used. The readings were taken only when they steadied relatively and consistently showed same pattern. The significance and the standard deviations were calculated following the routine statistical procedure with the help of computer program Sigma Stats.

Results

The heart of the control animals exhibited slow action potentials (SAPs), spike plateaus (SPs), isoelectric phases (IPs) (Fig. 1a), but there were no fast action potentials (FAPs) i.e. APs with sharp amplitudes. The salinity stress ranging from 1-30% dilution induced production of FAPs (Fig. 1b and c). The dilution of sea water from 1 to 10% induced gradual decrease in the number of SAPs per minute (Table 1). But dilution of sea water (1-30%) induced disproportionate increase in the number of FAPs, with reference to control. All the

*Correspondent author

dilutions except 30% induced two-fold increase in FAPs, with reference to the number of SAPs. The 10% dilution of sea water induced maximum number of SPs. The increases and decreases in the APs were not directly proportional to the dilution of sea water.

When the duration of the APs were studied (Table 2), it was observed that 30% dilution produced FAPs with 4.0 msec durations, but for the rest of the dilutions, the durations of the FAPs remained above 4.0 msec, but less than 4.7 msec. The SAPs did not show much alterations in duration. The SPs exhibited maximum duration for 10% dilution and a decrease for 1% dilution. The IPs were having reduced durations for 1, 10 and 30% dilutions.

The SAPs showed maximum amplitude at 1% dilution of sea water. The FAPs exhibited amplitudes in the range of 2.71 mV to 5.81 mV. The maximum amplitude (5.81±2.84 mV) was observed for 20% dilution of sea water.

When the electrical properties of the *Perna* were studied under thermal stress, it was found that thermal stress introduced FAPs (Fig. 2b and c; Table 3). Thermal stress induced reduction in the number of SAPs, with no SAP in the initial phase.

Table 1—Electrical properties of heart of *P. viridis* under salinity stress

Dilution (%)	SAPs (/min)	FAPs (/min)	SP (/min)
Control	31.0±2.77	nil	11.0±1.48
1	29.0±1.26	72.0±2.93	10.0±2.68
5	13.0±1.78	66.0±4.00	11.0±1.78
10	10.0±2.82	27.0±2.09	22.0±1.78
20	25.0±1.78	56.0±0.89	10.0±5.76
30	42.0±3.34	34.0±2.82	17.0±1.89

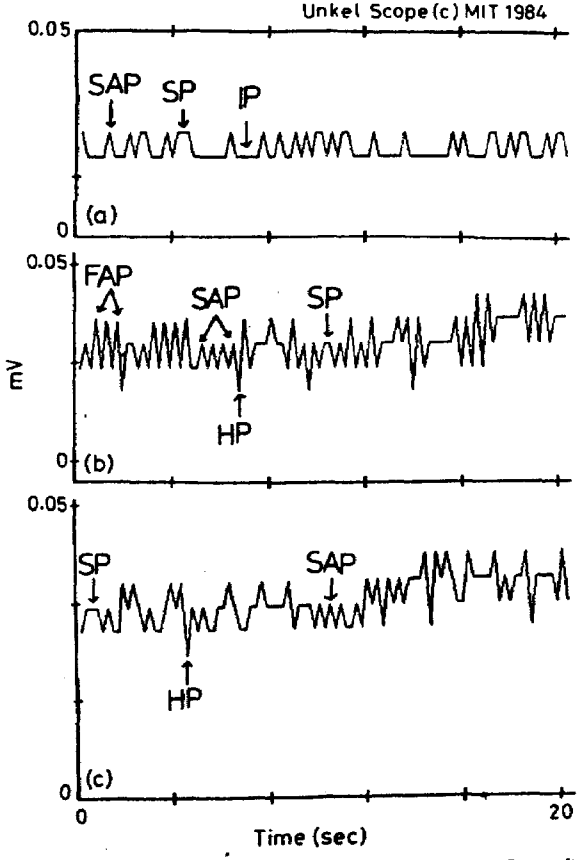

Fig. 1—Effect of different dilution of sea water on *P. viridis* heart mussels. [a. Control, b. 1%, c. 38%. SAP=slow action potential; SP=spike plateau; IP=isoelectric phase; FAP=fast action potential; HP=hyperpolarisation]

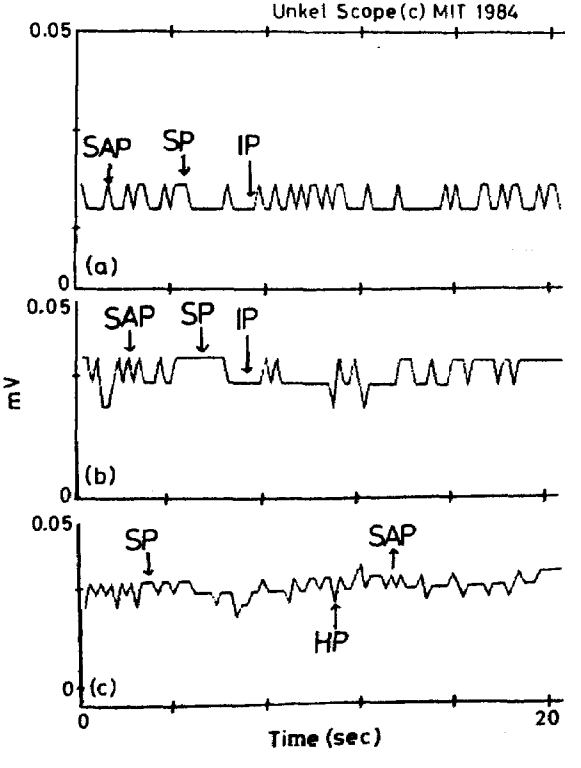

Fig. 2—Effect of different temperatures on *P. viridis* heart mussels. [1. Control, 2. 10°C, 3. 40°C. SAP=slow action potential; SP=spike plateau; IP=isoelectric phase; FAP=fast action potential; HP=hyperpolarisation]

Table 2—Duration and amplitude of action potentials under the ifnluence of salinity stress on *P. viridis*

Dilution (%)	C	1	5	10	20	30
BAPs (m sec)	4.25±0.50	4.50±0.00	–	–	4.34±0.45	4.73±1.18
FAPs (m sec)	–	4.14±0.36	4.12±0.30	4.60±0.54	4.50±0.00	4.00±0.00
SP (m sec)	3.24±1.25	2.50±0.00	–	7.08±4.69	5.25±4.59	4.00±0.00
IP (msec)	4.50±0.81	2.50±00	6.25±0.35	2.50±0.00	–	2.25±0.33
HP (msec)	–	2.50±0.00	–	–	2.25±0.35	4.00±0.00
SAPs (mV)	2.50±0.00	7.60±3.86	–	–	7.50±0.00	3.75±1.44
FAPs (mV)	nil	4.00±0.00	2.80±0.29	2.71±0.36	5.81±2.84	3.12±1.25

Table 3—Electrical properties of heart of *P. viridis* under thermal stress

Temp. (°C)	SAPs (/min)	FAPs (/min)	SP (/min)
Control			
27	31.0±2.77	–	11.0±1.48
0	4.0±1.49	24.0±2.87	28.0±3.10
5	6.0±1.35	24.0±5.54	18.0±1.78
10	10.0±0.05	17.0±1.67	24.0±3.40
20	12.0±2.09	20.0±4.38	10.0±3.05
30	30.0±1.41	10.0±1.41	20.0±3.74
40	8.0±1.01	65.0±2.96	19.0±1.41

Maximum number of FAPs were induced at 40°C, while the lowest numbers were found for 30°C. The thermal stress induced increase in the number of SPs for all the temperatures except 20°C. Duration of APs (Table 4) show a range of 1-7 msec. Maximum reduction in time course for FAPs was observed at 20°C. Maximum increase in the time course for spike plateau was seen at 60°C. Maximum reduction in time course of spike plateau was seen at 20°C. FAPs had an amplitude range of 2.5-6.3 mV.

Under salinity stress, the dominant changes were observed for 10% salinity dilutions, where the resting potentials (RPs) showed considerable increase. The 20% dilutions eliminated the SPs in the first 5 seconds (Table 5).

Under the influence of thermal stress, sharp upsurgence in the RPs and amplitudes were observed; the predominant changes were for 60°C (Table 5).

Discussion

Extracellular recordings from whole ventricle in molluscs exhibit only FAPs, IPs, SPs and hyperpolarisations, but not slow Aps[1-6]. But, in contrast in the present study, the control animals exhibited only SAPs. Hence, introduction of FAPs under thermal and salinity stress indicate the promotion of Na^+ and Ca^{2+} flux and stress on the heart, as FAPs are known to be caused by Ca^{2+} transport[6].

Under the condition of minimum to moderate salinity stress, rise in the frequency of FAPs could be compensatory response for the reduction in SAPs indicating that the heart exhibits a switch-over from Na^+ dependency to Ca^{2+} dependency, as FAPs and spike part of spike-plateau Aps are reported to be Ca^{2+} dependent for *Mytilus*[7], *Crassostrea gigas*[8], *Geukensia*[1,2], and *Helix*[9,10]. SAPs and plateau phase of spike plateau are reported to be sodium dependent[1,2] for *Guekensia* and for *Dollabella*[11]. Similar observations were reported by Deaton and Greenberg[12].

Reduction in the number of IPs also indicates stress on the heart. Salinity decrease appears to induce K^+ influx causing hyperpolarisations. Thus, salinity stress could be promoting opening of K^+ channels.

Table 4—Duration and amplitude of action potentials under the influence of thermal stress on *P. viridis*

	Temp.(°C)							
	C	0	5	10	20	30	40	60
SAPs (msec)	4.25±0.50	–	–	–	–	–	–	–
FAPs (m sec)	–	4.00±0.75	4.12±0.25	4.66±1.27	1.00±0.00	4.37±0.70	4.62±1.37	5.37±2.12
SP (msec)	3.25±1.25	4.00±0.00	2.50±0.00	5.25±3.88	1.85±0.00	–	4.66±0.57	7.00±0.00
SAPs (mV)	2.50±0.00	–	–	–	–	–	–	–
FAPs (mV)	–	2.50±0.00	2.50±0.00	3.08±0.19	2.51±0.00	3.00±0.00	5.50±2.07	6.30±2.10

Table 5—Amplitude of action potentials under the influence of salinity and thermal stress on *P. viridis*

Salinity : Dilutions (%)	SAPs (mV)	SPs (mV)	FAPs (mV)
Control	24.0	24.0	–
1.0	22.0	22.0	24.0
5.0	24.5	24.5	28.0
10.0	49.0	49.0	49.0
20.0	24.5	–	26.5
30.0	19.5	19.5	22.0
Thermal: Temp. (°C)	SAP (mV)	SPs (mV)	FAPs (mV)
Control			
27	24.0	24.0	–
0	39.0	39.0	39.0
5	36.0	36.0	36.0
10	32.0	32.0	32.0
20	36.5	36.5	36.5
30	32.0	–	32.0
40	78.0	78.0	78.0
60	120.0	118.0	124.0

The decrease in the number of SAPs and increase in FAPs under thermal stress indicate switch-over from Na^+ influx to Ca^{2+} influx and in the early phase there is even total loss of SAPs indicating non-operation of Na^+ channels, but the presence of spike plateaus indicate coupled operation of Na^+ and Ca^{2+} channels. The thermal stress from 0°-40°C appears to promote operation of K^+ channels. Decrease in the number of hyperpolarisations at 50°C and 60°C indicate reduction in the opening of K^+ channels.

The wide fluctuations in amplitudes under salinity and thermal stress may be due to the stretch during contraction although not due to the altered time course of contraction[13] and may partly be due to the impaired ion channels.

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