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Tkachenko S. S., Rodinsky O. G.

ELECTROPHYSIOLOGICAL STUDY OF THE LONG-TERM HYPOANDROGENISM EFFECT ON THE NEUROMUSCULAR APPARATUS

Dnipro State Medical University (Dnipro, Ukraine)

Tkachenkoss@i.ua

The aim of the research was to find out the effect of long term androgen deficiency on skeletal muscles and neuromuscular synapse by bioelectrical activity studying. A 4-month hypoandrogenic state model was created by bilateral orchiectomy on sexually mature male Wistar rats. The parameters of the excitability, evoked potentials of the skeletal muscle and functional mobility of the neuromuscular synapse were studied. As a result, after 4 months of a hypoandrogenic state, the threshold of the neuromuscular apparatus excitation increased, especially with indirect irritation (up to 300%). The latency and duration of the evoked response, as well as the amplitude of the muscle action potential, were increased. A lengthening of the refractory period manifested itself in the slowing down of the response amplitude recovery to the test stimulus in the experimental group when stimulation with double pulses was used. During stimulation by series of impulses with a frequency more than 200 Hz, a rhythm transformation was observed in castrated animals, while in the control group the synapse was able to transmit a signal with a frequency up to 400 Hz.

Thus, negative functional changes in the neuromuscular complex in conditions of long-term hypoandrogenemia can be explained mostly by bioelectrical changes of nervous structures of the neuromotor unit, namely, sharp decrease excitability, deterioration of the amplitude-time characteristics of the action potential and a decrease in the functional stability of the synaptic connection.

Key words: *androgens, neuromuscular synapse, muscle, excitation.*

The connection of the publication with planned research works.

The experimental study is a part of the department research work “Compensatory and adaptive reactions of the central and peripheral nervous system in normal and pathological conditions”. State registration number 0123U100034.

Introduction.

Testosterone take place in carbohydrates, lipid and proteins regulation of metabolism and modulation of the cells and tissues functions, particularly muscle. Testosterone increases synthesis of proteins in myocytes trough androgen receptors stimulation and causing rise of insulin-like growth factor-1. Moreover, it promotes the accretion of myonuclear cells and the involves of satellite cells. There are studies showing a relationship of the weakness development and low levels of testosterone [1, 2].

It`s known that testosterone replacement therapy of spinal injury cause increasing of muscle mass without traditional exercises. The mechanisms of muscular hypertrophy as a result of hormone treatment remain unclear [3].

Numerously clinical methods were used to investigation of pathologies of the neuromuscular system, especially electrophysiological methods, like electroneurography and electromyography, that enables to estemate the bioelectric activity of nerves and muscles as well as to evaluate the rate of excitation via nerve and neuromuscular junction [4]. However, the results of electrophysiological studies of the acute hypoandrogenemia effect on the neuromuscular apparatus in the long term were not found.

The aim of the study.

To find out the effect of androgen deficiency on skeletal muscles and neuromuscular synapse in the long term by studying changes in bioelectrical activity.

Object and research methods.

As a neuromuscular system classical model was used the complex of calf muscle and sciatic nerve with neuromuscular synapse. Researching was conducted in vivo to provide the realisms of model [5].

24 sexually mature Wistar white rat males were used in researching. Animals were divided into control (n=10) and experimental (n=14) groups. Age of animals was 6 months, weight 190-250 g. A model of a hypoandrogenism was made by total orchiectomy [5]. There was only the scrotum membranes section in the control group, followed by suturing. Animals were kept in vivarium (light / dark cycle – 12/12 h, t=22±2°C, a standard diet) during 120 days, then they were involved in an acute experiment. After anesthesia with thiopental sodium (50 mg/kg weight) and preparation of sciatic nerve last one was placed on bipolar electrodes for stimulation. The pair of needle electrodes, introduced in the calf muscle, was applied for evoked action potential (AP) registration and direct myostimulation [5].

We used standard equipment for data processing: an electronic stimulator, an electronic amplifier, an analog-to-digital converter, and a notebook.

Statistical data processing was performed with methods of biometric analysis from EXCEL-2003® and STATISTICA 6.1 (StatSoft Inc., Serial No. AGAR909E415822FA) licensed packages. The calculation of the arithmetic mean, the error of the mean (M±m) and percentages also were used. Probability was estimated with methods of parametric statistic (Student’s t-test). Changes in indicators were considered probable at $p<0.05$ [6].

Table – Indicators of the calf muscle excitability in indirect stimulation, M±m

EP parameters	Control group	Experimental group
Latent period	1.19±0.027 ms (n=10)	1.57±0.04 ms (n=14) ***
Duration	3.51±0.11 ms (n=10)	7.09±0.18 ms (n=14) ***
Amplitude	20.95±1.70 mV (n=10)	34.66±1.98 mV (n=14) ***

Notes: *** – confidence level p<0.001.

All experiments were performed according to with the European Council Directive of 24 November 1986 (86/609 / EEC).

Research results and their discussion.

We analyzed next indicators of excitability – the chronaxy and threshold of the calf muscle excitation during its direct and indirect stimulation; the latent period (LP), the amplitude and total duration of the evoked action potential (AP) – in indirect irritation. The refractoriness was studied with indirect double stimulation with an interstimuli interval from 1 till 20 ms, stimuli duration 0.3 ms, double threshold intensity. For estimation of homosynaptic depression processes was used stimulation of the sciatic nerve with a series of 10 impulses and a frequency of following from 50 till 500 Hz [5].

The value of the threshold of calf muscle excitation in indirect stimulation was 0.053±0.004 mA (n=10), in animals with androgen deficiency it increased to 433.96% (0.23±0.005; n=14, p<0.001). The chronaxy of the calf muscle in indirect stimulation was 45.5±4.54 μs (n=10), in rats of experimental group significant change wasn`t found, however tendency to decrease of the indicator was observed (43.79±1.23 μs; n=14).

We applied the technique of calf muscle direct stimulation for studing of its excitability. The threshold of excitation in the control group was 0.12±0.001 mA (n=10), and in the experimental group it increased to 141.67% (0.17±0.0075 mA, p<0.01, n=10).

The chronaxy value of the calf muscle in direct stimulation, was 70.6±3.1 μs in the control group and decreased in the group of animals with experimental hypoandrogenemia to 60.48% (42.7±1.48 μs, p<0.01, n=10).

About the parameters of the evoked potentials, the latent period increased by 31.93% in comparison with the same indicator in the control group, the amplitude of the AP rise by 65.44%, the duration of the AP increased by 101.99% (p<0.001) **(table)**.

In response to irritation with double impulses, with interval more than 3 ms, a trend to decrease the magnitude of second evoked potential in the group of animals with hypoandrogenemia was found. Value of changes ranged from 27.08% to 11.6% (p<0.001, n=14) relatively indicator value in control group (n=10) **(fig. 1)**.

Significant tendency to a more pronounced inhibition of the amplitude of the tenth AP was observed in animals with orchiectomy relatively the control group index in stimulation frequency over 50 Hz. at stimulation frequencies more than 200 Hz in the experimental group evoked response rhythm transformation with decrease in the frequency of induced AP by 50% was observed. In the control group the phenomenon was observed during stimulation of sciatic nerve with frequency over 400 Hz **(fig. 2)**.

Experimentally, complete repairing of muscle tissue in animals without androgen deficiency was accompanied by a short-term rise of the SDF CHEMOKINE receptor 4 and hepatocyte growth factor (HGF) expression. HGF probably is the muscle satellite cells (MSC) activation signal, and CXCR4 initiates chemotaxis of the activated MSC towards damage region. Analogically, the expression of follistatin altered, which is associated with the control of filling in the repairing process, and IGF-1, which is responsible for the MSCs differentiation [7].

Male sexual steroids also regulate the level of neurotrophic factor of the brain in muscles. The treatment with testosterone after orchiectomy normalized the value of this factor to the one of intact animals [8].

Probably, disorders of the processes of muscle tissue regeneration in natural renewal concerned with androgen lack led to decrease in excitability in the calf muscle, which expressed itself as a rise of the excitation threshold.

It`s known that testosterone applying increases the mRNA level encoding the synthesis of cholinacetyltransferase (CHAT) in the spinal motor neurons of mature male rats. A rise of CHAT mRNA can potentially cause in-

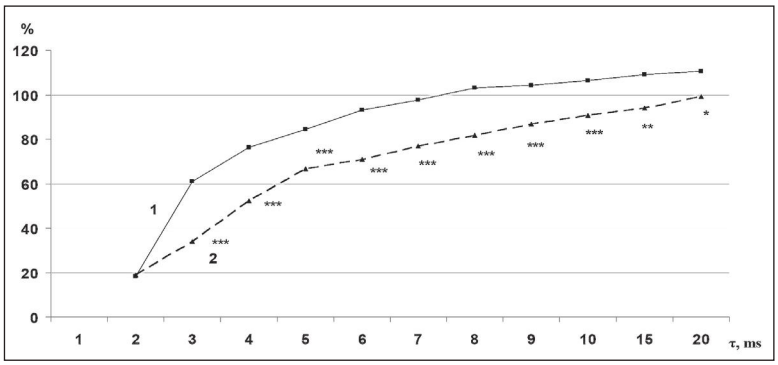

Figure 1 – Dynamics of changes in the action potential amplitude of the calf muscle evoked by a testing stimulus in indirect stimulation.

Notes: 1 – control animals; 2 – animals with experimental hypoandrogenemia. The confidence level * – p<0.05, ** – p<0.01, *** – p<0.001 in relation to the corresponding values of the control group.

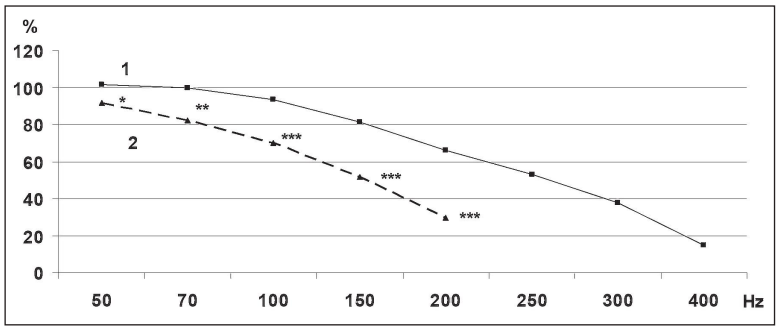

Figure 2 – The changes in the amplitude of calf muscle 10-th evoked potential during its indirect rhythmic stimulation.

Notes: 1 – control animals; 2 – animals with experimental hypoandrogenemia.

crease in the CHAT activity at the presynaptic terminals, increasing the synthesis of acetylcholine [9].

It is probable androgens deficiency impairs transmission of excitation through the neuromuscular synapse because of decrease in the acetylcholine synthesis. This can explain an increased phenomenon of homosynaptic depression, a decrease in the functional mobility of the neuromuscular apparatus and, therefore, dysfunction of motor units as a whole **(fig. 2)**.

Androgen deficiency can cause nerve degeneration, especially myelin coat, which is due to absence of testosterone neuroprotective effect [10].

Treatment with androgens stimulates the recovering of motor neurons after regressive changes and repairs both axons and dendrites, restoring normal neuromuscular function [11].

Deficiency of testosterone can lead to various types of nerve degeneration, which can cause morphological changes [12], that can explain the sharp rise of the excitation threshold of the calf muscle in indirect stimulation in the experimental group of animals.

It`s known testosterone has powerful remyelinating effect, which is due to interaction with androgen receptors in the curison model of sustained demyelination, in which spontaneous myelin sheath recovery is impossible [12].

Normal myelination of sciatic nerve fibers disorder due to low androgens level, in pair with a violation of their morphology, can lead to a decrease in the rate of action potential generation and, as result, cause prolongation of the latent period in experimental animals **(table)**. On the other hand, the rise of latency can be explained by decrease in the concentration of intracellular ionized calcium in the presynaptic ending of motor neurons axons caused by an androgens lack.

Moreover, androgen exposure lead to rapid change in intracellular [Ca2+] [13]. And since calcium levels modulation is a sharply fast reaction that lasts within a few seconds to several minutes, it is assumed that testosterone must bind receptors on the cell surface to provide such result [2].

Testosterone and its metabolites can affect flexibility of cellular membrane, interacting with its phospholipids bilayer, and subsequently alter the functioning of Ca-ATPase and Na / Ka pump [13]. There are evidences that molecules, that influence the lipid bilayer decrease the activity of both transport proteins [13]. It`s probably, testosterone increases the activity of both Sodium / potassium pump and Ca2+-ATPase [1]. Acute testosterone pharmacological therapy demonstrates a dose-dependent rise of the hydrolytic ability of Ca2+-ATPase in the synaptosomal membranes of rat cerebral neurons [13].

Therefore, a result of the androgens deficiency can be a slowdown in the restoring of the initial transmembrane ion gradient due to a dysfunction of ion pumps. The consequence of this is a prolongation of the refractory period, that was observed in animals with a deficiency of male steroid hormones **(fig. 1)**.

It is known that K+ channels in myocytes play a main role in the the duration of repolarization phase of the action potential. Testosterone can alter the function of K+ ion channels, modulating the gene expression and cellular signaling pathways. This can determine sexual differences in bioelectrical and contractile activity. Moreover, androgens directly bind to K+ ion channel or auxiliary subunits, modulating the activity of its activators and blockers. Testosterone increases repolarization current, ultra-fast K+ current, and expression of voltage-dependent K+ channels, that decrease the duration of the myocytes action potential [14].

Therefore, in hypoandrogenemia the opposite effect of refractoriness prolongation, and consequently increase in total duration of AP and reduce a lability can be observed **(table, fig. 1)**.

The metabolite of dihydrotestosterone-3α-diol can interact with the GABAA receptor and cause of increase in intracellular calcium and transmembrane potential gradient [13]. It`s logically that androgen lack can lead to increase in the transmembrane electric potential, that determines compensatory rise of the calf muscle evoked response amplitude in rats after orchiectomy.

Conclusions.

Thus, it was experimentally found that dysfunction of the neuromuscular system in the long term of androgen deficiency can primarily be caused by pathological changes in nerve structures, namely, deterioration of neuromuscular transmission due to disruption of mediator synthesis and its release from the presynaptic pool, as well as the processes of demyelination of efferent nerve fibers and degenerative changes in the axolemma. In favor of the above, there is an experimentally recorded decrease in the excitability of the neuromuscular apparatus, which is more pronounced when the sciatic nerve is irritated than directly in the calf muscle, an increase in the duration of the latent period of the response, a decrease in lability and a rapid exhaustion of the neuromuscular synapse during rhythmic stimulation. Unidirectional changes in the parameters of the excitability of the gastrocnemius muscle with indirect and direct irritation indicate the spread of the influence of low levels of male sex hormones in all parts of the neuromuscular complex. It is possible that the pronounced changes we found are due to a sudden and sharp decrease in the level of testosterone, when the compensatory mechanisms were not fully engaged. It is also not possible to exclude a mediated neurogenic effect of androgen deficiency through modulation of metabolism and electrolyte balance.

Prospects for further research.

It is advisable to continue research expanding it by studying of motor neuron and interneuron pools influences on peripheral link of somatic reflex arc in same conditions, and perspectives of nonhormonal replacement therapy of found disorders.

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ЕЛЕКТРОФІЗІОЛОГІЧНЕ ДОСЛІДЖЕННЯ ВПЛИВУ ТРИВАЛОЇ ГІПОАНДРОГЕНЕМІЇ НА НЕРВОВО-М'ЯЗОВИЙ АПАРАТ

Ткаченко С. С., Родинський О. Г.

Резюме. Метою роботи було дослідження впливу гострої андрогенної недостатності на нервово-м'язову систему у віддалених строках шляхом вивчення змін біоелектричної активності.

Дослідження було проведено на 24 щурах – самцях лінії Вістар віком 6 міс та вагою 190-250 г. Модель гіпоандрогенного стану було створено шляхом двобічної орхектомії. Через 4 місяці тварини були задіяні у гострому експерименті. Подразнення (електричні імпульси прямокутної форми) наносилось на сідничний нерв, викликаний потенціал дії відводили від литкового м'язу. Аналізували поріг, хронаксію, латентний період, рефрактерність, шляхом нанесення подвійних імпульсів, та функціональну стійкість нервово-м'язового синапсу стимуляцією сідничного нерву пачками з 10 імпульсів.

Поріг збудження литкового м'язу збільшувався більш ніж на 300% при непрямому, та на 42% при прямому подразненні. Латентний період збільшився на 32%, амплітуда відповіді зросла на 65%, тривалість потенціалу дії – на 102%, порівняно з контрольною групою. При подразненні парними стимулами, починаючи з інтервалу 3 мс виявлено достовірне зниження швидкості відновлення амплітуди відповіді на тестуючий стимул в групі тварин з експериментальною гіпоандрогенемією. Під час стимуляції пачками імпульсів, вже починаючи з частоти 50 Гц у тварин з орхектомією спостерігалось достовірне зменшення амплітуди відповіді, викликаної десятим стимулом. А при частотах понад 200 Гц спостерігалась трансформація ритму.

Таким чином, виражене зниження збудливості нервово-м'язового апарату за умов довготривалої гіпоандрогенемії відбувається як через підвищення порогу збудження посмугованої м'язової тканини, так і, насамперед, за рахунок нервових структур, зокрема порушення нервово-м'язової передачі, збільшення тривалості рефрактерного періоду та зниження лабільності.

Ключові слова: андрогени, нервово-м'язовий синапс, м'яз, збудження.

ELECTROPHYSIOLOGICAL STUDY OF THE LONG-TERM HYPOANDROGENISM EFFECT ON THE NEUROMUSCULAR APPARATUS

Tkachenko S. S., Rodynskyi O. G.

Abstract. The aim of the researching was to study the impact of acute androgen deficiency on the neuromuscular system in the long term by studying bioelectrical activity changes.

The study was conducted on 24 male Wistar rats, aged 6 months and weighing 190-250 g. The hypoandrogenic state was modeled by total orchiectomy. After 4 months, the animals were taken in the acute experiment. Bipolar electrical stimulation (rectangular-shaped impulses) was applied to the sciatic nerve and the calf muscle, the evoked response of calf muscle was registrated. Threshold, chronaxia, latent period, refractorines were analyzed by applying single, double pulses or bursts of 10 pulses for estimation of functional stability of the neuromuscular synapse.

The calf muscle excitation threshold increased by more than 300% with indirect stimulation and by only 42% with direct stimulation. The latency period increased by 32%, the amplitude of the response increased by 65%, the duration of the action potential – by 102% in comparison with the parameters of intact animals. Irritation by paired impulses with interval above 3 ms demonstrated a significant decrease in the speed of response amplitude recovery to the test stimulus in the group of animals with experimental hypoandrogenemia. In stimulation with group of

impulses with frequency more than 50 Hz, there was a significant reduction of the 10-th evoked response amplitude in castrated animals. The rhythm transformation was observed at stimulation frequency above 200 Hz.

Thus, a pronounced decrease in the excitability of the neuromuscular apparatus under conditions of long-term hypoandrogenemia occurs both due to an increase in the threshold of excitation of striated muscle tissue and, first of all, due to nervous structures, in particular, a violation of neuromuscular transmission, an increase in the duration of refractory period and reduced lability.

Key words: androgens, neuromuscular synapse, muscle, excitation.

ORCID and contributionship:
Tkachenko S. S.: 0000-0002-8828-8349 BCD
Rodynskyi O. G.: 0000-0002-8011-6104 AEF

Conflict of interest
The authors declare no conflict of interest.

Corresponding author
Tkachenko Serhiy Serhiyovych
Dnipro State Medical University
Ukraine, 49000, Dnipro, 9 Volodymyr Vernadsky str.
Tel.: +380500642471
E-mail: Tkachenkoss@i.ua

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