[^{3}H]tryptophan it was 867, 154, and 83 cpm, respectively (homogenate of myocardium of five rats, 5 μCi). [^{14}C]Formate was incorporated into RL-1, RL-2, and RL-3 in vivo also (331, 36, and 230 cpm, respectively; five rats, 200 μCi intraperitoneally, 18 h).

Consequently, the animal cell had an autonomous system for the biosynthesis of RL-1, RL-2, and RL-3.

The authors are grateful to A. F. Ushkalov and M. D. Machkovskii for discussing the results of this investigation.

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RESPONSE OF DIFFERENT TYPES OF CONNECTIVE TISSUE TO HORMONES

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UDC 615.357.015.4:612.75

A comparative study was made of the lysosomal glycosidases of the eye tissues (sclera and cornea) and also of bone tissue and cartilage from rabbits. Intraperitoneal injection of thyrocalcitonin (TCT), deoxycorticosterone (DOC), hydrocortisone (HC), and somatotropic hormone (STH) were shown to modify the activity of β-galactosidase, β-glucosidase, and hyaluronidase and the functional state of the lysosomal membranes in the tissues. HC and STH stabilize, whereas DOC and large doses of TCT labilize the lysosomal membranes. After injection of HC and STH the absolute activity of the enzymes in the tissue homogenates falls, whereas DOC has the opposite action.

KEY WORDS: *eye; bone; cartilage; lysosomal enzymes; hormones.*

Changes in the organ of vision are among the signs of various diseases of the skeletal system. Blue sclerotics are found in patients with osteogenesis imperfecta [1]; pathology of the organ of vision is characteristic of Marfan's syndrome, homocystinuria [2], mucopolysaccharidoses [14, 18], etc. Many pathological states of the organ of vision are evidently connected with changes in the activity and location of enzymes and also with disturbances of the functional state of the membranes of the lysosomes. High sensitivity of lysosomal enzymes of carbohydrate metabolism of the connective tissues of the eye (sclera, cornea) to the action of hormones has repeatedly been demonstrated [3, 4].

In this investigation the activity of the lysosomal enzymes of different types of connective tissue and its changes under the influence of certain hormones were compared.

Helmholtz Research Institute for Eye Diseases, Moscow. N. N. Priorov Central Scientific-Research Institute of Traumatology and Orthopedics, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR M. V. Volkov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 84, No. 7, pp. 38-41, July, 1977. Original article submitted December 17, 1976.

TABLE 1. Activity of Glycosidases (in μmoles/min/g protein) of Different Types of Connective Tissue (M ± 2.5m)

Tissue	β-Galactosidase		β-Glucosidase		Hyaluronidase	
	homogenate	lysosomal-mitochondrial fraction	homogenate	lysosomal-mitochondrial fraction	homogenate	lysosomal-mitochondrial fraction
Diaphysis	0,34±0,03	0,06±0,01	0,18±0,04	0,10±0,03	1,27±0,36	0,52±0,14
Metaphysis	0,45±0,06	0,14±0,03	0,28±0,01	0,07±0,02	4,5±1,04	1,66±0,2
Cartilage	0,4±0,01	0,17±0,03	0,50±0,11	0,15±0,02	2,8±0,48	1,19±0,1
Sclera	2,4±0,03	1,3±0,05	0,98±0,01	0,53±0,01	11,7±0,10	6,84±0,08
Cornea	0,79±0,09	0,42±0,03	0,68±0,01	0,42±0,03	38,2±9,4	23,5±0,83

TABLE 2. Effect of Steroid Hormones and STH on Glycosidase Activity (in μmoles/min/g protein) in Connective Tissue

Tissue	Control	DOC	HC	STH
		β-Galactosidase		
Bone	0,34±0,03	0,46±0,007	0,17±0,04	0,08±0,019
Cartilage	0,40±0,07	0,66±0,05	0,21±0,04	0,00
Sclera	2,4±0,03	1,19±0,01	1,3±0,01	—
Cornea	0,79±0,09	0,36±0,01	0,42±0,03	—
		β-Glucosidase		
Bone	0,18±0,04	—	0,08±0,01	0,15±0,07
Cartilage	0,50±0,11	1,9±0,5	0,15±0,03	0,1±0,02
Sclera	0,98±0,01	0,6±0,01	0,47±0,001	—
Cornea	0,68±0,01	0,36±0,001	0,32±0,01	—
		Hyaluronidase		
Bone	1,27±0,36	2,7±0,2	1,00±0,05	0,86±0,078
Cartilage	2,8±0,48	3,6±0,4	1,38±0,06	1,5±0,13
Sclera	11,7±0,1	20,13±8,5	26,8±1,5	—
Cornea	38,2±9,4	16,16±0,2	16,2±0,8	—

EXPERIMENTAL METHOD

Experiments were carried out on 50 male chinchilla rabbits weighing 2-2.5 kg. Enzymes splitting glycoside bonds in mucopolysaccharides were investigated: β-glucosidase, β-galactosidase, and hyaluronidase. The free and total activity of the enzymes was determined in compact (diaphysis) and cancellous (metaphysis) bony tissue, cartilage, sclera, and cornea [5]. The bone and cartilage were homogenized after the tissue had been ground to powder in a mortar cooled with solid carbon dioxide. Homogenates of eye, bone, and cartilage tissues were separated into subcellular fractions: nuclei, mitochondria, and cytosol [6]. The effect of thyrocalcitonin (TCT), deoxycorticosterone (DOC), hydrocortisone (HC), and somatotropic hormone (STH) on the activity of the connective tissue enzymes and the state of the lysosomal membranes was studied. Tests were carried out 1 and 3 h after intraperitoneal injection of the hormone. TCT was injected in doses of 2, 5, 10, and 60 MRC (Medical Research Council) units/kg, STH in a dose of 4 units/kg, and HC and DOC in doses of 10 mg/kg.

EXPERIMENTAL RESULTS

Activity of β-galactosidase, β-glucosidase, and hyaluronidase was found in the homogenates and the lysosomal-mitochondrial fractions of all the tissues tested (Table 1). The eye tissues contained greater activity of these enzymes than bone and cartilage, evidently because of the smaller numbers of cells in the bone and cartilage. The compact bone (diaphysis), cancellous bone (metaphysis), and cartilage also differed in enzyme activity. Least enzyme activity was found in the diaphyses.

In the experiments with TCT, no changes were found in the activity of these enzymes in any of the tissues tested 1 and 3 h after its administration to the animals in a dose of 2 MRC units/kg. On increasing the dose to 5 or 10 MRC units/kg a tendency was observed only for the total activity of β-glucosidase to increase 3 h after injection of the hormone. The ratio between free and total activity, reflecting the functional state of the lysosomal mem-

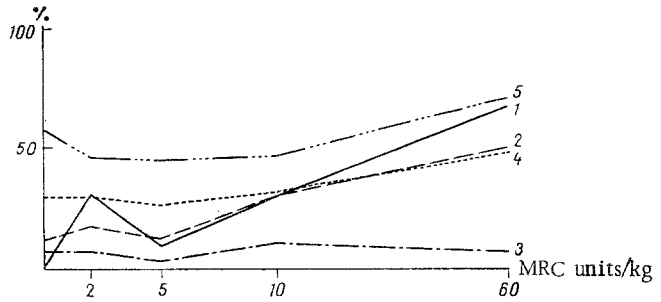

Fig. 1. Effect of TCT on lysosomal membranes of connective tissue: 1) diaphysis; 2) metaphysis; 3) cartilage; 4) sclera; 5) cornea. Abscissa, dose of TCT (in MRC units/kg); ordinate, free β-glucosidase activity as a percentage of total activity.

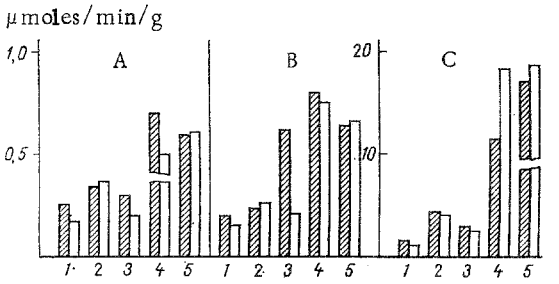

Fig. 2. Effect of large doses of TCT on glycosidase activity of connective tissue: 1) diaphysis; 2) metaphysis; 3) cartilage; 4) sclera; 5) cornea. Shaded columns — control; unshaded columns — injection of hormone in dose of 60 MRC units/kg. A) β-Galactosidase, B) β-glucosidase, C) hyaluronidase. Ordinate, activity of glycosidases (in μmoles/min/g protein).

branes, was clearly increased in the diaphysis and slightly reduced in the cornea under the influence of TCT in a dose of 2 MRC units/kg (Fig. 1). No significant changes in this ratio took place in the sclera, metaphysis, and cartilage. When the dose of the hormone was increased to 10 MRC units/kg, liberation of β-glucosidase from its membrane-bound state was increased only in the bone metaphysis.

Under the influence of large doses of TCT (60 MRC units/kg) β-glucosidase and β-galactosidase activity in the homogenates of the sclera, diaphysis, and cartilage fell. In the cornea and metaphysis, the activity of these enzymes was practically unchanged (Fig. 2). However, the fraction of free β-glucosidase activity was increased under these circumstances, especially in the diaphysis and rather less so in the metaphysis and sclera; no changes were found in the cartilage (Fig. 1).

These results suggest that TCT, in a dose of 10 MRC units/kg, induces labilization of the lysosomal membranes in bone tissue, the target tissue for this hormone. In a dose of 60 MRC units/kg TCT had a labilizing action on the lysosomal membranes of all the tissues studied except cartilage. Differences in the response of the eye tissues to injection of different doses of hormone have also been demonstrated in other investigations [7, 9, 15].

The marked labilizing action of high doses of TCT on the lysosomal membranes of bone tissue, sclera, and cornea must be taken into account whenever this hormone is used, as it is widely nowadays in clinical practice in various doses, sometimes high [10]. The action of TCT on connective tissue is evidently mediated through cyclic AMP [16, 17].

The study of the effect of steroid hormones on the lysosomal enzymes of connective tissue showed that HC (in a dose of 10 mg intraperitoneally) reduced their activity in all tissues except the sclera, in which there was characteristically an increase in hyaluronidase activity, evidently associated with increased biosynthesis of this enzyme (Table 2). Meanwhile, the liberation of enzymes from the lysosomes into the cytosol was reduced in the bone

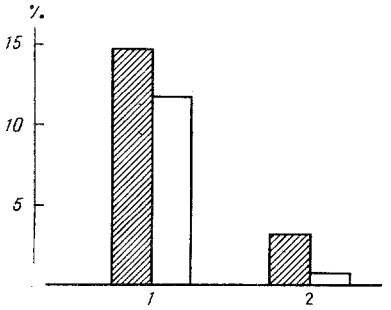

Fig. 3. Effect of hydrocortisone on state of lysosomal membranes of connective tissue (content of β-galactosidase in cytosol). Shaded columns — control; unshaded — injection of hormone. 1) Cornea; 2) bone. Ordinate, percentage of activity in homogenate.

tissue and cornea by HC (Fig. 3). Free glycosidase activity as a percentage of total was reduced in the homogenates of these tissues and also of the sclera. STH reduced enzyme activity and caused stabilization of the membranes, i.e., its action was analogous to that of HC. DOC increased the activity of the bone and cartilage glycosidases (Table 2). In view of observations on the lytic action of DOC on lysosomes of various tissues [8, 11, 12], it can tentatively be suggested that it has a similar action on bone tissue also [13].

Different types of connective tissue thus differ in the sensitivity of their lysosomal glycosidases to the action of hormones. Activation of the lysosomal enzymes and their penetration into the cytoplasm and extracellular space as a result of hormonal imbalance can evidently lead to the development of pathological changes in the connective tissue and to the onset of various diseases accompanied by disturbances of the state both of the organ of vision and of the skeletal system.

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