MORPHOLOGICAL AND FUNCTIONAL ANALYSIS OF REVERSIBLE CHANGES INDUCED BY CHRONIC COLD STRESS

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UDC 612.592.014.2

KEY WORDS: stress; reversibility; endocrine glands; mitoses.

The consequences of even short-term exposure to stress may not be manifested until some time later [5]. A long stay under uncomfortable conditions can evidently induce marked functional and morphological changes which subsequently determine the level of predisposition to various diseases and the course of ontogenetic development.

The object of this investigation was to study changes in various organs of animals exposed to the prolonged action of cold and their reversibility. The test objects were organs whose role in thermogenesis differs widely, so that it is possible to estimate the degree of specificity of the changes observed and the dependence of their reversibility on the contribution of the organ to adaptation to cold.

EXPERIMENTAL METHOD

Experiments were carried out on adult noninbred male albino rats of the same age, with a body weight before the experiment of 190-200 g, divided into the four following groups: 1) rats exposed to cold for 80 days and killed immediately after the end of exposure (eight rats); 2) intact animals kept under animal house conditions at 22-24°C, the control to group 1 (six rats); 3) rats exposed to cold for 80 days and kept for 35 days thereafter at 22-24°C (ten rats); 4) intact animals, the control to group 3 (seven rats). All the animals received the same varied diet and water ad lib. The experimental animals were kept for 23 of the 24 h in separate compartments of a refrigerator at 5-7°C. The compartments were big enough for the animals to move about freely. The rats were killed in the morning and their body weight and the weight of the test organs determined. Paraffin sections through the organs, stained with hematoxylin and eosin, and total preparations of the cornea were studied morphologically. The morphometric study of the adrenals was carried out by the method described previously [4]. The relative area of the various structures of the thyroid gland and also of the blood capillaries of the liver was determined by means of a morphometric grid [1]. To compare the dimensions of the hepatocytes, their number in a standard field of vision was counted. The number of binuclear hepatocytes per 1000 cells was counted. The glycogen and cholesterol concentrations in the liver and 11-hydroxycorticosteroids in the blood were determined biochemically and by the methods in [2, 3, 7]. Glycogen was detected histochemically by the PAS reaction. The number of mitoses and their phases were counted in 100 standard fields of vision in total preparations of the cornea and sections through the tongue. The percentage of pathological mitoses was determined in the corneal preparations.

EXPERIMENTAL RESULTS

As Table 1 shows, chronic cold stress caused changes in the organs responsible for adaptation to low temperatures. Important indices of tissue homeostasis (mitotic activity) also were changed in organs without any appreciable role in heat production, namely the cornea and tongue. The number of mitoses in the corneal epithelium was 170.5 ± 21.1 in 100 fields of vision, compared with 213.3 ± 55.1 in the control ($P > 0.1$). The percentage of metaphases was considerably increased under these circumstances (46.8 ± 4.7, compared with

Department of Histology and Central Research Laboratory, Khabarovsk Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR V. P. Kaznacheev.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 91, No. 6, pp. 657-658, June, 1981. Original article submitted November 4, 1980.

 0007-4888/81/9106-0722$07.50 © 1981 Plenum Publishing Corporation

TABLE 1. Changes in Organs of Rats under the Influence of Cold (M ± m)

Organ	Index	Experimental conditions			
		80 days at 5-7°C (group 1)	control (group 2)	35 days after end of exposure to cold (group 3)	control (group 4)
	Weight of rats, g	208±5,5*	247±4,6	244±8,0	254±6,0
	Width of adrenal cortex, μ	780±21	830±38	800±47	810±27
	Width of zona glomerulosa, mm	48±1,7*	36±3,5	63±5,4	53±2,8
Adrenal gland	Diameter of nuclei, μ:				
	Zona fasciculata	6,5±0,08	6,5±0,07	6,5±0,1*	7,0±0,06
	Zona reticularis	5,9±0,05	6,0±0,06	5,8±0,09*	6,1±0,07
	Medulla	7,2±0,06*	6,9±0,1	6,9±0,1	7,1±0,17
	11-Hydroxycorticosteroids, μg %	33,4±3,6*	56,1±8,8	19,0±2,4	21,9±4,5
	Weight:				
	Absolute, mg	27±1,9	29±3,2	28±2,0	26±2,3
	Relative, mg/100 g	13,2±0,9	11,1±1,1	12,0±0,9	10,5±1,1
	Percent of area occupied by				
	Follicular epithelium	34±2,8	31,4±1,3	27±0,8*	33,7±2,3
	Colloid	13±1,7	13,3±2,4	16,5±1,9*	22±2,1
Thyroid gland	Interfollicular islets of stroma	31±3,2	32,4±3,2	32,5±2,1*	25±2,7
		22±4,1	20,9±0,5	24±1,4*	19,3±1,4
	Weight:				
	Absolute, mg	26±1,0	24±1,2	28±0,2	26±1,2
	Relative, mg/100 g	12,6±0,4*	9,7±0,5	11,6±0,9	9,6±0,8
	Number of binuclear hepatocytes, ‰	32,6±2,45	39,1±3,6	39,2±3,6	42±3,47
	Number of hepatocytes in standard field of vision	35,3±1,46*	30,4±0,82	29,0±1,03	30,2±0,55
Liver	Percent of area of section occupied by vessels	9,4±0,43	9,1±0,5	3,7±0.39	3,4±0,57
	Content of:	—	359±70,2	267±23,3*	395±29,3
	Glycogen, mg %†	252,3±26,7	337,7±67,4	263±20,6*	325±16,7
	Cholesterol, mg %				
	Absolute, mg	6290±175*	8330±476	7370±252	7850±218
	Relative, mg/100 g	3025±77*	3369±146	3015±71	3133±42

*P < 0.05 for comparison of group 1 with group 2 and group 3 with group 4.
†Not discovered biochemically in the animals of group 1, found histochemically in single hepatocytes.

32.7 ± 6.1 in the control; P < 0.05) and the percentage of telophases was sharply reduced (11.0 ± 3.7, compared with 32.9 ± 9.2 in the control; P < 0.05). Changes in the ratio between the phases of mitosis in the lingual epithelium were similar. Besides this, the number of pathological mitoses in the cornea was doubled (10.0 ± 0.8%, 4.8 ± 0.91% in the control; P < 0.001), possibly due to a decrease in the corticoid concentration which has this effect under stress conditions [6]. The reversibility of some of the changes 35 days after the end of exposure of the rats to cold was incomplete. For instance, the glycogen and cholesterol concentrations in the liver were not back to the control levels (Table 1). Morphometric indices for the thyroid and adrenal glands differed from those both in intact animals and in the rats of group 1. Changes also were found in the ratio between the phases of mitosis in the corneal epithelium. The percentage of metaphases was 41.1 ± 1.7 and of telophases 24 ± 2.1; the corresponding values in the control were 29.6 ± 5.5 and 32.4 ± 4.0. The number of pathological mitoses was virtually indistinguishable from the control (6.4 ± 0.26 and 5.8 ± 0.8% respectively).

The results are evidence that prolonged exposure to cold causes changes in organs directly concerned with heat production, namely the liver, thyroid gland, and adrenal cortex, which respond nonspecifically to various forms of stress, and also in structures which have no evident role in these processes, such as the corneal epithelial cells.

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