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EFFECT OF HYDRA PEPTIDE MORPHOGEN ON LEVELS OF β-ENDORPHIN AND CERTAIN HORMONES IN ALBINO RAT BLOOD AND ADRENALS

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Analysis of the mechanisms of the high regenerative capacity of the freshwater *Hydra* has shown that repair processes are regulated by a group of morphogens [13]. One of these, *Hydra* peptide morphogen (HPM), regulates growth and differentiation of cells of the head part of *Hydra attenuata* [12]. The peptide has been isolated in the pure form and synthesized chemically [10]. HPM or substances closely similar to it in their immunoreactivity, are found in the rat hypothalamus and intestine [14] and also in human brain and blood plasma [11]. The physiological role of the neuropeptide is currently being comprehensively studied. It has been found, in particular, that HPM activates physiological and reparative regeneration [3, 7, 9].

The aim of this investigation was to assess the role of endocrine changes induced by HPM in stimulation of cell division of epithelial tissues. The aim was to study the phenomenologic character of the effect of HPM on levels of certain hormones: inhibitors of cell division (corticosterone and adrenalin) and stimulators β thyroxine (T_4), tri-iodothyronine (T_3), and insulin. β-Endorphin also acts as a stimulator of proliferative processes.

It must be admitted that the definition of "inhibitor" and "stimulator" is somewhat conventional, for the character of the effect of hormones depends on dose and conditions, but on the whole, this subdivision is in line with accepted opinion [1, 2].

EXPERIMENTAL METHOD

Experiments were carried out on 130 male albino rats weighing 180-200 g. HPM was injected intraperitoneally in doses of 10 μg/kg (dose A) and 100 μg/kg (dose B). The investigation was carried out 4 and 24 h after injection of the peptide. Rats receiving an equal volume of isotonic sodium chloride solution intraperitoneally served as the control. The β-endorphin level was measured by radioimmunoassay using kits from "INCSTAR" (USA). The T_3, T_4, and insulin levels were determined by means of kits from the Institute of Bioorganic Chemistry, Academy of Sciences of the Belorussian SSR,

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TABLE 1. Effect of HPM in Doses A and B on β-Endorphin and Hormone Levels in Blood and Adrenals of Albino Rats 4 and 24 h after Injection

Group	β-endorphin in blood, pmoles/liter	Corticosterone		Catecholamines in adrenals, μg/g	
		blood, pmoles/liter	adrenals, nmoles/g	adrenalin	NA
Control	12,6±2,5	152,9±14,9	9,62±1,2	630,1±50,8	40,2±10,1
Experiment, dose A					
4 h	8,5±2,8	224,7±16,7*	13,6±2,8	1018,1±94,2*	97,1±9,8*
24 h	4,81±1,8*	163,3±35,6	21,5±2,8*	883,8±77,5*	52,4±14,1
Control	20,8±3,7	136,2±6,5	10,9±2,2	592±54,9	210,4±36,3
Experiment, dose B					
4 h	—	98,5±18,0*	15,6±0,7*	872,3±70,3*	84,5±18,7*
24 h	43,8±8,4*	157,2±22,3	17,3±1,1*	217,6±22,8*	65,5±16,6*

Legend. Here and in Table 2, asterisk indicates significant differences compared with control at $p < 0.05$.

TABLE 2. Effect of HPM in Doses A and B on Blood Insulin and Thyroid Hormone Levels in Albino Rats 4 and 24 h after Injection

Group	Insulin, mU/ml	T_3, nmoles/liter	T_4, nmoles/liter
Control	14,6±3,2	1,4±0,3	35,3±4,5
Experiment, dose A			
4 h	13,5±2,8	1,98±0,2	44,8±4,6
24h	10,8±1,9	2,0±0,2	50,6±9,2
Control	14,6±3,2	0,44±0,08	35,3±4,5
Experiment, dose B			
4 h	8,6±1,4	0,7±0,09* 0,7±0,18 (C)	49,1±7,4
24 h	30,5±6,7*	1,1±0,1 (E)	38,2±13,9

Legend. C) Control, E) experiment (additional).

and corticosterone levels in the blood and adrenals were determined by the aid of kits from the Research Institute of Experimental Pathology and Therapy, Academy of Medical Sciences of the USSR, Sukhumi. Catecholamines and 11-HCS in the adrenals were determined fluorometrically, using standard methods [5, 6] on a "Hitachi" spectrofluorometer (Japan). HPM was synthesized in the Laboratory of Peptide Synthesis, All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR, by Professor M. I. Titov. The numerical results were subjected to statistical analysis by Student's test.

EXPERIMENTAL RESULTS

Previous investigations showed that in doses A and B, HPM stimulates cell division in epithelial tissues, and the effect is statistically significant and dose-dependent. The present results show that, unlike its similar action on cell division, HPM in doses A and B has different effects on the parameters of the endocrine status chosen for study.

The different character of changes in the blood β-endorphin level must be particularly mentioned. In dose A an almost threefold decrease was observed in the β-endorphin concentration 24 h after injection, whereas in dose B there was a twofold increase in the β-endorphin concentration at the same time (Table 1).

In dose A, HPM induced a 1.5-fold increase in the blood corticosterone concentration 4 h after injection, whereas in dose B there was a 1.5-fold decrease in the corticosterone concentration after the same time (Table 1). The study of changes in the corticosterone concentration in the adrenals showed them to be uniform in direction. Injection of HPM in dose A led to a twofold increase in the corticosterone concentration after 24 h, whereas in dose B its level was significantly higher by 1.5-1.7 times at both times of the investigation. Although the increase in the corticosterone concentration in the adrenals after 4 h with dose A was not significant, in the parallel experiment, in which 11-HCS was determined in the blood and adrenals, a significant rise in the 11-HCS level was found in the adrenal tissue, and it was accompanied by an almost twofold increase in the blood 11-HCS concentration.

Determination of adrenalin in the adrenals showed changes similar in direction (an increase in its concentration) after 4 h when doses A and B were used. After 24 h, however, in response to injection of the peptide in dose A the adrenalin concentration also was significantly higher than in the control, but after injection of HPM in dose B, the adrenalin concentration in the adrenal tissue was reduced by half. This difference in the effects of doses A and B on the noradrenalin (NA) levels may be determined not only by dose, but also by differences in the initial background levels. Injection of HPM in dose A led to accumulation of NA after 4 h, whereas injection of dose B led to a significant decrease in the NA concentration at both times.

Analysis of blood thyroid hormone levels revealed that injection of the peptide in dose A caused no significant changes in the T_3 and T_4 concentrations, although they showed a tendency to rise 4 and 24 h after injection of the peptide. Injection of HPM in dose B caused a significant increase in the T_3 concentration 4 h after injection of the peptide. The possibility cannot be ruled out that the different type of response may be due to a difference in the parameters in the control groups (Table 2). HPM in dose A caused no change in the insulin concentration; with an increase in the dose there was a significant increase in its concentration 24 h after injection (Table 2). These changes were evidently brought about by secondary mechanisms and, in particular, by changes in the catecholamine levels at this time of the investigation.

The mechanism of the change in hormone levels in response to HPM requires further study. Evidently some changes are due to peptide–hypothalamus–pituitary interaction. This conclusion was drawn by investigators who studied the effect of HPM on androgen metabolism in the gonads during sexual maturation in rats [4]. Meanwhile the results of the present investigation allow definite conclusions to be drawn. Changes in hormonal balance observed after injection of HPM into animals have a definite effect on the character of proliferative processes in epithelial tissues. Nevertheless, even at this stage it can be concluded that these changes do not play a determining role in the regulation of proliferation after injection of the peptide. This conclusion is supported by the opposite character of the changes observed following injection of different doses of HPM as regards both inhibitors (corticosterone) and stimulators (β-endorphin) of cell division. It will be recalled that the two doses of HPM studied caused dose-dependent activation of proliferation. The increase in the adrenalin concentration in the adrenals 4 h after injection of the peptide in both doses shows definite similarity to the picture observed in the alarm stage of the general adaptation syndrome. During stress, it is these changes which determine inhibition of cell division in the epithelium in the alarm stage [1, 8]. However, stress is characterized by inhibition of thyroid gland function, and in our experiments an increase in the concentration of T_3, a stimulator of proliferative processes, was observed in response to injection of HPM in dose B.

In our opinion the results of the investigation described above are further evidence in support of a direct effect of HPM on cell division. This hypothesis requires further experimental study.

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