pothesis on the role of nitric oxide produced by many vasodilators. Nevertheless, the marked protective effect of no-spa calls for further investigations of the antiviral activity of similar compounds.

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Effect of Mexidol on the Content of Transmitter Monoamines and Amino Acids in Rat Brain Structures

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The content of two major classes of neurotransmitters (monoamines and amino acids) and their main metabolites is measured in rats at certain intervals after *per os* administration of mexidol (150 mg/kg). The level of dopamine and of its metabolite dihydroxyphenylacetic acid is found to be considerably elevated in the frontal cortex, suggesting a pronounced cortical component in the mechanism of action of mexidol.

Key Words: *mexidol; frontal cortex; dopamine; dihydroxyphenylacetic acid; γ-aminobutyric acid*

Mexidol (3-hydroxy-6-methyl-2-ethylpyridine succinate) is a water-soluble biogenic antioxidant, one of the 3-hydroxypyridine derivatives, that are structurally analogous to the compounds of the vitamin B_6 family. Experiments on rodents have demonstrated that mexidol (25-100 mg/kg) exhibits antihypoxic, antiamnestic, anxiolytic, antistress, and anticonvulsant effects, possesses heropsychotropic activity, and potentiates hexenal-induced sleep. A course of mexidol treatment is reported to result in a stable rearrangement of lipid-protein complexes of neuronal membranes in the rat brain [4]. However, the effect of mexidol on the brain neurotransmitter systems has not yet been studied.

There are ample data on the involvement of neurotransmitter systems in the mechanism of action of nootropics [1,9,13,14]. The monoaminergic and acidergic components of neural transmission are usually evaluated by measuring the content of various neurotransmitters in certain brain structures of laboratory animals. We believe that it is more useful to study the dynamics of these parameters, since quantitative changes in the content of neurotransmitters may not always be captured from measurements at some arbitrary chosen time intervals.

In light of this, the aim of the present study was to evaluate the content of two major classes of neu-

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rotransmitters (monoamines and amino acids) and their main metabolites in rats at different terms after *per os* treatment with mexidol.

MATERIALS AND METHODS

The neurochemical experiments were carried out on random-bred male albino rats weighing 280-320 g. The rats were maintained under standard vivarium conditions with 12-hour daylight at 21-22°C in 2145 cm^2 cages (10 rats per cage), with free access to food and water. Mexidol (150 mg/kg) was administered intragastrally in one dose as an aqueous solution with a concentration of 30 mg/kg. Control animals (n=5) received water.

Animals were decapitated 30 min, and 1, 2, 4, and 6 hours after the treatment (n=4 for each time point). The brain was removed and the frontal cortex was isolated in the cold. The samples were homogenized in 20 volumes of 0.1 N $HClO_4$ and the homogenate was centrifuged at 10,000g for 10 min. Aliquots of filtered supernatant were used for parallel measurements of the content of biogenic amines and γ-aminobutyric acid (GABA). In the next experimental series the animals were given mexidol (150 mg/kg, intragastrally) and decapitated after 2 hours, after which the hypothalamus and striatum were isolated. The samples were treated as described above and used for simultaneous measurements of transmitter monoamines and amino acids.

The content of norepinephrine, dopamine, dihydroxyphenylacetic acid (DOPAC), serotonin, and 5-hydroxyindoleacetic acid (5-HIAA) was measured by high performance liquid chromatography with electrochemical detection [5] using precolumn derivatization with o-phthaldialdehyde and β-mercaptoethanol as the stabilizer of the reaction [3]. Glutamate, aspartate, taurine, glycine, and homocysteic acid (internal standard) were separated on a tandem of Separon C18 columns (8 μ, 150×4 mm, ELSIKO, Russia) with a mobile phase consisting of 0.57 M Na-acetate buffer (pH 3.85) and acetonitrile (82:18 v/v). The mobile phase for the determination of GABA consisted of 0.2 M Na-acetate buffer (pH 3.8) and methanol (40:60 v/v), and d-aminovaleric acid served as the internal standard [7]. A quantitative amino acid assay was performed using a fluorescent detector at 338 nm excitation and 450 nm emission wavelengths.

The experimental data were processed statistically using the Student t and Wilcoxon-Mann-Whitney U tests.

RESULTS

Figure 1 shows that mexidol considerably increased the content of dopamine and of DOPAC, the main

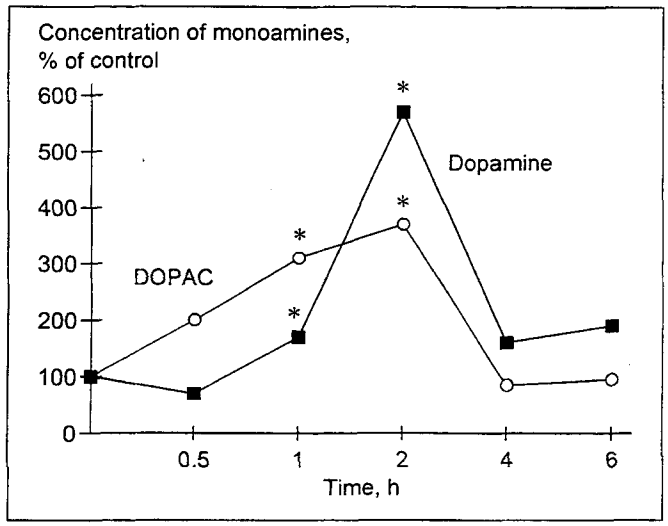

Fig. 1. Dynamics of the content of dopamine and DOPAC in the rat frontal cortex after a single *per os* administration of mexidol (150 mg/kg). Concentration of monoamines in the control (0.09±0.01 ng/mg dopamine and 0.014±0.003 ng/mg DOPAC) taken as 100%. Here and in Figs. 2 and 3: *p<0.05 in comparison with the control.

metabolite of dopamine in rodents, 1 and especially 2 hours after administration. The content of norepinephrine, serotonin, and its metabolite 5-HIAA in the frontal cortex was practically unaffected, whereas the content of GABA was lowered by 15-20% during the first two hours after administration and subsequently normalized (Fig. 2).

Recent investigations have demonstrated activation of GABAergic and suppression of dopaminergic neurotransmission in pathological states (naturally occurring and modeled) related to memory disturbances. The content of dopamine in the striatum and substantia nigra has been shown to decrease in aging [8,11]. Nootropic and antiamnestic preparations, on the other hand, reduce the content of GABA and activate the dopaminergic system. Stress increases

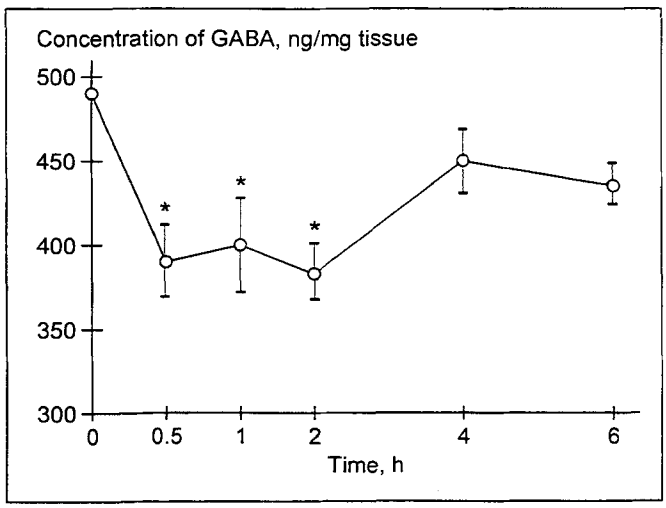

Fig. 2. Dynamics of GABA content in the frontal cortex after a single *per os* administration of mexidol (150 mg/kg).

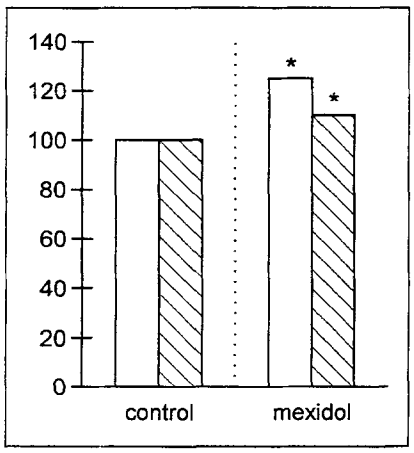

Fig. 3. Change in the content of dopamine (light bars) and glutamate (dark bars) in the rat striatum 2 hours after a single *per os* administration of mexidol (150 mg/kg). Ordinate: concentration of transmitters, % of control (4.70±0.35 ng/mg dopamine and 1399±51 ng/mg glutamate).

the content of GABA in the rat cortex [6]. Intraperitoneal injection of piracetam (250 mg/kg) and N-acetylaspartate (50 mg/kg) has been reported to abolish this stress-induced effect and to reduce the GABA content in the rat cortex [6]. A drop of GABA and elevation of DOPAC were observed in rat striatum 1 hour after a single intraperitoneal injection of piracetam (400 mg/kg). Chronic administration of piracetam and aniracetam (7 days) boosted the content of dopamine, norepinephrine, and serotonin in the cortex. This effect was more pronounced in senescent rats, in which the level of monoamines was noted to be markedly lowered [13]. Administration of piracetam, meclophenoxate, and vinpocetine during 3 weeks restored the initial level of dopamine secretion, reduced during aging, in the cells of the rat striatum [14].

Scopolamine is known to induce memory disturbances and serves for modeling amnesia. Intraperitoneal injection of scopolamine (0.5 mg/kg) has been shown to selectively reduce the content of dopamine metabolites DOPAC and homovanillic acid in the hippocampus and frontal cortex [10]. Mexidol (100 mg/kg) has been found to prevent electric shock- or scopolamine-induced amnesia in the conditioned passive avoidance test. The normalization of the scopolamine-induced drop in the content of dopamine and DOPAC probably underlies the antiamnestic activity of mexidol.

Thus, our findings suggest that mexidol affects the dopaminergic and GABAergic transmission in the rat frontal cortex and confirm the hypothesis on the dopaminergic effect of nootropics [9].

Despite the fact that mexidol readily crosses the blood-brain barrier and equilibrium between the central and peripheral chambers is established just 30 min postinjection, its drastic though transient neurochemical effect is delayed. This underscores the importance of recording the time course of the neurochemical effect. The following observation serves as a good illustration: injection of neuroleptics leads to a blockade of the dopamine receptors and, consequently, to the accumulation of DOPAC and homovanillic acid in the brain, the level of dopamine being unchanged after a short time span. At the same time, sulpiride (200 mg/kg, intraperitoneally) has been shown to increase the content of dopamine in the rat striatum drastically 2 hours postinjection [12].

In our experiments dopamine turnover was enhanced soon after injection, implying a receptor-mediated effect of mexidol on the dopaminergic system. Two hours later, mechanisms which enhance the biosynthesis of dopamine were presumably switched on, because the content of dopamine in the frontal cortex rose by 577%.

The pronounced dopaminergic component in the neurochemical activity of mexidol prompted us to study its effect on the content of transmitter monoamines and amino acids in subcortical structures of the rat brain. The content of monoamines and amino acids in subcortical structures was determined 2 hours after *per os* administration of 150 mg/kg mexidol. This time point corresponded to the maximal neurochemical effect of the preparation in the frontal cortex. As is seen from Fig. 3, the content of dopamine and glutamate increased considerably in the striatum but not in the hypothalamus. The levels of norepinephrine, DOPAC, serotonin and 5-HIAA, glycine, taurine, aspartate, and GABA did not differ reliably in the control and experimental groups in either of these structures.

Thus, mexidol moderately elevates the content of dopamine in the striatum, which suggests a pronounced cortical component in the mechanism of action of this drug. Our findings are in conformity with previous data [2]: chronic administration of mexidol (100 mg/kg, i.p.) results in increased stabilization of the dominant peak of the Fourier-transformed electroencephalogram power spectrum of the sensorimotor cortex and dorsal hippocampus of the rat brain. It is interesting that the drop in the content of the inhibitory transmitter GABA in the cortex is accompanied by a rise in the content of the activating neurotransmitter glutamate in the striatum. This may be interpreted as follows: the drop of GABA results in activation of the glutamatergic neurons of the frontal cortex (which account for 70% of the total population of cortical neurons) and enhanced impulse flow along the corticostriatal fascicle, which stimulates the release of the neurotransmitter by terminals of the pyramidal neurons in the striatum. Moreover, direct inhibition of glutamate decarboxylase, an enzyme converting glutamate into GABA, cannot be ruled out.

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