CONTENT OF ACID PROTEINS IN NEURON AND GLIAL CELL NUCLEI OF THE RAT HYPOTHALAMUS DURING COLD ADAPTATION

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The content of acid proteins in nuclei of neurons and glial satellite cells in the medial preoptic region and supraoptic nucleus of the rat hypothalamus was studied by two-wave cytospectrophotometry on the 1st, 3rd, 7th, and 15th days of adaptation of the animal to cold (temperature 2-4°C). Cooling led to an initial decrease in the content of nuclear proteins in the whole neuronal-neuroglial system of the medial preoptic region, followed by gradual restoration to normal by the 15th day of cooling. In the glial cells of this region, before the return to normal there was a temporary increase in the content of acid proteins above the control level. In the neuronal-neuroglial system of the supraoptic nucleus a gradual accumulation of acid proteins was followed by a return to the control level. By the 15th day of the rats' stay in the cold, the content of neuronal and glial acid proteins of this nucleus fell somewhat below the control level.
KEY WORDS: adaptation; cooling; hypothalamus; neuron and neuroglia; acid proteins.

The hypothalamus plays the leading role in the regulation of the body temperature of homoiothermic organisms [9, 10, 12, 13]. Changes in the functional activity of the hypothalamic structures are accompanied by readjustment of the metabolism of macromolecules (RNA and proteins) in neurons and glial cells [7, 11].

Our attention was drawn to acid proteins for they are present in the cell nucleus in high concentrations, and account for not less than half the total nuclear proteins [4]; they play an important role in the regulation of gene activity, reactivating parts of the genome that have been repressed by histones [1].

The object of the present investigation was to study changes in the content of acid proteins in the neuronal-neuroglia system of certain parts of the hypothalamus of rats during prolonged exposure to a low temperature.

EXPERIMENTAL METHOD

Experiments were carried out on sexually mature male rats weighting 130-150 g, which lived for two weeks in a cold room (temperature 2-4°C). The animals received a standard balanced diet. The rectal temperature of these rats remained unchanged throughout the experiment. Animals of the same sex, age, and weight, kept under ordinary animal house conditions at a temperature of 19-21°C served as the control.

After the experimental animals had been in the cold room for 1, 3, 7, and 15 days they were killed, with control animals at the same time, by rapid decapitation without anesthesia, the brain was removed, and the hypothalamus excised and fixed in cold Carnoy's mixture and then embedded in paraffin wax. Sections 5 μ thick were stained for acid proteins with Fast Green FCF, pH 2.6 [3, 5].

The total content of nuclear proteins, expressed per cell, was determined by two-wave cytospectrophotometry; the general principles and concrete details of the measurements are described in the monograph [2, 8]. All determinations were made at 600 and 650 nm on the MTsFV-1 digital-printing two-wave cytospectrophotometer [6]. Each arithmetic mean value of the content of acid proteins (in conventional units) was calculated from the results of photometry of 130-190 cells (neurons and the same number of perineuronal glial cells) from 5 or 6 animals. The whole of the numerical results were subjected to statistical analysis by Student's method (Tables 1 and 2).

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TABLE 1. Content of Nuclear Acid Proteins (in conventional units per cell) in Neuronal-Neuroglial System of Medial Preoptic Region of Rat Hypothalamus at Various Times of Adaptation of Animals to Cold (M ± σ)

Duration of adaptation, days	No. of animals	No. of cells	Content of nuclear acid proteins	Significance of difference from level							
				in control		after 1 day		after 3 days		after 7 days	
				%	P	%	P	%	P	%	P
Neurons											
Control	6	177	63±0,98	—12,8							
1	5	144	55±1,08	—12,8	<0,001						
3	6	189	59±0,95	—6,3	<0,01	7,3	<0,01				
7	5	155	62±0,79	—1,6	>0,1	12,7	<0,001	5,1	<0,02		
15	5	152	63±1,10	0,0		14,5	<0,001	6,8	<0,01	1,6	>0,1
Glia											
Control	6	175	59±0,89								
1	5	144	48±1,26	—18,6	<0,001						
3	6	188	63±0,87	6,3	<0,001	31,3	<0,002				
7	5	149	69±0,71	17,0	<0,001	43,8	<0,001	9,5	<0,001		
15	5	156	59±0,89	0,0		22,9	<0,001	—6,4	<0,01	17,0	<0,001

TABLE 2. Content of Nuclear Acid Proteins (in conventional units per cell) in Neuronal-Neuroglial System of Supraoptic Nucleus of Rat Hypothalamus at Various Times of Adaptation of Animals to Cold (M ± σ)

Duration of adaptation, days	No. of animals	No. of cells	Content of nuclear acid proteins	Significance of difference from level							
				in control		after 1 day		after 3 days		after 7 days	
				%	P	%	P	%	P	%	P
Neurons											
Control	6	176	76±1,50								
1	5	158	78±1,52	2,6	>0,1						
3	5	144	90±1,61	18,4	<0,001	15,4	<0,02				
7	5	151	84±1,63	10,5	<0,001	7,6	<0,01	—6,7	<0,01		
15	5	125	67±1,57	—11,8	<0,001	—14,1	<0,001	—25,6	<0,001	—20,2	<0,001
Glia											
Control	6	171	66±1,43								
1	5	137	73±1,42	10,6	<0,001						
3	5	138	81±1,68	22,7	<0,001	10,9	<0,001				
7	5	147	70±1,66	6,0	>0,05	—4,1	>0,1	—13,6	<0,001		
15	5	120	60±1,55	—9,1	<0,01	—17,8	<0,001	—25,9	<0,001	14,3	<0,001

EXPERIMENTAL RESULTS

The participation of the hypothalamus in mechanisms of thermoregulation is most closely linked with the medial preoptic region (MPR) [10, 12]. As Table 1 shows, the content of acid proteins in the neurons of MPR fell somewhat after the rats had been kept for 24 h in the cold, but later during adaptation it returned to the control level. In the glial cells surrounding the neurons the content of these proteins also fell 24 h after the beginning of the experiment. Later during the rats' stay in the cold the content of acid proteins rose sharply (above the control level), but fell again toward the 15th day, to reach the control value again (Table 1).

The neurosecretory supraoptic nucleus of the hypothalamus plays an important role in the activity of the whole hypothalamic-hypophyseal-adrenal system [9]. The content of acid proteins in the neurons of this nucleus increased significantly only by the third day of cold adaptation, but then returned gradually to normal, so that by the 15th day it was actually below the control level (Table 2). The dynamics of the content of acid proteins in the glial satellite-cells of these neurons was similar.

The functional-biochemical comparison of the two hypothalamic neuronal-neuroglial systems shows that the cells of MPR respond in the same direction to the action of cold, by a decrease in the content of acid proteins in the nuclei of both neurons and glial cells as early as after exposure of the rats to cold for 24 h. The neuronal-neuroglial system of the supraoptic nucleus, on the other hand, respond to the fall of temperature by

an increase in content of acid proteins in the nuclei of both neurons and perineuronal glia, which does not reach a maximum until the third day of adaptation. It might be supposed that this dynamics of the content of nuclear acid proteins in the cells of the hypothalamus is connected with the specificity of cold adaptation for MPR and its nonspecificity for the neurosecretory supraoptic nucleus.

The metabolic response in the neuronal-neuroglial system of MPR is probably linked more specifically with the direct effect of cooling: initial stimulation of cold receptors leads to excitation and, possibly, even to over-excitation of the thermosensitive neurons of MPR. The cytochemical manifestations of this over-excitation, as has often been observed during the investigation of other types of neurons [8], is a decrease in the content of RNA and proteins in the whole neuronal-neuroglial system. In the course of subsequent adaptation reparative biosynthesis of macromolecules takes place, more effectively in the cells of the perineuronal glia than in the neurons themselves [8]. As Table 1 shows, the dynamics of the content of nuclear acid proteins in the neuronal-neuroglial system of MPR accords well with this view.

Cooling can be regarded as one form of stress. Compared with deep hypothermia, induced by keeping rats at −20°C [11], the degree of cold used in the present case (2-4°C) is a weaker stress factor, capable of inducing moderate excitation but not overexcitation of the corresponding neurons. As was shown previously during an investigation of different neuronal-neuroglial systems, adequate excitation leads to the accumulation of RNA and proteins in the neurons and, in some cases, in the perineuronal glia also [8]. The accumulation of acid proteins in the cells of the supraoptic nucleus evidently fits in with this scheme, for this nucleus is primarily connected with the participation of the single hypothalamic-hypophyseal-adrenal system in the response to stress-producing factors.

Exposure to moderate cold (an ambient temperature above 0°C), if continued for long enough (up to two weeks) stimulates the development of cold adaptation [9]. It is accordingly worth noting that the changes observed in the content of acid proteins in these experiments takes place in the initial period of cooling; by the 15th day of the animals' stay in the cold the level of nuclear acid proteins in the neuronal-neuroglial system is back to normal. This confirms the principles discovered during the study of the effect of deeper cooling of rats [11] and during analysis of changes in the histone content in the hypothalamus of rats adapted to cold [7], namely the specific biochemical readjustment of the cells of the hypothalamus accompanied initial adaptive changes in the body and ceases at the moment when adaptation is complete.

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