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Research Article / Araştırma Makalesi

Papillary Muscle Action Potential Alterations After Abdominal Ischemia-Reperfusion

Abdominal İskemi-Reperfüzyon Sonrası Papiller Kas Aksiyon Potansiyelinde Meydana Gelen Değişiklikler

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Abstract: Abdominal aortic aneurysm (AAA) has a high prevalence and surgical treatment is still the best option for many reasons. Surgical treatment needs abdominal blood flow to be discontinued, which leads to secondary complications caused by ischemia-reperfusion (I/R) in different organs. Since distant organ damages are seen after surgery, abdominal ischemia reperfusion results in post-treatment deaths especially due to heart based problems. In this study, we aimed to investigate the possible alterations in action potential parameters of papillary muscle after abdominal I/R injury. Adult Wistar-Albino rats were divided into two groups randomly: SHAM group (only laparotomy was performed) and I/R group (abdominal aorta was clamped for 1 hour and reperfused for 2 hours). After the operational period, left ventricle papillary muscles were isolated and action potential (AP) recording experiments were carried out in-vitro. Significant hyperpolarization was seen in resting membrane potential in I/R group. There was no alteration in the general shape of the action potential after I/R. Some delayed-after-depolarizations were recorded, that suggests an impaired persistent Na+ channel activity when interpreted with resting membrane potential findings.This study shows that I/R does not affect the AP parameters except for resting membrane potential. However, it is also likely that the sodium-calcium exchanger (NCX) causes delayed arrhythmias after depolarization due to dysfunction of unknown causes.
Keywords: abdominal aortic aneurysm, action potential, Ischemia-reperfusion, papillary muscle

Özet: Abdominal aort anevrizması (AAA) yüksek prevalansa sahiptir ve cerrahi tedavi hala birçok nedenden ötürü en iyi seçenek olarak görülmektedir. Cerrahi tedavi abdominal kan akımının bir süreliğine kesilmesine neden olmakta ve iskemi-reperfüzyon (I/R) olarak adlandırılan bu durum farklı organlarda hasar meydana getirerek sekonder komplikasyonlara yol açmaktadır. Post-operatif komplikasyonlar arasında kardiyak olanlar en yüksek morbiditeye sahiptir. Bu çalışmada, abdominal I/R sonrası kalp yeterliliği bakımından en önemli role sahip papiller kasa ait aksiyon potansiyelinde meydana gelebilecek değişikliklerin araştırılması amaçlanmıştır. Yetişkin Wistar-Albino sıçanlardan abdominal aortu 1 saat süreyle klemplenip ve 2 saat süreyle reperfüzyon gerçekleştirilenler I/R, yalnızca laparotomi yapılanlar ise SHAM grubu olarak adlandırılmıştır. Reperfüzyon periyodunun hemen ardından sol ventrikül papiller kasları izole edilmiş ve aksiyon potansiyeli kayıtları yapılmıştır. Dinlenim zar potansiyelinin I/R grubunda anlamlı ölçüde hiperpolarize olduğu görülürken, aksiyon potansiyelinin genel görünümünde ise herhangi bir değişiklik meydana gelmemiştir. Kayıtlarda görülen gecikmiş-depolarizasyonlar, dinlenim zar potansiyeli bulguları ile yorumlandığında, Na+ kanal aktivitesindeki kalıcı bir değişimi düşündürmektedir. Sonuç olarak, bu çalışma I/R ile papiller kas hücre zarında hiperpolarizasyon meydana geldiğini fakat aksiyon potansiyeli parametrelerinde anlamlı bir değişikliğin olmadığını göstermiştir. Gözlenen gecikmiş aritmilerin sodyum-kalsiyum değiş-tokuşcusunun (NCX) disfonksiyonu sebebiyle ortaya çıkmış olabileceğini düşündürmektedir.
Anahtar Kelimeler: abdominal aort anevrizması, aksiyon potansiyeli, iskemi-reperfüzyon, papiller kas

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1. Introduction

Although there has been a significant increase in mortality from cardiovascular diseases in recent years, the mechanism of pathogenesis for such diseases has not yet been fully explained. Myocardial papillary muscle is vital in terms of atrioventricular valve adequacy, although it is relatively small in structure compared to cardiac tissue. A tendon of the cord tears, or if one of the myocardial papillary muscles is paralyzed, the tricuspid and mitral valves flex backward during ventricular contraction. A heart failure situation that can occur according to this level of perfection can produce fatal consequences (1). Given this situation, it can be understood that the papillary muscle has an important role in the search for heart diseases. Ischemia causes a sudden decrease in blood flow to the tissue, resulting in a lack of nutritional products, the inability to remove metabolic wastes and oxygen deficiency. Reperfusion is called restoring blood flow after ischemia. Although reperfusion is important to maintain the viability of the ischemic tissue, contrary to what is expected, more tissue damage or even death may occur during reperfusion (2). Abdominal aortic aneurysms (AAA) have a high prevalence rate between 1.0-2.2% among women and 1.1-8.9% among men (3). Morbidity rate of cardiac post-operative complication is 12% among patients treated with open surgery (4).

In this study, we have recorded in-vitro action potential and resting membrane potential values in order to investigate whether there is any electrical remodeling of the papillary muscle membrane after 1 hour of abdominal aortic ischemia following 2 hours of reperfusion.

2. Materials ve Methods

Animals and Ischemia-Reperfusion Model

This study was approved by Necmettin Erbakan University Experimental Medicine Research and Application Center (approval no. 2017-028). 8-week old adult male Wistar-Albino rats weighing 250-300 g were used in experiments. Animals were housed as 4 per cage at ambient temperature and humidity on 12/12h light/dark cycle. Food and water were given ad libitum to rats. Randomly, they were divided to two groups. First group was named as SHAM operated group (SHAM) and the other group as ischemia-reperfusion group (I/R). 8 rats were used for SHAM group and 6 animals were used for I/R group.

Rats were inhaled by using isoflurane (150-200 ml/min O_2 + %2-3 isoflurane) and body temperature was maintained at 37 ^{o}C using heating pad (MAY RTC9404-A Animal Rectal Temperature Controller, Commat Ltd., Turkey) during operations. After preparation of abdominal region approximately 3 cm vertical incision was made. Abdominal aorta was cleaned from connective tissue and was made clear in the intrarenal region. Then, it was occluded by using a microvascular clamp in order to stop blood flow. After 1 h ischemia, microvascular clamp was removed and incision was sutured with 4-0 silk. Rats were put into their cage for 2 h reperfusion period and they were sacrificed for I/R group immediately after reperfusion period. SHAM group was exactly subjected to same procedure except positioning of microvascular clamp on their abdominal aorta (5).

Papillary Muscle Isolation & Action Potential Recordings

Under inhalation anesthesia with isoflurane, the heart was removed from the thoracic cavity quickly, then transferred into the ice-cold Krebs solution (in mM: 135 NaCl, 5 KCl, 1 $MgCl_2$, 2 $CaCl_2$, 15 $NaHCO_3$, 1 $Na2HPO_4$, 11 glucose at pH 7.4, gassed with %95 O_2 and %5 CO_2 mixture). In this solution, the hearts were fixed in the right ventricle via a small steel pin in the position visible from the dorsal region. Both atriums are separated from the hearts with a microsurgical scissors avoiding any kind of mechanical pressures in the hearts. An incision was made from the level of the atrioventricular valve to the apex on the ventricular wall, from which the ventricle was opened to make the papillary muscles visible. Myocardial papillary muscles were isolated by cutting the ventricle tissue from the surrounding tissues without touching the

related muscles as much as possible. Then papillary muscle was attached to bath which was perfused with Krebs solution continuously and temperature was controlled with a water circulator at 37 ^{o}C. Papillary muscles were stimulated using a pair of tungsten electrodes with 0.2 ms duration supramaximal pulses having 1 Hz frequency, generated by stimulator (SIU5, Grass Ins.). Borosilicate microelectrodes fabricated using pipette puller (PN-31, Narishige Japan) were used for intracellular recordings. Electrodes have had 10-20 MΩ resistance when having filled with 3M KCl solution. Intracellular action potentials were recorded through intracellular pre-amplifier (IE-251A, Warner Instruments) using data acquisition unit (MP100, Biopac) at 25 kHz sampling rate.

Properties of the APs were defined with some parameters; amplitude (difference between resting membrane potential and peak voltage), overshoot (difference between 0 mV and the peak voltage), time to peak (time required for an AP to reach peak voltage from resting), maximal rate of rise (maximum derivative of an AP), and 25%, 50%, 75% and 90% decay times (APD25, APD50, APD75, APD90; times required for an AP to repolarize to 25%, 50%, 75% and 90% of peak voltage level, respectively).

Statistics

Data are presented as mean ± standard error of the mean (SEM). Data obtained for two different groups were compared using unpaired Student's *t*-test after testing of normal distribution by Kolmogorov-Smirnov. p values smaller than 0.05 are considered as significant.

3. Results

Results of experiment showed that significant change was only seen in resting membrane potentials (E_m) of the I/R group (Fig. 1) as compared with SHAM group. The E_m values for the SHAM and I/R groups are -67.19 ± 1.04 mV and -71.44 ± 1.13 mV, respectively. In addition to this change, no significant changes were found in APD25, APD50, APD75 and APD90 values of other AP parameters analyzed for 4 different points from repolarization phase (Fig. 2). There was not any significant change for average amplitude (mV) and overshoot (mV) values. Time to peak (ms) value tended to increase for I/R, but it was not significant as it was in maximal rate of rise (mV/ms) value (Table 1).

Table 1. Average values of parameters measured from action potential recordings for each group.

	Amplitude (mV)	Overshoot (mV)	Time to Peak (ms)	Maximal Rate of Rise (mV/ms)
SHAM (n=9)	87.58 ± 3.06	20.72 ± 2.95	11.46 ± 0.77	203.37 ± 32.41
I/R (n=6)	89.68 ± 2.89	21.24 ± 3.71	13.37 ± 0.68	186.67 ± 43.24
p	0.64	0.91	0.10	0.76

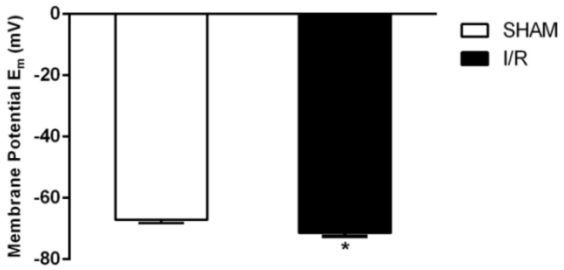

Figure 1 . *Effect of ischemia-reperfusion on resting membrane potential (mV) (SHAM, n =9 and I/R, n=6). * represents significance (p<0.05).*

For all of these values, abdominal ischemia-reperfusion did not cause a significant change in the papillary muscle action potential. In addition, we caught delayed after depolarizations (DAD) during recordings for I/R group as a remarkable finding. We observed a standard AP followed with DAD without any extra stimulus in 3 of the 6 records (%50) used for analysis (Fig. 2).

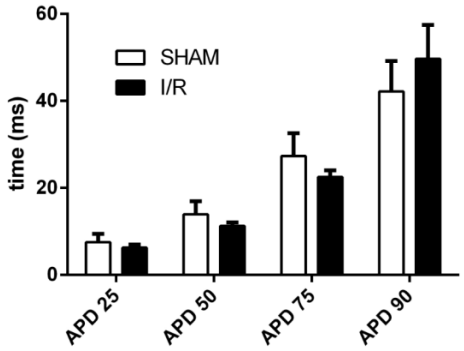

Figure 2. *Average values for repolarization phase of action potentials recorded from SHAM (n=9) and IR (n=6) groups.*

4. Discussion

Heart failure is secondary to abdominal aorta ischemia-reperfusion injury which mimics the systemic inflammatory response after AAA repair (4, 6-9). In this study, we used animal model of AAA repair. In a study by McLaughlin et al. (2000), under light anesthesia blood flow from rats' abdominal aorta was stopped by insertion of a clip into the infrarenal region. Clip insertion time was 60 minutes and the duration of reperfusion was determined as 24 hours, 48 hours and 7 days (10). In this study, it was aimed to reduce the damage caused by ischemia-reperfusion by temperature preconditioning. As a result of this study, with 60-day ischemia followed by 24-hour reperfusion procedure in rats, we have encountered similar findings with clinical findings (11). Blood flow from the abdominal aorta was stopped by insertion of a clip into the infrarenal region. In this study, the duration of clip placement was determined to be 30 minutes and the following reperfusion time was 2 hours. In the abdominal aorta, significant results were obtained in ischemia-reperfusion group compared to the healthy experimental group. It has been argued that these results are very close to the findings of McLaughlin et al (10). We also used this animal model in our previous study to understand underlying mechanisms of respiratory dysfunction after AAA repair (5). So, we determined the periods of ischemia-reperfusion according to that knowledge in this study. Post-operative complications such as heart failure should also occur in that animal model because they are associated with endotoxemia and sepsis conditions (12, 13). Most heart failure arise from a result of electrophysiological remodeling in cardiac cells. Electrophysiological remodeling means that there is something disrupting ionic homeostasis.

In cellular level, some fundamental mechanisms provide ionic homeostasis. These are ion pumps, leakage channels, rectifiers, junctions and exchangers. Na^+-Ca^{2+} exchanger (NCX) is one of the most important parts of resting membrane potential regulation mechanism. It has two working modes which depend on ionic concentrations of Na^+ and Ca^{2+} intracellular and extracellular. They are called as forward and reverse mode. If NCX is working on its forward mode, this means three Na^+ ions come inside the cell and one Ca^{2+} ion

goes out. In reverse mode of NCX same ionic equation works contrarily. After a contraction period, NCX is accepted as responsible for %5 Ca^{2+} regulation. This shows that any change in NCX working mechanism may affect the homeostasis. Our results showed that there is a significant hyperpolarization in I/R group papillary muscle resting membrane potentials. Backzo et. al. indicates this situation in their study that hyperpolarization of RMP occurred because forward mode of NCX was inhibited (14). NCX forward mode provides +1 ionic change for each exchange.

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If ischemia-reperfusion triggered an inhibition mechanism of forward mode of NCX, hyperpolarization could be caused by this phenomenon. This hypothesis need further and detailed studies. In addition to hyperpolarization, another interesting finding is delayed-after depolarizations of papillary muscle cell membrane in I/R groups. Some studies describe this situation as reverse mode dysfunction of NCX. In our study, as shown in Fig. 3, DADs are clearly occurred. It indicates a problem on NCX reverse mode working

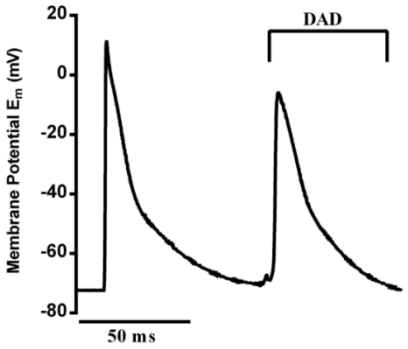

Figure 3. *Sample trace of an action potential followed by a delayed after depolarization (DAD) which was recorded from papillary muscle of I/R group and SHAM group*.

Hyperpolarization of membrane after ischemia-reperfusion injury could be due to conductance alteration of persistent Na channel and decreased activity of Na-K pump which is related to energy metabolism impairment. But in recent studies in diabetic rats which also have damaged energy metabolism, resting membrane potential of papillary muscle cells were found to be depolarized (15) or not changed significantly (16).

As a conclusion, our findings show that ischemia-reperfusion creates alterations papillary muscle ionic regulations. Previous studies showed that these alterations probably caused by NCX dysfunction in both working modes. So, that can be simply said that 1-hour ischemia and 2-hours reperfusion creates a NCX dysfunction according to our findings. This hypothesis need to be revealed with detailed studies which include changed ischemia-reperfusion durations and by using specific NCX inhibitors. Even, in order to get detailed information, molecular studies should be done. All aspects of abdominal ischemia-reperfusion and papillary muscle alterations need further and detailed studies.

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