RUSSIAN ACADEMY OF SCIENCES
SCIENTIFIC COUNCIL ON PROBLEMS OF BIOLOGICAL PHYSICS
INSTITUTE OF THEORETICAL AND EXPERIMENTAL BIOPHYSICS
INSTITUTE OF CELL BIOPHYSICS

BIOLOGICAL MOTILITY

Materials of International Symposium

Pushchino • 2016

УДК 577.3
ББК 28.07
В60

Biological Motility. – Pushchino: SYNCHROBOOK – 2016. – 280 p.

This volume contains the presentations that were made during the International Symposium "Biological motility". It took place in Pushchino, Moscow region and was devoted to new ashievements and perspectives in this area of knowledge as: the basics of muscle contraction, muscle plasticity and cytoskeleton, nonmuscle motility, new instrumentation and methodology.
Materials of the Symposium are of interest for biologists, medical and other specialists.

Responsible for the issue S.N. Udaltsov

Support of the Symposium by the following sponsors is gratefully acknowledged:

Russian Academy of Sciences

Russian Foundation for Basic Research, project 16-04-20104

ISBN 978-591874-042-2

muscle. There are four types of potassium channels in arterial smooth muscle: voltage-dependent K^+ channels (K_v, the most functionally relevant subtype in arterial smooth muscle is K_v7), Ca^{2+}-activated K^+ channels (BK_{Ca}), inward rectifier K^+ channels (K_{IR}), ATP-sensitive K^+ channels (K_{ATP}). We hypothesized that the role of K^+ channels in the regulation of vasocontractile responses changes during postnatal maturation.

Saphenous arteries were isolated from young (10-15-days old) and adult (2–3-month old) male rats, endothelium was denuded and arterial contractile responses were studied using wire myography. Blockers of K_{ATP} (glibenclamide, 3 μM), K_v7 (XE991, 3 μM), BK_{Ca} (iberiotoxin, 0.1 μM) and K_{IR} ($BaCl_2$, 30 μM) channels were used in order to evaluate the role of different K^+ channels in the regulation of basal tone and contractile responses to $alpha_1$-adrenoceptor agonist methoxamine. mRNA expression levels were determined by qPCR.

K_{ATP} blocker had no effect on basal tone and contractile responses to methoxamine in arteries in both groups. Blockade of K_v7 caused a significant increase of basal tone and contractile responses in young, but not adult rats; this was consistent with higher expression levels of $K_v7.1$ and $K_v7.5$ in arteries of young rat. The effects of BK_{Ca} blocker iberiotoxin were more prominent in arteries of adult animals. This was accompanied by higher expression level of beta-subunit of BK_{Ca} channel in their arteries, while the expression level of alpha-subunit was similar in saphenous arteries of young and adult rats. K_{IR} blockade augmented contractile responses in both age groups, however the effects were more prominent in arteries of young animals.

In conclusion, our results show that in saphenous arteries (i) K_{ATP} channels have no impact on contractile responses to alpha1-adrenoceptor agonist in both age groups; (ii) the influence of K_v7 and K_{IR} channels on the regulation of contractile responses decreases, while (iii) the contribution of BK_{Ca} increases during early postnatal ontogenesis.

This study was supported by the RFBR (grant № 16-04-01395-a).

EFFECTS OF ELEVATED HOMOCYSTEINE LEVEL ON THE RATE OF MATURATION OF RAT SENSORY-MOTOR REFLEXES

A.I. Gilmutdinov*, E.V. Gerasimova, *G.K. Ziyatdinova, N.N. Khaertdinov*, E.R. Ziganshina**, O.V. Yakovleva*, G.F. Sitdikova***

**Institute of Fundamental Medicine and Biology, Kazan Federal University, Kremlevskaya st.18, Kazan, 420008, Russia*

***Institute of Chemistry, Kazan Federal University, Kremlevskaya st.18, Kazan, 420008, Russia*

Homocysteine is a sulpho-containing endogenous amino acid, which is produced in the methylation cycle of protein metabolism and involved in maintaining the cells redox balance. Inherited deficiency of the enzyme methylenetetrahydrofolate reductase (MTHFR), transforming Homocysteine into methionine through remethylation, is associated with severe muscular hypotonia. The reduction in activity of this enzyme is one of the main reasons for homocysteine accumulation in the

body – hyperhomocysteinemia [1]. Extremely high homocysteine levels (up to 200 μM) has been found in some patients with disrupted homocysteine metabolism, and is believed to be at the root of certain vascular disorders including stroke and coronary occlusions [2]. Increased homocysteine concentrations lead to cardiovascular disease, renal dysfunction, associated with a number of neurodegenerative diseases, contributes to the pathologies of nervous system development, leading to disruption of cognitive functions, which one way or another will occur in roughly-motor activity and fine motor skills of the animal [3]. Significantly elevated plasma homocystein levels were detected in mothers of affected children and in maternal plasma and amniotic fluid in neural tube defects pregnancies [4]. The aim in our study was to reveal the rate of maturation of sensory-motor systems in rat's pups born from the rat females with elevated concentration of homocysteine in blood during pregnancy.

The work was carried out on Wistar rats, grown in vivarium of KFU. 88 pups between the ages of 0 and 16 days of life were used. Rats were obtained from 12 female's rat, divided into control and experimental groups. The females of the experimental group ($n = 6$) for 3 weeks prior to pregnancy, during and after delivery, while feeding have got methionine in the dose of 7.7 g/kg per day with food. The females of the control group ($n = 6$) were on a standard diet.

The homocysteine quantification is based on its 1,4-Michael addition reaction with *o*-quinone. The product formed undergoes electrochemical reduction at -0.16 V on glassy carbon electrode modified with multi-walled carbon nanotubes (MWNT/GCE) under conditions of square-wave voltammetry [5]. Voltammetric measurements were performed on potentiostat/galvanostat μAutolab type III with the software GPES, version 4.9.005 (Eco Chemie B.V., The Netherlands). Phosphate buffer solution pH 7.0 was used as supporting electrolyte. The electrochemical cell consisted of working MWNT/GCE, silver-silver chloride saturated KCl reference electrode and counter electrode (platinum wire). 3.66 mL of supporting electrolyte, 40 μL of catechol and 300 μL of plasma were added in electrochemical cell and square-wave voltammograms were registered within the potential range from 0.5 to –0.4 V using the frequency of 25 Hz, amplitude 50 mV, step potential 8.0 mV and scan rate 200 mV s^{-1}. Baseline correction by moving average algorithm included in GPES software has been applied for the better peaks identification. Homocysteine concentration was calculated using calibration graph.

Concentration of the homocystein on blood in the control group was 7±1 μM, in the experimental group was 124±23 μM.

The physical development of the animals of the experimental and control groups was assessed: the weight, the detachment of the ear, the appearance of hair and eye opening. To study somatosensory maturation in rat pups a standard battery of tests assessing the developing behavioral phenotype of rats (P2-P16) during the feeding was used [6].

In the experimental group the physical development of rat pups (the detachment of the ear, the appearance of hair and eye opening) did not significantly differ from the control animals. The most important indicator of somatic development of animals is the dynamic of the body weight. The analysis of the weight

Table 1. Dynamics of weight of newborn rat pups

Group/ Age	2 days	8 days	16 days
Control group	7.9± 0.6g	17.3± 0.5g	35.3± 0.2g
Homocysteine group	6.5± 0.2g	13.7± 0.5g	28.6± 1.6g

dynamics of rat pups has shown the lower body weight of experimental animals compare to control animals (table 1).

The prenatal damaging neurotoxic effects of homocysteine have been studied using sensorimotor reactions in the postnatal period. In tests conducted in early developmental stages of animals (P2-P9), there was a significant impairment in the formation of a number of reflexes (table 2).

In the test "rolling on a horizontal plane" no significant differences were observed in the day of the reflex formation (day 8 after birth), but the reliably faster reflex fulfillment was established in animals of the control group ($p<0.05$). "Pendulum" reflex in rat pups of both groups were formed by 8 days after birth, but the number of head rotations per minute was significantly lower in experimental group compare to the controls (table 2).

In the test, "avoiding the cliff" with a fixed time (10 sec), we observed a significant slowdown in the formation of the reflex in the experimental group. So in rat pups of the control group, the reflex was formed by day 6 after birth, and in the experimental group only by day 7 (table 2). The muscle strength in rat pups of the control group during the specified observation period was greater than in the experiment (table 2).

Table 2. The speed of sensory-motor reflexes maturation in rat pups

Index	Control group	Experimental group
Rolling on a horizontal plane (mean time in sec.) *P*=8	1.62±0.18	2.03±0.21*
Avoiding the cliff (mean day of formation of the reflex)	5.90±0.18	7.11±0.29*
"Pendulum" reflex (mean number of movements) *P*=8	6.52±0.68	2.31±0.30*
Muscle strength (sec)		
P=4	1.44±0.97	0.32±0.24*
P=10	5.06±1.16	1.31±0.32*
P=16	14.97±3.84	2.38±0.83*

* $P_u \leq 0{,}05$ – U-criterion Mann-Whitney

Thus, the obtained data suggest that in rat's pups born from the rat females with elevated concentration of homocysteine the lower weight and the delay of maturation of several sensory-motor reflexes were observed.

This work was supported by Russian Science Foundation №14-15-00618.

References

1 Skvortsov, I. Y. Homocysteine as a risk factor for coronary artery disease / Y. I. Skvortsov, A. S. Korolkova // Saratov scientific medical journal. 2011. Vol. 7, No. 3. P. 619-624.

2 Bukharaeva, E. Homocysteine aggravates ROS-induced depression of transmitter release from motor nerve terminals: potential mechanism of peripheral impairment in motor neuron diseases associated with hyperhomocysteinemia / Bukharaeva, E. A. Shakirzyanova, Khuzakhmetova V., Sitdikova G., Giniatullin R // Frontiers in Cellular Neuroscience – 2015 – Vol. 9 Article 391 – P. 1-8.

3 West, R. K. Homocysteine and cognitive function in very elderly nondemented subjects / R. K. West, M. S. Beeri, J. Schmeidler // Am. J. Geriatr. Psychiatry. – 2011. – Vol. 19 (7). – P. 673-677.

4 Greene N.D.E., Dunlevy L.P.E., Copp A.J. homocysteine is embryonic but does not cause neural tube defects in mouse embryos // Anat Embryol. – 2003 – Vol. 206 - P. 185-191.

5 Lee P. T., Lowinsohn D., Compton R. G. Simultaneous detection of homocysteine and cysteine in the presence of ascorbic acid and glutathione using a nanocarbon modified electrode // Electroanalysis - 2014. – V. 26. – P. 1488-1496

6 Guidance on experimental (preclinical) study of new pharmacological substances. Ed. by R. U. Habrieva. – 2-ed., revised and enlarged. – Medicine, 2005 – 832p.

COMPARISON OF THE PROPERTIES OF "NATURAL" MOLLUSCAN ACTIN WITH STRAUB-TYPE RABBIT ACTIN

U.V. Girich, N.S. Shelud'ko

A.V. Zhirmunsky Institute of Marine Biology,
Far Eastern Branch of the Russian Academy of Sciences,
Palchevsky str. 17, Vladivostok, 690041, Russia

Recently, we have proposed our method for isolation of "natural" actin from the molluscan catch muscle. The need for such a method is determined by the fact that the current method for isolation, designed for the skeletal muscles of vertebrates, is not valid for smooth muscles of invertebrates. Therefore, studies of the muscle contractile apparatus of invertebrates often use the Straub-type rabbit actin combined with muscle proteins of invertebrates. Although actin is a highly conservative protein, we have no full confidence in the correctness of this combination.

In this study, we compared the properties of the Straub-type actin from rabbit skeletal muscles and "natural" mussel actin isolated by us from the catch muscle of *Crenomytilus grayanus*.

We have obtained the following results:

1. Slight differences were detected in the degree of activation of MgATPase activity of the rabbit skeletal muscle myosin in solutions with a low ionic strength (30 mM KCl), while no difference between actins were observed