MORPHOMETRIC ANALYSIS OF REVERSIBILITY OF CHANGES IN THE RABBIT ADRENAL CORTEX AFTER CHOLESTEROL WITHDRAWAL

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Cholesterol is an invariable component of animal cells, and in some of them it is directly connected with the performance of specific functions. This applies in particular to the parenchymatous cells of the adrenal cortex — the adrenocorticocytes [9, 15]. This evidently explains the marked changes taking place in these cells in response to considerable fluctuations of their cholesterol concentration. There are some particularly interesting data to show that elevation of the blood cholesterol level, leading to an increase in its concentration in the adrenals, is reflected essentially in a number of their morphological and functional parameters. This was discovered during the study of the adrenal cortex of patients with atherosclerosis and also in animals with experimental hypercholesterolemia; depression of the parameters of functions of the gland was discovered [2-4, 10]. The study of reversibility of changes developing in the adrenal cortex under these conditions is of both theoretical and practical interest in connection with the possibility, in principle, of correcting metabolic disturbances caused by these changes. The result of the study of reversibility of changes in the liver and aorta developing in rabbits with experimental atherosclerosis showed that the cholesterol concentration in the animals' liver tissue was restored to normal when administration of cholesterol was discontinued [5], whereas atherosclerotic changes in the aorta did not undergo complete regression for a very long time [1, 5, 11]. No morphological or morphometric data on the state of the adrenal cortex after cholesterol withdrawal could be found.

The aim of this investigation was to study some morphological parameters of the rabbit adrenal cortex on withdrawal of cholesterol after administration for a short period of time, but which nevertheless, according to data in the literature, induces definite changes in histophysiology of the gland [10, 11].

EXPERIMENTAL METHOD

The adrenals of 32 adult rabbits, divided into three groups, were studied: Group 1 consisted of intact animals (10 rabbits), group 2 of animals receiving cholesterol for 14 days in a dose of 25 mg/100 g body weight (10 rabbits), and group 3 of animals receiving cholesterol for 14 days and killed 1.5 months after withdrawal (12 rabbits). Rabbits of different groups were killed simultaneously during the morning by air embolism. The cholesterol concentration in the blood and adrenals was determined by the method described previously [13]. The adrenals were weighed, sections were cut through their central part, and these were stained with hematoxylin and eosin. The area of section of the organ, of its medulla and cortex, and of the different zones of the cortex was determined gravimetrically. The mean area of cross section of the adrenocorticocytes also was determined gravimetrically. For this purpose, in all cases the area of cross-section of 50 cells was measured in each zone. In preparations stained for total protein with bromphenol blue and for nucleic acids with gallocyanin by Einarson's method, the concentrations of protein and RNA in the cytoplasm of the adrenocorticocytes were determined cytospectrophotometrically. Cytophotometry was carried out in each zone in all cases on 50 cells by the plug method at a wavelength of 546 nm. The numerical results were subjected to statistical analysis.

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TABLE 1. Changes in the Adrenal Cortex of Rabbits during Cholesterol Administration and 1.5 Months After Its Withdrawal

Parameter studied	Experimental conditions					
	intact animals (1)	administration of cholesterol (2)	P_{1-2}	1,5 months after cholesterol withdrawal	P_{1-3}	P_{2-3}
Blood cholesterol, mg%:						
adrenal blood	42±5,7	461±67	<0,001	50±9,9	>0,2	<0,001
	837±109	1279±123	<0,05	646±47	>0,1	<0,001
Weight of adrenal						
absolute, mg	204±23,3	308±30	<0,02	276±41,9	>0,1	>0,5
relative (mg/100 g)	8,4±0,31	109±1,01	<0,05	10,0±1,23	>0,2	>0,5
Area of section, mm^2						
of cortex	8,1±0,56	15,4±2,37	<0,01	11,8±1,9	<0,1	>0,2
zona glomerulosa	1,3±0,11	1,4±0,13	>0,5	1,4±0,19	>0,5	—
zona fasciculata	6,2±0,43	13,3±2,1	<0,01	9,6±1,7	<0,1	<0,2
zona reticularis	0,6±0,1	0,7±0,2	>0,5	0,8±0,17	>0,5	>0,5
medulla	0,5±0,07	0,53±0,07	>0,5	0,6±0,06	>0,2	>0,2
Area of cross-section of adrenocorticocytes, μ^2						
zona glomerulosa	52±4,9	53±5,5	>0,5	48±4,1	>0,5	>0,5
zona fasciculata	113±7,0	139±7,1	<0,02	111±6,8	>0,5	<0,02
zona reticularis	81±7,8	101±5,5	<0,05	78±3,6	>0,5	<0,01
Concentration in cytoplasm, conventional units: protein						
zona glomerulosa	317±21,2	220±19,4	<0,01	307±19,4	>0,5	<0,01
zona fasciculata	222±16,6	118±11,0	<0,001	197±11,0	>0,2	<0,001
zona reticularis	224±16,7	136±15,5	<0,02	200±25,5	>0,5	<0,05
RNA						
zona glomerulosa	194±11,9	168±20,7	>0,2	204±9,3	>0,5	>0,1
zona fasciculata	95±6,6	73±7,1	<0,05	94±6,4	>0,5	<0,05
zona reticularis	114±5,0	99±6,9	>0,1	109±6,6	>0,5	>0,2

EXPERIMENTAL RESULTS

Administration of cholesterol to the rabbits for 14 days caused marked hypercholesterolemia, which caused a marked increase in the cholesterol concentration in the adrenal (Table 1). Under these circumstances the weight of the organ increased. Determination of areas of cross section showed that the increase in area of the cortex (by 90%) on account of hypertrophy of the zona fasciculata was the main cause of the increase in weight of the adrenal. The area of cross section of the zona glomerulosa and zona reticularis was virtually indistinguishable from the control. The mean area of section of the adrenocorticocytes was increased in z. fasciculata and z. reticularis. Comparison of the degree of increase of this parameter (by 1.23 times in z. fasciculata, by 1.25 times in z. reticularis) and the increase in area of section of the cortex as a whole (by 1.9 times) indicates that the increase in size of the cells could not evidently be responsible for such a marked degree of hypertrophy of the cortex, although it made a significant contribution to that process. Besides the increase in cholesterol concentration noted above, there were also other changes in parameters of adrenocortical tissue metabolism: a significant fall in total protein concentration in the cytoplasm of adrenocorticocytes in all zones and of RNA in z. fasciculata. Usually such deviations ofhistochemical and biochemical parameters are regarded as evidence of depression of the functional state of the gland [8]. Morphological changes of this kind evidently induce a decrease in the steroid-producing powers of the adrenal cortex in experimental hypercholesterolemia [2, 6, 14]. A fall in the blood cholesterol level virtually to that found in intact animals, with a parallel decrease in its concentration in the adrenals, were observed (Table 1) 1.5 months after the end of cholesterol administration. The morphometric parameters characterizing the weight of the adrenals and the area of cross section of the cortex and of its zona fasciculata occupied intermediate positions between those for intact rabbits and animals killed immediately after the end of a 14-day period of cholesterol administration. Meanwhile the mean area of section of the adrenocorticocytes in all zones was virtually indistinguishable from the corresponding parameters in intact animals. This applies also to the RNA concentration in the cytoplasm of the adrenocorticocytes, whereas the protein concentration in them was a little lower than in intact rabbits. After administration of cholesterol to rabbits the degree of hypercholesterolemia and of the atherosclerotic changes in the aorta reveal considerable individual differences [1, 5]. This also was observed in a study of the adrenal cortex of the experimental animals of groups 2 and 3. Although many average parameters for animals of group 3 differed appreciably from those for intact rabbits, differences were not statistically significant ($P > 0.05$). On the whole the results indicate that changes in the adrenal cortex of rabbits, arising during short-term cholesterol administration are largely reversible. This evidently depends on the histophysiological features of the gland, changes in the functional state of which are accompanied by considerable fluctuations in the concentration of this steroid [7, 15], which become (within certain limits) "usual." Meanwhile the reversibility of the changes can be regarded as evidence that during a brief increase in the cholesterol concentration in the gland gross changes leading to their destruction do not develop in most of its cells. Accordingly, it would seem to be very interesting to study the possibility and degree of reversibility of changes developing in the adrenal cortex during long-term administration of cholesterol to animals. The answer to this question will evidently be a matter of concern to both experimental researchers and clinicians studying different aspects of the pathogenesis of atherosclerosis.

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