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Sedative and Antistressor Effects of a Complex of Peptides Isolated from Human Amnion

M. I. Borshchevskaya, E. A. Vasil'chenko, L. N. Vasil'eva, and V. P. Georgievskii

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Sedative and antistressor effects of a complex of peptides isolated from human amnion are studied. The peptides are found to exert a marked sedative effect and a certain antistressor effect on rats with immobilization stress, the degree of manifestation of these effects being dose-dependent.

Key Words: *peptide complex; sedative effect; antistressor effect*

Peptides are now considered to be among the most important functional regulators. Many regulatory peptides, such as adrenocorticotropic hormone, vasopressin, interleukins, endorphins, enkephalins, etc., have now been studied in detail [5,7]. Peptide complexes (PC) representing tinctures or extracts from various tissues of the body are attracting special attention. Among such substances are extracts from the thymus, bone marrow, spleen, lymph nodes, retina, cerebral cortex and white matter, pineal body, prostate, placenta, and some other organs [8-11].

Amniotic tissue containing peptides, polysaccharides, glycosaminoglycans, numerous enzymes, etc. is an important source of bioactive substances. This study explores the sedative and antistressor effects of PC isolated from human amnion.

MATERIALS AND METHODS

Experiments were carried out with albino Wistar rats of both sexes weighing 190 to 230 g. PC were injected to experimental animals in doses of 10, 20, and 50 mg/kg once daily for 10 days. To controls aqua pro injection in matching volumes was administered. The sedative action of PC was assessed by changes in the orienting-exploratory activity of animals, which was recorded in an Animex chamber by counting all types of movements and body changes in the environment, as well as by the open field test, which characterizes the total activity and orientational and emotional reactions of animals. Besides motor activity, the number of defecations (pellets) and urinations was counted, as these reflect the emotional status of the animals and permit assessment of the autonomic function of the central nervous system.

Emotional stress was induced by the well-known immobilization method of tying the animals in the supine position to a board for 2 hours. The influence of PC on resistance to emotional stress was assessed from the content of glucose in the blood and of glycogen in liver tissue [1-3,6]. Glucose was measured in the blood by the cuprometric method [5] before the course of PC injections (intact control), after a 10-day course of injections (control level of glucose before stress induction), and after stress. The content of glycogen in liver tissue was

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TABLE 1. Effect of PC on the Motor Activity of Rats ($M \pm m$)

Experimental conditions	Number of experiments	Dose, mg/kg	Integral expression of activity	
			beginning of experiment	end of experiment
Control	6	–	710.9±44.7	719.5±39.4
PC	12	10	707.3±50.0	525.8±20.4
Control	6	–	612.4±19.2	623.6±21.1
PC	11	20	609.1±31.0	375.0±25.3
Control	6	–	787.5±14.5	766.0±15.9
PC	14	50	744.5±19.5	689.0±14.9

measured by the anthrone method [5] in animals of all groups after the induction of stress and in a separate group of intact rats of the same population.

RESULTS

The data on the motor activity of animals presented in Table 1 provide evidence that it was virtually the same in control rats over the entire period of study.

PC in a dose of 10 mg/kg caused an approximately 25.6% reduction in the number of movements in comparison with the initial level (p=0.01) and a 26.9% decrease in comparison with the respective control (p=0.001). A twofold increase of the PC dose led to an appreciable increase of the sedative effect: the number of movements dropped by 38.4% on average *vs.* the initial level and by 35.3% *vs.* control values by the end of the experiment (p=0.001 for both cases). A further increase of the PC dose (2.5 times) did not enhance the sedative effect established for the 20 mg/kg dose: the number of movements decreased by just 10% in comparison with the initial level (p<0.05).

Hence, PC had a dose-dependent sedative action. This permitted us to determine the effective dose, equal to 23±1.99 mg/kg.

Data on the motor activity of animals in the open field are presented in Table 2. We see that all 5 parameters of the orienting-exploratory behavior of control rats remained virtually the same over the entire experiment. PC in a dose of 20 mg/kg reduced the motor activity of rats (locomotions+standstills) by 55 and 60%, on average, in comparison with the initial level, and the orientational reaction was approximately 25% reduced (p=0.001). A 2.5-fold increase of the PC dose did not augment the effect: the motor activity of animals was 28.5% reduced, and the orientational reaction 22% reduced (p=0.2). PC did not influence the number of fecal pellets and urinations.

Hence, the results indicate that the studied PC is characterized by a pronounced sedative effect on the motor and orienting activity of rats without affecting the autonomic functions of animals.

Results on the antistressor effect of PC, presented in Table 3, showed that glucose levels in

TABLE 2. Effect of PC on the Total Activity and Orientational and Emotional Reactions of Rats (Number of Various Physiological Parameters, $M \pm m$)

Parameter of assessing sedative effect	Control		PC effect	
	beginning of experiment	end of experiment	beginning of experiment	end of experiment
Dose 20 mg/kg (n=15)				
Locomotions	48.2±9.5	43.9±15.2	52.5±7.7	21.00±2.3
Standstills	17.9±2.6	15.4±2.4	17.9±1.8	8.00±0.6
Grooming	7.1±1.0	7.8±1.5	7.1±0.8	5.32±0.5
Pellets	2.0	2.2	2.0	2.0
Urinations	1.0	0.8	1.0	0.8
Dose 50 mg/kg (n=17)				
Locomotions	50.4±4.15	59.4±3.8	50.3±4.15	35.20±1.7
Standstills	10.3±1.57	10.7±0.7	12.0±1.30	8.76±0.9
Grooming	8.0±0.64	8.5±0.5	9.2±1.40	7.17±0.7
Pellets	1.8	1.4	1.9	1.8
Urinations	0.6	0.6	1.0	0.8

TABLE 3. Effect of PC on Resistance of Rats to Emotional Stress ($M \pm m$)

Experimental conditions	Glucose content, mmol/liter	Glycogen content, μg/ml
Intact control	5.81±0.51	57.11±5.40
Before stress		
Control	5.79±0.35	–
PC in doses of:		
20 mg/kg		5.20±0.50 –
50 mg/kg		5.45±0.35 –
After stress		
Control	9.37±0.66	39.36±3.89
PC in doses of:		
20 mg/kg	6.37±0.68	45.58±5.67
50 mg/kg	10.15±0.48	30.00±7.37

the blood of intact rats and in those after a 10-day course of injections of PC and its solvent (pre-stress level) were virtually the same. The development of stress in control rats was characterized by an appreciable increase of the blood glucose concentration: by an average of 61.9% in comparison with the initial level ($p<0.001$). In animals injected PC blood glucose also increased, but to a different degree, the increase being dose-dependent. For example, injection of peptides in a dose of 20 mg/kg led to an approximately 22% rise of the blood glucose level in comparison with the control ($p=0.1$), whereas in animals injected PC in a dose of 50 mg/kg this value surpassed the control level by 85.9% on average ($p<0.001$). These changes indicate that PC in a dose of 50 mg/kg does not protect the rats from stress, whereas in a dose of 20 mg/kg it exerts a patent antistress effect: blood glucose is approximately 32% lower than in the respective control ($p=0.01$).

As shown in Table 3, the development of stress in control animals was characterized, among other things, by a drop of the glycogen level in the blood by 31%, on average, as against the intact control ($p<0.05$). Changes in the content of glycogen in the liver of animals injected PC were variously directed. For instance, the dose of 20 mg/kg caused an approximately 18.8% increase of glycogen, whereas the dose of 50 mg/kg brought about a further reduction of its level (by an average of 23.8%) in comparison with the control for stress development. These changes indicate that, just as with changes in glucose concentrations in the blood, PC in a dose of 50 mg/kg exerts no stress-protective effect, whereas at 20 mg/kg such an effect is expressed to a certain degree.

Our study of the effects of PC on the central nervous system, involving assessment of its sedative action, showed that the peptides investigated possess an appreciable sedative and antistress action. The clear-cut dose dependence of these properties is worthy of note.

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