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Changes in the Levels of Inhibitory and Excitatory Amino Acids in the Brain Structures of Female Rats with Cobalt Epileptogenic Focus during Different Phases of the Estrous Cycle

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We studied changes in the levels of inhibitory and excitatory neurotransmitters in female rat brain structures during different phases of the estrous cycle in health and after creation of a cobalt epileptogenic focus at stage I of epileptogenic system development. The most pronounced shifts were found in the contralateral cortex, where the levels of GABA and glycine decreased significantly during the diestrus-2 phase (corresponding to menstruation), which attests to a convulsive threshold decrease during this period.

Key Words: *amino acids; cobalt epilepsy model; estrous cycle*

Neuronal excitability and convulsive readiness are changing in epileptic women during different phases of the menstrual cycle because of fluctuations of gonadotropin secretion throughout the cycle [1,13]. Estrogens increase the numbers of excitatory neuronal synapses, which leads to a decrease in the convulsive threshold; they also reduce GABA-mediated inhibition and enhance glutamatergic neurotransmission [9,10,12]. By contrast, progesterone potentiates inhibition by activating GABA synthesis and increasing the number of GABA receptors; it also reduces glutamate-induced stimulation, thus reducing neuronal excitability and elevating the convulsive threshold [8]. Therefore, estrogens can be regarded as proconvulsants and progesterone as an anticonvulsant. Inhibitory neurotransmitters GABA and glycine modulate gonadotropin secretion [4] and hence, can be involved in the regulation of the menstrual cycle and neuronal excitability.

We studied changes in the levels of inhibitory and excitatory amino acids in brain structures of female rats during different phases of the estrous cycle in health and after creation of a cobalt epileptogenic focus at stage I of the epileptic system development.

MATERIALS AND METHODS

The experiments were carried out on adult outbred female albino rats (250-290 g) from Lytkino Breeding Center, Russian Academy of Medical Sciences. The animals were kept in a vivarium with 12-h daylight regimen with free access to water and standard fodder. Experiments were carried out in accordance with the

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ethical requirements to manipulations on laboratory animals.

An epileptogenic focus was created by application of metal cobalt powder on the surface of the left motor cortex through a trephination opening with a glass cannula [2].

On day 4, a determinant cobalt epileptic focus (CoEF) appeared in the contralateral right sensorimotor cortex (stage I of the epileptic system development). At this stage we observed clear-cut epileptiform activity characterized by solitary spikes, spike–wave complexes, and bursts of synchronized acute waves with the maximum values in electrograms of the contralateral cortex and hippocampus [3,6]. The control group consisted of sham-operated animals, in which a trephination hole was drilled in the same area, but no cobalt application was carried out. The phase of estrous cycle was determined by microscopic examination of vaginal smears. The diestrus-1 (D-1) corresponds to the premenstrual phase in women, diestrus-2 (D-2) to the menstruation phase, and the proestrus (Pro) to the preovulation phase [4]. Three control and 3 experimental groups were then formed, each of 10 animals, with the corresponding stages of the estrous cycle. The animals were decapitated and brain structures were removed in the cold: the right and left parietal cortex, hypothalamus, right and left hippocampus, which were frozen in liquid nitrogen [11]. The isolated structures were fragmented in a homogenizer (Teflon glass) in 20 volumes of 0.1 n $HClO_4$. The samples were centrifuged at 10,000*g* for 10 min. The levels of inhibitory (glycine, taurine, GABA) and excitatory (aspartate, glutamate) amino acids were measured by HPLC/PD on an Agilent 1100 chromatograph with HYPERSIL ODS analytical column (4.6×250 mm, 5 μ) at λ_{ex}=230 nm and λ_{em}=392 nm.

The data were statistically processed by the standard statistical methods using Student's *t* test and Excel 7.0 software.

RESULTS

Studies of the dynamics of amino acids throughout the estrous cycle in sham-operated (control) females showed an elevation of GABA level from stage D-1 to Pro in the right motor cortex. The differences between GABA levels during the Pro and D-1 and D-2 stages were significant (p<0.05; Table 1). In the left motor cortex, the level of GABA in the Pro stage was also significantly higher than in the D-1 stage. A significant elevation of GABA content in the hypothalamus of control rats was found during D-1 and Pro stages in comparison with the D-2 stage. No appreciable fluctuations in the GABA levels in the left and right hippocampus were found throughout the estrous cycle. The concentrations of glutamate, glycine, and taurine in the left and right cortex, hypothalamus, and hippocampus of control rats virtually did not change throughout the estrous cycle. Hence, clear-cut changes in GABA concentrations throughout the cycle were seen in the rats, with the maximum level during the Pro stage in the cerebral cortex and hypothalamus, but not hippocampus. Our results correlated with the previous data [7], according to which GABA levels were increasing in the hypothalamus throughout the proestrus *vs.* diestrus.

Studies of the dynamics of amino acid levels during the estrous cycle in the brain of rats with CoEF showed the maximum GABA levels in the right motor cortex in Pro stage and significant differences between the Pro and D-2 stages in the right and left cortex (similarly as in sham-operated rats). In addition, significantly higher levels of glycine and taurine were found in the right cortex in comparison with

TABLE 1. Changes in the Neurotransmitter Levels (mmol/liter) in the Contralateral (Right) Cerebral Cortex of Female Rats with CoEF and Sham-Operated Rats Depending on Estrous Cycle Phases (*M*±*SEM*)

Neurotransmitter		Estrous cycle phase		
		D-1	D-2	Pro
Sham-operated rats	aspartate	0.178±0.010	0.264±0.030	0.275±0.021
	glycine	0.107±0.007	0.123±0.004	0.145±0.019
	GABA	0.276±0.023	0.330±0.004	0.442±0.037
Rats with CoEF	aspartate	0.182±0.022	0.177±0.018*	0.272±0.085
	glycine	0.100±0.014	0.070±0.009*	0.174±0.073
	gabas	0.235±0.029	0.229±0.027*	0.463±0.238

Note. *p<0.05 in comparison with sham-operated animals.

D-2 stage. No appreciable fluctuations in the GABA and taurine levels in the hypothalamus, left and right hippocampus were found throughout the estrous cycle in rats with CoEF; however, glycine level in the hypothalamus reduced significantly during D-2 *vs.* D-1 stage. Glutamate concentrations in the left and right cortex, hypothalamus, and hippocampus of rats with CoEF changed negligibly throughout the estrous cycle (similarly as in sham-operated animals).

Comparison of amino acid levels in brain structures of sham-operated rats and rats with CoEF during the same phases of the estrous cycle revealed some significant differences. The levels of inhibitory neurotransmitters GABA and glycine and of excitatory neurotransmitter aspartate in the right contralateral cortex of rats with CoEF were significantly lower than in the control during the D-2 phase, while GABA level in the hypothalamus was lower during the D-1 phase.

Fluctuations of the inhibitory amino acid levels in rats with CoEF throughout the estrous cycle were found mainly in the cortex. In the right contralateral cortex, these changes involved not only the GABA level (as in the control), but also taurine and glycine levels. The most pronounced shifts in GABA and glycine levels in the rats with CoEF during stage I of the epileptic system development were found in the contralateral cortex, in which a determinant secondary epileptogenic focus appeared by that period. These data indicated that fluctuations in the inhibitory amino acid levels throughout the estrous cycle were more pronounced in the right cortex with determinant secondary epileptogenic focus in comparison with the left cortex with the primary focus. The most pronounced changes in rats with CoEF, characterized by a significant reduction of GABA and glycine levels in comparison with the control, were found during phase D-2 (corresponding to the menstruation phase in women).

Hence, modulation of the levels of GABA, but not excitatory amino acids, was detected in sham-operated rats and rats with CoEF: high levels of endogenous estrogens were associated with low level of GABA, while low levels thereof with its high level. This indicated determination of GABA level by the sex cycle phase. Fluctuations in the levels of inhibitory amino acids, depending on the estrous cycle phases, were more pronounced in animals with CoEF in comparison with sham-operated ones and involved not only GABA, but also glycine and aspartate and were found not only in the cortex, but in the hypothalamus as well. The differences in GABA levels in sham-operated animals and rats with CoEF were particularly manifest during the diestrus phase. The data attest to close cooperation of the ovarian hormonal and GABAergic systems in the mechanisms of convulsive state development.

REFERENCES

1. G. N. Avakyan, S. V. Denisova, O. M. Oleinikova, and S. A. Kurbanova, *Zh. Nevrolog. Psikhiatr.*, **107**, No. 9, 85-92 (2007).
2. O. L. Badalyan, L. N. Nerobkova, T. A. Voronina, *et al.*, *Eksp. Klin. Farmakol.*, No. 2, 7-10 (2002).
3. T. A. Voronina and L. N. Nerobkova, *Manual of Experimental (Preclinical) Studies of New Drugs*, Ed. R. U. Khabriev [in Russian], Moscow (2005), 277-295.
4. T. M. Mishunina, *Vopr. Med. Khim.*, **36**, No. 2, 22-24 (1990).
5. J. B. Becker, A. P. Arnold, K. J. Berkley, *et al.*, *Endocrinology*, **146**, No. 4, 1650-1673 (2005).
6. R. S. Dow, A. Fernandez-Guardiola, and E. Manni, *Electroencephalogr. Clin. Neurophysiol.*, **14**, 383-198 (1962).
7. M. Frankfurt, E. Fuchs, and W. Wuttke, *Neurosc. Lett.*, **50**, No. 1-3, 245-250 (1984).
8. A. G. Herzog, M. N. Friedman, S. Freund, and A. Pascual-Leone, *Epilepsy Behav.*, **2**, No. 4, 367-369 (2001).
9. N. Jette and M. J. Morrell, *Am. J. Electroneurodiagnostic Technol.*, **45**, No. 1, 36-48 (2005).
10. J. Logothetis, R. Harner, F. Morrell, and F. Torres, *Neurology,* **9**, No. 5, 352-360 (1959).
11. W. Loscher, D. Honack, and M. Gramer, *J. Neurochem.*, **53**, No. 6, 1737-1750 (1989).
12. M. J. Morrell, *Am. Fam. Physician*, **66**, No. 8, 1489-1494 (2001).
13. M. J. Morrell, G. E. Sarto, P. O. Shafer, *et al.*, *J. Women's Health Gend. Based Med.*, **9**, No. 9, 959-965 (2000).