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Seasonal comparison of active v/s passive electrical characteristics of skeletal muscle fiber membranes of the reptile, *Uromastix hardwickii*

Abstract

Introduction: This comparative study deals with the observation of changes with temperature variations of the seasons in the muscular active versus passive electrical excitability characteristics in the reptile *Uromastix hardwickii*.

Objectives: The present study was carried out to investigate seasonal comparison of active electrical characteristics (resting membrane potential, maximum depolarization potential, duration of depolarization and repolarization potentials) and passive electrical characteristics (threshold potential, after-potential {AHP} & its duration), which are not studied earlier.

Methods: 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 (active and passive electrical potentials). Resting membrane potentials and action potentials of reptilian skeletal muscle cell membranes were measured in whole cell current mode. The glass microelectrodes, with a tip diameter 2-3μm and tip resistance 5-6 MΩ (when filled with intracellular solution) were used in these experiments.

Results: The average mean values of the active electrical characteristics were stable and showed no any significant change (P>0.05) with season, but the passive electrical characteristics were found to fall significantly (P<0.05) from winter towards summer & vice versa; therefore the passive electrical characteristics/parameters affect muscular excitability and probably muscular activities of the animal from winter towards summer.

Conclusion: Seasonal temperature changes in passive electrical characteristics of the skeletal muscles of the *Uromastix hardwickii* are responsible for the change in the active behavior & homeostasis of these reptiles from summer towards winter by raising their muscular electrical excitability to cause some required movements during winter sleep/hibernation.

Keywords: patch-clamp technique, *Uromastix hardwickii*, gastronomies muscle, seasonal temperature, active and passive electrical parameters

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Abbreviations: RMP, resting membrane potentials; GRB, general reptilian buffer; THP, threshold potential; AHP, after-hyper polarization potential; ANOVA, analysis of variance

Introduction

The reptile, *Uromastix hardwickii*, looks like a long spiny-tailed lizard, is found in the desert areas of Asian & African countries; and is herbivorous. Local people hunt it to have its fat. Which is boiled down and the resulting oil is used to relieve pain, and also as a cure for impotency.[1–3] The skeletal muscle membranes of these reptiles are found to exhibit membrane potentials, as being permeable to Na+, K+ and Cl–.[4–7] These muscle cell membrane electrical potentials/characteristics have been divided into active and passive categories; the passive electrical characteristics show some thermal dependency in different species of the animals.[8,9] As the, passive electrical properties are important in determining the excitability of muscle fibers.[8]

Electro physiologists, now-a-days use patch-clamp technique, that is a refinement of voltage clamp; for studying single and multiple ion channels in cells to record the membrane potentials/currents of the cells, especially in excitable nerve & muscle cells.[10–13] Very little is known about the active and passive electrical characteristics of this reptile/lizard *Uromastix hardwickii*, except a few works on some other lizard species skeletal muscles by Adams BA[8] observed temperature effects on membrane chloride conductance and recorded decreased electrical excitability of lizard skeletal muscle fibers with increasing temperature to 45°C. He recorded resting membrane potentials (RMP) of -80mV and -70mV of the skeletal muscle fibers of those lizards. Seasonal temperature effects potentials in other species of animals are also studied by various workers.[14–16]

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. Fresh animals (adults) of both the sexes were used in all the experiments. In the laboratory, the animals were kept at room temperature (25°C). Since their biochemistry is reported to change with season,[17] 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.[2] 1gm muscle sample was digested in 10ml digestive fluid, containing 1% (w/v) pepsin (0.1gm) and 1% (v/v) HCl (0.1ml) and 9.9ml water. Magnetic stirring of the mixture was carried out for 3hours at 37°C, the digest was settled for 20minutes and 2/3rd supernatant removed. The deposit was filtered through 355µm mesh, settled again for 20minutes and then supernatant fluid removed. Afterwards the sediment was washed with warm (37°C) water, settled and supernatant removed till the whitish fluid volume of 1ml was left, having the clear muscle cells for electrical recording.[18–20] The membrane potentials were measured by the patch-clamp method as described by Hamill OP et al.[10] the glass microelectrodes used in patch-clamp technique had tip diameter 2-3µm,[9,20] tip resistance 5-6 MΩ.[11] The microelectrodes were filled with intracellular solution (in mM 145 KCl, 10 NaCl, 10 EGTA, 1 $MgCl_2$, 2 CaCl2 and 10 Hepes buffer; by using 1ml syringe.[11,21] The muscle cells mounted in microscope were perfused with general reptilian buffer (GRB), as described by Khalil F[22] The intracellular solution, used in patch-pipette had lower osmolality (266 mOsm/L) than that of extracellular solution (GRB) (307mOsm/L); in order to improve seal formation[10] and prevent cell swelling which may occur during long time recordings.[21] The parameters measured by patch-clamp technique, were active electrical characteristics/parameters: resting membrane potential (RMP), action potential (maximum depolarization potential & its duration, repolarization potential), and passive electrical parameters: threshold potential (THP), after-hyper polarization potential (AHP)/+ve after-potential & their durations. 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". Hypothesis was rejected for P-value smaller than 0.05.[23]

Results

By comparing the electrical parameters (active & passive) obtained during peak winter (Figure 1) and summer (Figure 2) 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) (active electrical parameter): The average values of this phase of action potential (Table 1), showed insignificant difference in between peak winter and summer months.

b) Duration of depolarization (ms) (active electrical parameter): According to the average values of this electrical parameter (Table 1), which was measured from threshold potential toward the peak, were found to have insignificant difference in between peak winter and summer months.

c) Threshold potential (mV) (passive electrical parameter): 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-hyper polarization Potential (AHP) (mV) (passive electrical parameter): 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 hyper polarization potential.

e) Duration of repolarization (ms) (active electrical parameter): According to Table 1, 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) (passive electrical parameter): The average values of this electrical parameter mentioned in Table 1, highly significant ($P<0.01$) fall was noted, this fall was calculated to be 12% toward summer.

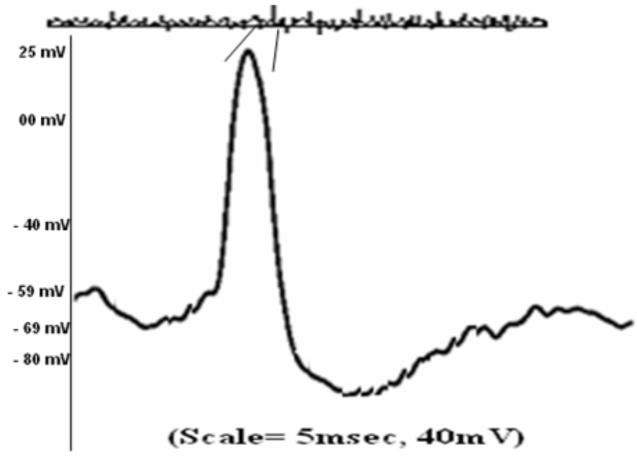

Figure 1 Patch-clamp winter recording of electrical parameters (resting membrane potential: -69mV, threshold potential: -59mV, maximum depolarization potential: 25mV) of gastrocnemius muscles of *Uromastix hardwickii*.

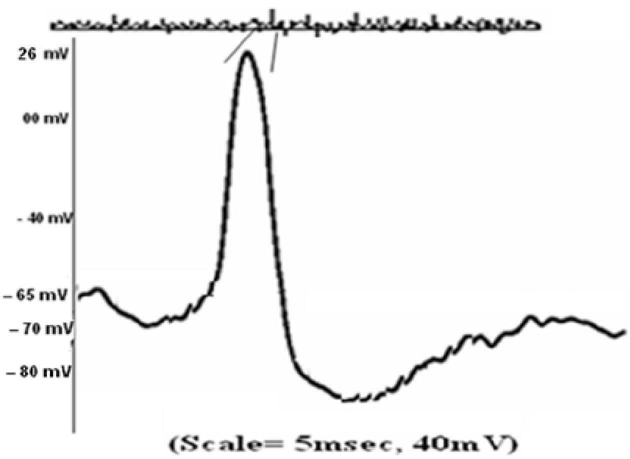

Figure 2 Patch-clamp summer recording of electrical parameters (resting membrane potential: -70mV, threshold potential: -65mV, maximum depolarization potential: 26mV) of Gastrocnemius muscles of *Uromastix hardwickii*.

Table 1 Seasonal comparison of active v/s passive electrical characteristics (parameters) of skeletal muscle membranes, of *Uromastix hardwickii*, obtained by patch-clamp technique during winter and summer months

S. no.	Parameters	Winter	Mean ± S.E.M	Summer	Mean ± S.E.M	Significance level winter v/s summer
	Active electrical parameters					
1	Resting membrane potential(RMP) (mV)	– 69	± 0.4	– 70	± 0.63	P>0.05
2	Action potential					
a)	Maximum depolarization potential(mV)	25	± 0.4	26	± 0.57	P>0.05
b)	Duration of depolarization(ms)	2	± 0.28	2	± 0.07	P>0.05
c)	Duration of repolarization(ms)	1.77	± 0.11	2.16	± 0.16	P>0.05
	Passive electrical parameters					
d)	Threshold potential(mV)	– 59	± 0.4	– 65	± 0.49	P<0.01
e)	Maximum after-hyper polarization potential (AHP)/after-potential(mV)	– 91.8	± 0.6	– 95	± 0.57	P<0.01
f)	Duration of AHP/after- potential(mV)	12.4	± 0.15	14	± 0.4	P<0.01

Note RMP recorded as holding potential

P<0.05 denotes the significant values

P>0.05 denotes the insignificant values

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 (active & passive) of reptilian muscle fiber membrane is rather scanty, except few works by some researchers[8] demonstrated the effects of experimental temperatures on electrical excitability by chloride conductance of lizard/desert iguanas (Dipsosaurus dorsalis) skeletal muscle, and observed decreased electrical excitability with increasing temperature.[9] 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 flat fish[24] also observed increased action potential discharge rate of pacemaker cells in cold conditions of fish heart.[25] Observed increased excitatory junction potential amplitudes in cold acclamation in ectothermal crab[26] demonstrated effects of season on the conduction velocity of action potential that increased with decreased temperature; in squid giant axon.[27] Also observed increased firing rate (excitability) in a songbird pre-motor nucleus. While studying the electrical characteristics/parameters of the skeletal muscle fiber membrane of our experimental animal/lizard *Uromastix hardwickii*, recorded during the peak winter December & January and peak summer months of June & July; it was obvious that active electrical characteristics: resting membrane potential (RMP), action potential (Figure 1) (Figure 2), their durations were stable with the change of season from summer towards winter (Table 1); with insignificant changes. But the passive electrical characteristics: threshold potential (THP), after-potential (after-hyper polarization potential/AHP) and the duration of after-potential were influenced and changed significantly with season (Table 1). That these two potential (THP, AHP) values decreased in negativity/increased positivity and the duration of AHP also remarkably decreased with the fall of temperature in the peak winter months of December & January, thus showing higher muscular electrical excitability in these colder months as compared to hotter season of June & July. Therefore it is suggested that gastrocnemius (skeletal) muscles of *Uromastix hardwickii* undergo excitability changes (because of changes in passive electrical characteristics) between winter and summer; especially increased muscle electrical excitability, due to changes in passive electrical characteristics, during fall of the temperature in winter, which is helpful to cause some sluggish movements during winter sleep/hibernation. Also all of the above mentioned references guiding the increased electrical excitability, during fall of the temperature in winter in other species of the animals; support our findings, of seasonal changes in the passive electrical parameters which are studied and compared with active electrical characteristics (not influenced by seasonal temperature changes) for the first time in the skeletal muscle of *Uromastix hardwickii*. Our study on skeletal muscle tissues of this animal studied are very much relevant with humans, 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 & very much resistant to the conditions of food shortages, and lives for several days without any food or water because of its fat storages.[21]

Conclusion

Seasonal temperature changes in all the above mentioned passive electrical parameters of the skeletal muscles of the *Uromastix hardwickii* are definitely responsible for the change in the active behavior & homeostasis of these animals from summer towards winter by raising their muscular electrical excitability to cause some required movements during winter sleep/hibernation.

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None.

Conflict of interest

Author declares that there is no conflict of interest.

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