EFFECT OF HYDRA PEPTIDE MORPHOGEN ON CYCLIC NUCLEOTIDE LEVELS IN INJURED TISSUES

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A new neuropeptide, namely hydro peptide morphogen (HPM), consisting of 11 amino-acid residues, has recently been isolated and identified. HPM has been shown to stimulate androgen metabolism in the gonads [2], thereby accelerating sexual maturation in rats [6]. It is suggested that this neuropeptide may be involved in growth regulation [2]. These indirect data suggest a possible influence of HPM on tissue repair. To test this hypothesis, the investigation described below was undertaken. The concentration of cyclic nucleotides, absolute and relative, in the tissues was taken as marker of the course of repair [1].

EXPERIMENTAL METHOD

Experiments were carried out on 155 male and female BALB/c mice weighing 15-17 g. Liver and striated muscle tissue was injured by the standard methods [5]: muscles by repeated subcutaneous pricking of both hind limbs, the liver by injection of carbon tetrachloride in a dose of 5 mg/kg body weight. Concentrations of cAMP and cGMP in samples of muscle and liver tissue were determined by the use of kits from "Amersham International" (England). Radioactivity in the processed samples was counted on a Mark III liquid scintillation counter (USA). The results were subjected to statistical analysis on the "Iskra 226" computer, by Student's test. The HPM used was synthesized in the All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR, naloxone was from "Sigma" (USA). The HPM (30 μg/kg) and naloxone (2 mg/kg) were injected intramuscularly 30 min after injury to the tissues. In series of experiment in which naloxone and HPM were given simultaneously, naloxone was injected after 30 min and HPM 1 h later.

EXPERIMENTAL RESULTS

Investigation of samples of striated muscles taken 6 and 24 h after injury revealed an increase in the cAMP concentration, a decrease in the cGMP concentration (after 6 h), and an increase in the cAMP/cGMP ratio, which was especially marked after 6 h (Table 1). Separate administration of naloxone and HPM or their combined use did not cause any significant changes in the parameters studied at all times of observation.

In samples of liver tissue taken 6 h after injury the cAMP concentration also was increased and the cGMP concentration reduced, with a significant increase in the cAMP/cGMP ratio. Injection of HPM led to a significant fall of the cAMP level, a rise of the cGMP level, and a significant (almost fourfold) fall of the cAMP/cGMP ratio. Preliminary administration of the opiate receptor blocker naloxone did not abolish the effect of HPM. Injection of naloxone alone produced changes similar to those caused by HPM (Table 1).

In samples of liver tissue taken 24 h after injury, a sixfold drop of the cAMP level and a rise of the cGMP level were observed, so that the cAMP/cGMP ratio was considerably reduced (Table 1).

Administration of HPM, naloxone, or a combination of both led to an increase in the cAMP concentration, but no change in the cGMP concentration; the cAMP/cGMP ratio was increased, although not up to the level found in intact animals.

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Table 1. Cyclic Nucleotide Concentrations in Liver and Striated Muscle of Rats under the Influence of HPM and Naloxone

Variant of experiment	cAMP, pmoles/kg tissue	cGMP, pmoles/kg tissue	cAMP/cGMP
Muscle			
Intact animals (n = 10)	422,0±40,0	24,6±2,8	17,0±1,1
Injury, after 6 h (n = 10)	562,0±50,8*	11,1±1,0**	51,0±6,2**
Injury + HPM, after 6h (n = 8)	460,0±40,0	10,1±1,2**	46,0±3,4**
Injury + naloxone, after 6 h (n = 8)	446,1±40,0	10,4±2,6**	44,0±1,9**
Injury + naloxone + HPM, after 6 h (n = 8)	491,0±38,6	10,7±2,0**	47,5±4,2**
Injury, after 24 h (n = 8)	591,0±42,0*	25,6±3,0	22,8±1,6
Injury + HPM, after 24 h (n = 8)	461,0±45,1	19,6±2,4	23,6±2,1
Injury + naloxone, after 24 h (n = 8)	431,0±28,0	28,0±3,2	15,4±1,0
Injury + naloxone + HPM, after 24 h (n = 8)	468,0±28,7	34,0±4,1	13,8±1,2
Liver			
Intact animals (n = 10)	191,0±19,6	17,4±1,8	11,0±1,0
Injury, after 6 h (n = 10)	333,8±34,2*	14,1±1,2	24,0±2,6**
Injury + HPM, after 6 h (n = 8)	141,0±12,1	23,6±2,1	5,9±0,8**
Injury + naloxone, after 6 h (n = 8)	145,8±14,6	23,9±2,4	6,0±0,8**
Injury + naloxone + HPM, after 6 h (n = 8)	130,0±14,1*	24,6±2,8*	5,2±0,8**
Injury, after 24 h (n = 8)	33,8±3,8**	23,7±1,9*	1,4±0,2**
Injury + HPM, after 24 h (n = 8)	104,0±11,2*	25,1±1,9*	4,2±0,4**
Injury + naloxone, after 24 h (n = 8)	73,5±8,3**	17,2±1,9	4,3±0,4**
Injugry + naloxone + HPM, after 24 h (n = 8)	68,0±7,2**	17,4±1,9	3,9±0,4**

Legend. *p < 0.05, **p < 0.001.

As a result of injury, the cAMP concentration in striated muscle rose and the cAMP/cGMP ratio increased, reflecting inhibition of repair processes [3]. This phenomenon was particularly marked in the early period after injury to the muscle, when the cGMP concentration fell at the same time. Injection of HPM caused virtually no change in the cyclic nucleotide levels or in the ratio between them in striated muscle. Changes affecting cyclic nucleotides, reflecting the process of inhibition of repair, also were observed in the liver tissue in the early period after injury. Conversely, after 24 h the opposite picture was observed: a considerable fall of the cAMP concentration, a rise of the cGMP level, and a sharp fall in the cAMP/cGMP ratio, which can be interpreted as activation of tissue repair processes in response to injury [3]. Regeneration of liver cells is known to take place much more rapidly than of striated muscle cells [5], and this evidently explains the temporary differences observed in the cyclic nucleotide levels. Injection of HPM restored the normal cyclic nucleotide levels in liver tissue in both cases, i.e., a modulating effect was observed on changes in the repair process of the liver cells: normalization in the case of both inhibition and marked activation. The increase in the rate of anabolic processes in the injured liver, mediated through the polyamine system and under the influence of HPM [4] also supports the view that processes of reparative regeneration are intensified in this organ also, and are stimulated by HPM. Since naloxone, an opiate receptor blocker, did not abolish the effects of HPM, it can be tentatively suggested that the effect of the morphogen on repair processes is not realized through opiate receptors.

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PHOSPHOLIPASE A_2 ACTIVITY DETERMINES RATE OF MITOCHONDRIAL RESPIRATION IN HIBERNATING ANIMALS

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When animals sink into a state of hibernation the intensity of their oxidative metabolism decreases by 50-100 times within a few hours, and it increases just as quickly to the normal level on awakening [5]. In full agreement with the physiological state of the animals, isolated mitochondria (MCh) from the liver of hibernating susliks are characterized by a greatly reduced rate of respiration compared with the MCh of active animals [1]. No sufficiently convincing explanation of inhibition of mitochondrial respiration in hibernating animals has yet been given. One probable cause of the slowing of respiration may be a change in the physicochemical properties, microviscosity for example, of the membranes of MCh, leading to disturbances of the working of the membrane-bound enzymes. In turn, changes in the physicochemical properties of the membranes may be based on a change in mitochondrial phospholipase A_2 (PLA_2) activity [3].

To test this hypothesis, an investigation was carried out to study the role of PLA_2 in regulation of the rate of mitochondrial respiration in hibernating susliks.

EXPERIMENTAL METHOD

Susliks of the species Citellus undulatus were used in the experiments. MCh were isolated from the liver and incubated as described previously [1]. The rate of mitochondrial respiration was determined with the aid of a Clark's oxygen electrode in a thermostatically controlled cell with a volume of 2 ml at 27°C, with constant mixing. To assess the microviscosity of the membranes, polarization of fluorescence of 1,6-diphenylhexatriene was used [2]. The rate of ATP synthesis was determined enzymatically, by measuring accumulation of glucose-6-phosphate in the presence of 0.1 U hexokinase and 10 mM glucose, and recording fluorescence of NADPH [12]. The rate of substrate transport was estimated from swelling of MCh in 100 mM solutions of ammonium succinate, β-hydroxybutyrate, and glutamate [7]. Swelling of MCh was judged by the decrease in optical density of the mitochondrial suspension at 520 nm. The protein concentration was determined by Lowry's method.

EXPERIMENTAL RESULTS

The measurements showed that the microviscosity of the mitochondrial membranes in hibernating animals was higher than in active susliks: 0.252 ± 0.028 compared with 0.194 ± 0.017 P, respectively (n = 3). The increased microviscosity of the mitochondrial membranes

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