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The Effects of Magnetic Field on the Electrophysiological Parameters of Soleus Muscle in Streptozotocin-Induced Diabetic Rats

Streptozotosın İle Deneysel Diyabet Oluşturulan Sıçanların Soleus Kaslarının Elektrofizyolojik Parametrelerine Elektromanyetik Alanın Etkileri

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ABSTRACT

Purpose: To investigate the effects of magnetic field (MF) on the resting membrane potential and action potential parameters of the Soleus muscle preparations in streptozotocin (STZ) induced diabetic rats.

Material and Methods: Forty male wistar rats were used in this study. The rats were divided into four groups; Group I (control), Group II (control with magnetic field), group III (diabetic), group IV (diabetic with magnetic field). The diabetes was induced by a single intravenous injection of STZ (45 mg/kg) in citrate buffer to the jugular vein under the ketamine and xylazine anesthetic combination. Resting membrane potential (RMP), muscle action potential (MAP), depolarization and half-repolarization time, MAP integral, depolarization and repolarization rates were recorded by means of the conventional microelectrode technique.

Results: When group III and IV compared to group I, a significant decrease was found in the RMP ($p<0.05$). When control group compared to the other groups, MAP values found to be significant. When the depolarization time was compared there was a significant increase difference between group I and group III and group IV. Furthermore, half-repolarization time, MAP integral, depolarization and repolarization rates showed significant decrease results as occurred in MAP.

Conclusion: MF increased the excitability of the cell membrane in RMP, which revealed that cell membrane had been more depolarized. It was assumed that this increase in depolarization may have been due to the changes in the conductivity of K^+ and/or Cl^- ion channels.

Key Words: Soleus muscle, streptozotocin, resting membrane potential, action potential, magnetic field

ÖZET

Giriş: Streptozotosin (STZ) ile deneysel diyabet oluşturulan sıçanlar elektromanyetik alana maruz bırakıldıktan sonra soleus kas preparatlarından dinlenim zar potansiyeli ve kas aksiyon potansiyelinin ölçülerek incelenmesi.

Materyal ve Metod: Bu çalışmada 40 adet Wistar türü erkek sıçan kullanıldı. Sıçanlar dört gruba bölündü; Grup I (Kontrol), Grup II (Manyetik alan kontrolü), Grup III (Diyabetik), Grup IV (diyabet uygulanmış manyetik alan grubu). Diyabet tek doz halinde (45 mg/kg) ketamin ve ksilazin kombinasyon anestezisi altında citrat tamponu içerisine karıştırılmış STZ olarak jügüler venden verildi. Konvensiyonel mikroeletrod tekniği ile dinlenim zar potansiyeli, aksiyon potansiyeli, depolarizasyon ve repolarizasyon süresi, kas aksiyon potansiyeli integrali kayıtlandı.

Tartışma: Manyetik alan hücre membranında dinlenim zar potansiyelinin uyarılabilirliğini arttırdı. Bu da hücre membranlarının daha fazla depolarize edilmiş olduğunu göstermektedir. Bu depolarizasyon durumunun artması K ve/veya Cl iyon kanallarının iletkenliğindeki değişimlerden kaynaklanabileceği varsayılmaktadır.

Sonuçlar: Dinlenim zar potansiyeli, Grup I, III ve IV arasında anlamlı bir azalma bulunmuştur ($p<0.05$). Kas Aksiyon Potansiyelleri diğer gruplarla karşılaştırıldığında sonuçlar anlamlı bulundu. Depolarizasyon süresini karşılaştırdığımızda Grup I, III ve IV ararsında anlamlı bir artış gözlendi. Bunun yanında yarı repolarizasyon süresi, kas aksiyon potansiyeli integrali, depolarizasyon ve repolarizasyon sürelerinin anlamlı bir şekilde düştüğü görüldü.
Anahtar Kelimeler: Soleus kası, streptozotosin, Dinlenim zar potansiyeli, Aksiyon Potansiyeli, Manyetik Alan.

INTRODUCTION

Diabetes is a metabolic disorder that affects various organ systems, including skeletal muscles. These changes are thought to involve both structural and metabolic defects[1]. Experimentally induced diabetes is associated with changes in the contractile and electrical properties of skeletal muscles.

Electricity and magnetic field (MF) may positively influence the outcome of the healing process of different tissues like skin, bone, and peripheral nerves[1-4]. Biological effects of MF raise the question of whether imposed MF constitutes a hazard in terms of physiological processes[2-4]. Besides, the development of diagnostic and therapeutic applications of MF draws attention to possible effects. McArdle et al have demonstrated that resting membrane potential, contraction and relaxation rates were showing changes at different temperatures[5].

Microelectrode technique is one of the indispensible techniques of electrophysiological signal recordings in intracellular recordings of excitable cells. The soleus is an important skeletal muscle, which plays an important role in standing to the gravity force and exercises. However, the effects of MF on the electrophysiological characteristics of streptozotocin-induced diabetic rat soleus muscles have not been investigated yet.

Most previous electrophysiology experiments in the diabetic muscle have focused on resting membrane potential changes rather than action potentials[1,6-8]. Grossie noted that action potential amplitude and rate of depolarization were lowered, and action potential duration was prolonged, by severe diabetes[9]. However, studies were done in animals whose survival was only 5 days in the absence of insulin, so that these data most likely reflect the effects of acute severe and terminal diabetic ketoacidosis rather than the consequences of a chronic diabetic state.

The aim in this study was to determine the effect of the MF on the electrophysiological features of the soleus muscle in diabetic rats.

MATERIALS and METHODS

Animals

Mature male Wistar Albino rats were used (aged 19 wk at start of study) in our experiments ranged between 250 to 300 g. First of all, male Wistar rats (n=80) were randomly divided into two groups; one of which was given a single injection of 0.1 M sodium cold citrate buffer alone (pH 4.5) and the other one was given a single injection of streptozotocin (STZ) (45 mg/kg i.v.) in cold citrate-buffered (pH 4.5) under the ketamine (Ketalar, Pfizer, TURKEY) and Xylazine (Rompun, Bayer Animal Health, GERMANY) anesthetic combination. Rats (n=40) were again randomly divided into two groups; the group was not exposed to MF was called Group I (n=20), the other group exposed to MF 165 minutes (30 minutes working, 15 minutes unworking) a day, was called Group II (n=20). Then, STZ induced group was divided into two groups; the one was not exposed to the MF was called Group III (n=20), the other one exposed to MF 165 minutes (30 minutes working, 15 minutes unworking) 2a day was called Group IV (n=20). Rats were housed in standard metal cages, five animals per cage, and were allowed normal cage activities. All animals were fed with water, available ad libitum and kept in an environmentally room at 23±1 °C, with a light/dark cycle of 12/12 h. Rats were obtained from the Laboratory Animal Research Center of

Cukurova University, Adana, Turkey. All animals received human care according to the criteria outlined in the Guide for the Care and Use of Laboratory Animals prepared by the National Academy of Sciences and published by the National Institutes of Health, and the study protocol was approved by the Animal Ethical Committee of Cukurova University.

Diabetic recordings

Diabetes was verified 48 hours later by the presence of hyperglycemia detected in a drop of urine by using glucotest sticks (Chemstrip 900Udx, Roche Diagnostics, SWITZERLAND). Then 5 mL intracardiac non-fasting blood was taken to determine the final plasma glucose concentration before the rats were sacrificed by decapitation. Final plasma glucose levels were determined by the glucose oxidase method with Olympus AU5200 autoanalyzer.

Magnetic Field

The magnetic field was generated in a pair of Helmholtz coil of 60 cm diameter and 30 cm distance[4,10]. The coils were placed in a 90×90×50 cm sized Faraday cage in order to prevent environmental electromagnetic interaction. Helmholtz coils were connected to a power supply (220V, 50 Hz) and a microprocessor controlled frequency generator developed in Department of Biophysics, University of Cukurova, Adana, TURKEY. For measuring the intensity of the MF, an axial probe of a Teslameter (PHYWE-Germany) was placed inside the MF cage. Magnetic field intensity was 1.5 mT (miliTesla) and there was no heat changes caused by the MF. The rats were put into a 40×40×25 cm plastic cage between the two coils where they can freely wander. The exposure was applied four weeks for groups II and IV. Five experimental animals were placed, in order to be MF exposed on in this cage and exposure was always applied in a separate department apart from the group I and III, and the animals were not exposed to electrical transients when the field was turned on and off.

Solutions

After the animals were killed by decapitation, soleus muscles with sciatic nerve were promptly isolated. Then the tissue was placed into the microelectrode places containing Krebs solution (in mM/ NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 0.6, $KH_2PO_4$1.17, $NaHCO_3$ 25, glucose 11.1). The bath solution was continuously gassed by a mixture of 95% O_2 5% CO_2. The pH of which was maintained 7.4 and temperature was 28 °C. Muscle tissues were perfused at 2.5 mL/min and after a period of equilibrium recording was performed by microelectrode technique.

Electrophysiological Recordings

Microelectrodes were made by pulling capillary glasses, brand name is intrafil with borosilicate filament (outer diameter is 1.0 mm, inner diameter is 0.58 mm). The outer tip of the pulled microelectrode was measured as 0.5-0.6 µm through a microscope scaled. They were filled with 3M KCl, which were placed in microelectrode holder. Ag-AgCl agarose gel electrode was used as a reference electrode. The membrane potentials were recorded through both microelectrode amplicator (Nihon Kohden) and electrophysiological recording system. Using Nihon Kohden stimulator and isolator manually triggered by 0.2 ms duration with rectangular pulses the action potentials were recorded through indirect stimulating nerves of the muscles. The data were recorded from the oscilloscope by the special program to the computer.

Statistical Analysis

The Action potential of soleus muscle curves transferred to the computer. The electrophysiological parameters of the muscle determined. All the results are given as Means ± Standard Error of Means (SEM). Resting membrane potential (RMP), muscle action potential (MAP), depolarization and half-repolarization time, MAP integral, depolarization and repolarization rates were compared by using two-way analysis of variance (ANOVA). The differences were considered significant at $p<0.05$.

RESULTS

The resting membrane potentials and muscle action potentials were recorded by microelectrode technique. The amplitude, depolarization and half repolarization time were obtained from the muscle action potential curves (Table 1). Action potential integrals, depolarization and repolarization rates (mV/ms) were obtained the same curves (Table 2).

Table 1. Action Potentials Parameters in the four different groups. N: Number of the animals, n: Resting membrane potentials number. The datas are mean ± standard error of mean (SEM), Vp-p: Muscle Action potential value, Vo: restiing membrane potential values, Tdep: Action potentials depolarization time, ½RT: Action potentials Half repolarization time.

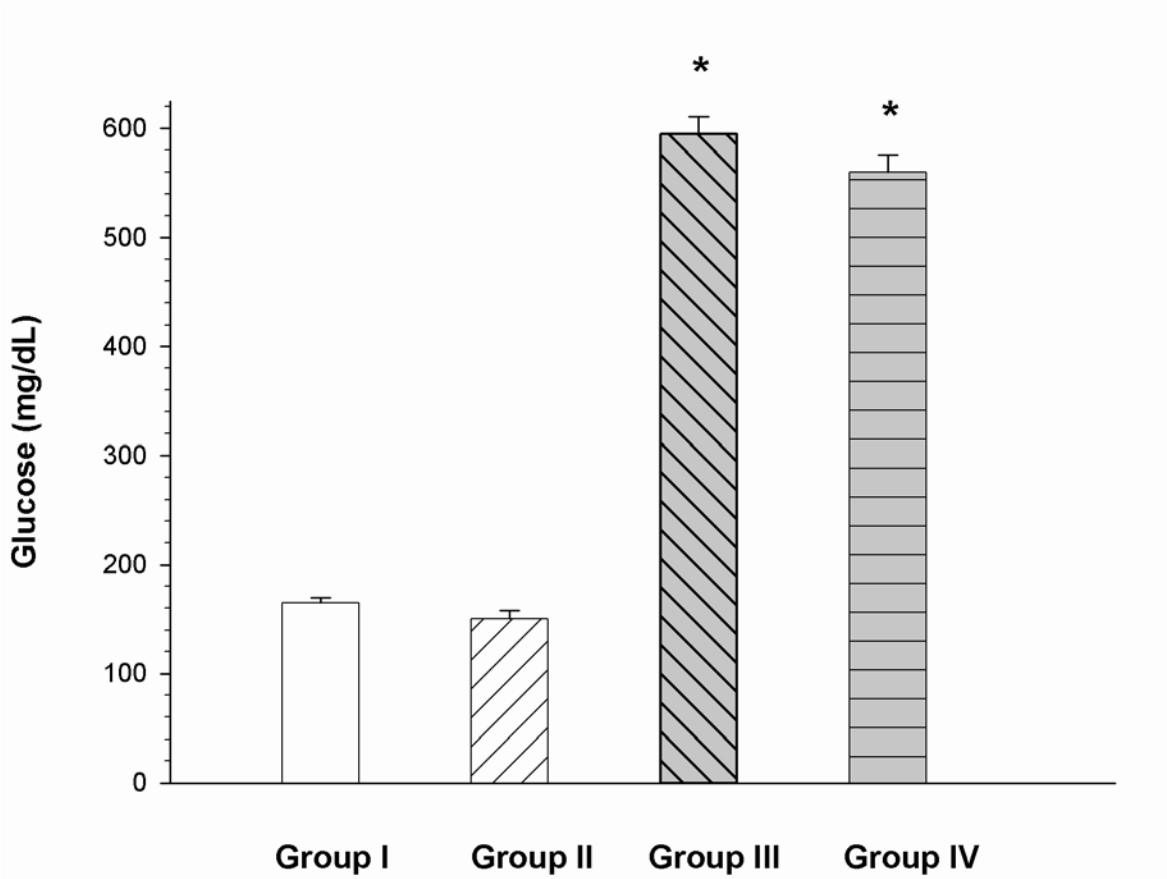
	MUSCLE ACTION POTENTIALS PARAMETERS			
	V_o (RMP) (mV)	**V_{P-P} (MAP) (mV)**	**T_{dep} (ms)**	**½RT (ms)**
Group I (N=20, n=150)	-87,9±0,8	100,3±0,8	0,36±0,008	0,40±0,01
Group II (N=20, n=150)	-88,2±0,6	107,3±1,4*	0,37±0,01	0,31±0,01*
Group III (N=20, n=150)	-82,7±0,7*	93,5±0,7*	0,59±0,01*	0,62±0,009*
Group IV (N=20, n=150)	-82,9±0,7*	95,3±0,7*	0,44±0,006*	0,50±0,008*

*Significantly different from control (Group I), $P < 0.05$.

Table 2 . Soleus muscle - sciatic nerve action potentials integral and derivatives in the four different groups. The values are mean ± standard error of mean (SEM).

Groups	Action Potential Area (Integrals) (mV.ms)	Depolarization Rate (positive derivative) (mV/ms)	Repolarization rate (negative derivative) (mV/ms)
I	160,6 ±1,1	545,5±20,6	170,2±4,1
II	130,8 ± 3,2*	399,4 ±11,9*	119,5±3,1*
III	71,3±0,4*	298,9 ±8,1*	108,1±4,3 *
IV	118,7±1,5*	366,5± 4,6*	110,9±1,4 *

*Significantly different from control (Group I), $P < 0.05$.



* Significantly different from control (Group I), *P*< 0.05.

Figure 1. Plasma Glucose Level of the Four Groups.

The following findings were determined in the cross comparison of the groups in terms of RMP. Significant decrease was found between the group I and group III and group IV in the RMP ($p<0.05$). When we compared the control group and other groups MAP of the control group found to be significant. In addition, when the depolarization time was compared there was a significant increase between group I and group III and group IV. Furthermore, half-repolarization time, MAP integral, depolarization and repolarization rates showed improvement results as occurred in MAP. Magnetic field was corrected all of the diabetic rat parameters.

We compared the plasma glucose level of Group I (165.1 ± 4.7 mg/dL) and Group II (150.5 ± 7.5 mg/dL) we found lower plasma glucose levels in Group II. We also compared plasma glucose levels of Group III (595.0 ±15.6 mg/dL) and Group IV (560.3 ±15.8 mg/dL) and found lower plasma glucose levels in Group IV (Figure 1).

DISCUSSION

There are some studies showing some bioelectrical parameters from diabetic soleus

muscles in the rats. There have been no study on bioelectrical parameter affected by both MF and diabetes. In the analysis of MF, effects on the diabetic soleus contraction rates the effects on particularly contraction and relaxation time could be explained by the changes in ion and/or ion channels.

Action potential depolarization results from the opening of Na^+ channels, whereas repolarization is due to both the closure of Na^+ channels and the opening of K^+ channels[11]. These changes should have altered the rate of action potential depolarization, which was not the case. An exercise-induced slowing of Na^+ channel inactivation could have resulted in a slowing of action potential repolarization.

Skeletal muscle contains many types of K+ channels, each with different roles in the regulation of cellular electrophysiological function. Inward rectifier K^+ channels contribute importantly to resting membrane potential, whereas delayed rectifier K^+ channels play the predominant role in action potential repolarization[12-16]. Other K^+ channel types (e.g., ATP-sensitive K^+ channels and Ca^{2+}-activated K^+ channels) appear to have minor electrophysiological roles in relatively quiescent muscle but could conceivably be more important in actively contracting muscle[16-18].

Several mechanisms have been hypothesized to account for these diabetic changes. One proposal is that low intracellular pH is responsible for the force reduction in diabetic skeletal muscle[19]. Additionally; diabetes has been found to elicit an atrophy of hind limb muscles[20], decrease muscle fiber cross-sectional area resulting in a decrease in muscle oxygen delivery and exchange[20-21], and impair vasoreactivity[22-23].

During an action potential Na^+ ions flow in and K^+ ions flow out of the muscle, based on the concentration gradients across the membrane and the degree to which ion channels open. The duration of the action potential is regulated by Na^+ and K^+ channels, with longer action potentials increasing Ca^{2+} influx and thereby force. If diabetes affects the transmembrane ion concentrations, or if diabetes has direct effects on Na^+ or K^+ channels, the resultant alterations of action potential duration could provide an additional mechanism for impaired muscle force and fatigue resistance in diabetes.

Resting membrane potential was significantly depolarized in diabetic compared with normal muscle in the present study. The resting membrane potential modulates the processes of the action potential by influencing its height and the membrane excitability. Mild depolarization of resting membrane potential diminishes action potential height, whereas more severe maintained depolarization inactivates Na^+ channels, which if sufficiently severe may render the membrane refractory to action potential generation. Most previous evidence as well as the present study suggests that diabetes depolarizes muscle resting membrane potential[1,9,13,24].

The present study indicates that diabetic action potential repolarization is significantly faster and action potential area is significantly lower than in normal muscle. it most likely is due to changes in K^+ ion conductance in diabetes. The action potential repolarization phase is due to the closure of Na^+ channels plus the opening of one of more types of K^+ channels, in particular voltage-gated K^+ channels. The opening of Na^+ channels is primarily responsible for the action potential depolarization phase. Since depolarization was not affected by diabetes (as measured by action potential peak height, rate of rise and rise time), it is unlikely that the change in repolarization properties is due to changes in Na^+ channels. On the other hand, diabetes-induced increases in K^+ channel conductance and/or K^+ channel numbers would result in a faster influx of K^+ and a faster action potential repolarization phase in diabetic muscle. The faster repolarization would result in a decrease in action potential area, a decrease Ca^{2+} release and a decrease in the magnitude and/or duration of muscle contraction.

This study extends these observations by noting that in diabetic muscle the prolongation of the action potential repolarization was significantly less than that in normal muscle. This provides further evidence for alterations in K^+ channels produced by diabetes. An increase in K^+ channel conductance and/or the number of K^+ channels in diabetic muscle would allow more K^+ to flow in during repolarization.

This is a follow up study based upon the findings of our previous study carried on the mechanic features of the soleus and edl muscles[25].

In conclusion, the current study has shown that magnetic field has not enough correction on the resting membrane potential parameters, but there was a significant decrase on the diabetic MAP (Group III), This significant decrase was increased by the magnetic field effects (Group IV). Depolarization time and half relaxation time was prolonged by the effects of diabetes but magnetic field was significant contracted this prolongation. These data furthermore suggest that K^+ channel regulation of action potential is altered by diabetes. Blocking K^+ channels pharmacologically prolongs the muscle action potential duration, which increases Ca^{2+} influx, thereby increasing muscle contraction force.

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