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THE EFFECT OF ESTROGEN DEFICIENCY ON THE FUNCTIONING OF ARC CENTRAL LINK SEGMENTAL REFLEX

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The purpose of this study was to analyze the influence of the spinal cord interneuronal pool due to the lack of estrogens on the functioning of the central link of the reflex arc. The study was performed on 51 mature rats (females). Estrogen deficiency was induced by ovariohysterectomy. L5 dorsal root was stimulated by single and paired square-wave impulses. The response was recorded on the dorsal surface of the spinal cord and the ventral root at the same level. The overall effect of estrogen deficiency is an increase in the excitability of spinal cord motor neurons, activity of the interneuronal pool, inhibition of nerve transmission at low frequencies due to inhibitory influense of the gelatin substance neurons. First time in vivo results of a study of the modulating effect of the interneuron pool on the motoneuron apparatus of the spinal cord in acute hypoestrogenemia will help to understand the pathophysiological mechanisms of impaired sensitivity, motor activity and seizures that are part of menopausal syndrome, especially in ovarian disease.

Key words: menopause, estrogen, spinal cord, interneuron, motoneuron.

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ВПЛИВ ДЕФІЦИТУ ЕСТРОГЕНІВ НА ФУНКЦІОНУВАННЯ ЦЕНТРАЛЬНОЇ ЛАНКИ СЕГМЕНТАРНОЇ РЕФЛЕКТОРНОЇ ДУГИ

Метою даного дослідження було проаналізувати вплив інтернейронного пулу спинного мозку внаслідок нестачі естрогенів на функціонування центральної ланки рефлекторної дуги. Дослідження проводили на 51 дорослому щурі (самиці). Дефіцит естрогену був спричинений оваріогістеректомією. Дорсальний корінець L5 спинного мозку стимулювався одиночними і парними прямокутними імпульсами. Реакцію реєстрували на дорсальній поверхні спинного мозку та вентральному корінці на тому ж рівні. Загальним ефектом дефіциту естрогенів є підвищення збудливості мотонейронів спинного мозку, активності інтернейронного пулу, пригнічення передачі нервових імпульсів на низьких частотах за рахунок гальмівного впливу збоку нейронів желатинозної субстанції. Вперше отримані in vivo результати дослідження модулюючого впливу пулу інтернейронів на мотонейронний апарат спинного мозку при гострій гіпоестрогенемії допоможуть зрозуміти патофізіологічні механізми порушення чутливості, рухової активності та судом, що є частиною клімактеричного синдрому, особливо при захворюваннях яєчників.

Ключові слова: менопауза, естроген, спинний мозок, інтернейрон, мотонейрон.

The study is a fragment of the research project "Mechanisms of compensatory-adaptive reactions of the central and peripheral nervous system in normal and altered conditions", state registration No 0119U100957.

The well-known modeling effect of female sex hormones on the central nervous system [3, 7] is realized through the activation of nuclear estrogen receptors α and β (Erα/β). It is known that nociceptive transmission may be facilitated by blocking ERα with a methylpiperidine-pyrazole selective antagonist (MPP). It has also been shown that in the dorsal horns of the spinal cord of animals ERα is involved in the modulation of pain Aδ and C afferent transmission [12]. Studies have shown that ERα is particularly pronounced in the I, II and IV plates of the gray matter of the dorsal horn of the spinal cord, and is most common in the lower lumbar and sacral segments [11]. It is also known that the use of methylpiperidino-pyrazole dose-dependent increases the frequency of spontaneous emergence of excitatory postsynaptic potentials in neurons of gelatinous substance [12]. However, not only sensory but also motor systems of the spinal cord are exposed to interneuronic chains.

The purpose of the study was to analyze the influence of the activity of the interneuronal pool of the spinal cord due to the lack of estrogens on the functioning of the central link of the segmental somatic reflex arc.

Materials and methods. Studies were performed on 52 mature Wistar rats aged 6–8 months, weighing 180–240 g, from "Dali-2001" PC (Kyiv, Ukraine). All the rats were divided into 2 groups. The first group – intact animals (n=25), the second group – animals with experimental menopause (n=27). Menopause was induced by a minimally invasive ovariohysterectomy through midline laparotomy. The control group of animals underwent midline laparotomy. Both group were kept under standard vivarium conditions with a 12-h/12-h light/dark cycle and a controlled temperature of 20±2 °C on a standard diet for 120 days before they were taken into an acute experiment [1]. After the experiment was over, the rats were devitalized.

To verify the menopause model, microscopic cytological examination of the vaginal smears was performed 120 days after the surgical procedure (Gill GW, 2015), and plasma level of 17β-estradiol was measured selectively using automated elecrochemiluminiscence immunoassay method (ECLIA).

Under general thiopental sodium anesthesia (Sigma, USA, 50 mg/kg), laminectomy and autopsy of the dura mater of spinal cord were performed. Spinal cord was crossed at the level of Th12–L1 segments and filled with vaseline.

To eliminate the manifestations of spinal shock, the animal was kept for 3 hours at 36°–37°C [1]. The dorsal spine of L5 was stimulated by bipolar electrodes with rectangular single pulses of 0.3 ms duration and an amplitude of 1 to 5 thresholds (T), as well as paired stimuli with an application interval of 2 to 1000 ms. The potential of the dorsal surface of the spinal cord (PDSSC) was registered in the focus of maximum activity with a monopolar silver ball electrode, and the reference electrode was placed on the muscles of the lower limb [2]. The monosynaptic induced response of motoneurons (the monosynaptic discharges of the ventral root –MDVR) was recorded at the proximal area of the ventral root. Standard electrophysiological equipment was used for stimulation. The registration was carried out using an analog-to-digital converter and a personal computer. The threshold, chronaxy, duration of the latent period (LP), and the total duration of the induced response and its components were studied. The statistical processing of the study materials was carried out using biometric analysis methods implemented in license packages EXCEL-2003® and STATISTICA 6.1 (StatSoft Inc., Serial No. AGAR909E415822FA). To process the results, we used the computation of the indicators of visibility in percent, the average arithmetic error and the error of the mean (M±m). Probability was estimated using parametric statistics methods (Student's t-test). Changes in the indices were considered reliable at p <0.05. All the xperimental procedures were carried out in accordance with the European Council Directive of the Council of Communities of November 24, 1986 (86/609/EEC).

Results of the study and their discussion. For analysis of peculiarities of interneuron and motoneuron pulls activity in spinal cord under estrogen deficiency, we studied parameters of excitability and characteristics of evoked responses. For PDSSC amplitude and duration of its components were analyzed also. The study was conducted in comparison with value of analogous parameters in control group. In the control group of animals, the threshold for the occurrence of PDSSC, which was assessed on the appearance of AP, was 0.71±0.09 μA (n=13). In the group of animals with experimental menopause, this indicator was significantly increased by 100 % (1.42±0.21 μA, n=16, p<0.001). Chronaxy did not change reliably. LP of the incidence of PDSSC in the control group was 0.17±0.017 ms (n=13), whereas in terms of animals with experimental menopause it was increased to 194.12 % (0.33±0.02 ms, n=13, p<0.001).

Analysis of the amplitude of PDSSC components in terms of animals with EM showed a significant increase in the amplitude of N1, N2, N3 components and P-wave (table 1).

Table 1

Amplitude of PDSSC components under EM conditions, M±m

Amplitude	Control	Animals with EM	Difference
AP	2.01±0.07 mV (n=13)	2.06±0.17 mV (n=13)	1 %
N_1-component	2.07±0.08 mV (n=13)	2.28±0.06 mV (n=13)*	10 %
N_2- component	2.03±0.06 mV (n=13)	2.27±0.06 mV (n=13)*	12 %
N_3- component	0.58±0.06 mV (n=13)	0.86±0.04 mV (n=13)**	48 %
P-wave	0.57±0.04 mV (n=13)	0.75±0.06 mV (n=13)*	31 %

Note: level of probability * – p<0.05; ** – p<0.01.

The amplitude of afferent peak, demonstrating electrical activity of afferent nerve fibers and neurons of dorsal root ganglions, as well as duration of PDSSC components, has not undergone any significant changes.

To study the changes in presynaptic inhibition, paired stimuli with a different interval between stimuli from 1 ms to 1 s were applied. At intervals from 2 to 3 ms, a significant (p<0.01) increase in amplitude of N1 component was observed (fig. 1).

Applying stimulation with value of interstimulus intervals from 6 to 30 ms demonstrated inhibition of N1 component of the second PDSSC (p<0.01). The change of second response amplitude in other intervals between stimuli was unreliable.

Monosynaptic responses of the ventral roots of the spinal cord are an indicator of the activity of the motoneuron apparatus and synaptic transmission. The threshold of their occurrence in animals with EM decreased to 54.17±19.8 % (p<0.05, n=10) in comparison with the control group of animals. Absolute values were 1.30±0.26 μA and 2.40±0.35 μA (n=11) respectively. Chronaxy in the EM group increased in comparison with the control by 7.24±1.38 % (p<0.05, n=10) (97.50±1.35 μs and 90.91±2.59 μs (n=11) respectively). Shortening of LP and increase in the amplitude of the induced responses in EM conditions were also revealed (table 2).

Table 2

Parameters of monosynaptic discharges of the ventral root of the spinal cord under the conditions of experimental menopause, M±m

Parameters	Control	Animals with EM	Difference
LP	0.89±0.035 ms (n=11)	0.76±0.02 ms (n=10)*	15 %
Duration	1.23±0.04 ms (n=11)	1.33±0.03 ms (n=10)*	8 %
Amplitude	1.45±0.086 mV (n=11)	1.85±0.07 mV (n=10)**	28 %

Note: level of reliability * – p<0.05; ** – p<0.01.

When paired stimuli were applied to animals with EM, a slower recovery of the response amplitude to the test stimulus was observed at application intervals shorter 4 ms and from 6 to 100 ms (Fig. 2). The second response amplitude changes in other interstimulus intervals were unreliable.

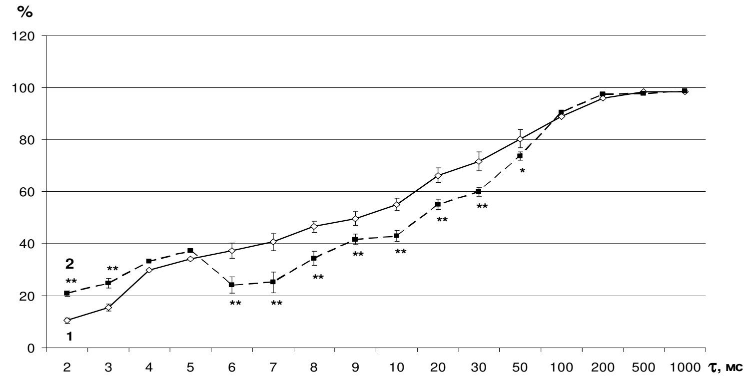

Fig. 1. Recovery of N1-component in terms of control animals and animals with experimental menopause while applying paired stimuli. (Amplitude of second response in percentage of the first one)

1 – Control group; 2 – Animals with experimental menopause. Level of reliability: * – p<0.01, ** – p<0.01 compared with the control group.

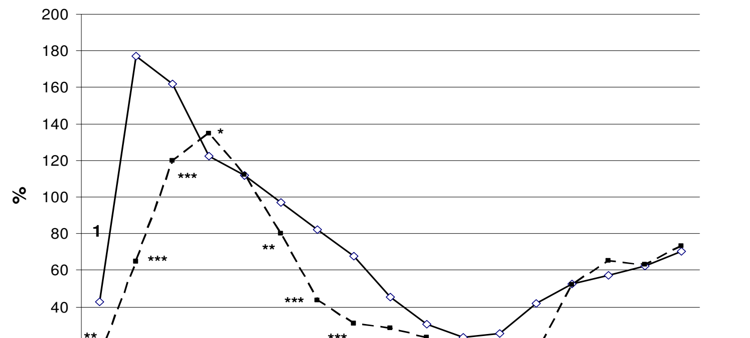

Fig. 2. Nature of inhibition of monosynaptic reflex responses of the ventral root of the spinal cord to the testing stimulus (amplitude of second response in percentage of the first one). 1 – control group; 2 – animals with experimental menopause. Level of reliability: * p<0.05, ** – p<0.01, *** – p<0.001 compared with the control group. Level 17β-estradiol was sharply decreased in comparison with the animals of the control group (< 5 pg/ml (n=5) and 210.6 ± 32.66 pg/ml (n=6)).

Increase in the amplitude of N-components characterizing the activity of segmental (N1, N2) and nonsegmental interneurons (N3) [2] can be explained by the increase in the excitatory glutamatergic transmission during stimulation of afferent fibers in the absence of inhibitory effect of estrogens on this process [12]. In addition, in the absence of excitatory influence of estrogen on ERα, the incidence of spontaneous exciting postsynaptic currents in neurons of gelatinous substance increases [5, 12]. The result of their summation on the interneurons of the dorsal horn of the gray matter of the spinal cord can promote an increase in the activity of these neuronal groups, as evidenced by the change in the amplitude of the corresponding N-components [2]. In addition, a low level of estrogen causes hypocalcaemia due affection of this ion absorption in gastro-intestinal tract [10]. The ionic imbalance leads to an increase in the excitability of interneurons, and, consequently, to increase in their number in the excitation state. Moreover, there is information, that ovariectomy increased mechanical pain sensitivity and 17β-estradiol replacement restored it to basal levels in mice [7]. Besides, It is postulated that plasma membrane calcium ATPase 2 (PMCA2) is essential for adequate ERα signaling in the female and that impaired ERα signaling in the female mice with genotype PMCA2+/– hinders the analgesic effects of E2 leading to increased sensitivity to mechanical stimuli [7], which means increase in electrical activity of segmental interneurons.

Increase in the amplitude of the P-wave in animals with estrogen deficiency indicates an increase in the activity of neurons of gelatinous substance [2] due to the alleviation of the exciting synaptic transmission under the conditions of reduced inhibitory effect of estrogens. The same effect was observed during the selective ERα antagonist (MPP) applying [12].

Inhibition of the second induced response at intervals between stimuli of 6 – 100 ms can be considered to include the presynaptic inhibition of interneurons IV plate of the gray matter [4], including on the part of the gelatinous substance, whose neurons are mainly involved in the formation of P wave [2] and increase the frequency of background impulse in the absence of estrogenic action [9, 11]. Correspondence of the interval from 6 to 100 ms of the duration of positive component of the first PDSSC (114.14±1.78 ms), amplitude of which in the experimental group was significantly increased in comparison with the control, proves it as well.

The reason for a significant increase in the amplitude of N1 component of the second PDSSC at intervals of 2 ms and 3 ms can be explained by synaptic potentiation, and the absence of inhibition at these intervals is explained by the inertia of the dorsal horn braking system due to the presence of a synaptic delay in the interneuron synaptic junctions [8].

Decrease in the threshold of MDVR occurrence can be explained by a shift in the activation potential of the sodium channels of the membrane in such a way that lower depolarization levels are required to generate normal sodium flow [6] due to the decreased calcium level in the blood [10]. The decrease in LP duration, primarily due to the synaptic component, may depend upon the accelerated release of the mediator due to the increase in the intracellular concentration of calcium ions in the neurons of the spinal ganglia under the conditions of estrogen deficiency [9]. Intracellular calcium stimulates synthesis of a retrograde messenger (nitric oxide) which increases the ejection of the glutamate mediator from the presynaptic ending [8], which may explain the increase in the amplitude of the MDVR.

Slower recovery of the response amplitude to the test stimulus may be due to an increase in presynaptic inhibition in the interneurons of the spinal cord dorsal horn in the 6 ms–100 ms interval, which may contribute to a decrease in segmental motor efferentation while explaining the decrease in the amplitude of the second induced MDVR at the indicated interstitial intervals.

Conclusions

The overall effects of the long-term estrogen deficiency are next:

1. An increase in the excitability of spinal cord motoneurons, the proof of which is halving the excitability threshold (1.30±0.26 μA and 2.40±0.35 μA respectively) on the background of decrease in the excitability of spinal cord interneurons, that was showed by significant rise of the excitability threshold from 0.71±0.09 μA in control group to 1.42±0.21 μA in experimental animals.

2. The marked inhibition of high-frequency impulses, that was demonstrated with decrease in amplitude of the second MDVR during pair stimulation at frequency more than 250 Hz, due to deepening of the processes of presynaptic inhibition from neurons of gelatinous substance, activity of which increases significantly at analogous values of interstimulus intervals.

3. Attenuation of middle frequency nerve impulse transmission in the structures of the spinal cord posterior horn due presynaptic inhibition, that was displayed with decrease in amplitude of N1 component of the second PDSSC during pair stimulation at interstimulus interval 6–30 ms

4. Attenuation of middle frequency nerve impulse transmission in the structures of the spinal cord anterior horn due inhibitory influence of substantia gelatinoza neurons, displaying with decrease in amplitude of the second MDVR during pair stimulation at interstimulus interval 6–30 ms, that coincides with duration of P-component of PDSSC.

The prospect of further research is the study the possibility of pharmacological correction of detected changes, mainly with the assistance of non-hormonal drugs.

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INFLUENCE OF BEHAVIORAL PHENOTYPING ON THE DEVELOPMENT OF PATHOLOGICAL CHANGES IN THE SALIVAL GLANDS OF RATS AGAINST THE BACKGROUND OF OBESITY AND STRESS

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Pathological changes in rat salivary glands depending on behavioral phenotyping were analyzed in a model of glutamate-induced obesity and the combined effects of obesity and stress. The study found that under conditions of modeling immobilization stress and the combined effects of obesity and stress, probable changes in the concentrations of thiobarbituric acid reactants, oxidative modified proteins and catalase in the salivary glands of stress-resistant and non-stress-resistant rats compared to controls. A probable difference between the studied parameters in the salivary glands is observed between rats with different stress resistance only in the content of thiobarbituric acid reactants. Thus, under conditions of glutamate-induced obesity and chronic stress in the salivary glands of rats with different behavioral phenotyping, a significant increase in the number of thiobarbituric acid reactants, an increase in oxidative modified proteins and a decrease in catalase activity, indicating a decrease in antioxidant system activity.
Key words: stress resistance, oxidative stress, sodium glutamate.

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ВПЛИВ ПОВЕДІНКОВОГО ФЕНОТИПУВАННЯ НА РОЗВИТОК ПАТОЛОГІЧНИХ ЗМІН У СЛИННИХ ЗАЛОЗАХ ЩУРІВ НА ТЛІ ОЖИРІННЯ ТА СТРЕСУ

На моделі глутамат-індукованого ожиріння та поєднаної дії ожиріння і стресу проаналізовані патологічні зміни у слинних залозах щурів в залежності від поведінкового фенотипування. Дослідження виявило, що за умов моделювання іммобілізаційного стресу та сполученої дії ожиріння і стресу були виявлені вірогідні зміни концентрацій реактантів тіобарбітурової кислоти, окисно-модифікованих білків та каталази у слинних залозах щурів стресостійкого та стресонестійкого типу порівняно з контролем. Вірогідна різниця між досліджуваними показниками у слинних залозах спостерігається між різними за стресостійкістю щурами лише за вмістом реактантів тіобарбітурової кислоти. Таким чином, за умов глутамат-індукованого ожиріння та хронічного стресу у слинних залозах щурів з різним поведінковим фенотипуванням визначено достовірне зростання кількості реактантів тіобарбітурової кислоти, підвищення вмісту окисно-модифікованих білків і зниження активності каталази, що свідчить про зниження активності антиоксидантної системи та розвиток оксидативного стресу.
Ключові слова: стресостійкість, оксидативний стрес, глутамат натрію.

The study is a fragment of the research project "Features of pathological changes development in the digestive system under different conditions and the development of methods for their correction", state registration No. 0120U100502.

The World Health Organization and other international organizations consider obesity a pandemic of this century. Obesity plays a crucial pathophysiological role in the development of insulin resistance, dyslipidemia and hypertension, which leads to type 2 diabetes and increased risk of cardiovascular disease, obstructive sleep apnea, osteoarthropathy and others [7, 8, 11].

New accents of the classical concept of stress, which were proposed at the end of the last century by Sudakov K.V. argue that the basis of stress syndrome in addition to non-specific mechanisms of its implementation are specific systemic hormonal and tissue mechanisms. In the works of most researchers analyzing the various manifestations of stress syndrome are used, as a rule, averaged physiological and biochemical parameters without taking into account the individual typological characteristics of the body.

However, back in 1992 Simonov P.V. proved that stress is an indicator of individual-typological differences. Fedorenko Y.V., Grzegotski M.R. [6] note that the formation of adaptive reactions of the body