

THE MORPHO-FUNCTIONAL STATE OF THE BRAIN UNDER CONDITIONS OF HYPOKINESIA AND ITS POSSIBLE PHARMACOLOGICAL CORRECTION BY GABA-ERGIC SUBSTANCES

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ABSTRACT

In this paper it has been shown that deterioration of the brain cortex capillary system and negative dynamics of cerebral tissue morphology occur under conditions of hypokinesia. Simultaneously, gamma-aminobutyric acid (GABA) and piracetam have been shown to favor the development of vasodilation and prevent further worsening of the cerebral blood supply. During the experiment, it was also established that among the substances investigated, the specific antagonist of GABA-receptors—bicuculline—displays the strongest cerebroprotective effect in early hypokinesia.

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INTRODUCTION

Hypokinesia has obtained wide biological and social importance due to its prevalence and various defiant causes in the epoch of scientific and technical progress. Hypokinesia, as well as stress factors, remains an important clinical problem which can cause premature aging, development of cardiovascular diseases^{5,6,9,10,18} and cerebrovascular disorders, giving researchers reason enough to carry out investigations in order to discover some physiologic and morphologic aspects of cerebral blood flow and metabolism in conditions of hypokinesia and possible ways for their pharmacological correction. Taking into account the possible cerebrovascular effects induced by gamma-aminobutyric acid (GABA), which were first discovered in our laboratory as far back as the 1960's, its significant effect on the hypothalamus, cortex and to a lesser degree the white matter became obvious. The clearly marked effects of GABA on the quantitative indices of local and regional cerebral circulation were revealed^{13,14} and GABA-ergic substances were thus evaluated ever since.

MATERIALS AND METHODS

In order to study the parameters characterizing cerebral

blood supply in normal conditions and in conditions of hypokinesia, changes were induced in the morpho-functional state of the capillary system of the brain cortex by calcium-adenosine-triphosphate (ATP) method without injection in order to discover the intraorganic microcirculatory channels.⁴ This was based on hydrolysis of ATP, incubation with calcium salts, precipitation of calcium phosphate in structures of microcirculatory channels and production of black sulfide, which was revealed by microscopy.

The rats were decapitated and the brains placed in a solution of 1% neutral formalin for 24 hours. Thick slices of cortex (150-200 μm) were obtained via a cryomicrotome and kept in physiologic solution for 4 hours and then placed in incubation media with the following chemical composition: a) 2 mL of glycine buffer (5.3 mL of 1 M NaOH was added to 4.7 mL of glycine and a 5.8% solution of NaCl); b) 2 mL of 0.1 M solution of calcium acetate; c) 1 mL of 0.1 M solution of ATP.

After incubation the slices were irrigated with distilled water and kept in plumbic mixture for 2 hours. The latter was made by adding 2 drops of acetate and 2g of plumbic acetate to 100 mL of distilled water; 100 mL of 1M acetate buffer (pH=6.2) and 15 mL of an 8% solution of ammonium acetate were then added. The slices were irrigated with

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Table I. The number of sharply narrowed capillaries (SNC) in brain cortex in 100 visible areas in conditions of hypokinesia and under the influence of GABA-ergic substances ($M \pm m$), $n = 57$.

Condition	Control	Hypokinesia			
		15th day	30th day	45th day	60th day
Intact	10 ± 2	24 ± 0.8*	22 ± 0.4	24 ± 0.8*	25 ± 1.2
GABA	8 ± 1.4*	8 ± 0.7*	16 ± 0.97	14 ± 0.57*	20 ± 0.7
Piracetam	9 ± 1.3	12 ± 1.1*	10 ± 1.2	14 ± 0.56	18 ± 0.73*
Bicuculline	17 ± 2.4*	30 ± 1.0	26 ± 0.67*	28 ± 0.65	26 ± 0.24

* $P < 0.05$

Table II. Middle diameter of functioning capillaries of brain cortex in conditions of hypokinesia & under the influence of GABA-ergic substances (μm).

Condition	Control	Hypokinesia			
		15th day	30th day	45th day	60th day
Intact	5.7 ± 0.4*	5.15 ± 0.9*	5.32 ± 0.7*	5.19 ± 0.8*	5.096 ± 0.3*
GABA	8.7 ± 1.1	7.27 ± 1.02*	5.15 ± 0.83	6.2 ± 0.91*	5.17 ± 0.7
Piracetam	7.2 ± 0.5	5.31 ± 0.9*	6.2 ± 0.94*	5.81 ± 0.8	5.76 ± 0.87
Bicuculline	4.7 ± 0.36	4.0 ± 0.9	4.79 ± 0.8*	4.2 ± 0.8	4.12 ± 1.0*

* $P < 0.05$

distilled water twice and kept in a 20% solution of ammonium acetate for a few seconds. Each slice was then fixed on subject and cover glass by glycerin-gelatin mixture.

The diameter of the capillaries was measured by an MOB - 115 (Ob-40, Ok-15) micrometer.

Two main parameters were studied in order to discover the functional capacity of the microcirculation of brain cortex: the mean diameter (MD) of functioning capillaries and the number of sharply narrowed capillaries (SNC) in 100 visible areas.

In order to carry out pathomorphological investigations in cortical and subcortical structures, one of the hemispheres was fixed in 96° alcohol and covered with paraffin. The histochemical stains used during investigation included Nissle, Brashee, Jasuma, Iticava and hematoxylin and eosin.¹⁵

The experiments were carried out on 127 male mature Wistar rats weighing 160-180g. Hypokinesia was modelled in individual small cages.⁵

The experiments were carried out in both early (from the 15th day till the 60th day) and late (from day 60 et seq.) periods of hypokinesia.

During the study GABA (San Diego, California), nootropil (piracetam) (Polfa, Starograd) and bicuculline (Sigma Chemicals Co, USA) were used. These GABA-ergic substances were administered intraperitoneally in doses of 2 mg/kg, 20 mg/kg and 0.2 mg/kg, respectively. The results were processed by methods of variation statistics.^{8,16} The Boeing Graph program was used during statistical analysis on an IBM computer.

RESULTS

The phasing of changes are undoubtedly evident. The general trend of changes was as follows: as demonstrated in Fig. 1, in early hypokinesia (3-30th day), the number of SNC sharply increases approximately 1.5 times, and the middle diameter of the functioning capillaries (MDFC), as shown in Fig. 2, decreases 17.5% (from 5.7 ± 0.7 to 4.7 ± 0.26 μm , $P < 0.05$) as compared to controls.

There is a tendency from the 30th day of hypokinesia toward normalization of the functional capability of

microcirculatory channels. We also observed a tendency towards re-establishment of MDFC and the number of SNC.

Late stage hypokinesia (2 months and later) was accompanied by sudden worsening of the functional capability of brain cortex, which was displayed as a significant increase of the number of SNC (Table I) and decrease of FC (Table II); i.e., the number of SNC, which was 10 ± 2 in controls, increased to 25 ± 1.2 ($P < 0.05$), that is 1.5 times on the 60th day of hypokinesia and MDFC decreased 10.1% compared to controls (from 5.7 ± 0.4 to $5.09 \pm 0.3 \mu\text{m}$, $P < 0.05$).

Simultaneously, our aim was to investigate the morphological status of the capillary system in brain cortex. During the experiment it was established that the morphologic picture changed significantly, as there were registered deformed capillaries which obtained shoe-like forms with peripheral contractions and regression in capillary channels.

Proceeding from the unique compensatory role of the GABA system as the most important link of neurochemical mechanisms regulating cerebral blood circulation, we deemed it necessary to continue our investigations to evaluate the influence of GABA-ergic substances on the functional capability of the capillary system of brain cortex.

In control conditions, the vasodilating effect of GABA was more pronounced than piracetam. GABA and piracetam interact identically on the functional state of brain cortex. By analyzing results of the influence of GABA and piracetam on the capillary channels of brain cortex, it was seen that these two substances display a unidirectional influence, i.e., MDFC increases with the intraperitoneal administration of GABA (2 mg/kg) and piracetam (20 mg/kg) for one week in control rats by 52.6% and 26.3% ($P < 0.05$), and the number of SNC decreases by 20% and 10%, respectively ($P < 0.05$).

We then attempted to detect the influence of the specific antagonist of GABA receptors—bicuculline—on MDFC and the number of SNC of brain cortex. It was discovered that a 0.2 mg/kg dose of bicuculline in control rats leads to vasoconstriction, and therefore an increase in the number of SNC in 100 visible areas by $17.5 \pm 2.0\%$ and a 1.7 fold decrease in MDFC ($P < 0.05$).

Significant changes were observed in the microcirculatory system on the 30th and 60th days of hypokinesia under the influence of GABA, piracetam and bicuculline as shown in Figs. 1 and 2.

It has been established that hypokinesia leads to morphologic changes in cerebral tissue in rats. These changes were observed in cerebral tissue from the 15th day of hypokinesia, and became more progressive during the experiment. These changes are not similar in neurocytes of cortical and subcortical structures. The structural damage grows progressively worse in cortical neurocytes, and is accompanied by the appearance of glial nodules in cerebral tissue (Fig. 3). Pyknosis of giant pyramid cells prevails during the experiment on day 45, as these cells become deformed and small. Signs of vacuolization and peripheral

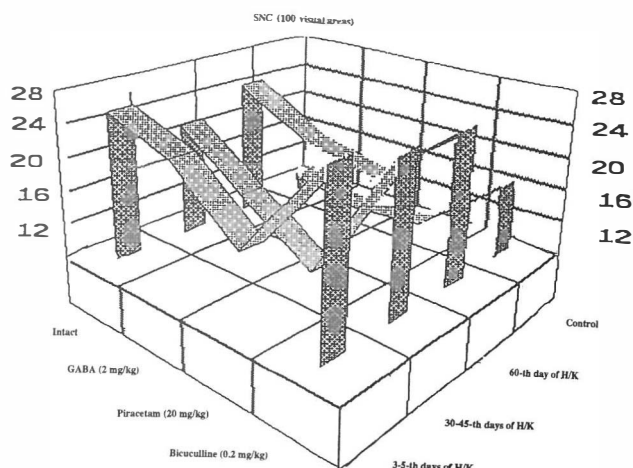


Fig. 1. Number of sharply narrowed capillaries (SNC) in brain cortex.

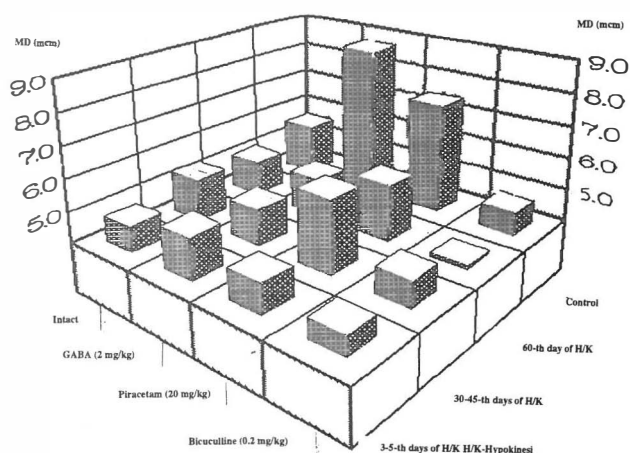


Fig. 2. Middle diameter of the functioning capillaries in brain cortex.

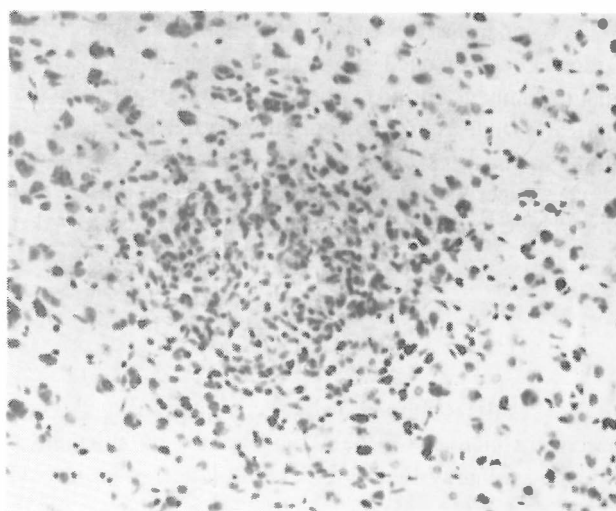


Fig. 3. Diffuse glial node in the cerebral cortex (on day 45). (H&E, 100 \times).

chromatolysis are seen in neurocytes of brain cortex terminal slices (Fig. 4). Chromatophilic substances decrease in neurocytes of the subcortical nucleus on the 15th day of hypokinesia, which often accumulate under the plasmolemma as a compact homogenous layer. During the experiment, peripheral chromatolysis and later (on day 45) neuronophagia were observed.

The hyperemia of vessels and edema of cerebral membranes gradually increase. On the 45th day of hypokinesia areas of hemorrhage in cerebral membranes, brain cortex and cerebral ventricles (Fig. 5), as well as intravascular thrombosis, are detected.

The specific antagonist of GABA receptors—bicuculline—was shown to express greater cerebroprotective activity compared to other GABA-ergic substances when evaluated on the 30th day of hypokinesia, and the effect on cytoarchitectonics of the cortex and its cellular elements were observed. Peripheral chromatolysis was observed in only some neurocytes. The vessels of the cerebral membrane, brain and vascular plexus had not changed. However, the use of bicuculline on the 45th day of hypokinesia displayed significantly less protective effect. Vacuolization and chromatolysis of the perikarion of neurocytes progressively developed and were seen especially in the giant pyramid cells. An increase in vascular permeability was observed as well.

Following the administration of GABA on the 15th day of hypokinesia, peripheral chromatolysis was observed in neurocytes of terminal slices of cortex. On the 30-45th days of the experiment, pyknosis and neuronophagia had developed with formation of glial nodules and areas without neurocytes. Hyperemia of pial and intraorganic vessels developed during the experiment, accompanied by an increase in permeability of vascular walls (Fig. 6), perivascular edema, and edema of cerebral tissue.

Similar but more pronounced negative dynamics were observed under the influence of piracetam. The application of piracetam worsened the hemodynamic and cellular structural damage during hypokinesia, which was expressed in the early stage as diffuse formation of glial nodules, areas without neurocytes and vascular abnormalities. Diffuse vascular hyperemia, edema of cerebral membranes and hemorrhagic foci in cerebral tissue and ventricles were also observed.

DISCUSSION

The results obtained in early hypokinesia show a consequent increase in the activity of the hypothalamic-pituitary-adrenal system, as this condition acts as an extreme stress factor.^{5,10,18}

It is possible that in the re-establishment of MDFC and the number of SNC on days 30-45 of hypokinesia, adaptive

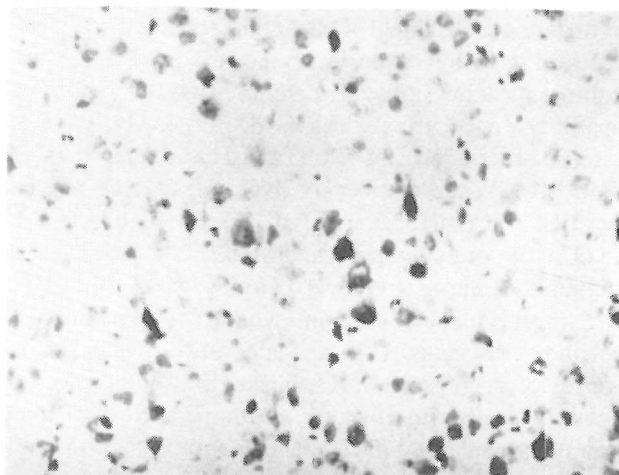


Fig. 4. Pyknosis of giant pyramid cells. Signs of vacuolization and peripheral chromatolysis in neurocytes of brain cortex terminal slices (on day 45). (Brashee, 400×).

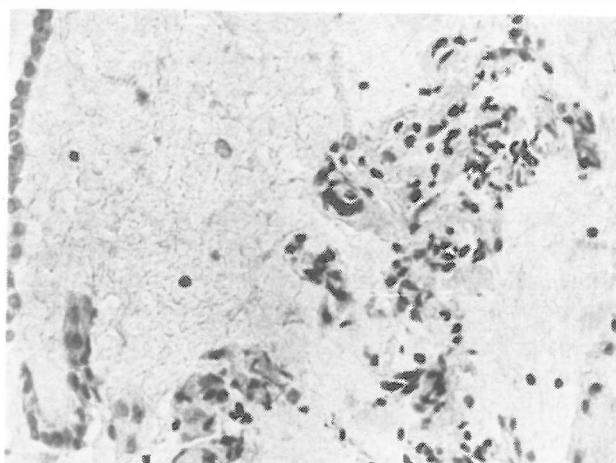


Fig. 5. Area of hemorrhage in cerebral ventricle (on day 45). (H&E, 160×)

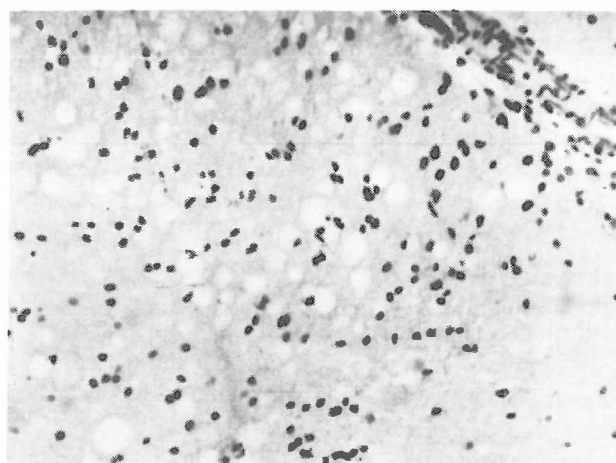


Fig. 6. Edema of cerebral tissue (on day 30). (H & E, 100×).

reorganization occurs in the organism, in which the GABA system takes a great part. This is expressed by increased activity of glutamate-decarboxylase (GDC) due to stress interaction, which leads to an increase of GABA levels.¹ This is accompanied by suppression of the sympathetic adrenal system.²

In order to explain the obtained changes in the functional capability of the microcirculatory system of brain cortex, it must be noted that two factors take part in the mechanisms of capillary dilation and constriction: intracapillary pressure and tension of capillary walls.¹¹ It is known that a great number of α -adrenoreceptors have been discovered in precapillary sphincters of the brain, stimulation of which leads to significant spasm of precapillary sphincters and derived arterioles, favoring a decrease in intracapillary pressure. It is believed that capillaries, due to the presence of special cells—pericytes—in their walls, can contract spontaneously.¹⁹ Changes in pericyte activity and their number are a result of stressful conditions.¹⁷ These changes are reflected as a decrease in MDFC and an increase in the number of SNC in 100 visible areas. Therefore, the above mentioned changes in the functional activity of the capillary system are a classic picture of chronic stress in the late period of hypokinesia.

The above mentioned effects of piracetam confirm the results obtained by Bhattacharya S.K. et al.,³ according to which piracetam usually leads to an increase in vasoconstrictive prostaglandins (PGE, PGF_{2 α}) which, however, do not fully reverse the vasodilation induced by piracetam itself.

Analysis of the possible mechanisms of piracetam in conditions of hypokinesia suggests that the level of GABA in cerebral tissue is increased in conditions of cerebral hemodynamic abnormalities under the influence of piracetam. On the other hand, the cycling of GABA in brain tissue with formation of the pyrrolidonic ring, intensifies the vascular effect.¹⁴ As it was shown, the functional capability of the brain cortex microcirculatory system becomes worse in conditions of hypokinesia, which is accompanied by the development of ischemia, causing acidosis. Piracetam, a cyclic analogue of GABA containing a pyrrolidonic ring, represents a GABA prototype which is split in conditions of acid pH.⁷ Under the influence of piracetam, cyclic AMP accumulates²¹ and its level increases in hypokinetic conditions.²⁰

Agonists of GABA receptors, in conditions of early hypokinesia, prevent the further worsening of cerebral blood flow by leading to vessel relaxation and preventing marked vasospasm of capillaries, and decrease the number of nonfunctioning capillaries and may provide an adequate level of blood supply, leading to improvement of cerebral metabolism.¹⁴

Possibly, the observed effects are mediated via GABA receptors located in the wall of cerebral vessels. GABA and

piracetam express vasodilating activity in conditions of hypokinesia, which leads to an increase of vascular permeability. As a result, plasma accumulates in the pericellular space, leading to the development of pyknosis, chromatolysis, neuronophagia and glial nodules. Bicuculline, as a selective antagonist of GABA receptors, leads to the elimination of cerebral vasodilation, thus preventing the development of subsequent cell damage.

The obtained data allow us to conclude that locomotor hypoactivity leads to worsening of hemodynamic and morphologic states of the brain, and as a result hemorrhagic and necrotic foci may develop in various structures of the brain. Simultaneously, these findings are evident of possible participation of the GABA system as an endogenous modulator in adaptive reorganization of the cerebral blood circulation in conditions of hypokinesia.

REFERENCES

1. Acosta GB, Otero Losada ME, Rubio MC: Chemical stress and GABA-ergic central system. *J Gen Pharmacol* 21 (4): 517-520, 1990.
2. Allykmets LC, Polevoy LG, et al: Pharmacology and Toxicology—Moscow 6: 603. 1979.
3. Bhattacharya SK, Upadhyay SN, Jaiswal AK: Effect of piracetam, a nootropic agent, on rat brain monoamines and prostaglandins. *Indian J Exp Biol* 27 (3): 261-264, 1989.
4. Chilingarian AM: The calci-ATP-ic method for discovery of intraorganic microcirculatory channel. *J Experim and Clinical Medicine-Yerevan* 5: 19-28, 1977.
5. Feodorov IV: In: Metabolism in conditions of hypodynamia. Moscow, pp. 228-251, 1982.
6. Hakopian VP, Melkonian KV, Kocharian AG, Mirzoyan NR: Therole of GABAergic substances on cerebral blood circulation in conditions of hypokinesia. 37th Annual World Congress of the International College of Angiology. Helsinki, Finland, pp. 89-90, 1995.
7. Herrshaft H: Effect of piracetam on the blood circulation in the human brain: quantitative regional circulation measurements in acute cerebral ischemia. *J Med Klin* 73 (60): 195-202, 1978.
8. Kamensky LS: In: Medical and Demographic Statistics—Moscow, p. 351, 1974.
9. Kovalenco EA, Gurovsky NN: Hypokinesia. Moscow: Medicine, p. 320, 1980.
10. Macho L, Kvetnansky R, et al: Is prolonged space flight of rats a stressful stimulus for the sympathoadrenal system? Catecholamines and Stress: Recent Advances. New York: Elsevier, pp. 399-408, 1980.
11. Mchedlishvily GI: Blood Microcirculation. Leningrad, p. 296, 1989.
12. Melkonian KV, Hakopian VP, Kanayan AS: Possible pharmacological correction ways of the cerebral hemodynamic changes in hypokinesia. *J Pharmacological Research. Proceedings of the 1st European Congress of Pharmacology—Milan, Italy*, p. 341, 1995.
13. Mirzoyan SA, Hakopian VP: The influence of GABA and its

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- derivatives on cerebral blood circulation. *J Pharmacol Chem: Mat. of 2nd Conf. for Pharmacol. in USSR-Moscow*, p. 210, 1965.
14. Mirzoyan SA, Hakopian VP: In: *GABA and Cerebral Hemocirculation*. Edited by the Scientific Academy of Armenia. Yerevan, pp. 17-38, 1985.
 15. Pirs E: In: *Theoretical and Practical Histochemistry*. Moscow, pp. 475-489, 1962.
 16. Plochinsky NA: In: *Biometry*. Moscow, p. 357, 1970.
 17. Tagami, et al: Ultrastructural changes in cerebral pericytes and astrocytes of stroke-prone spontaneously hypertensive rats. *Stroke-Japan* 21 (7): 1064-1071, 1990.
 18. Tigranian RA: In: *Stress metabolic aspects in space flight*. Moscow, p. 138, 1985.
 19. Ushiwata I, Ushiki T: Cytoarchitecture of the smooth muscles and pericytes of rat cerebral blood vessels. A scanning electron microscopic study. *J Neurosurg Japan* 73 (1): 82-90, 1990.
 20. Weltmann AS, Sachler AM, Sparber SB: Endocrine, metabolic and behavioral aspects of isolation stress on female albino mice. *J Ibid* 37 (8): 804-810, 1966.
 21. Weth GMD: Effects of piracetam on the second messenger. 2nd Intern Symp on Nootropic Drugs. Mexico, pp. 34-45, 1981.