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Original Article

Effects of season (summer & winter) on electrical characteristics of skeletal muscle membranes of the spiny-tailed lizard, *Uromastix hardwickii*

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Abstract

This study deals with the observation of changes with temperature variations of the seasons in the muscular electrical excitability in the reptile *Uromastix hardwickii*. Freshly captured adult animals of both the sexes were used in all the experiments, and the gastrocnemius (skeletal) muscles were dissected out. The muscle samples were digested with digestive fluid (pepsin & Hcl), stirred, settled and supernatant was removed, till whitish fluid having clear cells obtained for patch clamp recording of ionic currents and potentials. Resting membrane potentials and action potentials of reptilian cell membranes were measured in whole cell current mode. The glass microelectrodes, with a tip diameter 2–3 µm and tip resistance 5–6 MW (when filled with intracellular solution) were used in these experiments.

The present study was carried out to investigate the electrical characteristics of the skeletal muscles of this species of *Uromastix*, which are not studied earlier.

The average mean values of resting membrane potential, action potential and its durations showed no significant changes with the change in the season, but other components of action potential including threshold potential, after-potential and its duration were found to be increased significantly ($P<0.05$) in summer as compared to winter.

Temperature dependency of these parameters with seasonal variation, are studied for the first time in the gastrocnemius (skeletal) muscles of *Uromastix hardwickii*. Hence seasonal changes in the components of action potential are invariably associated with changes in environmental temperature, and may be responsible for changes in the activities and homeostasis of these animals; and possibly indicating underlying mechanism of hibernation.

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Introduction

Uromastix hardwickii, a spiny-tailed lizard (Fig. 1), is found in the desert areas of Asian & African countries. These lizards are mainly herbivorous; and are hunted by local people to extract oil from its fat. This oil is generally used to relieve pain and as a cure for impotency (1–3).

Fig. 1 : *Uromastix hardwickii.*

The cell membrane of reptilian skeletal muscle has been demonstrated for being permeable to Na^+, K^+ and Cl^-. Hence, an electrical potential difference is thereby generated across the cell membrane making outside of the cell membrane positive and inside negative that gives rise to the resting membrane potential (4–6).

Electrophysiologists use "*patch-clamp technique*" (Fig. 2), a refinement of voltage clamp, to study single and multiple ion channels in cells to record the membrane currents/potentials of the cells, especially in excitable nerve & muscle cells (7–9).

Electrophysiological characteristics of skeletal muscles of lizard species', male desert iguanas (Dipsosaurus dorsalis) and of motor neurons of the song birds, white crowned sparrows (Zaonotrichia leucophrys gambelii) have been investigated (10, 11), but the electrical characteristics of the excitable tissues (nerves and muscles) of *Uromastix hardwickii* are lacking.

Fig. 2 : Patch-clamp technique set-up to record the electrical parameters.

Purpose of study

Our interest in this cold-blooded reptile, *Uromastix hardwickii* is that it has undergone certain specific adaptations to survive in hot desert environment (12) where it can maintain its muscular contractile activity at temperatures even above 40°C (13). Also is a hibernator and undergoes winter sleep but occasionally shows moderate activity during colder seasons (12). Secondly, this animal is economically very cheap to obtain in the laboratory and is easily available throughout the year.

The survey of literature has revealed that this animal *Uromastix hardwickii*, has been neglected and scarce investigations related to its osmolal (14) and mechanical (2) characteristics of the skeletal muscles (excitable tissues), and some ecological aspects (1) are available, that are not enough to understand its physiological characteristics; unless it's electrical properties are not investigated. Therefore the present study was carried out to investigate the electrical characteristics/parameters of the skeletal muscles of *Uromastix hardwickii*.

Materials and Methods

The study was carried out in peak winter months of December & January, the temperature ranged between 20 to 24°C; and summer months of June & July between the temperature range of 32 to 36°C,

at University of Karachi, Pakistan.

Freshly captured/supplied animals (adults) of both the sexes were used in all the experiments. In the laboratory, the animals were kept at room temperature ranging from 20–38°C (av: 25°C). Since their biochemistry is reported to change with season (15), all the animals of a fresh batch were used up within a week, and for experimental purpose, the animals were killed by decapitation and the gastrocnemius (skeletal) muscles of both the limbs were dissected out (Fig. 3), the dissected muscles were kept in buffer/bath solution (2), that was gassed with oxygen to maintain its oxygen and carbon dioxide concentrations (7–9). The international standard was followed for handling animals, including decapitation. In addition, the animal research ethics committee, Faculty of science, University of Karachi, Pakistan has approved the procedures and handling of animals.

1 gm muscle sample was digested in 10 ml digestive fluid, containing 1% (w/v) pepsin (0.1 gm) and 1% (v/v) Hcl (0.1 ml) and 9.9 ml of Deionized water. Magnetic stirring of the mixture was carried out for 3 hours at 37°C, the digest was settled for 20 minutes and 2/3rd supernatant removed. The deposit was filtered through 355 µm mesh, settled again for 20 minutes and then supernatant fluid removed. Afterwards the sediment was washed with warm (37°C) Deionized water, settled and supernatant removed till the whitish fluid volume of 1 ml was left, having the clear muscle cells for electrical recording,

Fig. 3: Dissection of the animal, Uromastix hardwickii.

the protocol for enzymatic digestion for isolating cells was used as described by (16). The isolated muscle cells were placed in a bath solution gassed with oxygen before electrophysiological recordings to maintain proper oxygen concentration; for electrical recordings, only healthy cells were patched. Those cells that showed less than – 50 mV resting membrane potential after whole cell, and cell capacitance change more than 10% during experiment were excluded from the data (7–9).

The membrane potentials were measured by the patch-clamp method as described by (7), the glass microelectrodes used in patch-clamp technique had tip diameter 2–3 µm, tip resistance 5–6 MΩ (9). The microelectrodes were filled with intracellular solution (in mM 145 KCl, 10 NaCl, 10 EGTA, 1 MgCl, 2 CaCl and 10 Hepes buffer; by using 1ml syringe (8). The muscle cells mounted in microscope were perfused with extracellular or general reptilian buffer solution (GRB) (2), gassed with oxygen; the intracellular solution, used in patch-pipette had lower osmolality (266 mOsm/L) than that of extracellular buffer solution (307 mOsm/L); in order to improve seal formation (7, 8) and prevent cell swelling which may occur during long time recordings (17). The currents were injected from pulse generator (300 pA – 500 pA) to excite cells to produce action potential (7–9).

The parameters measured by patch-clamp technique, were: resting membrane potential (RMP), threshold potential (THP), action potential (maximum depolarization potential, repolarization potential), after-hyperpolarization potential (AHP) /+Ve after-potential and their durations. Na^+ Inward currents during the elicitation of action Potential using K^+ channel blocking drug, tetraethylammonium (18); K^+ outward currents during elicitation of action potential using Na^+ channel blocking drug, tetradotoxin (19).

All of the calculations including multiplications, divisions, averages, standard errors and 't' tests and P-values done in the present work were carried out on MS Office Excel and Minitab version 13.30. The one way analysis of variance (ANOVA) was evaluated. P-Value approach was adopted which suggested the evidence in favor of or against the null hypothesis; keeping in consideration the degree of freedom for

variation associated “between the treatments” (peak winter & summer) and “within the treatment”. P<0.05 considered statistically significant (20).

Results

Patch clamp recordings of electrical parameters (resting membrane potential, action potential) were carried out in winter & summer, given in Table I, and also shown in Fig. 4 and 5.

By comparing the electrical parameters obtained during peak winter and summer months, it was observed that :

i) Resting Membrane Potential (RMP) (mV): Average mean values mentioned in Table 1, showed insignificant difference, from winter to summer.

ii) Action Potential:

a) Maximum depolarization/action Potential (mV): The average values of this phase of action potential (Table I), showed insignificant difference in between peak winter and summer months.

b) Duration of depolarization (ms): According to the average values of this electrical parameter (Table I), which was measured from threshold potential toward the peak, were found to have insignificant difference in between peak winter and summer months.

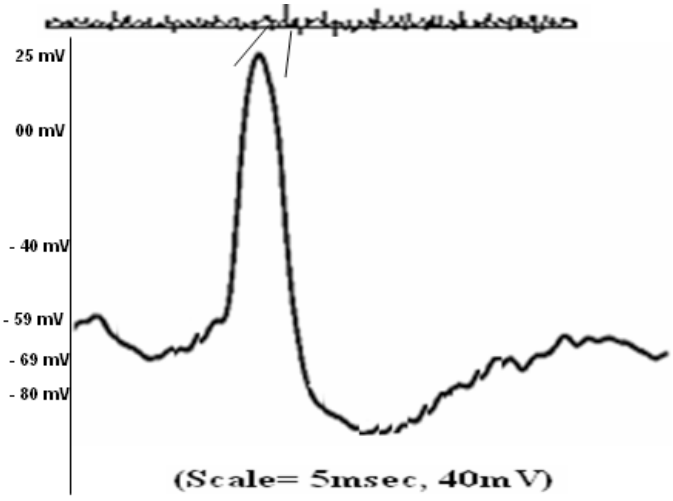


Fig. 4 : Patch-clamp recording of membrane potentials in winter.

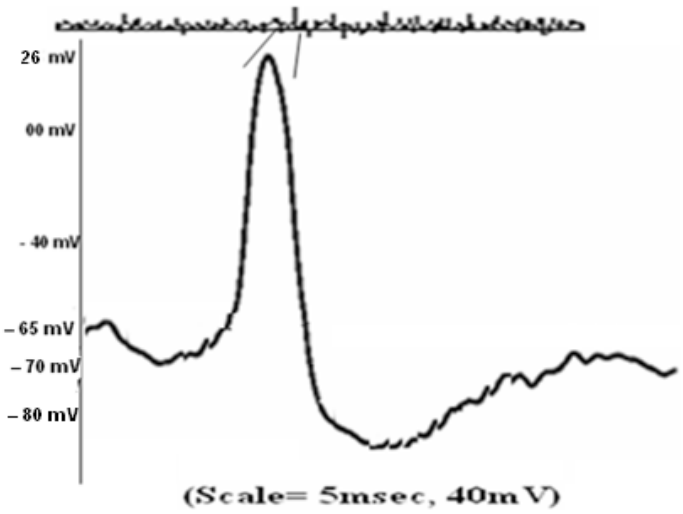


Fig. 5 : Patch-clamp recording of membrane potentials in summer.

c) Threshold potential (mV): According to Table 1, the average values of this electrical parameter, were found to have highly significant (P<0.01) difference in between peak winter and summer months. This difference demonstrated 10% lesser values of this parameter in summer months.

d) Maximum After-hyperpolarization Potential (AHP) (mV): The average values of this electrical parameter mentioned in Table 1, demonstrated highly significant (P<0.01) fall from peak winter towards summer months. This fall was calculated to be 9% from winter in the average values of maximum hyperpolarization potential.

e) Duration of repolarization (ms): According to Table I, the average values of this electrical parameter were found to decrease insignificantly in between peak winter and summer months.

f) Duration of AHP/+Ve after-potential (ms): The average values of this electrical parameter mentioned in Table I, highly significant (P<0.01) fall was noted, this fall was calculated to be 12% toward summer.

TABLE I : Seasonal effects on electrical characteristics (parameters) of skeletal muscle membranes, of *Uromastix hardwickii*, obtained by Patch-Clamp technique during peak summer and winter months.

S. No.	*Parameters*	*Summer*	*Mean ±S.E.M*	*Winter*	*Mean ±S.E.M*	*Significance level summer v/s winter*
1	Resting membrane potential (RMP) (mV)	–70	±0.63	–69	±0.4	P>0.05
2	Action potential:					
	a Threshold potential (mV)	–65	±0.49	–59	±0.4	P<0.01
	b Maximum depolarization potential (mV)	26	±0.57	25	±0.4	P>0.05
	c Duration of depolarization (ms)	2	±0.07	2	±0.28	P>0.05
	d Duration of repolarization (ms)	2.16	±0.16	1.77	±0.11	P>0.05
	e Maximum after-hyperpolarization potential (AHP) (mV)	–95	±0.57	–91. 8	±0.6	P<0.01
	f Duration of AHP/+ve after- potential (mV)	14	±0.4	12.4	±0.15	P<0.01

P<0.05 denotes the significant values
P<0.025 denotes the more significant values
P<0.01 denotes the highly significant values
P>0.05 denotes the insignificant values
Note: RMP recorded as holding potential

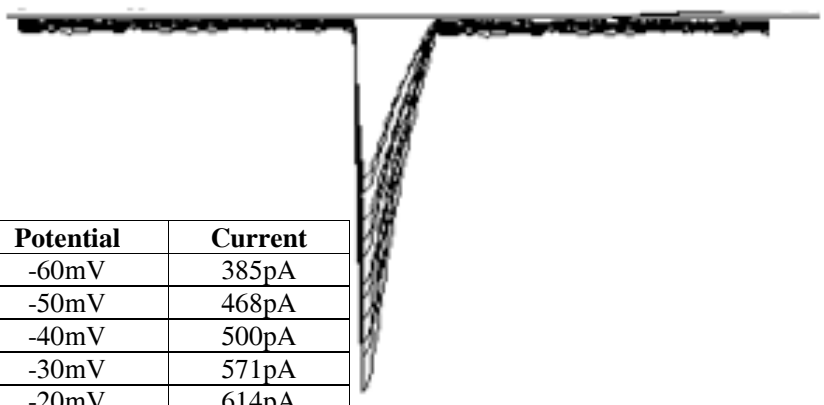
iii) Inward and outward membrane currents :

a) Na^+ Inward currents during the elicitation of action Potential were recorded by patch-clamp using K^+ channel blocking drug, tetraethylammonium; in winter (Fig. 6) and in summer (Fig. 8).

b) K^+ outward currents were also recorded during elicitation of action potential by using Na^+ channel blocking drug, tetradotoxin in winter (Fig. 7) and summer (Fig. 9).

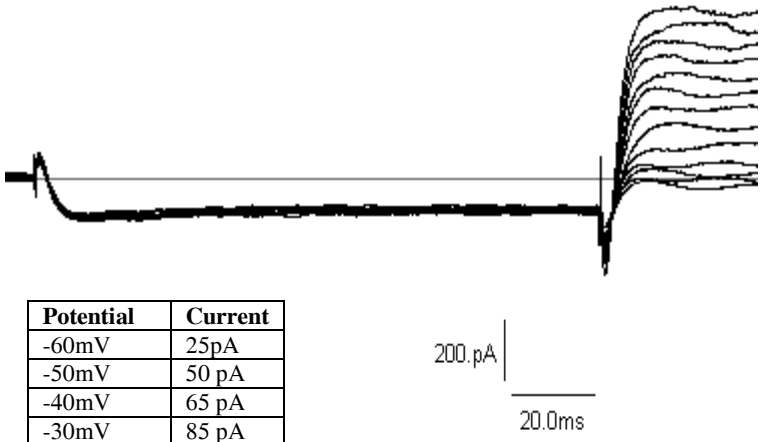
c) Na^+ inward currents recorded at membrane potentials from –60 mV to +60 mV were found to have higher values in peak winter as compared to summer (Fig. 5 & 7).

d) But K^+ outward currents, at the same potentials had lower values in winter as compared to summer (Fig. 6 & 8).



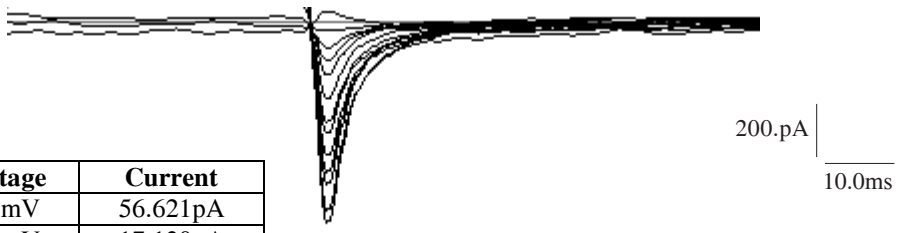
Potential	Current
-60mV	385pA
-50mV	468pA
-40mV	500pA
-30mV	571pA
-20mV	614pA
-10mV	685pA
0mV	785pA
10mV	842pA
20mV	910pA
30mV	830pA
40mV	772pA
50mV	663pA
60mV	583pA

Fig. 6 : Na^+ inward currents recorded in winter by patch-clamp, during elicitation of action potential using K^+ channel blocking drug; tetraethylammonium Pulse protocol: Holding potential at RMP = –69 mV; steps from –60 to +60 mV.



Potential	Current
-60mV	25pA
-50mV	50 pA
-40mV	65 pA
-30mV	85 pA
-20mV	200pA
-10mV	295pA
0mV	352pA
10mV	438pA
20mV	542pA
30mV	628pA
40mV	676pA
50mV	742pA
60mV	847pA

Fig. 7 : K^+ outward currents recorded in winter during elicitation of action potential, by using Na^+ channel blocking drug; tetradotoxin. Pulse protocol: Holding potential at RMP = –69 mV; steps from –60 to +60 mV.



Voltage	Current
-60mV	56.621pA
-50mV	17.130pA
-40mV	58.772pA
-30mV	125.65pA
-20mV	189.48pA
-10mV	261.55pA
0mV	314.04pA
10mV	377.31pA
20mV	459.28pA
30mV	509.85pA
40mV	589.71pA
50mV	641.61pA
60mV	714.91pA

Fig. 8 : Summer recording of Na^+ inward currents. Pulse protocol: Holding potential at RMP = –70 mV; steps from –60 to +60 mV.

Discussion

It is worth to mention that seasonal changes are invariably associated with changes in environmental temperature, and are analogous to changes in experimental temperature. The information on thermal dependence regarding the electrical properties of reptilian muscle fiber membrane is rather scanty, except few works by some researchers (10). Demonstrated the effects of experimental temperatures on electrical excitability by chloride conductance of lizard/ desert iguanas (Dipsosaurus

Voltage	Current
-60mV	128.55pA
-50mV	122.48pA
-40mV	259.48pA
-30mV	451.60pA
-20mV	102.10pA
-10mV	175.58pA
0mV	251.10pA
10mV	341.09pA
20mV	428.35pA
30mV	512.17pA
40mV	582.44pA
50mV	607.04pA
60mV	642.08pA

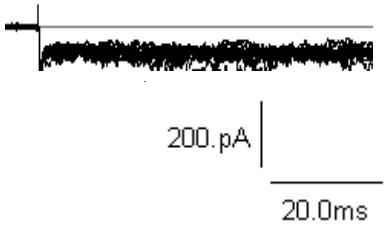


Fig 9 : Summer recording of K^+ outward currents. Pulse protocol: Holding potential at RMP = –70 mV; steps from –60 to +60 mV.

dorsalis) skeletal muscle, and observed decreased electrical excitability with increasing temperature (21). Worked on both the season & the experimental temperature, and observed changes in the generation of spontaneous action potential discharge that increased with the fall of temperature & vice versa in sinus venosus (heart muscle) of European flatfish (22). Also observed increased action potential discharge rate of pacemaker cells in cold conditions of fish heart (23). Observed increased excitatory junction potential amplitudes in cold acclamation in ectothermal crab (24). Demonstrated effects of season on the conduction velocity of action potential that increased with decreased temperature; in squid giant axon (11). Also observed increased firing rate (excitability) in a songbird pre-motor nucleus in a breeding winter season.

While studying the electrical characteristics of the skeletal muscle fiber membrane of our experimental animal *Uromastix hardwickii*, recorded during the peak winter December and January and peak summer June and July; it was obvious that resting membrane potential (RMP), action potential (Fig. 4 & 5), & their durations were stable with the change of season from summer towards winter (Table I) as the difference in changes were found to be insignificant. But the threshold potential (THP), after-potential (AHP) and the duration of after-potential were influenced and changed significantly with season (Table I). That these two potentials (THP, AHP) voltages increased and the duration of AHP remarkably decreased with the fall of temperature in the peak winter months of December and January, thus showing higher muscular electrical excitability in these colder months as compared to hotter season of June and July.

Na^+ inward currents recorded at membrane potentials from –60 mV to +60 mV were also found to have higher values in peak winter as compared to summer (Fig. 6 & 8), thus proving the higher electrical excitability of skeletal muscles of this animal in peak winter months (December and January), as compared to summer June and July.

Hence it is observed that gastrocnemius (skeletal) muscles of *Uromastix hardwickii* undergo electrical excitability changes between peak winter and summer; especially increased muscle electrical excitability in peak winter, which is helpful to cause some sluggish movements during peak winter sleep/ hibernation. Above mentioned references, especially (10–11) who worked on seasonal temperature effects on electrical excitability of lizard Dipsosaurus dorsalis skeletal muscle, and avian (white crowned sparrows) forebrain song-control neurons guiding the increased electrical excitability, during fall of the temperature in winter in these species of lizards and birds; support our findings, of seasonal changes in the electrical characteristics of our experimental animal. However these electrical characteristics are studied for the first time in the skeletal muscles of this lizard species, *Uromastix hardwickii*.

Our study on skeletal muscle tissues of this animal studied are very much relevant with the studies in humans as explained by (4, 5, 9), hence hints to expand further research through the living tissues of *Uromastix*; which can't be carried out on living human beings. This animal is desert-adapted and very much resistant to the conditions of food shortages, and lives for several days without any food or water because of its fat storages (25).

Conclusion

Seasonal temperature effects on electrical characteristics, as observed in skeletal muscle tissues of Uromastix hardwickii that raised electrical excitability in peak winter as compared to summer is definitely responsible for the performance of some required activity/ movements during winter-sleep/ hibernation and hence this activity though sluggish, helps to maintain homeostasis to continue the life of the animal.

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