© by PSP Volume 27 – No. 12A/2018 pages 8876-8881 Fresenius Environmental Bulletin

EFFECTS OF DIFFERENT TEMPERATURES ON ELECTRICAL CHARACTERISTICS OF RAT DIAPHRAGM MUSCLE

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ABSTRACT

The electrical properties of the diaphragm muscle low and high temperatures have been studied at previous studies, but there is very little information in the literature as to what changes occur during temperature changes. Therefore, this experimental study was designed to investigate the effects of temperature changes on electrical properties of Wistar rat diaphragm muscle in vitro. Muscle strips were removed from the ventral-costal regions of rat diaphragm muscles, placed into the organ baths containing Krebs solution and recorded at 22, 25, 30, 35 and 37ºC. Parameters (depolarization time, half repolarization time, amplitude, overshoot, and latency) of action potential and resting membrane potential measured by using the conventional glass capillary electrodes at different temperatures. Though not significant, amplitude of resting membrane potential was observed to decrease when temperature was increased from 22ºC to 37ºC. Also, the significant decrements in parameters of action potential occurred with increasing temperature. Our results demonstrated that in rat diaphragm muscle, action potential parameters are temperature-sensitive because depolarization time, half repolarization time, amplitude, overshoot, and latency were significantly reduced with rising temperature.

KEYWORDS:
Action potential parameters, diaphragm muscle, microelectrode, rat, temperature changes

INTRODUCTION

Diaphragm, dome-shaped, is a membranous and muscular structure that separates the abdominal and thoracic cavities. The diaphragm is a skeletal muscle and consists of three types (I, IIa and IIx) of muscle fibers [1]. Crural and costal regions of the diaphragm receive motor innervation from a phrenic nerve [2]. Diaphragm is the most important and basic respiratory muscle, and responsible for more than 60% of the vital capacity [3]. Regulation of the function of the diaphragm muscle is associated with ion channels, membrane excitability and muscle activation. There are many factors such as long-term mechanical ventilation support [4] and chronic obstructive pulmonary disease [5] that affect the function of the diaphragm muscle. Due to these factors, the diaphragm muscle is adversely affected and cannot perform its electrical functions properly.

Ion channels play an important role in contraction and relaxation of the diaphragm muscle. K^+ and Cl^- channels act to determine membrane potential to control muscle activation. In addition, Na^+ channel together with the K^+ and Cl^- channels controls the release of Ca^{+2} ion from sarcoplasmic reticulum by determining the duration and amplitude of the action potential. Thereby the released Ca^{+2} ions determine strength of the muscle contraction [6]. At the same time, the sensitivity of ion channels to temperature change has been expressed in previous studies [7-10]. Previous studies showed that decreasing or increasing temperature affect functions of the rat diaphragm muscle [6, 11, 12]. Therefore, action potential parameters and thus functions of the rat diaphragm muscle can be expected to change with temperature by the kinetics of ion channels.

In previous studies, the parameters of the action potential have been observed at different (low or high) temperatures [6, 13-15] and at increasing/decreasing temperature [11, 16]. In other studies, the effects of increasing/decreasing or different (low or high) temperatures on the ion channels in the diaphragm muscle were investigated [7-10]. Thus, in this experimental study, we aimed to determine the effects of increasing temperature changes from cold to warm on electrical properties of rat diaphragm muscle.

METHODS

Animals. Twenty male Wistar albino rats (220–260 g) were obtained from the Animal House of the School of Medicine, Çukurova University. Rats were housed in specific cages. A 12-h light/dark cycle was maintained and the rats were

feed ad libitium. The study was approved by the local ethics committee of Çukurova University. Animal care and experiments conformed to the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health, and approval of the ethics committee of our institution was obtained before the commencement of the study.

Tissue Preparation. The diaphragm muscle preparations were prepared according to the method described by Kelsen and Nochomovitz [17]. After rats were killed by decapitation, diaphragm muscles were rapidly removed and muscle strips (n=20) were cut from the ventral-costal regions of diaphragm muscles. Weight and length of the muscle strips were 53.4±6.2 mg and 1.8±0.2 cm, respectively. They were placed between platinum electrodes and kept in organ bath containing 20 ml of Kreb's solution (Fig 1). The composition of the Kreb's solution was as follows: 118 mM NaCl, 4.69 mM KCl, 2.5 mM $CaCl_2$, 0.6 mM $MgSO_4$, 1.17 mM KH_2PO_4, 25 mM $NaHCO_3$ and 11.1 mM glucose. The organ bath solution was continuously bubbled with a gas mixture (5% CO_2 - 95% O_2). The pH was kept between 7.35 and 7.45. Temperatures set to 22, 25, 30, 35 and 37ºC, respectively.

Electrophysiologic Recordings. Transmembrane potentials were recorded using conventional glass capillary electrodes. Electrodes (outer diameter 1.0 mm and tip resistance 10-30 MΩ) were filled with 3 M KCl and were electrically coupled to the input of a high-impedance amplifier (Nihon Kohden, Japan) equipped with capacitance compensation facilities. The exploring electrode with an angle 50° was mounted on a micromanipulator (vertical movement accuracy: 0.01 mm). The indifferent electrode at 2.0 cm away from the exploring electrode was placed at the incubation bath.

Records were digitized and analyzed using a computer-based data acquisition and analyzing system allowing for online determination of the resting membrane potential and action potential parameters (latency, depolarization time, amplitude, half repolarization time and overshoot) after a stabilization period of about 30 minutes for each temperature.

Action potential properties were characterized as follows: amplitude (difference between resting membrane potential and the peak positive voltage), overshoot (amount by which voltage exceeded 0 mV at the peak of the action potential), depolarization time (time required for the depolarizing phase to go from 10 to 90% of action potential height), half repolarization time (time required for the action potential to repolarize 50% of the way back to resting membrane potential) and latency (duration of action potential).

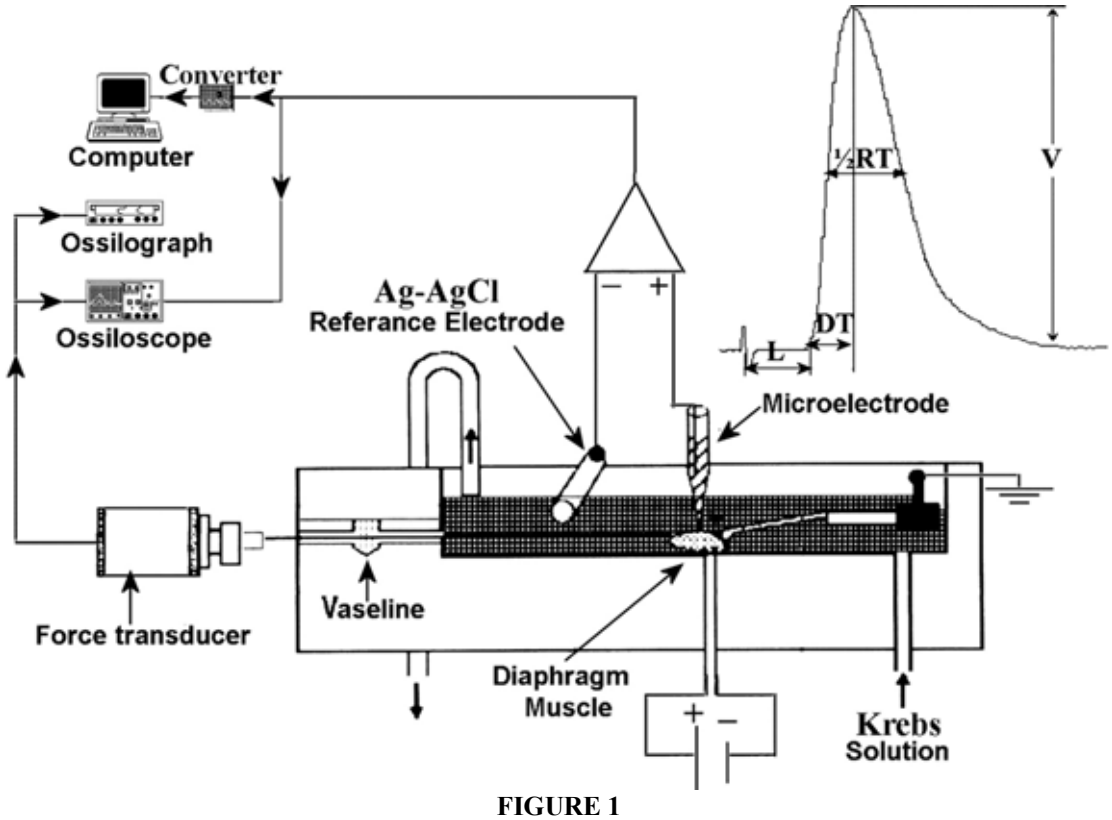

FIGURE 1
Scheme of the organ bath and recorder for measurement of resting membrane potential and action potential of isolated rat diaphragm muscle.
DT: Depolarization Time, ½ RT: Half Repolarization Time, V: Amplitude, L: Latency

Statistical Analysis. Statistical analysis performed using SPSS 11 software (Lead Technologies, Charlotte, NC). All data represent means ± standard error of the mean (SEM). Statistical analysis was performed by one way analysis of variance (ANOVA) and subtest was paired-t-test. The differences were considered to be significant at $p<0.05$.

RESULTS

This study examined resting membrane potential and action potential parameters from isolated rat diaphragm muscle fibers at 22, 25, 30, 35 and 37ºC. The effects of temperature were studied in 20 fibers at each of the five study temperature.

Table 1 shows that resting membrane potential and action potential parameters (amplitude, overshoot, depolarization time, half repolarization time and latency) of isolated rat diaphragm muscle were recorded using conventional glass capillary electrodes.

Resting membrane potential did not affect at temperature between 22 and 37°C (Table 1 and representative waveform in Fig 2). When temperature was increased, resting membrane potential was decreased, but this decrement did not significantly ($p>0.05$).

When temperature was increased from 22°C to 37°C, the amplitude (6%) and overshoot (18%) of action potential markedly reduced ($p<0.001$) (Table 1 and Fig 3). Similarly, latency (%56), depolarization time (65%) and half repolarization time (64%) of action potential significantly decreased with increasing temperature ($p<0.001$) (Table 1 and Fig 4).

DISCUSSION

In the present study, we studied the effects of temperature changes on electrical properties of the rat diaphragm muscles. In our study, diaphragm muscle was selected in our study because of the fact that the most important muscle of the respiratory system. There are limited studies on temperature effects on the rat diaphragm muscle [6, 11, 12]. Therefore, different temperatures as hypothermia (22 and 25°C), mild hypothermia (30 and 35°C) and isothermia (37°C) were selected. However, the mechanism of electrical properties of the diaphragm muscle at different changes could not to be clearly explained. For this reason, we aimed to determine the effects of temperature changes on the diaphragm muscles' electrical characteristics.

TABLE 1
Resting membrane potential and parameters of action potential recorded from isolated rat diaphragm muscle at different temperatures (n=20).

	Temperature (°C)					
	22	**25**	**30**	**35**	**37**	**p value**
Resting Membrane Potential (mV)	-75.7±2.4	-75.0±2.2	-74.2±2.4	-74.0±2.7	-73.8±2.6	p>0.05
Amplitude (mV)	100.4±5.1	98.0±5.2	95.2±5.4	93.0±5.9	92.3±6.0	p<0.001
Overshoot (mV)	25.0±2.4	24.6±3.3	21.7±3.0	20.1±3.4	19.0±3.1	p<0.001
Depolarization Time (ms)	1.7±0.5	1.2±0.3	0.8±0.2	0.7±0.1	0.6±0.1	p<0.001
Half Repolarization Time (ms)	1.1±0.2	0.9±0.2	0.6±0.1	0.4±0.1	0.4±0.07	p<0.001
Latency (ms)	4.6±0.7	3.8±0.3	3.1±0.3	2.2±0.2	2.0±0.4	p<0.001

Values were calculated as mean±SEM

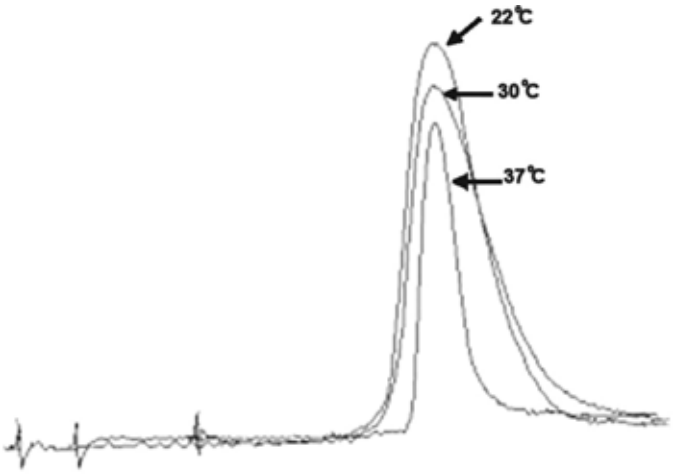

FIGURE 2
Representative waveforms of action potentials recorded from isolated rat diaphragm muscle at 22ºC, 30ºC and 37ºC temperatures.

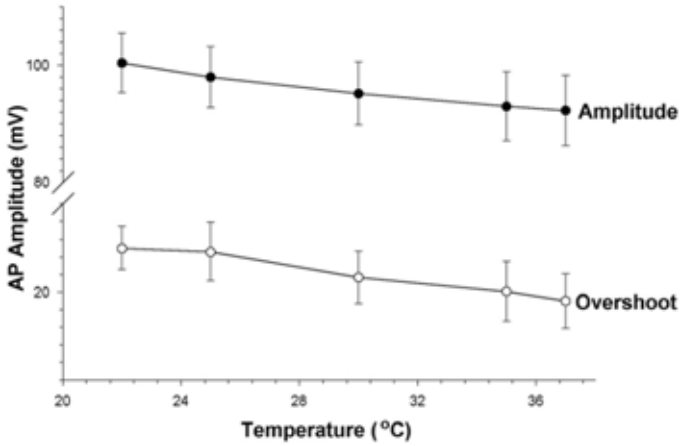

FIGURE 3
Overshoot and amplitude of action potential (AP) recorded from isolated rat diaphragm muscle at different temperatures.

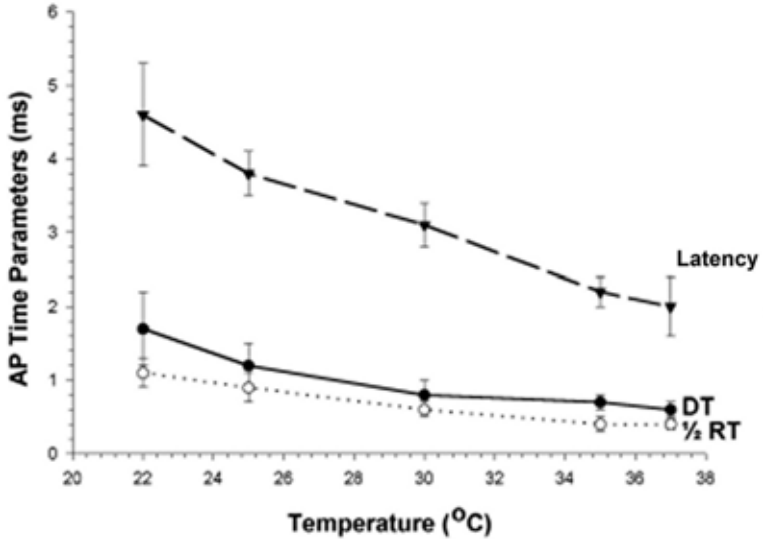

FIGURE 4
Latency, Half Repolarization Time (½RT) and Depolarization Time (DT) of action potential (AP) recorded from isolated rat diaphragm muscle at different temperatures.

Our results showed that parameters of action potential were markedly reduced with increasing temperature, although resting membrane potential did not significantly change.

The present study, as well as others, has demonstrated that resting membrane potential did not affect at temperature between 22 and 37°C. From our findings [13, 18] together with previous results [6, 11, 15], it could be concluded that different temperatures between 22 and 37°C were not altered on resting membrane potential of the diaphragm muscle. Van Lunteren et al. [6] found that resting membrane potentials were 71.1±0.9 mV and 73.6±1.7 mV at 37°C and 20°C, respectively. Delbono and Kotsias [15] (75.6±3.2 mV at 30°C) reported similar finding. Contrary to these studies, Ruff RL [16] found that resting membrane potential was 98.3±1.1 mV at 25°C and when temperature was increased, resting membrane potential was increased but this increment did not significantly. Hence, our data and earlier investigations [6, 11, 15] indicating that an increment of temperature between 22 and 37°C had no effect on the amplitude of the resting membrane potential of the rat diaphragm muscle.

In the present study, we found that when temperature was increased, the amplitude and overshoot of action potential markedly reduced. Similarly, depolarization time, half repolarization time and latency of action potential significantly decreased with changing temperature between 22°C and 37°C. Many researchers showed that whether changes in action potential parameters may be influenced by increasing temperature because of the sensitivity of ion channels to temperature [7-10].

Amplitude, overshoot and latency of action potential were determined between 22 and 37°C in our study. Similar results were found in other stud-

ies; Alkis et al. [13] found that amplitude level is 96.0±0.52 at 30°C, Van Lunteren et al. [6] found that amplitude levels are 93.8±2.9 and 101±3.3 at 37°C and 20°C, respectively. Obtained results indicated that increased temperature markedly decreased the amplitude and latency of action potential. The results of our study seem to be consistent with other studies [6, 16]. The formation of action potential can be limited by the temperature. The flow of ionic currents and then conduction of membrane enhanced with raising temperature. On the contrary, membrane conduction is blocked at low temperatures. The results of temperature changes on parameters and hence shapes of action potential indicate temperature effects on conductance and interactions. When the temperature rises, the duration and amplitude of the action potential is reduced and also the membrane becomes hyperpolarized. Conversely, membrane at low temperatures becomes polarized [19].

Depolarization time is one of the parameters of action potential. In the depolarization process of the membrane, the cell becomes more depolarized by entering Na^+ into the cell. In the cell membrane, which is more depolarized, it causes more Na^+ channels to open and hence potential of the membrane quickly rises to equilibrium potential of Na^+. Meanwhile, K^+ channels activated and potassium ions are out of the cell from these channels. Therefore, potential of the membrane cannot reach to equilibrium potential of Na^+ [20]. In this study, depolarization time of action potential were determined at different temperatures and significantly decreased with increasing temperature from 22°C to 37°C. Similar finding was found at another study [6]. The shortened depolarization time may be due to change of the structure and the channel function of the Na^+ channels with increasing temperature.

In the present study, we found that half repolarization time was significantly decreased with increasing temperature. Our results are in accordance with other studies [6, 11]. Potassium ions, potassium channels, and Sodium – Potassium pumps play an important role in the half repolarization phase of the action potential process. In addition, calcium ions are involved in many biological processes such as effects on ion channels, neurotransmitter uptake and muscle contraction [21]. As the known effects of temperature on biological processes and channel conduction were estimated, the half repolarization time of the action potential was found to be shortened with increasing temperature. The shortening of the repolarization time means that the time required for the formation of a new action potential is reduced when the cell receives another stimulus. In this process, with the increasing temperature, the input of calcium ions into the cell increases and consequently the number of opened potassium ion channels and potassium channel currents increases. Then more potassium ions move out of the cell and the pump activity increases. Thus, the cell is ready for a new potential for action.

In conclusion, this report suggests that when temperature was increased, while the resting membrane potential of diaphragm muscle didn't change, parameters (depolarization time, amplitude, overshoot, half repolarization time and latency) of action potential significantly reduced in the rat diaphragm muscle. Changes in action potential parameters with increasing temperature has been tried to be explained by the sensitivity of the ion channels to the temperature. Further investigation is needed to elucidate kinetics and roles of ion channels with changing temperature in the rat diaphragm muscle.

ACKNOWLEDGEMENTS

The manuscript has not been submitted to more than one journal for simultaneous consideration.

The authors declare that they have no conflict of interest.

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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Received: **19.03.2018**
Accepted: **04.04.2018**

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