Mechanism of Protective Effect of Energostim during Catalepsy Produced by Single Treatment with Trifluoperazine

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NAD, cytochrome *c*, and energostim modulated the fluorescence emission spectrum of trifluoperazine in the solution and in microsomal suspension. The data suggest that NAD and energostim modify structural and conformational characteristics of the dopamine receptor-trifluoperazine complex. These changes probably underlie the anticataleptic effect of energostim.

Key Words: *trifluoperazine; energostim; nigrostriatal microsomes; fluorescence*

The psychotropic effects of various drugs are realized via the dopaminergic and other transmitter systems [10-12]. Homeostasis is maintained by mechanisms of transmitter interactions. Changes in the functioning of one system are followed by compensatory responses of others. This mechanism allows the organism to react adequately to a stimulus and return to the initial state of functional activity [11]. The involvement of transmitter interactions in the pathogenesis of neurodegenerative disorders is confirmed by similar neurophysiological changes produced by psychoactive substances (ligands of transmitter systems) [2,11-13].

We first studied medicinal proteomics and showed the role of posttranslational ligand-induced changes in the drug-receptor interaction and initiation of the cascade of mediated signal mechanisms.

MATERIALS AND METHODS

Experiments were performed with the microsomal fraction of the substantia nigra from male albino rats weighing 150-190 g. The animals were maintained under standard conditions. After decapitation the brains were placed in liquid nitrogen. The samples and microsomes were isolated as described elsewhere [1]. To this end, 1 mg tissue was dissolved in 7-fold volume of cold solution containing 0.05 M $CaCl_2$, 0.32 M sucrose, and 20 mM Tris-HCl buffer (pH 7.1). The fluorescence emission spectrum of trifluoperazine was recorded on a Hitachi MPF 4A spectrofluorometer at an excitation wavelength of 358±2 nm. The spectra were recorded in the presence of trifluoperazine in various concentrations and after treatment with energostim, cytochrome *c*, and inosine. The corrected spectra were derived as described elsewhere [5]. The results were analyzed by Student's *t* test.

RESULTS

Energostim is an antihypoxant and antioxidant preparation with direct action [4,5]. This substance markedly reduced the symptoms of catalepsy produced by trifluoperazine in a single dose of 3 mg/kg. Moreover, energostim prevented imbalance in the dopaminergic system and in the interactions between dopaminergic, sympathoadrenal, and serotoninergic systems [1]. The mechanism of this effect remains unclear. Taking into

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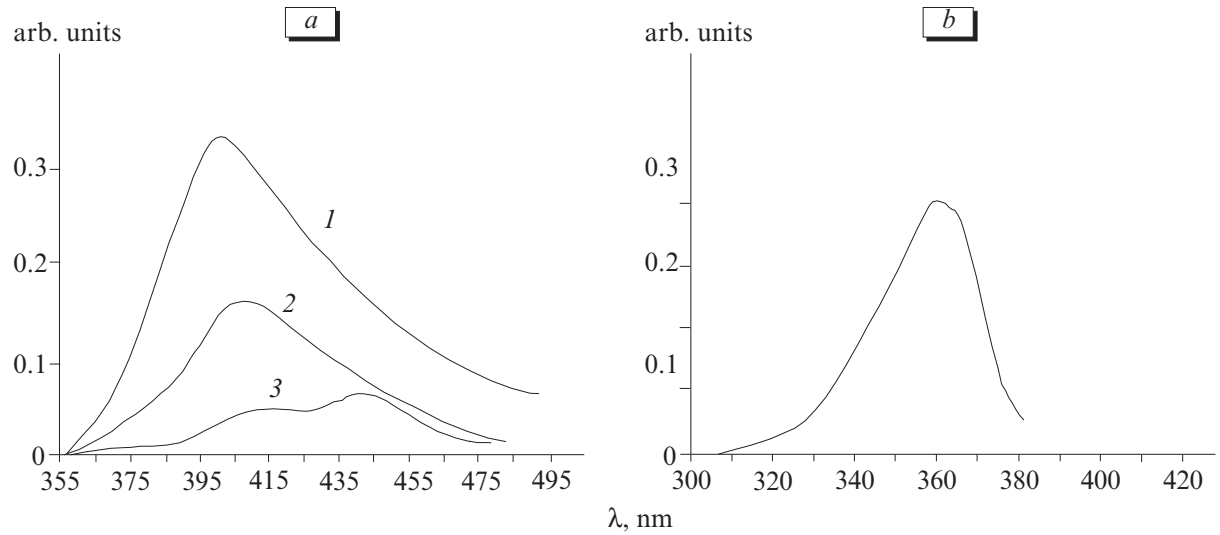

Fig. 1. Corrected spectra of fluorescence emission (*a*) for 1 μM trifluoperazine at the excitation wavelength (*1*) and after treatment with 0.1 mM NAD (*2*) and energostim (0.1 mM NAD, *3*); trifluoperazine absorption spectrum (1 μM, *b*).

account the composition of energostim (NAD, cytochrome *c*, and inosine) and structure of trifluoperazine, it cannot be excluded that these compounds interact with or competitively bind to dopamine receptors. The increase in blood trifluoperazine concentration is accompanied by a decrease in hemoglobin affinity for oxygen due to conformational changes in hemoglobin molecule [2,7].

The fluorescence emission maximum for trifluoperazine in the solution and microsomal suspension was detected at 397±2 nm and linearly depended on the concentration of this substance (Fig. 1).

The addition of NAD to the suspension of microsomes and trifluoperazine *in vitro* decreased the fluorescence emission maximum by 2.2 times. The fluorescence peak became more flat. Energostim shifted the fluorescence emission maximum towards 412±2 nm. Moreover, we revealed the second peak at 445 nm. It should be emphasized that the intensity of the first peak decreased by 8 times compared to fluorescence of pure trifluoperazine. Addition of cytochrome *c* and inosine induced the appearance of a weak peak, which also decreased trifluoperazine fluorescence emission. It is important that we observed unidirectional, but different changes in the suspension of microsomes and solution of trifluoperazine (medium for microsomes). These data reflect complex interaction between trifluoperazine and ingredients of energostim in the medium with brain structures. Previous experiments showed that megosin and rometin decrease the intensity of electron paramagnetic resonance signals of trifluoperazine and chlorpromazine in the solution of hemoglobin [7]. Trifluoperazine acts as a potent inhibitor of Ca^{2+}-dependent calmodulin and affects the amount of membrane-bound Ca^{2+} [2]. Moreover, trifluoperazine in a concentration of 2-5 μM can induce cell death [13]. Trifluoperazine and chlorpromazine produce reverse changes in hemoglobin affinity for oxygen and modify the conformation of hemoglobin into the T-state. These substances interact with tryptophan residues of hemoglobin (6 on protomer), which reflects the formation of trifluoperazine-oxygen complexes. Trifluoperazine and inositol hexose phosphate act synergistically, which indicates that they interact at a site differing from the binding site for 2,3-diphosphoglycerate [2].

Chemically, trifluoperazine acts as an electron donor. Similarly to serotonin, trifluoperazine interacts with NAD. The pyridine ring of NAD or NADPH, phosphate residues, and amine group of purine serve as acceptors [8]. Therefore, trifluoperazine can interact with NAD. This is confirmed by changes in trifluoperazine fluorescence observed after addition of 0.001 mg/ml NAD. It should be emphasized that the solution of energostim containing NAD in the same concentration produced a more pronounced decrease in the fluorescence maximum for trifluoperazine.

As regards energostim, cytochrome *c* capable of undergoing transition from the oxidized state to the reduced state and, probably, inosine interact with single electron pairs of N and S atoms in trifluoperazine.

Energostim has anticataleptic activity and prevents the development of extrapyramidal disorders produced by trifluoperazine. Moreover, energostim maintains the balance between the content of biogenic amines and transmitter interaction. This effect is probably related not only to the involvement of NAD into the regulation of synthesis and degradation of catecholamines and serotonin, but also to conformational variations and changes in affinity of trifluoperazine bound to dopamine receptors.

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