PREVENTION OF EXPERIMENTAL STRESS-INDUCED HEART LESIONS BY ENKEPHALINS

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The close connection between the pathogenesis of stress-induced and ischemic heart lesions [4] has stimulated the active search for measures of reducing the harmful action of the stress syndrome on the myocardium. The principle of simulation of the natural antistressor systems of the body by the use of metabolites of these systmes or their synthetic analogs, suggested by Meerson [4], is very promising in this respect.

TABLE 1. Accumulation of ^{99m}Tc-PP in the Heart and Serum CPK Activity of Rats during Stress (M ± m)

Experimental conditions	Time after stress, h	^{99m}Tc-PP concentration in heart, % of injected dose per gram of tissue	Serum CPK activity, U/liter
Intact control (n=25)	—	0,076±0,0052	84,21±4,94
Stress control	2 (n=13)	0,257±0,025	104,29±3,34
	P	<0,001	<0,01
	4 (n=9)	0,434±0,017	91,67±2,62
	P	<0,001	>0,05
	8 (n=8)	0,575±0,08	161,43±6,29
	P	<0,001	<0,001
	12 (n=7)	0,570±0,046	130,20±5,40
	P	<0,01	<0,001
Experiment	2 (n=8)	0,206±0,022	95,18±4,19
	P	<0,001	>0,05
	P_1	>0,05	>0,05
	4 (n=10)	0,240±0,015	83,57±3,68
	P	<0,001	>0,05
	P_1	<0,001	>0,05
	8 (n=9)	0,267±0,029	136,57±4,33
	P	<0,001	<0,001
	P_1	<0,001	<0,01
	12 (n=8)	0,254±0,022	114,11±3,8
	P	<0,001	<0,001
	P_1	<0,001	<0,05

Legend. P) Significance of differences relative to intact control, P_1) significance of differences relative to stress control. n) Number of observations.

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The aim of this investigation was to study the effect of an enzyme-resistant analog of endogenous Leu-enkephalin, with antistressor properties [2, 3], on the degree of stress-induced heart damage in rats.

EXPERIMENTAL METHODS

Experiments were carried out on 97 male albino rats weighing 160-180 g. Stress-induced heart lesions were produced by an experimental anxiety neurosis as described in [4]. Before the experiment began animals of the experimental group were given an intraperitoneal injection of a hexapeptide arginine-containing analog of Leu-enkephalin in a dose of 1.25 nmole/100 g body weight (obtained in the Laboratory of Peptide Synthesis, All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR, by Dr. Chem. Soc. M. I. Titov). Animals of the first control group (stress control) received an injection of the equivalent volume (200 µl) of physiological saline. The rats were decapitated under superficial ether anesthesia 2, 4, 8, and 12 h after a 6 h period of stress. The second control group consisted of intact rats (intact control). The degree of stress-induced damage was assessed as the percentage of uptake of the injected dose of ^{99m}Tc pyrophosphate (^{99m}Tc-PP) taken up by 1 gram of myocardial tissue and by the serum creatine phosphokinase (CPK) level. The ^{99m}Tc-PP was injected 1.5 h before decapitation in a dose of 0.5 MBq/100 g body weight. Radioactivity was measured, allowing for the half-life, by means of Tracor Analytic Gamma-spectrometer (USA). CPK activity was determined on the SF-26 spectrophotometer (USSR) and by means of BIO La-test kits (Czechoslovakia).

The results were subjected to statistical analysis (M ± m) using tables [7] and Student's t test.

EXPERIMENTAL RESULTS

The degree of accumulation of ^{99m}Tc-PP in the rats' heart was significantly greater than in intact animals: by 3.4 times after 2 h, by 5.7 times after 4 h, by 7.6 times after 8 h, and by 7.5 times after 12 h (Table 1). These changes could be evidence of increased entry of PP into the cardiomyocytes as a result of damage to their membrane apparatus [8]. This is also indicated by elevation of the serum CPK activity: by 24% after 2 h, by 9% after 4 h, by 91.7% after 8 h, and by 54.6% after 12 h. The results confirm the common pathogenesis of ischemic and stress-induced heart lesions, for changes of this kind are classical signs of cardiac ischemia [6]. The fact that maximal values of changes in these parameters did not occur until 8 h after discontinuation of stress suggests that the lesions which developed are connected with progressive disturbances of homeostasis that are characteristic of the stress syndrome.

Preliminary injection of enkephalin was followed by a significant decrease in ^{99m}Tc-PP uptake by the myocardial cells 4 h after cessation of exposure to stress. The percentage accumulation of injected radioactive label by the heart tissue of rats receiving the peptide was 1.8 times less after 4 h and 2.2 times less after 8 and 12 h than in the stress control. As regards the CPK level, this same pattern was found only 8 and 12 h after stress, when the activity of this enzyme was 15.4 and 12.4% less respectively than in the control. The evident explanation of this fact is that the injected enkephalin had no direct membrane-stabilizing action on the myocardium, but inhibited the development of an excessively strong stress reaction [3] and, in particular, it contributed to the reduction of secretion of an excess of adrenal substances [1], capable of stimulating lipid peroxidation during stress, and also of causing activation of lipases and phospholipases, which damage cardiomyocyte membranes [4]. For this reason the effects of the enkephalin were not manifested in the early stages of the investigation.

The writer previously obtained data to show that enkephalins can prevent adrenal-induced heart lesions in rabbits [5]. However, in a separate series of experiments, the enkephlin which was studied had no significant effect on myocardial ^{99m}Tc-PP uptake or serum CPK activity in rats with isoproterenol-induced cardiac necrosis. This latter effect can be explained by differences in the type and functions of the myocardial opiate receptors in rats and rabbits.

The results thus suggest that it may be possible, in principle, to prevent stress-induced heart lesions by the use of stable synthetic enkephalin analogs.

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EXPERIMENTAL AMYOTROPHIC LEUKOSPONGIOSIS IN GUINEA PIGS WITH RETROBULBAR INFECTION

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Among human neuroinfections a group of slow infection of the CNS caused by nonclassical viruses is distinguished. This group includes kuru, Jakob–Creutzfeldt disease (JCD), amyotrophic leukospongiosis (ALSP), and the Gerstmann–Streissler syndrome [3, 9, 11]. All these diseases are characterized by a long incubation period, measured in months or years, absence of inflammatory reactions in the CNS, and they are inevitably fatal. The agents of slow infections of the CNS are nonclassical viruses, which constitute a new class of subviral pathogens which, as Prusiner has suggested [11], may be called prions.

ALSP, found in the territory of the Belorussian SSR, like the Gerstmann–Streissler syndrome, has been included in the group of slow infections of the human CNS as a result of proof of its infectious nature and of isolation of the causative agent which, in its biological and physicochemical properties, has the features of a nonclassical virus. However, the particular features of the pathogenesis of the disease have so far been inadequately studied, and this is holding up the development of methods of intravital diagnosis, specific chemotherapy, and prevention of this fatal infectious disease in man. The undertaking of such investigations has been retarded by the absence of a convenient laboratory model with a short incubation period. The study of the spectrum of sensitivity of laboratory animals (squirrel, monkeys, rabbits, guinea pigs, hamsters, and rats) to the agent of ALSP has shown that the animal most susceptible to infection is the guinea pig. Nevertheless, the incubation period of the disease after intracerebral or intramuscular infection of the animal, has been found to be 3.5-8.2 and 5.3-11.1 months respectively [2, 4].

For the reasons given above, it was decided to attempt to develop a method of modeling ALSP in guinea pigs with a short incubation period and to study some aspects of the pathogenesis of the disease.

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