Comparison of Behavioral Effects of Cortexin and Cerebrolysin Injected into Brain Ventricles

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We compared central effects of polypeptide preparations cortexin and cerebrolysin injected into brain ventricles of Wistar rats in doses of 1, 10, and 100 μg. Both drugs exhibited moderate psychoactivating effect, the effects cortexin were more pronounced compared to those of cerebrolysin in all tests.

Key Words: *neuropeptides; cortexin; cerebrolysin; central effects; rats*

Tissue-specific biogenic stimulators are used for activation of metabolism in organs and tissues, mainly in those from which they were derived. Preparations isolated from the brain tissue, cortexin (CT) and cerebrolysin (CL), attract special attention.

Cortexin is a polypeptide drug isolated from cattle and porcine brain cortex and created on the basis of modern pharmaceutical technologies [5, 7,8]. Cortexin is effective in monotherapy and in combination with traditional methods of treatment. Cortexin produces tissue-specific, regulatory, and reparative effects on the brain cortex and contains active low-molecular-weight neuropeptides (≤10 kDa) penetrating through the blood-brain barrier. The main tissue-specific characteristic of CT manifests in neuroprotective, neuromodulating, nootropic, and anticonvulsant effects [3,4,9]. Cortexin increases the efficiency of energy metabolism in neurons, improves intracellular protein synthesis, regulates neurotransmitter metabolism and lipid peroxidation processes in the cerebral cortex, optic nerve, and retinal neurons, stabilizes cerebral bloodflow, prevents excessive formation of free radicals, and attenuates neurotoxic effects of stimulatory amino acids [5,8,9].

The effects of CL (concentrated low-molecular-weight bioactive neuropeptides with a molecular weight ≤10 kDa) are similar. Cerebrolysin is characterized by organ-specific multimodal effect on the brain, acts as metabolic regulator, functional neuromodulator, neurotrophic activator, and neuroprotector. Cerebrolysin is regarded as a nootropic peptidergic drug with proven neuron-specific neurotrophic activity, similar to the effects of natural neuron growth factors, but manifesting, in contrast to latter, under conditions of peripheral treatment. Cerebrolysin stimulates the formation of synapses, growth of dendrites, and prevents activation of microglial cells and induction of astrogliosis [6,12,13].

We compared the effects of CT and CL in animal experiments.

MATERIALS AND METHODS

Experiments were carried out on 144 male Wistar rats (200-220 g), grown in groups of 5 animals. Males and females were kept separately in standard plastic cages with free access to water and food under conditions of inverted light (8.00-20.00) at 22±2°C. All behavioral experiments were carried out on adult (90-100 days) animals in the fall and winter.

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Cortexin (Gerofarm) and CL (Ebewe Pharma) were infused into the lateral brain ventricle through an implanted cannula. Guide metal cannulas (200 μ in diameter) were implanted into the left ventricle of the brain unipolarly by coordinates: AP=0.9 mm behind the bregma, SD=1.4 mm laterally from the sagittal suture, and H=3.5 mm from the skull surface. For intraventricular infusion of the test drugs, metal microcannulas (100-μ) with tips 0.2 mm longer than the guides were inserted into the guides. The drugs were infused into the brain ventricles in doses of 1, 10, and 100 μg. The choice of doses was based on the preferable use of these doses in behavioral experiments. All substances were infused 5-10 min before the experiment. Infusion of 0.9% NaCl solution served as the control.

Free motor activity of animals was recorded in the open field test [10,11] (open field was a round area 80 cm in diameter with 16 holes 3 cm in diameter each). The duration of 1 experiment was 3 min. Elementary motor acts and postures were registered: horizontal and vertical activities, grooming, explored holes, defecation, and urination. The data were mathematically processed.

Elevated plus-maze consisted of 2 open arms (50×10 cm) and 2 closed arms (50×10 cm) perpendicular to each other [10] at a height of 1 m above the floor. The animal was placed into the center of the maze. The time spent in open and closed arms, number and duration of peeping down from the maze platform and closed arms were recorded by pressing an appropriate key of the etograph connected to computer. The duration of the test was 5 min.

Aggressive behavior was studied in the intruder-resident test [11] in adult rats. A smaller animal (intruder) was placed into the cage with a large male (resident). The number of behavioral manifestations of aggressiveness and defense and total number of behavioral acts describing the interrelationships between the animals were registered.

Stereotaxic implantation of electrodes into the rat brain was carried out under nembutal narcosis (50 mg/kg) using a stereotaxic device (Medicor). Monopolar nichrome glass-insulated electrodes (electrode diameter 0.25 mm, tip length 0.25-0.30 mm, thickness 0.12 mm) were bilaterally implanted into the lateral hypothalamic nucleus according to coordinates: AP=2.5 mm behind the bregma, SD=2.0 mm laterally from the saggittal suture, and H=8.4 mm from the skull surface. An indifferent nichrome wire electrode was fixed on the skull. All electrodes were commutated on a plug-and-socket microunit fixed on the skull with a self-hardened plastic. Behavioral experiments were started no earlier than on day 10 postoperation. After all experiments the location of electrode tips was verified morphologically.

Ten days after implantation of the electrodes into the brain the rats were trained to press a lever in the Skinner cell for electrical stimulation of the brain (rectangular pulses of negative polarity, 1 msec, 100 Hz, 0.4 sec duration, threshold current values in the "fixed pack" mode). The frequency and duration of pressing episodes were registered automatically. The frequency and duration of each lever pressing were analyzed. The "dissociation" coefficient [10] was estimated from these data; this coefficient is a convenient accessory indicator for evaluating drug effects.

Each group consisted of at least 10-12 animals. The results were statistically processed using Student's *t* test.

RESULTS

Open field test demonstrated a moderate activating effect of CT, manifesting in increased horizontal and vertical activity of animals; the maximum CT dose (100 μg) moderately reduced their emotionality. No dose-dependent effect of CT was observed (Table 1). Similar regularities were observed after CL treatment, but manifestation of the effects were less pronounced.

In the elevated plus-maze, CT infusion increased the number of open-arm entries and peeping down from the platform (Table 2). The maximum effect was produced by CT in a dose of 1 μg. The anxiolytic effect sharply decreased with increasing the dose. Treatment with CL also led to moderate anxiolytic effect, but, in contrast to CT, it was less pronounced and increased in a step-wise mode attaining the maximum in the dose of 100 μg.

Cortexin in doses of 10 and 100 μg caused a moderate reduction of the communicative activity in the intruder-resident test (Table 3). Aggressive manifestations were most frequent after the dose of 1 μg, after which aggressiveness decreased in a dose-dependent manner. The defense behavior was similar. The effects of CL in doses of 10 and 100 μg were similar. The only difference was the absence of aggressiveness increase after CL infusion, though defense behavior increased 2-fold after the dose of 1 μg. Hence, the drug effects were to a certain measure similar. Importantly, CT and CL stimulated the aggressiveness and defense system, which attests to their psychoactivating effects.

Infusion of saline into the brain ventricles virtually did not modify the frequency of the hypothalamus autostimulation (Fig. 1). Cortexin in doses

TABLE 1. Effects of CT and CL on Rat Behavior in the Open Field Test (*M*±*m*)

Parameter	Control	Dose, μg		
		1	10	100
CT				
Number of crossed squares	15.67±2.02	21.33±2.76	23.67±3.06*	20.33±2.63
Rearings	9.00±1.16	12.00±1.56	11.33±1.46	11.00±1.42
Explored holes	10.00±1.33	11.67±1.56	11.67±1.56	11.67±1.56
Grooming	9.67±1.25	11.00±1.42	12.00±1.56	11.00±1.42
Number of defecation boluses	5.83±0.77	4.33±0.57	4.00±0.53	3.33±0.44*
CL				
Number of crossed squares	14.33±1.90	17.33±2.30	19.67±2.61*	17.33±2.30
Rearings	10.00±1.29	10.33±1.34	12.00±1.56	10.67±1.38
Explored holes	10.00±1.29	10.00±1.29	11.67±1.51	11.00±1.42
Grooming	9.00±1.19	10.00±1.33	9.67±1.28	10.33±1.37
Number of defecation boluses	4.33±0.56	3.67±0.47	3.33±0.43	2.67±0.34*

Note. Here and in Tables 2 and 3: *$p<0.05$ compared to the control.

TABLE 2. Effects of CT and CL on Rat Behavior in Elevated Plus-Maze (*M*±*m*)

Parameter	Control	Dose, μg		
		1	10	100
CT				
Time in open arms, sec	8.33±1.09	24.33±3.19*	9.00±1.18	14.00±1.84*
Peeping down from central platform (number of acts)	1.00±0.13	2.67±0.35*	1.67±0.22	1.67±0.22
Peeping out from closed arms (number of acts)	0.00±0.00	0.00±0.00	0.33±0.04	0.00±0.00
CL				
Time in open arms, sec	5.00±0.26	4.00±0.52	9.00±1.18*	17.00±2.23*
Peeping down from central platform (number of acts)	0.00±0.00	0.00±0.00	1.00±0.13*	2.00±0.26*
Peeping out from closed arms (number of acts)	0.00±0.00	0.00±0.00	0.00±0.00	0.50±0.07*

of 1 and 10 μg did not change, while in a dose of 100 μg considerably increased the reinforcing effects of electrical stimulation of the hypothalamus. Cerebrolysin exhibited less pronounced reinforcing effect, which was maximum after administration of 10 μg (Fig. 1). Hence, both substances, infused into brain ventricles exhibited a psychoactivating effect on the reinforcement systems of the brain.

The maximum stimulation was observed after administration of 100 μg CT and 10 μg CL. The absence or slight psychoactivating effects of the drugs in other doses indicate a typical effect of the peptide preparations, which, as a rule, work in a strictly definite range of doses, characteristic of this or that peptide(s) [1].

The results suggest that CT and CL produce a moderate psychoactivating effect, the effect of CT were more pronounced than those of CL. The data were obtained in experiments with direct infusion of the drugs into the brain ventricles, that is, avoiding the blood-brain barrier. This does not rule out possible differences in the effects of these drugs in systemic administration.

The mechanism of the action of peptide bioregulator can be explained from the viewpoint of the regulatory cascade. They produce a direct information impact on cell structures of the brain, and then promote release of the cerebral regulatory peptides, which in turn, induce the release of the next group of peptides.

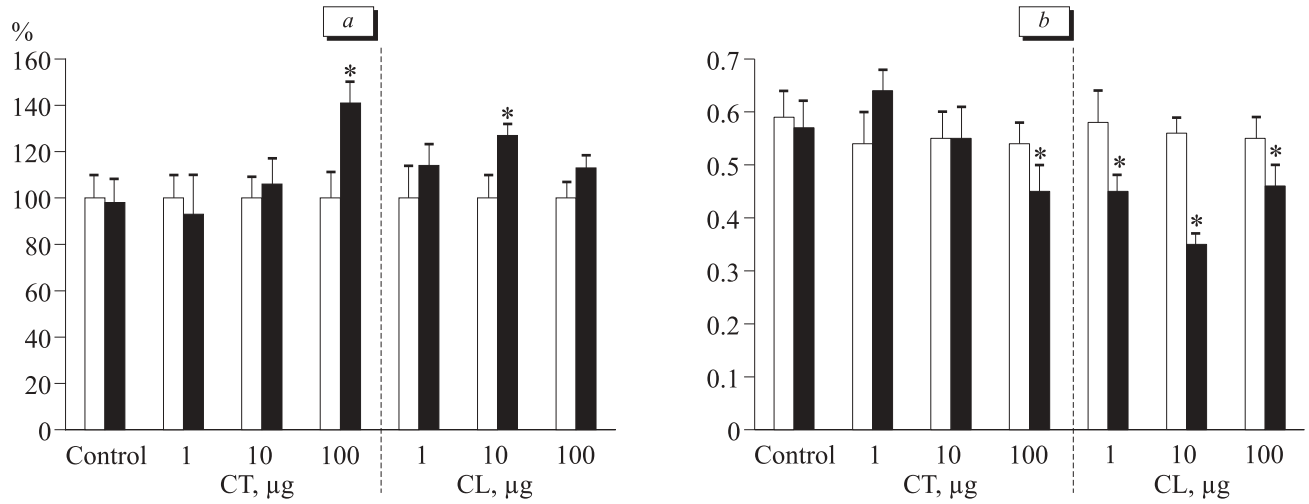

Fig. 1. Effects of CT and CL on rat behavior in the test of autostimulation of the lateral hypothalamus. *a*) number of lever pressings over 5 min; *b*) dissociation coefficient. Light bars: before drug infusion; dark bars: after infusion. **p*<0.05 compared to values before drug infusion.

Clinical and biochemical studies demonstrated a neuromodulating effect of CT on the neurons; it abolishes (or appreciably reduces) NMDA receptor blockade, thus preventing further development of the cascade pathological processes [5]. Used in therapy of destructive diseases (neuroinfections, neurotrauma, severe hypoxia), CT supports the damaged neuron and reduces activity of autoimmune processes.

The neuroprotective effect of CL manifests in protection of neurons from the destructive effect of lactate acidosis, prevention of free radical formation, and reduction of LPO products concentration on the ischemia—reperfusion model, improvement of neuron survival and prevention of their death under conditions of hypoxia and ischemia, reduction of the destructive neurotoxic effect of stimulating amino acids (glutamate), suppression of apoptosis by caspase inhibition [12,13].

Hence, the main metabolic effects, intrinsic of the cerebral organ preparations CT and CL, are neuroprotective with a psychoactivating trend. The intensity of the drugs effects is different: by the central effects CT is on the whole superior to CL under experimental conditions.

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TABLE 3. Effects of CT and CL on Rat Behavior in the Intruder-Resident Test (*M*±*m*)

Parameter	Control	Dose, μg		
		1	10	100
CT				
Individual behavior	39.00±5.07	42.67±5.55	25.00±3.25*	22.33±2.90*
Communicative behavior	38.33±4.98	38.67±5.03	11.33±1.47*	12.00±1.56*
Aggression manifestation	1.67±0.22	3.67±0.48*	0.33±0.04*	0.00±0.00*
Defense behavior	1.67±0.09	5.67±0.74*	1.67±0.22	2.00±0.26
CL				
Individual behavior	44.50±5.79	40.67±5.29	26.67±3.47*	24.67±3.21*
Communicative behavior	42.60±5.79	39.67±5.16	22.00±2.86*	25.33±3.29*
Aggression manifestation	0.50±0.07	1.33±0.17	0.00±0.00	0.33±0.04
Defense behavior	2.50±0.33	4.33±0.56*	1.00±0.13	0.33±0.04

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