in activity of the μ-enkephalinergic system in the course of chronic alcoholization, and these changes may play an important role in the development of dependence on ethanol.

LITERATURE CITED

1. Yu. V. Burov, V. N. Zhukov, and A. B. Kampov-Polevoi, Technical Recommendations of the Experimental Pharmacological Study of Preparations Suggested for the Treatment and Prevention of Alcoholism [in Russian], Moscow (1980).
2. Yu. V. Burov, P. I. Absava, A. B. Kampov-Polevoi, et al., Farmakol. Toksikol., No. 1, 50 (1981).
3. I. P. Viglinskaya, in: Experimental and Clinical Psychopharmacology [in Russian], Moscow (1980), pp. 104-115.
4. I. A. Sytinskii, Biochemical Principles of the Action of Ethanol on the Central Nervous System [in Russian], Moscow (1980), pp. 70-98.
5. K. Blum, M. G. Hamilton, and J. E. Wallace, in: Alcohol and Opiates, K. Blum, ed., New York (1977), pp. 203-236.
6. M. R. Dzoljic and A. L. von der Poel-Heisterkamp, Brain Res. Bull., 8, 1 (1982).
7. H. Frenk, B. C. McCarty, and K. Liebeskind, Science, 200, 335 (1978).
8. J. Glowinski and L. L. Iversen, J. Neurochem., 13, 655 (1966).
9. H. Pollard, C. Llorens, J. C. Schwartz, et al., Brain Res., 151, 392 (1978).
10. R. R. Goodman, S. H. Snyder, M. J. Kuhar, et al., Proc. Natl. Acad. Sci. USA, 77, 6239 (1980).
11. R. S. Zukin and S. R. Zukin, Life Sci., 29, 2681 (1981).

MOTOR ACTIVITY AND NEUROACTIVE AMINO ACID CONCENTRATIONS IN BRAIN TISSUES

V. P. Akopyan and L. S. Balyan

UDC 612.822.1:547.466]-06:612.766

KEY WORDS: neuroactive amino acids; gamma-aminobutyric acid; cerebral blood flow.

The discovery of gamma-aminobutyric acids (GABA) and enzymes of its metabolism in the walls of the cerebral blood vessels [4-6] and the discovery of changes in neuroactive amino-acid levels in cerebrovascular insufficiency [1, 7] led to the establishment of a link between elevation of the GABA concentration in the brain and its vessels and manifestations of compensation of disturbances of the cerebral hemodynamics. Another basis for this conclusion was the results of experiments [2, 5] which demonstrated the dynamics of changes in concentrations of neuroactive amino acids in brain tissues and vessels with age and also under the influence of vasoactive drugs.

The object of the present investigation was to study the effect of forced motor activity on quantitative changes in neuroactive amino-acid levels in the brain tissues of normal rats and rats with experimental disturbances of the cerebral blood flow. For comparison, changes in neuroactive amino-acid levels also were investigated in the cerebrospinal fluid (CSF) of cats.

EXPERIMENTAL METHOD

Experiments were carried out on sexually mature rats of both sexes (120 animals) weighing 180-240 g, under ether anesthesia, and on 18 cats weighing 3-4 kg, under pentobarbital anesthesia (50 mg/kg, intraperitoneally).

Department of Pharmacology, Erevan Medical Institute.

(Presented by Academician of the Academy of Medical Sciences of the USSR A. V. Val'dman.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 96, No. 7, pp. 51-54, July, 1983. Original article submitted January 31, 1983.

TABLE 1. Concentrations (in mg %) of Neuroactive Amino Acids in Brain Tissues (M ± m)

Experimental conditions	Number of animals	GABA		Glutamic acid		Aspartic acid	
		cortex	hypothalamus	cortex	hypothalamus	cortex	hypothalamus
Control	15	10,0±0,8	21,5±2,2	90,2±0,7	99,5±1,7	21,0±0,3	30,0±1,2
1 h after ligation	12	13,5±0,5*	23,4±0,9*	38,5±0,3*	43,5±0,64*	21,0±0,4*	25,6±1,2*
3rd day after ligation	21	13,7±0,6*	30,3±0,3*	55,3±1,3*	74,6±1,2*	23,4±1,3*	36,7±2,8*
6th day after ligation	18	12,6±0,6*	35,7±5,1*	76,4±2,7*	62,7±1,7*	70,5±0,4*	24,7±0,9*
Swimming, control	15	18,2±0,4	24,7±0,8	80,4±0,6	98,8±0,7	24,4±0,7	27,4±0,9
Swimming from 3rd day after ligation	22	13,1±0,4**	23,5±3,5**	40,1±1,5**	47,5±0,6**	21,3±2,3**	27,2±1,7**
Swimming from 6th day after ligation	18	23,1±0,3**	46,7±3,1**	99,0±2,3**	110,0±1,7**	30,25±1,2**	32,7±2,7**

Legend. *$P < 0.05$ compared with control, **$P < 0.05$ compared with swimming only.

The concentrations of neuroactive amino acids were determined in rat brain tissues (cortex and hypothalamus) and cat CSF by electrophoresis on paper [11]. A disturbance of the cerebral circulation was produced by unilateral ligation of a carotid artery in rats and of carotid and vertebral arteries simultaneously in cats. Forced motor activity was induced by swimming carrying a load (5% of body weight for 30 min daily for 3 days) in the case of intact rats and rats with disturbance of their cerebral blood flow (3 days after arterial ligation). In all the experiments parallel determinations were made of the acid-base balance (ABB) of arterial blood on a blood microanalyzer (from Radiometer, Denmark) [10]. The results were subjected to statistical analysis by the Fisher–Student method.

EXPERIMENTAL RESULTS

The experiments showed that after swimming for 3 days definite changes were observed in the neuroactive amino-acid levels (Table 1). An increase in the GABA concentration in the cerebral cortex of the rats by 80% (from 10.1 ± 0.8 to 18.2 ± 0.4 mg %, $P < 0.05$) will be noted, whereas the aspartic acid level remained unchanged. Similar changes also were observed in hypothalamic tissues. A parallel study of ABB in arterial blood revealed no evident changes in its parameters.

The results suggest that elevation of the GABA level in the brain during intensive muscular activity could be effective. This hypothesis is based also on data [9, 12-14] according to which GABA is a mediator of inhibition, and that elevation of its level in the brain prevents excessive excitability of the CNS and the development of a hypoxic state. This assumption acquires a firmer basis if the simultaneous fall in the level of glutamic acid, an essential agent in excitation processes [3], is taken into account.

The writers showed previously [1, 7] that after brief (under 30 min) experimental disturbance of the cerebral blood flow considerable changes are observed in the GABA level in the tissues and blood vessels of the brain. In this connection data showing a combined effect of a longer disturbance of the cerebral blood flow and swimming on the neuroactive amino-acid concentrations in the brain become highly significant.

Ligation of a carotid artery in rats for 1 h was shown to be accompanied by elevation of the GABA level by 26.5%, whereas there was no significant change in the aspartic acid level. It is an interesting fact that on the 3rd day after ligation the changes mentioned above not only continued to take place, but were intensified. After 6 days a tendency was observed for the GABA concentration in the cerebral cortex to return to its initial values, whereas its level in the hypothalamus remained higher than the control.

It is evident that elevation of the endogenous GABA concentration and lowering of the glutamic acid level during experimental disturbance of the cerebral blood flow may help to restore the disturbed blood supply to the affected part of the brain [1].

Experiments to study the effect of the combined action of muscular activity and experimental disturbance of the cerebral blood flow showed that motor activity leads to a more marked change in neuroactive amino acid levels in the brain tissues. For instance, whereas on the 6th day after ligation of the carotid artery the cortical

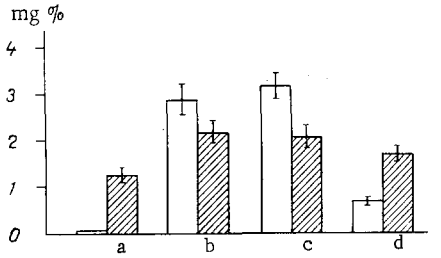

Fig. 1. Concentrations of neuroactive amino acids in the CSF. Unshaded columns – control, shaded columns – 60 min after ligation. a) GABA, b) glycine, c) glutamic acid, d) aspartic acid.

GABA level was 26% higher than the control, when ligation was combined with muscular activity its level was 130% higher than the control (Table 1). A similar but more moderate change in the GABA level was observed in the hypothalmic region.

Definite changes were found in the concentrations of glutamic and aspartic acids: their levels were raised on the 6th day after swimming.

Consequently, the concentrations of the various amino acids studied in rat brain tissues rise during experimental disturbance of the cerebral blood flow if this is combined with motor activity. It can be postulated that the changes described above contribute to compensation of the hypoxic state of the affected part of the brain. This is due not only to participation of neuroactive amino acids in the functional state of the brain, but also to the fact that they are structural elements of proteins and are intensively utilized by the brain as an important source of energy.

The logical sequel to the results described above was to study changes in the amino-acid concentration in the CSF of animals with a disturbance of the cerebral blood flow, for CSF is known to contain neuroactive amino acids, with the exception of GABA [13].

The results of these experiments indicate that the glutamic acid concentration was highest (3.2 ± 0.2 mg %) in the CSF of the control cats, followed by glycine and aspartic acid (Fig. 1). A fall in the glutamic acid level was observed 1 h after arterial ligation, whereas the concentrations of aspartic acid and glycine rose. These changes in the concentrations of neuroactive amino acids in the CSF were observed more distinctly 24 h after ligation.

The appearance of GABA in the CSF when the cerebral blood flow was disturbed will be noted; its concentration reached 1.7 ± 0.2 mg % on the 2nd day after unilateral ligation of the carotid and vertebral arteries. Changes described in the neuroactive amino-acid levels were observed also on the 3rd-4th day after disturbance of the cerebral circulation, but later, starting with the 6th day, a tendency was observed for their concentrations to be restored.

It can be concluded from the effects of neuroactive amino acids on the cerebral circulation during disturbance of the intracranial hemodynamics that neuroactive amino acids help to maintain homeostasis of the blood supply of the brain as a whole, as a single functional unit.

An essential factor in the mechanisms of adaptation and compensation of the disturbed cerebral blood flow is thus an increase in the concentrations of most neuroactive amino acids and, in particular, of GABA, in the brain tissues and CSF, and an important role in these mechanisms is played by motor activity.

LITERATURE CITED

1. V. P. Akopyan, "Neuroactive amino acids and the cerebral circulation," Author's Abstract of Doctoral Dissertation, Kazan' (1977).
2. V. P. Akopyan, Zh. Éksp. Klin. Med., No. 5, 26 (1976).
3. I. E. Dyablova, Byull. Éksp. Biol. Med., No. 1, 66 (1962).
4. S. A. Mirzoyan, B. A. Kazaryan, V. P. Akopyan, et al., in: Problems in Brain Biochemistry [in Russian], Vol. 6, Erevan (1970), p. 109.

5. S. A. Mirzoyan, B. A. Kazaryan, V. P. Akopyan, et al., in: Problems in Brain Biochemistry [in Russian], Vol. 6, Erevan (1970), p. 45.
6. S. A. Mirzoyan, B. A. Kazaryan, and V. P. Akopyan, Dokl. Akad. Nauk SSSR, 214, 456 (1974).
7. S. A. Mirzoyan and V. P. Akopyan, Byull. Éksp. Biol. Med., No. 1, 41 (1978).
8. R. U. Ostrovskaya, "Neuropharmacology of the gamma-aminobutyric acid shunt," Author's Abstract of Doctoral Dissertation, Moscow (1977).
9. I. A. Sytinskii, Gamma-Aminobutyric Acid – A Mediator of Inhibition [in Russian], Leningrad (1977).
10. P. Astrup, Clin. Chem., 7, 1 (1961).
11. W. Grossmann, E. Hanning, and M. Plocke, Z. Physiol. Chem., 299, 258 (1955).
12. D. R. Curtis and A. R. Jonston, Ergebn. Physiol., 69, 98 (1974).
13. J. Logothetis, Neurology (Minneapolis), 8, 299 (1958).
14. C. Perez and M. Rubio, Gen. Pharmacol., 12, 489 (1981).

SUBARACHNOID ANALGESIA INDUCED BY SEROTONIN AND GAMMA-AMINOBUTYRIC ACID

I. I. Abramets and S. I. Komissarov

UDC 615.211:547.466.3].015.2:615.357:577.175.823]

KEY WORDS: gamma-aminobutyric acid; analgesia; serotonin; morphine.

An essential component in the analgesic action of narcotic analgesics is narrowing of the afferent input for nociceptive impulses at the spinal level. This action is based on the ability of opiates to enhance depolarization of high-threshold spinal afferents and to inhibit interneuronal activity in laminae V-VI of the gray matter of the spinal cord [3].

Changes in the functional state of neurons concerned with transmission of nociceptive information at the spinal level can be induced not only by opiates and endogenous opioids [6, 10, 11], but also by certain neurotransmitters: serotonin [7, 9], noradrenalin [9], and gamma-aminobutyric acid (GABA) [1, 12].

In this paper the effects of morphine, GABA, and serotonin on function of primary afferents and the analgesic activity of these substances when injected by the subarachnoid route are compared.

EXPERIMENTAL METHOD

The effect of morphine, serotonin, and GABA was studied on neurons and synaptic transmission in preparations of isolated spinal cord from rats aged 9-14 days. Electrotonic dorsal root potentials arising during superfusion of the isolated spinal cord for 30 sec with solutions of morphine, serotonin, or GABA were recorded. To discover whether the drugs tested act directly on primary afferents or through spinal interneurons, in the experiments of series I synaptic transmission in the spinal cord was blocked by superfusion with a solution containing an excess (10 mM) of Mg^{++} ions and a deficiency (0.2-0.4 mM) of Ca^{++} ions.

The effect of morphine, GABA, and serotonin on synaptic transmission in the spinal cord was studied by recording changes in dorsal root potentials evoked by electrical stimulation of the dorsal root of the neighboring segment (the DR_2-DR_1 potential, see diagram in Fig. 1), or in the orthodromic polysynaptic ventral root potential (DR_2-VR_1). Both potentials were evoked by stimulation of dorsal root L3 by square pulses of current with a duration of 0.3 msec, frequency 0.1 Hz, and intensity 8-10 thresholds. Potentials were derived respectively from dorsal root L4 and ventral root L4. Details of the technique were described previously [1].

Department of Pharmacology, Donetsk Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR A. V. Val'dman.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 96, No. 7, pp. 54-56, July, 1983. Original article submitted February 14, 1983.

