Anticataleptic Effect of Energostim during Single Treatment with Trifluoperazine

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 137, No. 3, pp. 288-292, March, 2004
Original article submitted October 29, 2003

Administration of trifluoperazine in a single dose of 3 mg/kg induced catalepsy and locomotor disorders in 86% intact animals, which persisted for 4 h. Catalepsy developed in only 15% animals pretreated with antihypoxic and antioxidant agent energostim in a dose of 230 mg/kg. The protective effect of energostim was associated with its ability to maintain the balance between dopaminergic, cholinergic, and adrenal activity in the substantia nigra and medulla oblongata during administration of neuroleptics.

Key Words: *catalepsy; biogenic amines; trifluoperazine; energostim*

Fine mechanism underlying the extrapyramidal effect of neuroleptics is unknown. It was hypothesized that this effect is related to an imbalance between 2 antagonistic neurotransmitter systems: dopaminergic (transmitter dopamine) and cholinergic systems (transmitter acetylcholine) [5,6,9]. Drug-induced extrapyramidal disorders are often observed in clinical practice and associated with a variety of side effects and imbalance between neurotransmitters that modulate activity of dopaminergic systems and functional state of dopamine receptors [6,9]. Considerable differences were revealed in the type and degree of neuroleptic-induced changes. They are related to differences in activity of neurotransmitter systems in the brain of animals with different predisposition to catalepsy [5].

Single administration of classical or atypical neuroleptics enhances dopamine turnover in the brain. It serves as a compensatory mechanism for activation of dopaminergic systems in the brain (primarily nigrostriatal and mesolimbic systems). This negative feedback response is related to blockade of postsynaptic dopaminergic receptors [5]. Severe and inadequate catalepsy is associated with dopamine deficiency in the striatum and serves as a syndrome of pathological behavior observed during schizophrenia [6]. However, previous experiments demonstrated the involvement of serotonin into the pathogenesis of schizophrenia. Neurons containing serotonin are primarily located in the midbrain [11].

Here we studied whether the antihypoxic agent energostim possessing antioxidant and antisympathomimetic properties [2,4,7] can correct the imbalance between catecholamines and increase dopamine content in various brain structures.

MATERIALS AND METHODS

Experiments were performed on 96 male albino rats weighing 150-200 g and kept under standard conditions. Group 1 animals (n=32) intraperitoneally received trifluoperazine in a dose of 3 mg/kg. Energostim in a dose of 230 mg/kg was administered to group 2 rats (n=32) before treatment with trifluoperazine. The control group included 32 animals receiving 1 ml physiological saline.

Catalepsy was studied as described elsewhere [11]. The test for catalepsy was considered to be positive when the rat retained posture >20 sec. The animals

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0007-4888/04/1373-0255

were tested for 120 sec and then placed in the same cage. Testing was performed 30, 60, 120, and 240 min after administration of preparations (alone or in combination). The rats were decapitated under light ether anesthesia. The blood was collected from cervical vessels and put in centrifuge tubes. Brain structures (hypothalamus, thalamus, and substantia nigra) were frozen in liquid nitrogen and homogenized. Microsomes were isolated from various brain regions by homogenization (tissue/solution ratio 1:7) [1]. The contents of catecholamines (epinephrine, norepinephrine, and dopamine), DOPA, and serotonin in brain structures and blood were measured spectrofluorometrically [3]. Acetylcholinesterase (AChE, EC 3.1.1.7) activity was estimated as described previously [8]. The amount of acetylcholine (μmol) degraded by microsomes (per 1 mg protein) or 1 ml blood plasma for 1 min was taken as a unit of AChE activity.

The results were analyzed statistically. Correlation analysis was performed.

RESULTS

Administration of trifluoperazine in a single dose of 3 mg/kg induced catalepsy and locomotor disorders (akinesia, rigidity, and rigid/shaky tremor) in 86% intact animals. These disorders persisted for 4 h. Dopamine content and AChE activity in the substantia nigra sharply decreased over the first 30 min after administration of trifluoperazine. These changes were accompanied by a decrease in DOPA and serotonin content (Table 1). Norepinephrine content increased, while the content of epinephrine remained unchanged. Dopamine content progressively decreased 1 h after administration of trifluoperazine. In this period the concentrations of norepinephrine and epinephrine did not differ from those observed 30 min after treatment.

Four hours after treatment epinephrine content sharply increased and 6.5-fold surpassed the control. The concentration of norepinephrine progressively decreased starting from the 2nd hour and was 4.4-fold below the control 4 h after trifluoperazine injection. It should be emphasized that over the first 30 min we observed a decrease in the content of not only dopamine, but also DOPA. These changes reflect disturbances in the synthesis of different dopamine substances. It is important that the concentration of DOPA remained low even 4 h after administration of trifluoperazine. Therefore, trifluoperazine abolished the inhibitory effect of dopamine. These changes were followed by hyperactivation of cholinergic neurons and development of locomotor disorders. A negative correlation was found between activity of AChE and contents of dopamine and DOPA (r=0.68 and r=0.67, respectively, p<0.05). It cannot be excluded that the striatal serotoninergic system plays a role in the development of catalepsy. Previous studies showed that catalepsy is accompanied by a considerable increase in tryptophan hydroxylase activity, key enzyme of serotonin biosynthesis [10]. The amount of serotonin during trifluoperazine-induced catalepsy sharply decreased (Table 1).

The degree of motor coordination correlated with the contents of dopamine (r=0.97, p<0.0001), norepinephrine (r=0.965, p<0.0005), epinephrine (r=0.954, p<0.001), and serotonin (r=0.96, p<0.0005). Correlations were found between catecholamine concentration, locomotor activity, pathochemical changes, and functional state, which corresponds to published data [3,10,12].

Trifluoperazine increased AChE activity, which has an adaptive role and is related to the strain in the nervous system and accumulation of acetylcholine. It should be emphasized that blood contents of DOPA and dopamine decreased at the early stage, but 2-fold surpassed the control 1 and 2 h after treatment. After 4 h the content of dopamine returned to normal, while the concentration of DOPA was 2-fold below the control level (Table 2). Blood concentrations of epinephrine and norepinephrine were below the control level at various terms of observations. Only 2 h after treatment norepinephrine content surpassed the normal, and epinephrine concentration reached the control level.

Energostim caused catalepsy in only 16% animals pretreated with 230 mg/kg energostim (over the first 2 h). Catalepsy in these rats was less pronounced than in control animals. Energostim reduced oligokinesia, rigidity, and tremor. These changes were not accompanied by the decrease in the amount of dopamine in the substantia nigra and increase in the content of DOPA and activity of ACHE. However, dopamine concentration markedly decreased over the first 30 min (Table 1). These results indicate that the dopaminergic regulation was not exhausted in animals receiving energostim. DOPA content decreased by 18 times in intact rats (4 h after treatment), but did not differ from the control in energostim-treated animals. The concentrations of serotonin and norepinephrine increased 1 h after administration of trifluoperazine to energostim-receiving rats. AChE activity remained high in these animals. Our results show that energostim decreased the severity of catalepsy, which can be related to disappearance of the imbalance between the contents of catecholamines and serotonin and stimulation of acetylcholine degradation in response to the increase in its concentration in the substantia nigra. Energostim maintains homeostasis in the nervous tissue and prevents degeneration of dopaminergic neurons, which is probably associated with its contribution to transmitter interactions [13]. Under normal conditions changes in

TABLE 1. Effect of Energostim on the Contents of Biogenic Amines (nmol/g wet tissue) and Activity of AChE (nmol/mg protein/min) in Various Brain Structures (*M*±*m*)

Index, period, min		Hypothalamus		Thalamus		Substantia nigra	
		trifluoperazine	trifluoperazine and energostim	trifluoperazine	trifluoperazine and energostim	trifluoperazine	trifluoperazine and energostim
Epinephrine	control	0.85±0.15		0.7±0.1		0.77±0.09	
	30	0.14±0.04*	0.37±0.03*+	0.32±0.06*	0.59±0.06+	1.1±0.2*	0.51±0.05*+
	60	0.36±0.03*	0.39±0.04*	0.85±0.06	0.69±0.09	1.1±0.1*	0.59±0.11+
	120	1.1±0.2	0.6±0.1+	0.010±0.003*	0.37±0.03*+	1.1±0.2*	0.63±0.09+
	240	4.6±0.3*	0.9±0.1+	0.15±0.03*	0.94±0.12+	4.6±0.3*	0.87±0.30+
Norepinephrine	control	0.75±0.08		0.55±0.15		0.89±0.09	
	30	0.69±0.06	0.7±0.1	0.24±0.04*	0.6±0.1	3.0±0.3*	0.13±0.03*+
	60	0.21±0.04*	0.9±0.1+	0.22±0.03*	0.69±0.09*+	2.1±0.3*	0.13±0.05*+
	120	0.09±0.02*	0.8±0.1+	0.22±0.04*	0.15±0.03*+	0.36±0.03*	0.14±0.03*+
	240	0.12±0.03*	0.6±0.1+	0.15±0.05*	0.26±0.05*+	0.20±0.03*	0.14±0.3*+
Dopamine	control	4.2±1.3		3.5±0.8		6.8±1.6	
	30	0.7±0.1*	2.6±1.3*+	0.9±0.1*	2.6±0.2*+	1.9±0.1*	4.4±0.9+
	60	1.9±0.8*	3.2±0.7*+	1.7±0.5*	4.0±0.7+	0.98±0.05*	4.8±1.2+
	120	2.1±0.3*	6.5±1.1*+	6.5±1.1*	4.1±0.3+	0.9±0.1*	5.8±1.1+
	240	1.2±0.2*	4.1±0.3+	4.1±0.3	3.8±0.3	0.9±0.1*	5.6±1.2+
DOPA	control	0.09±0.03		0.13±0.03		0.07±0.02	
	30	0.07±0.02	0.09±0.03	0.011±0.003	0.08±0.01	0.03±0.01*	0.09±0.03
	60	0.04±0.01*	0.08±0.02+	0.022±0.003*	0.13±0.01	0.027±0.005	0.08±0.02
	120	0.05±0.01*	0.09±0.03+	0.021±0.004*	0.10±0.01	0.03±0.01*	0.05±0.01
	240	0.010±0.002*	0.03±0.01*+	0.08±0.02	0.10±0.01	0.004±0.001*	0.04±0.01
Serotonine	control	3.2±0.3		2.8±0.6		3.2±0.3	
	30	1.3±0.4*	4.9±0.3*+	2.45±0.45	3.9±0.4	1.7±0.3*	2.5±0.3
	60	0.6±0.2*	2.5±0.4+	1.3±0.3*	2.0±0.3+	0.6±0.1*	4.9±0.3
	120	0.27±0.04	2.5±0.4+	0.44±0.06*	4.2±0.3*+	0.19±0.03*	4.2±0.5
	240	0.10±0.02*	2.2±0.3*+	0.39±0.06*	5.6±0.3*+	0.10±0.03*	2.2±0.3
AChe	control	14.5±1.8		13.2±1.3		11.2±1.3	
	30	7.3±0.4*	24.9±1.9*+	8.3±0.4*	14.9±0.3+	6.3±0.9*	21.3±2.4*+
	60	8.6±0.2*	22.5±1.4*+	9.6±0.9*	13.5±0.9+	5.6±0.8*	19.6±3.8*+
	120	4.3±0.4*	17.5±2.9+	10.3±0.9*	17.5±1.4*+	5.4±0.8*	20±2*+
	240	3.1±0.4*	15.2±0.8+	10.1±0.9*	14.2±1.3+	7.3±0.5*	17.3±3.5+

Note. Here and in Table 2: $p<0.05$: *compared to the control; +compared to trifluoperazine.

TABLE 2. Blood Content of Biogenic Amines (nmol/ml) in Rats Receiving Trifluoperazine (*M*±*m*)

Index, period, min	Trifluoperazine	Trifluoperazine and energostim
Epinephrine		
control	0.76±0.07	
30	0.26±0.04*	0.34±0.04*
60	0.17±0.02*	0.26±0.04*+
120	0.61±0.07	0.17±0.02*+
240	0.25±0.03*	0.30±0.03*
Norepinephrine		
control	0.36±0.06	
30	0.09±0.02	0.14±0.03*
60	0.25±0.04	0.14±0.03*+
120	0.49±0.07	0.21±0.03*+
240	0.14±0.02	0.49±0.07+
Dopamine		
control	0.05±0.01	
30	0.021±0.003*	0.065±0.007+
60	0.13±0.02*	0.065±0.006+
120	0.11±0.03*	0.043±0.005+
240	0.075±0.007	0.043±0.004
DOPA		
control	0.065±0.015	
30	0.013±0.002*	0.055±0.003
60	0.16±0.04*	0.052±0.006
120	0.16±0.04*	0.052±0.007
240	0.039±0.007*	0.039±0.006*

functional activity of a system induces an immediate response in other systems (*e.g.*, serotoninergic system) [14,15]. This mechanism allows the organism not only to react adequately to a stimulus, but also to return to the initial state of functional activity. It is observed after administration of 3 mg/kg trifluoperazine to animals pretreated with energostim. Energostim increases the content of oxidized NAD, which plays an important role in this process. This substance acts not only as a coenzyme of dopamine decarboxylase and tryptophan decarboxylase, but also as a key coenzyme during biosynthesis of serotonin and regulation of norepinephrine synthesis and degradation. It cannot be excluded that NAD and trifluoperazine produce a direct effect (similarly to NAD and serotonin). Under these conditions the contents of DOPA and dopamine in the blood practically do not differ from the control. Norepinephrine concentration decreases in the initial period, then progressively increases, and 1.5-fold surpasses the normal by the 4th hour. However, the amount of epinephrine remained low (Table 2).

Energostim induces similar changes in the hypothalamus and thalamus. Trifluoperazine causes an adaptive increase in activity of AChE, which is probably related to strain in the nervous system and rise in the amount of acetylcholine. Our results suggest that energostim produces a direct anticataleptic effect via dopamine receptors or indirectly affects the conformation of trifluoperazine and abolishes its interaction with receptors.

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