STABILITY OF THE NATIVE AND IMMOBILIZED INULINASE TO VARIOUS DENATURIZING AGENTS

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Today enzyme inulinase (2,l-β-D-fructanfructanohydrolase, EC 3.2.1.7), which splits off fructose residues from the nonreducing end of the molecule of inuline, is of great interest in connection with the possibility of its using in the production of fructose from inuline and inuline-containing materials. When using inulinase for reducing inuline-containing materials, it has received the product-95% fructose syrup, which doesn't demand of special purified methods. Another direction of using of this enzyme is the direct fermentation of inuline into ethanol.

Endoinulinase Aspergillus awamori has been purified by ammonium sulfate precipitation, gel-chomatograhy on sephadex G-100, SDS-PAGE electrophoresis. The immobilization of inulinase by ion exchange AV-26 and AV-17-2P has been made by adsorption and glutaraldehyde methods. The effect of UV-radiation and carbamide on the stability of native and immobilized enzyme has been investigated. DRT-400 lamp has been used in UV irradiation.

It has been determined that UV irradiation in doses 75.5-151.0 J/m^2 leads to the inactivation of soluble inulinase both immobilized inulinase preparations preserve 96 % of catalytic activity in dose 151.0 J/m^{2}min. Doses 906-1400 J/m^2 cause disulfide bonds degradation and the photolysis of catalytic site as a result of amino acid radicals formation. We have observed an increase oh the adsorptively immobilized inulinase stability at the 302 J/m^2. Covalently bonded protein preserves hydrolytic activity in high doses (1300 J/m^2). Thus, covalent immobilization provides high stability for the inulinase to UV irradiation. The type of a way of binding influences significantly on the heterogeneous enzyme preparations stability.

It is shown, that the thermo stability of adsorb immobilized inulinase, bound with anionite AV-26, increases in comparison with native inulinase: the immobilized enzyme has the max catalytic activity at temperature 70°C. For immobilized and native enzymes optimums pH are practically the same, only there is a wider range of meanings pH from 4.5 to 5.0. Activity of the native preparates is preserving completely the thermo stability of covalent bound inulinase is more higher than at the adsorb immobilization. So, after heating to 100°C inulinase, immobilized by the chemical method, shows 19% of the catalytic activity of the native enzyme. The fixation of the triple structure by the multipoint interaction between active groups of the carrier and of the protein takes place after immobilization of the enzyme on anionites. It was shown, that the immobilization leads to the increasing of the activation energy (E_{act}), ΔH of the hydrolysis reaction of inulinase in comparison with diffusion difficulties of the high molecular substrate during the approaching to bounding and catalytic groups of the active center. The negative value ΔS for the hydrolysis reaction of inulinase, realized by the native enzyme, means that the breaking up of the polymeric substrate proceeds with high speed and is characterized by the high order. After the immobilization of inulinase ΔS of the enzyme breaking up of inulinase decreases, apparently at the expense of the direct interaction on of the enzyme with the substrate.

It is shown, that the incubation of soluble inulinase with carbamide in concentration 8 mol/L leads to the total denaturation of the enzyme, and its activity isn't registered. After the interaction of immobilized inulinase with the solution of carbamide in concentration 8 mol/L for 60 min with constant mixing the enzyme showed the catalytic activity (30% of activity of immobilized unmodified inulinase).

Thus, the stability of inulinase in relation to denaturizing agents has been shown to increase with the immobilization of ion exchange. The character of binding with the matrix affects greatly the stability of immobilized enzyme to physical factors.

The article is admitted to the International Scientific Conference «Innovation Technologies», USA, New-York, December 19-27, 2007, came to the editorial office on 12.11.07

LOW-FREQUENCY NEUROMUSCULAR ELECTRICAL STIMULATION TRAINING OF HUMAN SKELETAL MUSCLES IN CONDITIONS OF GRAVITATIONAL UNLOADING

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A number of studies have indicated that sudden exposure to microgravity environment causes a decrease in the tone of the skeletal muscles [Kakurin et al., 1971b; Kozlovskaya et al., 1984], reduction of muscle strength [Cherepakhin & Pervushin, 1970; Kakurin et al., 1971a; Mitarai et al., 1980; Grigor'yeva & Kozlovskaya, 1985; Koryak, 1998; 2002], perceptual and coordination disorders in the neuromuscular systems [Ross et al.,

1984; Grigor'yeva & Kozlovskaya, 1985; Kirenskaya et al., 1985], shift of the spinal reflex mechanisms [Cherepakhin & Pervushin, 1970; Kakurin et al., 1971b; Kozlovskaya et al., 1982], and degradation of joint position sense [Bock et al., 1992; Bock, 1994]. It is accepted that the major factor responsible for all of these changes is the sudden elimination of the proprioceptive information from the muscle and tendon in response to absence of load-bearing. Gravitational loading appears to be necessary for the maintenance of human lower limb skeletal muscle size and force [Kawakami et al., 2000; Kubo et al., 2000; Koryak, 2001]. Studies simulating microgravity have shown that exercise countermeasures can attenuate, but not completely prevent the loss of muscle mass and force [Kawakami et al., 2001; Koryak, 2001]. The muscle groups most affected by exposure to microgravity appear to be the antigravity extensors of the knee and ankle [Akima et al., 2001]. Among these, the plantarflexors seem to be the most affected [Akima et al., 2000], likely due to their greater mechanical loading under normal gravitational conditions. Most notable after exposure to microgravity is a disproportionate loss of force as compared to that of muscle size [Akima et al., 2000; Kawakami et al., 2001], indicating that factors other than atrophy contribute to muscle weakness. The internal architecture of a muscle is an important determinant of its functional characteristics (*force–velocity* relationships, *force–length*, and maximum isometric force [Gans & Bock, 1965; Lieber & Frieden, 2000]. There is a paucity of studies on the effects of disuse [Maganaris et al., 1998] or simulated microgravity [Kubo et al., 2000; Kawakami et al., 2000] on muscle architecture.

Purpose. The purpose of the present study was to investigate the internal architecture of the triceps surae [medial (GM) and lateral (LG) gastrocnemius and soleus (SOL) muscles] in relation to the functional characteristics of the plantarflexors after 6 days of «*dry*» water immersion (DI) with exercise countermeasures [term-long low-frequency neuromuscular electrical stimulation (NMES) trainings].

Methods. To simulate microgravity the DI model has been used [Shulzhenko & Vil-Villiams, 1976]. Four subjects (men-volunteers; 22.8 ± 0.8 yr, 1.84 ± 0.1 m, and 79.3 ± 4.2 kg) gave their written, informed consent to participate in this study, after the Ethics Committee of the Institute of Biomedical Problems RAS had approved the procedures involved. All the experimental procedures were performed in accordance with the Declaration of Helsinki. NMES is applied to four muscle groups of both lower extremities. "*Dry*" electrodes (Ltd. "Axelgaard", USA) are placed on the skin above the quadriceps femoris muscles, the hamstrings, the tibialis anterior, the peroneal, and the triceps surae muscles. The synchronous stimulation of antagonistic muscle groups prevents unwanted joint movements. The NMES-training is performed during 3 hours per day with 1 s « *on* » and 2 s « *off* » trains at intensity levels of 20-30 % of maximum tetanic force and a frequency of 25 Hz and amplitude of stimulus from 0 up to 45 V. The electrical stimulus was provided by the "STIMUL LF-1" stimulator (RUSSIA). The technical equipment consists of electrode trousers carrying stimulation electrodes for the 12-channels, and 2 interconnected 6-channel stimulators caned on a belt. The NMES-training of muscles of the examinee was carried out directly in a bath. Subjects performed a series of isometric plantarflexion contractions on an isokinetic dynamometer ("Biodex", USA) at ankle angles of 0° (neutral ankle position: the footplate of the dynamometer perpendicular to the longitudinal axis of the tibia). All measurements were carried out with the knee joint flexed at 90 deg. A real-time B-mode ultrasound apparatus ("SonoSite MicroMaxx", USA) with a 7·5 MHz linear-array probe, and length of a scanning surface 60 mm and thickness of 10 mm was used to obtain sagittal images of the GM, GL and SOL at rest and at 50 % of plantarflexor MVC at the neutral ankle position. The fascicle pennation angle (Θ) was measured from the angles between the echo of the deep aponeurosis of each muscle and interspaces among the fascicles of that muscle. The length of fascicles (L) across the deep and superficial aponeurosis was measured as a straight line [Abe et al., 2000]. Shorter fascicle L fibres (ΔL_{muscle}) was determined as a delta between L and cos Θ fibres in the active comparison with the passive condition. In the present study, ultrasonic measurement was repeated three times for each subject and averaged values were used. All ultrasonic images were processed with use of the software package "Dr. ReallyVision" (Ltd. "Alliance – Holding", RUSSIA).

Results. After the 6-day DI with application by NMES-training, maximal plantar flexion torque by three subjects has increased on the average by 11.3 % (150 ± 17.3 *vs* 167 ± 6.7 N) and at one has decreased for 9.6 % (155 *vs* 140 N). After DI, in the passive condition, L fibres in the MG, and LG, and SOL has decreased for 12 (from 32 ± 2 to 28 ± 1 mm), 13 (from 36 ± 2 to 31 ± 2 mm), and 13 % (from 36 ± 3 to 32 ± 2 mm) but in the active condition by 18 (from 26 ± 3 to 22 ± 2 mm), 22 (from 36 ± 3 to 28 ± 2 mm), and 21 % (from 32 ± 2 to 26 ± 2 mm), respectively. The Θ angles, in the passive condition, was decreased by 22, 20 and 16 %; but in the active condition by 17, 22 and 17 %, respectively. Shorter fascicle lengths and

steeper fascicle angles in the active compared with the passive condition show internal shortening of fascicles by contraction. Before DI ΔL_{muscle} the MG has found 7.9 mm after has decreased and has made 7.8 mm, and in SOL 5.9 *vs* 5.6 mm. Significant increased in ΔL_{muscle} from 0.9 to 3.3 mm were found by LG.

Conclusion. This study describes, for the first time, the architecture of the human triceps surae [medial (MG) and lateral (LG) gastrocnemius and soleus (SOL) muscles] *in vivo*, both at rest and during graded (50 % MVC) isometric plantar flexions. The results obtained *in vivo* indicate that human MG, LG, and SOL architecture drastically changes both as a function of ankle joint angle at rest and as a function of the force developed during isometric contractions at a fixed joint angle. At rest, when changing the ankle joint angle from -15 to +30 deg, GM pennation angle increased from 31 to 49 deg, LG – from 20 to 28,5 deg, and SOL – from 22.8 to 34 deg; fibre length decreased from 35.5 to 26.8 mm, LG – from 46.8 to 31.2 mm, and SOL – from 39.2 to 28.2 mm. These results indicate that fibre length and pennation angle of the human triceps surae cannot be assumed to remain constant with changing muscle length [Huijing & Woittiez, 1985]. The decrease in fibre length and increase in pennation angle with increasing muscle length may be ascribed the taking up of the slack characterizing these structures [Huijing & Woittiez, 1985]. In the present study, the decrease in fibre length occurring from -15 to +30 deg of passive plantar flexion also suggests that muscle fibres became progressively slack with increasing ankle joint angles. The major findings of this study were that, after 6 day DI with of NMES-training, isometric maximal voluntary torque by the plantar flexor muscles increased. Previous studies have documented decrease of the contractile properties of skeletal muscles during DI (Grigor'yeva & Kozlovskaya, 1985; Koryak, 1998a; 2002, 2003). The present exercise training resulted small increased (~11 %) in maximal voluntary plantar flexion torque in the triceps surae muscle what is antigravitational the triceps surae muscle whereas absence of preventive actions results in decrease in MVC more than on 50 % [Grigor'eva & Kozlovskaya, 1985; Koryak, 1998a, b; 2002, 2003) and in P_o more than on 30 % (Koryak, 1998a,b; 2001, 2003].

Efficacy of NMES-training for increased the contractile properties of skeletal muscles has been suggested in previous studies [Koryak, 1995; Mayr et al., 2000; Koryak et al., 2002]. The insignificant increase in force of contraction in the present study can be assumed it is defined by slack intensity impulses.

It is well known that the smaller motoneurons innervating muscles are more readily activated than the larger cells innervating units [Henneman et al., 1965; Burke & Edgerton, 1975], as the strength of the contraction increases progressively. The smaller units consist of slow twitch muscle fibres (type I) and the larger units consist of fast twitch fibres (type II). In submaximal voluntary contractions, type I fibres the motor units are activated by the synaptic current impinging on the motor neuron. The situation is completely different in contractions triggered by NMES, because the muscle fibres of the motor units are activated by an electric current which is applied extracellularly to the nerve endings, and larger cells with lower axonal input resistance are more excitable [Blair & Erlanger, 1933; Solomonow, 1984]. In fact, when the stimulus is applied from outside the cell, the electric current must first enter through the membrane before it depolarises the cell, but the extracellular medium shunts the current, and the smaller motor units will not be activated during submaximal NMES because of their higher axonal input resistance. Therefore, the smaller motor units do not adapt to training with submaximal NMES. However when use electrical stimulation high training intensity, larger force NMES-training to be more efficient exercise [Almekinders, 1984].

Internal architecture of the GM, LG, and SOL muscle was altered and this was only partially prevented by exercise countermeasures. Both fascicle length and pennation angle were reduced after DI with NMES, this strongly suggests a loss of both in-series and in-parallel sarcomeres, respectively. The functional consequence of the decreased fascicle length was a reduced shortening during contraction. The loss of in-series sarcomeres would mean that this is likely to have implications both on the *force-length* and *force-velocity* relationships of the muscle. The observation of a smaller pennation angle during contraction after DI with NMES will partially compensate for the loss of force, because of a more efficient force transmission to the tendon. The reduced initial resting pennation angle probably, grows out reduction decreased tendon stiffness or of the muscle-tendon complex that finds confirmation in substantial growth ΔL_{muscle} of LG (with 0.9 up to 3.3 mm after DI) during contraction. This observation is consistent with the findings of Kubo et al. [2000]. In conclusion, NMES-training was partially successful in mitigating the loss of function and architecture induced by prolonged DI. Apparently, by ascending during NMES-trained a flow muscular afferentation [Gazenko et al., 1987]. In summary, from the present results, follows, first, that the architecture different lead the triceps surae muscle considerably differs, reflecting,

probably, their functional roles, second, various changes fibre length and pennation angle between different muscles, probably, are connected to distinctions in ability to develop force and elastic characteristics of sinews or muscle-tendon complex and, at last, in the third, NMES -training has preventive an effect on stimulated muscles: in part reduces loss of force of reduction of the muscles, the caused long unloading. The received data, allow concluding, that use of NMES-trained renders the expressed preventive action, essentially reduces depth and rate of atrophic processes in muscles.

Acknowledgements

The author are particularly grateful to Mrs. M. Kuz'mina, M.D., Ph.D. (Clinical Hospital N 1 President Medical Center, Moscow, RUSSIA) for the executed ultrasonic researches. Special gratitude the author expresses to Mrs. V. Kovalenko, Head of Moscow Representation «Dalco International» (Moscow, RUSSIA) for providing the ultrasound apparatus «SonoSite MicroMaxx» (USA). They thank Dr. Berezhinski I., Ph.D., Head of Moscow Ltd. «Alliance - Holding» for development of the software.

At last, the author express gratitude to all volunteers which participated in this research and without their participation there would be no opportunity to receive an actual material.

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The article is admitted to the International Scientific Conference «Present day problems of experimental medicine», Thailand, (Pattaya), February 20-28, 2008, came to the editorial office on 17.12.07

ABOUT INDICATORY ROLE OF THE BIOSUBSTRATES CRYSTALLOGENESIS

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History of the biocrystallization phenomenon scrutiny includes over 30 years [1, 3, 4], but the first mention about it had been written in 1804 [2]. There are many papers, which demonstrated diagnostic function of crystalloscopic and tezigraphic facia [4]. In that time, the unitary publications devote to ability of crystallographic methods for indication of treatment effectiveness [1, 3]. This thesis was aim of our investigations.

Materials and methods. We study the dynamics of the transformation of teziocrystalloscopic picture of some human biofluids (saliva, urine, blood serum, teardrops etc.) in the management process. Free crystallization of biomaterial (classic crystalloscopy) was examined by using the special identification table, which consist of 5 classes of crystal and amorphous structures and half-quantitive additional criteria, such as facia destruction degree [FDD], regularity [R], cellularity [C] and marginal belt [MB]. Tezigraphic facia was evaluated by complex of basic (initiation coefficient [IC]; belt coefficient [BC]) and additional parameters [1, 3]. We used two variants of tezigraphic test. There are comparative and differential teziography, which discrepant by number of the basic substances. Data were processed with statistic programs (SPSS 11.0; Primer of biostatistics 4.03).

Results. On the base of our data it was demonstrated, that the dynamics of the biofluids' teziocrystalloscopic picture correlates with patient common condition and his clinic-functional status. This thesis was verificated on patients, which have gastroenterological, neurological, traumatologinal, cardiological and nephrological diseases. We tested the dynamics of free and initiated biosub-