ARAŞTIRMA YAZISI / RESEARCH ARTICLE

ŞAŞILIKTA EKSTRAOKÜLER KASLARDA KALSİYUM ADENOZiN 5' TRiFOSFATAZ AKTİVİTESİ

CALCIUM ADENOSINE 5'TRIPHOSPHATASE ENZYME ACTIVITY IN EXTRAOCULAR MUSCLES IN STRABISMUS

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ÖZ

AMAÇ: Bu çalışma extraoküler kaslardaki kalsiyum adenozin 5'-trifosfataz Ca^2+-ATPaz enzim aktivite düzeyinin şaşılık ve kontrol gruplarında değerlendirilmesi ve karşılaştırılması amaçlamıştır.

GEREÇ VE YÖNTEM: Şaşılık grubu (grup 1), horizontal kaslarına rezeksiyon yapılan 19 hastanın 20 gözünden oluşmuştur. Daha önce organ nakli için gözünü bağışlamış, ölüm nedeni belli, herhangi bir sistemik ya da göz hastalığı olmayan 5 göz ise kontrol grubu (grup 2) olarak alınmıştır. Her iki gruba aynı miktarda rezeksiyon uygulanmıştır. Enzim aktivitesi, Ca^2+-ATPaz aktivitesi sırasında açığa çıkan inorganic fosfat (Pi) düzeyi ile ölçülmüştür.

BULGULAR: 'Grup 1'de ortalama aktivite 100.07 ± 12.75 Pimg-1 0.90 min-1 olarak ölçülürken, Grup 2'de 165.94 ± 13.60 nmolPi mg-1 0.90 min-1 olarak ölçülmüştür. Grup 1'de enzim aktivitesi, grup 2'ye gore istatistiksel olarak anlamlı düzeyde daha düşük bulunmuştur (p < 0.01).

SONUÇ: Şaşılık cerrahisi sırasında rezeke edilen kaslarda düşük Ca^2+-ATPaz enzim aktivitesinin bulunması şaşılık patofiyolojisindeki olası rolünün bir göstergesi olabilir.

ANAHTAR KELİMELER: Şaşılık, Extraoküler kas, Ca^2+-ATPase

ABSTRACT

OBJECTIVE: This study aims to assess and compare the level of the calcium adenosine 5'-triphosphatase (Ca^2+-ATPase) enzyme activity in resected extraocular muscles of strabismus and control groups.

MATERIAL AND METHODS: The level of Ca^2+-ATPase enzyme activity was evaluated in the extraocular horizontal muscles resected from twenty eyes of 19 patients who underwent strabismus surgery (group 1) and extraocular horizontal muscles samples taken from five eyes of 5 corneal transplant donors (control group - group 2). Enzyme activity was measured via inorganic phosphate (Pi) levels released during Ca^2+-ATPase activity.

RESULTS: The average activity was calculated as 100.07±12.75 nmol Pi mg^{-1} 0.90min^{-1} in group 1 and as 165.94±13.60 nmol Pi mg-1 0.90 min^{-1} in group 2. The enzyme activity in group 1 was significantly lower than in group 2 (p <0.01).

CONCLUSIONS: The low level of Ca^2+-ATPase enzyme activity in muscles that were resected during strabismus surgery might be an indicator for their possible role in pathophysiology of strabismus.

KEYWORDS: Strabismus, Extraocular muscle, Ca^2+-ATPase

INTRODUCTION

Strabismus may develop due to various reasons and is a disease group that affects the binocular vision due to deterioration of the parallelism of the visual axis of the eye (1). Independent of the etiologic factors in strabismus, the mechanism initiating the muscle weakness at the cellular level and the resulting dislocation are the issues of concern (2). Ca^{2+} ion hemostasis, which has significant importance in muscle contraction, and the enzyme system conducting this may be the first topic for investigation. There might be deterioration in the Ca^{2+} ion pump if a decrease is detected in the function of a muscle. Ca^{2+} ion hemostasis and Ca^{2+}-ATPase activity levels may have a role in the pathogenesis of strabismus.

However, little is known about its relation with extra ocular muscles (EOM) and strabismus.

Based on this opinion, Ca^{2+}-ATPase enzyme activity in EOM samples obtained from resection surgeries performed on the weak muscle was measured with the detection of inorganic phosphate (Pi) that was released during the activity. These measurements were compared with the Ca^{2+}-ATPase enzyme activities of healthy horizontal EOM samples resected from donors that were used in corneal transplant. In order to investigate whether Ca^{2+}-ATPase enzyme activity was affected in the EOMs of patients with strabismus, the activity levels were measured.

MATERIAL AND METHODS

Muscle specimens were extracted from twenty eyes of 19 patients who underwent planned resection of the horizontal muscles (group 1) and specimens were resected from the horizontal muscles of five eyes of 5 corneal donors to serve as the control group (group 2).

Patients with esotropia or exotropia with resection in medial rectus or lateral rectus muscles were included in this study. Patients with a history of other ocular disease or who had undergone previous ocular surgery were excluded.

The patients in the control group were selected from those who donated their eyes with no ophthalmological diseases or chronic diseases (e.g.,hepatitis, tumor, sepsis).

Strabismus surgery was performed under general anesthesia by making a conjunctival incision from the limbus. Incisions continued posteriorly in parallel with the upper and lower side of the global fibers of the EOM. The muscle to be resected was taken out via a crochet and marked from its adhesion point by fixing the part that was going to be resected. Later, the muscle was cut from its adhesion point. The muscle that was resected was frozen instantly (-20℃) in a dry tube.

First, the death registrations were made of the patients in the control group regardless of their age and sex, then 5 mm of global EOM was resected and taken into a dry tube and frozen (-20℃).

In the EOM homogenate preparation process; homogenization, pH measurements, and spectrophotometric measurements were performed using a Heidolph Type 50110, Beckman SS.1 pH meter, SchmadzuUV-260 and Teknikon AMES RA-50, respectively. Homogenate was prepared from EOM cells and 10mL 0.3M sucrose was used for 1 gram of tissue. Homogenization was made mechanically by passing the piston 15 times. The Ca^{2+}-ATPase enzyme activity measurement is made based on a certain principle: inorganicphosphate (Pi) released in one hour per milligram protein from 3 mM disodium ATP added to the environment during incubation (3, 4). A sucrose solution was used to prepare homogenates and separate the particulate fractions. After *homogenisation*, the muscle homogenates were added to the incubation environment with 0.15 mM Ca^{2+}, 6 mM Mg^{2+}, 0.3 mM EDTA, and 1 mM tris– HCl buffer (pH =7.4).

After pre-incubation for 5 min at 37 ℃ disodium ATP was added to each tube to reach a final concentration of 3mM. The tubes were incubated for 5 minutes at 37℃, and then ATP was added to reach a final concentration of 3mM. The blank, standard, and samples were incubated at 37 ℃ for 30 min. After that, they were placed into an ice bath and the reaction was stopped. After stopping the reaction, inorganic phosphate was determined on 1 ml aliquots of the incubated mixtures by addition of 1 mL lubrol-molybdate and further incubated for 10

min at room temperature. After that, incubated samples were measured at 240 nm against blank on the spectrophotometer for determining the amount of released Pi levels. The Pi value corresponding to this absorbance value was evaluated from a standard KH2PO4 curve (5).

The protein amounts in the samples were measured using the Folin phenol reagent methods performed by Lowry et al (6). The Ca^2+-ATPase activities expressed as nmol Pi release per minute, per mg protein.

Average and standard deviation values were measured and the difference between the group averages was examined using Student's t-test.

ETHICAL COMMITTEE

This prospective and comparative study was conducted in Hospital, Çukurova University Medical Faculty Department of Ophthalmology in accordance with the Declaration of Helsinki, and it was approved by the local ethics committee of Çukurova University Medical Faculty with protocol number TF 97 U9.

RESULT

Ca^2+-ATPase enzyme activity was measured in the extraocular horizontal muscles resected from twenty eyes of 19 patients who had strabismus surgery (group 1) and 5 eyes of corneal donors as the control group (group 2). Of the 19 patients in group 1, 10 were male (52.6%) and 9 were female (47.4%). The average of age of the patients in group 1 was 16.7 ± 7.6 (range, 5-50) years.

Of the 19 patients in group 1, 12 had exotropia, and 7 had esotropia **(Table 1)**.

Resection only was performed to the horizontal muscles in 11 of the 20 eyes; resection to one muscle and recession to other muscle was performed in 9. The average resection amount among the patients in group 1 was 4.9 (range, 3-6) mm. The resection amount of the horizontal muscles taken from corneal donors in group 2 was 5 mm (p= 0.061), **(Table 2)**.

The average and standard deviation of Ca^2+-ATPase enzyme activity of the 20 horizontal muscles in group 1 was 100.07 ±12.75 nmol Pi mg^{-1} 0.90 min^{-1}, and the average and standard deviation of the 5 horizontal muscles in group 2 was calculated as 165.94 ± 13.6 nmol Pi mg^{-1} 0.90 min^{-1}. The enzyme activity in group 1 was significantly lower than in group 2 (P =0.032), **(Table 2)**.

Table 1: $Ca+^2$ – ATPase enzyme activity, diagnosis and surgery type of patients in strabismus group (group 1)

Patient No	Diagnosis	Operation	Ca-ATPase Specific activity (nmol Pi . mg-1 .90 min^{-1})
1	Right XT	Right MR 6.0 mm res.	112.2
2	Left XT	Left MR 6.5 mm res.	96.0
3	Right ET	Right MR 5.0 mm rec. + LR 5.0 mm res.	104.4
4	Left ET	Left MR 5.0 mm rec. + LR 6.0 mm res.	94.5
5	Left ET	Left LR 5.0 mm res.	112.4
6	Right ET	Right MR 3.5 mm rec. + LR 4.0 mm res.	102.3
7	Right dominance alternating ET	Bil.MR 5.5 mm rec. + Right LR 5.5 mm res.	87.8
8	Right XT	Right MR 6.0 mm res.	95.4
9	Right dominance alternating XT	Right LR 5.0 mm rec. + MR 5.0 mm res.	97.7
10	Right XT	Right MR 5.0 mm res.	96.5
11	Left XT	Left MR 4.5mm res.	114.7
12a	Alternating XT	Right MR 5.0 mm res.	126.0
12b	Alternating XT	Left MR 5.0 mm res.	89.0
13.	Left XT	Left LR 5.0 mm res. + MR 5.0 mm res.	68.4
14.	Left dominance alternating XT	Left LR 6.0 mm rec. + MR 5.5 mm res.	87.5
15	Right XT	Right MR 3.0 mm res.	91.5
16.	Right XT	Right MR 4.0 mm res.	114.0
17.	Right XT	Right MR 3.0 mm res.	105.6
18.	Right dominance alternating ET	Bil. MR 5.0 mm rec. + Right LR 5.0 mm res.	107.5
19.	Right dominance alternating ET	Bil. MR 5.0 mm rec. + Right LR 4.5 mm res.	98.0

Bil, bilateral; ET, esotropia; XT, exotropia; LR, lateral rectus; mg, milligram; mm, millimeter; MR, medial rectus; nmol, nanomole; Pi, inorganic phosphate; rec, recession; res, resection

Table 2: Characteristics and Ca-ATPase spesific activity levels in patients and control groups

Characteristic	Strabismus group (n=19 patient, 20 eyes)	Control group (n=5 patients, 5 eyes)	p-value
Resection muscle length	4.9 mm	5 mm	.061 (p>.05)
Ca^{+2} – ATPase spesific activity (nmol Pi mg-1 .90 min-1)	100.07 ± 12.75	165.94 ± 13.6	.0032 (p<.05)

Data are expressed as the mean ±standard deviation. mg, milligram; mm, millimeter; nmol, nanomole; Pi, inorganic phosphate

DISCUSSION

Extraocular muscles show many differences with regard to the morphologic and functional specifications of other striated muscles in human body. The diameters of the fibers of EOMs are thinner compared with skeletal system muscles, and there is a greater number of nerve sper muscle fiber (7 - 10).

The fibrils of muscles are surrounded by a surface membrane with tubule-like invaginations.

Active contraction and relaxation of the muscle unit depends on free and bound Ca^2+ concentration (11). Ca^2+ transport is performed through calcium adenosine 5'-triphosphatase (Ca^2+-ATPase) in sarcoplasmic reticulum (12). Ca^2+-ATPase is quite important for the contraction and relaxation of muscles. Kjellgren et al. found that sarco (endo)plasmic reticulum Ca^2+-ATPase (SERCA) enzyme expression pat-

tern in EOMs was more complex and different compared with other muscles (13). These differences in the structure of EOMs might be the reason why they work more flawlessly and faster compared with other muscles. On the other hand, in concomitant strabismus, the histology of eye muscle fibers is also basically the same, but changes have been observed in the cellular and biochemical machinery of the fibers (14).

Although cellular level studies were conducted in various diseases where pathologic muscle contraction was observed, the changes in the EOM chemistry in strabismus has not yet been completely clarified. The studies have shown that Ca^{2+} pump activity decreases in muscular dystrophy and Brody diseases (15 - 16). The main cause of Brody disease is the decrease in Ca^{2+}-ATPase enzyme activity depending on the uptake of Ca^{2+} from cytosol. In this study, a cell culture was prepared from skeletal muscle samples of 10 patients with Brody disease, and the Ca^{2+}-ATPase enzyme activity was examined. There was no decrease in Ca^{2+}-ATPase concentration, where as a 50% decrease in enzyme activity was observed (16). In our study, enzyme concentrations from the muscle extracts were not controlled together with enzyme activity.

The enzyme activity of patient group was significantly lower than control group (p < .001). The decrease in the activity was not believed to be due to an uptake defect in cytosol because no such clinical symptom that might be implicative of a systematically genetic disease existed in our patient group. From rare studies of EOM histochemistry, the cause of inferior oblique overaction (IOOA) has been studied at a molecular level more than anatomically, and the histologic mechanism (17). In that study, muscle samples from primary IOOA, secondary IOOA, and a control group were examined. In the samples taken, the ligation ratio to ryanodine receptor, calcium uptake, the relation between SERCA, calcium uptake and the calsequestrin (Ca^{2+} storage) ratios were examined in all three groups. Ryanodine binding and Ca^{2+} uptake rates were significantly decreased in primary IOOA (p < 0.05). Ca^{2+} pump levels, which are one of the factors that impact the Ca^{2+} uptake rate, were assayed using western blotting for SERCA.

However, no significant difference was found between the three groups. Thus, it was inferred that a reduction in Ca^{2+} uptake in IOOA was not due to reduced Ca^{2+} pump levels, but rather it was probably due to a function an abnormality in SERCA or in phospholamban, a SERCA regulator, or alternatively to reduced level of ATP phosphorylation. This study hypothesized that increased intracellular Ca^{2+} concentrations due to reduced sarcoplasmic reticulum Ca^{2+} uptake might play a role in primary IOOA (17). From this point of view, the finding of a low level of ATPase activity compared with the patient and control group in our study is in agreement with the findings of the aforementioned study. However, one of the limitations of our study was that Ca^{2+} ion concentrations could not be measured due to having inadequate material.

EOMs are divided into two parts. The first part consists of thin muscle fibers including multiple mitochondria in the peripheral orbital layer, and extending through the orbital surface in the muscle layer. The second part involves the central or bulbar layer, and constitutes thick muscle fibers close to the globe with different numbers of mitochondria (18). One other inadequacy in our study was that only the global parts were resected in obtaining the EOM samples. However, Lennerstrand et al. stated that orbital and global fibers in EOMs showed differences according to myosin, heavy chain isoforms, mitochondria numbers, fiber numbers, and fatigue resistance (14). In another study, Jacoby et al. examined the distribution of sarcoplasmic Ca^{2+}-ATPase enzyme in rat and rabbit EOMs by using Ca^{2+}-ATPase – specific monoclonal antibodies. They showed that the contraction-relaxation characteristics of the orbital fibrils were not the same throughout the fibril length. They revealed that this was organized by gene expression and developed due to multiple innervations (19). Therefore, the simultaneous measurements of intracellular Ca^{2+} concentrations, muscle contraction, and Ca^{2+}-ATPase enzyme activity analyses must be performed using similar EOM fibers in a similar anatomic location. Further studies are needed to resolve this issue. One other weak point is the limited number of patients in both the control and study groups.

In the present study, activity levels of Ca^2+-ATPase enzyme which plays a role in muscle contraction, and enzyme system activity that directs this were examined. Ca^2+-ATPase activity in the EOM samples of the weak muscle of patients in strabismus was calculated according to determination of Pi that was released during the activity. A statistically significant decrease was determined in average enzyme activity in the study group compared with the control group (p< .001). No similar study was found on human EOMs in a literature review.

CONCLUSION

This is the first study that examined Ca^2+-ATPase enzyme activity which plays a crucial role Ca ion levels in strabismus. Both active factors and treatment were considered mechanically, and we demonstrated the importance of specialized Ca^2+-ATPase enzyme activity in EOMs.

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