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Effects of Taurine Application on the Prostaglandin and Malondialdehyde Levels In Skeletal Muscle Atrophy Induced by Denervation

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Summary : *The effects of taurine as an antioxidant and Ca^{2+} stabilizer on the denervated fast-twitch gastrocnemius and slow-twitch soleus muscles of the rats were investigated. Transport of taurine into skeletal muscle was performed by the intraperitoneal injection of 150mg/kg/day taurine, beginning 6 hours before neurotomy and lasting 10 days. After 10 days following neurotomy the animals were sacrificed and malondialdehyde (MDA) and prostaglandin E_2 like activity (PGLA) levels of both denervated and non-treated muscles and denervated and taurine-treated muscles were measured.*

MDA levels (X±SE)of the denervated and taurine-treated gastrocnemius muscles were (17.3±2.2 nmol/g) lower than denervated and non-treated controls(67±4.8 nmol/g).But there were no significant differences between taurine-treated and non-treated soleus muscles. Also the PGLA levels (X±SE) of denervated and taurine-treated gastrocnemius muscles were (24.4±3ng/g) lower than denervated and non-treated controls (39.2±4.2ng/g); but the PGLA levels of denervated and taurine-treated soleus muscles were higher than denervated and non-treated controls (41.1±6.5, 25.2±3ng/g respectively).

The protective effects of taurine against lipid peroxidation and PGE_2 production in the denervated muscles were found to be much greater in fast-twitch gastrocnemius muscles than slow-twitch soleus muscles.

Key words: *Skeletal muscle atrophy, taurine, oxidative stress.*

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Denervasyona Bağlı İskelet Kası Atrofisinde Taurin Uygulamasının Prostaglandin ve Malondialdehid Düzeylerine Etkisi

Özet : *Bir antioksidan ve Ca^{2+} stabilizatörü olan taurinin ratlarda, denerve hızlı-kasılan gastroknemius ve yavaş-kasılan soleus kaslarına etkileri incelendi.*

Nörotomiden 6 saat önce başlayıp, 10 gün süre ile günde 150 mg/kg taurin intraperitoneal olarak enjekte edilerek; iskelet kaslarına taurinin transportu gerçekleştirildi. Nörotomiden 10 gün sonra hayvanlar feda edilerek; tedavi görmeyen denerve kaslar ile taurin-uygulanmış denerve kaslarda malondialdehid (MDA) ve prostaglandin E_2 benzeri aktivite (PGLA) düzeyleri ölçüldü.

Taurin uygulanan denerve gastroknemius kaslarında MDA düzeyleri (X±SH) (17.3±2.2nmol/g), tedavi uygulanmamış denerve kontrollara göre (67±4.8nmol/g) düşük bulundu. Fakat taurin uygulanmış ve uygulanmamış soleus kaslarında anlamlı bir fark gözlenmedi. Aynı şekilde taurin-uygulanmış denerve gastroknemius kaslarında PGLA düzeyleri (X±SH) (24.4±3ng/g), tedavi yapılmamış denerve kontrollara nazaran (39.2±4.2ng/g) düşük idi. Fakat taurin-uygulanmış soleus kaslarında PGLA (41.1±6.5ng/g), kontrollere nazaran (25.2±3ng/g) yüksek bulundu.

Denerve kaslarda taurinin lipid peroksidasyonu ve PGE_2 üretimine karşı koruyucu etkisinin, hızlı-kasılan gastroknemius kaslarında, yavaş-kasılan soleus kaslarına nazaran daha fazla olduğu saptandı.

Introduction

It is well known that differentiation and maintenance of skeletal muscle fibers are intimately regulated by the nerve supply of the muscle[1]. If skeletal muscle is denervated, the nature and extent of denervation atrophy may vary with regard to fiber type[1,2]. Neurotomy in adult animals causes a preferential atrophy of type II fibers, because these fibers might be more dependent on neural influence than type I fibers[2,3]. With denervation or disuse, skeletal muscles undergo rapid atrophy leading to a profound decrease in size,

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protein content and contractile strength[4]. The primary cause of muscle wasting in denervation atrophy is the enhancement of protein breakdown. Following denervation, the activities of lysosomal protease have been reported to increase[4]. Muscle also contains a large amount of Ca^{2+}-dependent protease. Calcium induced an increase in protein breakdown consistent with the structural changes observed in muscle, and may be linked to a Ca^{2+}-stimulated release of lysosomal enzymes and sarcoplasmic proteinases[5,6]. Also denervation of skeletal muscle causes degenerative changes leading to alterations of the sarcolemmal and mitochondrial membranes[7,8,9,10]. The decreased respiratory activity found in denervated mitochondria is possibly due to inner membrane damage caused by Ca^{2+} induced swelling. The increased cytosolic Ca^{2+} concentrations observed could result in Ca^{2+} release from the sarcoplasmic reticulum due possibly to a deficit of energy or a specific neurotrophic factor in denervated state[11]. Ca^{2+} is known to be important in regulating many cellular functions. Deficiencies in normal regulation and increasing the amount of free Ca^{2+} in skeletal muscle may cause some abnormalities, such as stimulation of protein breakdown[10], leading to increased synthesis of PGE_2 6 and stimulation of lipid peroxidation[15,16,17]. Rodeman et al. (1982), proposed that PGE_2 mediates the protein catabolic action of elevated cellular Ca^{2+} in skeletal muscle. According to this concept, increase in cellular concentration of calcium stimulates phospholipase A_2, which is Ca^{2+} dependent. This enzyme releases arachidonic acid from membrane phospholipids. This in turn leads to increased synthesis of prostaglandins (PGs) that then activate the lysosomal and nonlysosomal enzymes, cathepsin B and D and Ca^{2+} activated neutral protease. Probably, PGE_2 promotes autophagic vacuole formation[6,10]. PGE_2 levels of denervated and nontreated muscles were found higher in accordance with the degree of the degenerative changes[12].

Oxygen free radicals cause cellular damage by inducing lipid peroxidation. In pathological states free radicals in many tissues are derived from xanthine oxidase metabolism[13,14]. Kondo et al (1993), demonstrated that xanthine oxidase activities in atrophied muscles were significantly higher than in controls and it is known that calcium activated neutral protease participates in producing xanthine oxidase from the xanthine dehydrogenase[15,16,17].

Taurine (a sulphur containing aminoacid) is a nonessential aminoacid found in high concentrations in muscle, nerve, brain and other organs. Taurine concentration in skeletal muscle is markedly dependent on fiber type distribution. It is more abundant in slow oxidative type I fibers than in the type II fibers in normal muscles[18,19]. Taurine decreases the rate of loss of calcium transport and increases the ATPase activities of sarcoplasmic reticulum[20]. So it is suggested that it may function as a membrane stabilizer on sarcoplasmic reticulum. Apart from this, it has been shown that taurine has antioxidant effects. As a direct antioxidant, taurine significantly reduces lipid peroxidation and as an indirect antioxidant, it acts to stabilize the plasma membrane[21,22,23].

Taurine concentration tends to be higher in denervation, muscular dystrophy and myotonia[18,19]. Iwata and Baba(1985) found that chronic taurine administration prevented catabolic changes of fast twitch muscle after denervation, suggesting a protective role of taurine against proteolytic digestion[19].

The aim of this study was to determine the effects of taurine on lipid peroxidation and PGE_2 levels, which are cell-damaging agents, in denervated gastrocnemius and soleus muscles.

Materials and Methods

Both male and female Wistar Albino rats (200±10 g) were anesthetized with Nembutal (30 mg/kg, I.P, Sodium Pentobarbital) and denervated in both hindlimbs as follows. Muscles in the middle third of the length of the thigh were bluntly separated and 1cm segment of the sciatic nerve was excised about 1cm above the popliteal fossa. There was no bleeding and the skin wound was closed with stitches[10,22].

Controls received no treatment and were left in ongoing atrophy for ten days. The experimental group received 150 mg/kg taurine intraperitoneally [24], daily for ten days, beginning six hours before neurotomy.

Animals were sacrificed following an overdose of Nembutal and all the gastrocnemius and soleus muscles of both controls and experimental groups were removed, cleared of fat, nerve and connective tissue. Small tissues samples were immediately dissected and taken for MDA and PGE_2 levels measurements.

MDA for "Thiobarbituric Acid Reactive Substance" was assayed by the spectrophotometric method of Uchiyama and Mihara[25]. Tissue samples were homogenized in ice-cold %1.15 KCl. After centrifugation, the supernatant was added to 1%phosphoric acid and %0,6 thiobarbituric acid. Then mixture is heated for 45 minutes water bath. Then n-butanol is added and centrifugated. The n-butanol layer is taken for spectrophotometric measurement at 535nm and 520 nm excitation[25].

Prostaglandin E_2 Like Activity (PGLA) of both the gastrocnemius and the soleus muscles was measured by bioassay methods of Gillmore and Vane[26]. Two sets of assay tissues that were a rat stomach strip and muscle extracts were prepared and superfused in polygraph channel for recording the activation prostaglandin E_2. The small muscle samples were acidified with hydrochloric acid and the PG_s in them were extracted with ethyl acetate. The responses to the standard PGE_2 of the stomach strips compared with the responses to sample extracts. The changes in length of the assay tissues were detected by strain gauges attached to auxotomic levers and were recorded on the Grass 7G model of polygraph.

Statistical analysis: Data shown in tables and graphics are typical of results obtained in at least two independent experiments, in the statistical analyses, "The Mann-Whitney U Test" was used for paired data, based on means ±SE of at least six animals.

Results

We compared the atrophied soleus and muscles with the gastrocnemius muscles of each rat in the denervated non-treated controls with the muscles of denervated and taurine-treated experimental groups.

MDA levels in the denervated gastrocnemius and soleus muscles of controls and taurine- treated experimental groups are shown in the Figure 1. By ten days after neurotomy the mean MDA level in the gastrocnemius muscles of taurine treated group was significantly lower than the gastrocnemius muscles of non-treated controls ($p<0.05$). But there were no significant differences between the MDA levels in soleus muscles of taurine treated and non-treated groups ($p>0.05$).

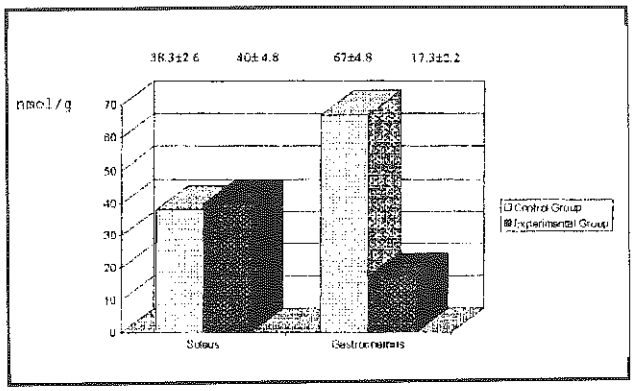

Figure 1. MDA levels (X±SE) of denervated gastrocnemius and soleus muscles of taurine - treated experimental group (n=6) and non-treated controls (n=6); *p>0.05.

Figure 2 compare the PGLA of denervated gastrocnemius and soleus muscles of taurine treated experimental group and non-treated controls. Levels of PGLA in the gastrocnemius muscles of taurine treated groups were found lower than non-treated controls. The difference was statistically significant

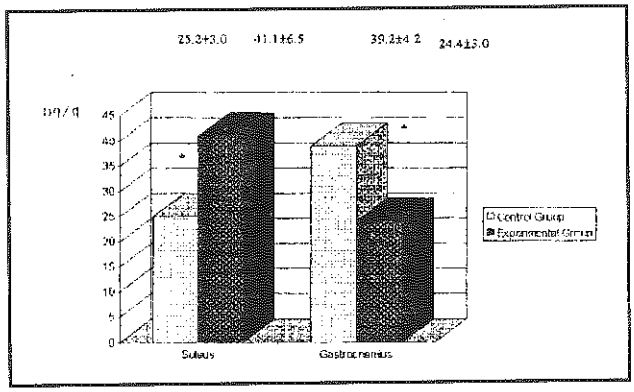

Figure 2. PGLA levels (X±SE) of denervated gastrocnemius and soleus muscles of taurine-treated experimental group (n=6) and non-treated controls (n=6); *p>0.05.

($p<0.05$). In the soleus muscles of taurine-treated experimental group there was an increase in PGLA levels compared with the non-treated controls ($p<0.05$).

Discussion

MDA and PGLA levels have been found high in denervated and non-treated gastrocnemius muscles of controls, but not in soleus muscles. Because it was known that their synthesis increase in skeletal muscles due to pathological states such as atrophy and dystrophy; our findings were in accordance with previous reports that denervation caused a preferential atrophy of type II fibers[1,3].

Physiologically, slow reacting red muscles contain more taurine than fast reacting white muscles[18]. However Iwata and Baba showed that denervation of skeletal muscles increased the transport and the content of taurine in fast twitch muscles but not in slow twitch muscles. They injected tracer amounts of 3(H) taurine intraperitoneally to rats and showed that its transport into fast twitch skeletal muscles seemed to be time-dependent and reached a maximum at about six hours after the injection, and the increase of taurine in denervated muscle was restricted preferentially to fast muscles rather than to slow muscles[19].

In our study it was found that, gastrocnemius muscles in the denervated and taurine-treated experimental group had lower PGLA and MDA levels. These findings encouraged us to suggest the protective and antioxidant effects of taurine in proteolytic digestion of atrophied muscles.

Taurine enhances the capacity for calcium uptake by sarcoplasmic reticulum. Thus it is likely that calcium fluxes in skeletal muscle could be regulated or modified by taurine[20]. Taurine has been shown to protect the guinea pig heart against hypoxic and reoxygenation damage and to attenuate Ca^{2+} influx during ischemia in the rabbit brain[27]. Also taurine administration to patients with dystrophy can markedly reduce the electrical signs of myotonia [28,29].

It was reported that the increase in ion and water permeability due to the membrane damage caused by lipid peroxidation was prevented by taurine probably with a calcium dependent mechanism and in this way it stabilized the membrane[23]. According to another report it was shown that, tissue MDA content was diminished in the taurine treated rats [22,23,30].

In our study the effect of taurine on lipid peroxidation in atrophied skeletal muscle by denervation was determined by MDA production, and we found that taurine decreased the MDA levels in gastrocnemius muscles of taurine-treated group, but not in soleus muscles. Because of the decreased level of lipid peroxidation in gastrocnemius muscles of taurine-treated group we suggest that this aminoacid exerts its beneficial effect by acting as an antioxidant.

We observed that the PGLA levels in the gastrocnemius muscles of taurine-treated experimental groups were lower than non-treated controls. In contrast, the PGLA levels in the soleus muscles of taurine-treated experimental groups were a little higher than the non-treated group. Although we expected that in slow-twitch muscles, taurine did not exert a beneficial effect, perhaps because these denervated muscles could not reserve enough taurine to be effective as mentioned by Iwata and Baba previously[19]; the high PGLA levels of the experimental soleus muscles must be investigated in forward studies.

The present study demonstrated the possible protective effects of taurine in the oxidative stress and in PGE_2 stimulated protein breakdown of fast-twitch muscles following denervation. We suggest that taurine plays an important role in the protection of cells from Ca^{2+} dependent proteolytic activity and oxidative damage by stabilizing the cellular membrane and regulating intracellular Ca^{2+} concentration in denervation.

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