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THE ALTERATION OF SLEEP-WAKING PHASES CAN BE INDUCED BY QUANTITATIVE CHANGES IN EXCITATIRY AMINO ACIDS

G. BERADZE, L. SHANSHIASHVILI, D. MIKELADZE
Department of Neurochemistry, I.S. Beritashvili Institute of Physiology, Georgian Academy of Sciences, Tbilisi

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Summary

The role of excitatory amino acids - glutamate and aspartate - in the sleep-waking cycle was investigated in the rats. Experiments were carried out in chronic preparations with stereotaxically implanted recording electrodes and cannulae. The latter was used for the lateral ventricle liquor sampling. Experiments have shown that the content of aspartate is maximal in slow-wave sleep and decreases during paradoxical sleep and, especially, during active waking. Concentration of glutamate was found to be increased during slow-wave sleep and in narcotized animals, and like aspartate in decreases in paradoxical sleep and waking. It was founded as well that arginine concentration fluctuates in a similar way. Besides the amino acids, concentration of ammonia was assessed in various phases of sleep-waking cycle. Increase of ammonia was found during paradoxical sleep and active waking.

Key Words: Waking, Paradoxical sleep, Slow-wave sleep, Aspartate, Glutamate, Arginine, NO, Ammonia, Rats.

Introduction

Elucidation of molecular bases of the sleep-waking cycle (SWC) is one of the most pressing problems of current neurobiology and neuropathology. Already in the 70-s T.Oniani suggested that alteration of the sleep phases and waking is determined by the biologically active substances, accumulated in metabolic processes, changes in concentration of which in the brain have regulate the SWC (Oniani 1976). It is undoubtful today that in information of the SWC various neurotransmitter systems do participate, which, via the thalamo-cortical pathways, regulate the course of sleep and awakening phases (McCormick and Bal 1997).

The main impact on the waking and sleep phases produce ascending cholinergic neurons of pedunculopontine tegmentum and lateral dorsal tegmentum, noradrenergic neurons of locus coeruleus, serotoninergic neurons of the raphe nuclei, and histaminergic neurons of hypothalamic tuberolaminar nuclei (McCormick 1992). Among the above structures the leading part belongs to the cholinergic neurons of pedunculopontine tegmentum, excitation of which is concerned with generation of waking and paradoxical sleep (PS) phases, and with cortical activation and PGO-wave genesis (Datta and Siwek 1997; Hobson 1988).

Existence of excitatory projections to the above brain structures is not determined as yet. According to investigations by Datta and Siwek (Datta and Siwek 1997), microinjections of small doses of glutamic acid into pedunculopontine tegmentum elicit PS induction, while administration of the higher doses determines awakening of the animal. These data indicate that excitation of pedunculopontine tegmentum cholinergic neurons may be executed by information coming from the glutamate receptors, activation volume and duration of which depends on concentration of glutamic acid.

Correspondence: Beradze G., PhD., Department of Neurobiology of Sleep-Wakefulness Cycle, I.S. Beritashvili Institute of Physiology, Georgian Academy of Sciences, 14, L. Gotua str., Tbilisi, 380060, Georgia. E-mail: nswc@gateway.ge

It should be noted as well that the rostral pons contain glutamate NMDA-receptors, which, via the swich-off mechanism, regulate the inspiration processes (Fung et al. 1994). The NMDA-receptor antagonists induce decrease of PS duration but do not affect the slow-wave sleep (SWS) and waking duration (Prospero-Garcia et al. 1994), decrease acetylcholine release from pedunculopontine tegmentum neurons and induce synchronization of the electrocorticogram (Rassmusson et al. 1996).

Aspartate and glutamate, being the major excitatory neurotransmitters, at the same time belong to the universal energetic substances, concentration of which depends on the neuron-glia interrelation and maintenance of the brain metabolism (Fillenz 1995). Non-vesicular fund of these amino acids in the intercellular liquid is changed during microcirculation disorders, hypoxia, ischemia, hypoglycemia, and oxidation stress, which impacts the glutamate-, especially the NMDA-receptor activity (Coyle and Puttfarcken 1993). It was shown that in different phases of sleep certain microcirculation alterations do occur (Nikolaishvili and Mitagvaria 1988). In the process of PS the content of vasodilatatory substance, NO, production does increase, while in the process of SWS its content decreases, which correlates with the cholinergic neurons' activity and PGO-waves' frequency (Williams et al. 1997). It was found, as well, that deprivation of NO biosynthesis elicits decrease of sleep duration and increase of SWS incidence (Dzoljic et al. 1996). These changes, obviously, are determined by production of nitric oxide in the neurons of pontine redicular formation, because NO-inhibitors determine significant decrease of acetylcholine release (Leonard and Lydic 1997).

Afore mentioned data indicate that in the various phases of sleep the blood flow and metabolic maintenance of brain undergo fluctuation, which may be reason of quantitative alteration of the main excitatory neurotransmitters - glutamate and aspartate. Quantitative fluctuations of the latter may determine an alteration of the sleep phases, their duration and depth. All the above mentioned prompted us to endeavour our investigation with an aim to evaluate quantitatively glutamate, aspartate, and NO-donor amino acid-arginine, in the lateral ventricle of the brain during waking and different phases of sleep.

Methods

Chronic experiments were carried out in adult Wistar rats. The SWC was assessed by recording of electrical activity of brain cortex field 17 (visual), neck and oculomotor muscles. Standard stainless steel electrodes were used for this purpose.

Special microcannulae (Push-Pull Cannulae, ID-0,5 mm) was implanted in the lateral ventricle of the brain. The microcannula tip was aimed according to the rat stereotaxic atlas coordinates by Paxinos and Watson (1986). During different phases of the SWC 10 μl portions of liquor were collected under negative pressure. The liquor obtained was further used for analysis of amino acids. The liquor samples were collected during both immediately after the surgery, under anesthesia (Equitisin, 3 ml/kg), and two weeks after the surgery, in freely behaving animals. Those samples only, which did not contain the blood cells, were used for analyses. Following termination of the experiments, animals were sacrificed and the brains were fixed in 10% formalin solution. The microcannulae localization was verified histologically in serial slices of the brain.

The amino acid analyses, after their derivatization, were performed on the amino acid analyzer (Pico Tag, Waters), according to the producer manual. Correction of glutamate, aspartate, arginine, and ammonia concentration was made against leucine, alanine and tyrosine quantities. The internal standard (2 μmol/l norleucine) was added to the sample instantly after its extraction.

Results

Conducted experiments have shown that aspartate content is the highest during the slow sleep phase, while its concentration in the intracranial liquor decreases during the phase, and especially, in the active waking. Concentration of glutamate was increased during the SWS, as well as during the narcosis, and like aspartate, decreased during PS and waking periods (Table 1). The contents of arginine was increased in SWS as well as in narcosis, while it was found to be decreased during PS and active waking. Contrariwise, to SWS and narcosis states, in PS and active waking phases a significant increase of ammonia was found.

The results obtained indicate that in SWS and narcosis state, an accumulation of excitatory amino acids - aspartate and glutamate - does occur. Decreased metabolism of the latter is certified by decreased ammonia production velocity. Besides, it should be noted that in SWS arginine accumulation does occur, which points at its low expenditure and at inhibition of the nitric oxide biosynthesis. In the PS and active waking an intense metabolism of aspartate and glutamate does occur, as result of which their concentration in the pontine neurons decreases, while the ultimate product of their decay - toxic ammonia - increases. In these phases the content of arginine decreases, which indicates an intense production of nitric oxide. The latter is the reason of vasodilatory effect.

Table 1. Quantitative changes in aspartate, arginine, and ammonia in the lateral ventricle of the brain, during different phases of sleep (Table contains M±m of three independent experiments)

PHASES OF SWC	GLUTAMATE, μMOL/L	ASPARTATE, μMOL/L	ARGININE, μMOL/L	AMMONIA, μMOL/L
Waking	0.63±0.08	0.37±0.09	0.25±0.05	0.25±0.07
SWS	3.34±0.22	2.20±0.18	4.44±0.26	0.04±0.01
PS	0.85±0.15	1.46±0.28	0.87±0.09	0.18±0.06
N	3.23±0.46	2.88±0.32	2.55±0.15	0.06±0.02

Discussion

Therefore, all the above results indicate that in the SWS phase an accumulation of excitatory amino acids - asparate and glutamate - does occur, increase of which in the lateral ventricle liquor, presumably, should be due to decreased maintenance of the neuronal metabolism. Insofar in the SWS process there is also found arginine accumulation, it could be suggested that decrease of metabolic processes should be due to decreased nitric oxide and initiation of vasoconstriction processes (Williams et al. 1997).

Excitatory amino acids accumulated in SWS, following reaching a certain level, determine incorporation of the pontine cholinergic neurons' NMDA-receptors, which induce formation of PS with cortical desynchronization and PGO-wave induction (Datta and Siwek 1997). These changes occur on the background of excess production of nitric oxide (Lonard and Lydic 1997), which speaks in favor of altered vasodilatory processes and, respectively, of increased metabolic maintenance of the neurons. We have shown that in such a case in the intracranial liquid there is decreased concentration of glutamate, asparate, and arginine and increased concentration of ammonia. It could not be excluded that as a result of enhanced metabolism, on the one hand, in the biosynthesis reactions the amino acids consumption increases, and, on the other hand, their intense exchange does occur, which is indicated by an excess production of ammonia. Dynamic decrease of the excitatory amino acids fund in PS determines decrease of their concentration in the intercellular liquid, which elicits switch-off of the NMDA-receptors. The latter results in inhibition of the pontine cholinergic neurons, decrease of nitrogen oxide production, and SWS induction. As a result of vasoconstriction process and decreased blood-flow in this brain area, concentration of glutamate and aspartate starts to increase again, which in a time, again recruits the NMDA-receptors and, hence, determines an onset of the next PS phase.

Thus, in our opinion, alteration of the SWC phases should be attributed to the non-vesicular glutamate and aspartate, present in the intercellular liquid, concentration of which fluctuates according to intensity of microcirculation. In so far these amino acids themselves regulate the blood-flow intensity, it could be assumed that they may produce the cyclic processes, which underlay the phase nature of the SWC.

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