EFFECT OF HYDROCORTISONE ON ACTIVITY OF LYSOSOMAL ENZYMES OF THE THYMUS AND SPLEEN

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The effect of hydrocortisone on β-glucosidase and β-galactosidase activity in the thymus and spleen of rabbits was studied. During the first 30-60 min of the experiment the free activity of the enzymes fell and then rose again. Bound activity, on the other hand, rose appreciably. This indicates increased strength of the bond between the β-glucosidase and β-galactosidase with the lysosomal membranes of the lymphoid organs in the early stages of the experiment. During the first few hours of hydrocortisone administration the hyaluronidase activity in the thymus and spleen increased and then fell appreciably. Changes in all enzymes studied were proportional to the dose hydrocortisone administered and were expressed to a greater degree in the thymus than in the spleen.

Steroid hormones are known to have regulatory action on the permeability of cell membranes, including lysosomal membranes [1, 6, 9, 10]. Interaction between lysosomal enzymes [3] and the corresponding substrates takes place through molecular reorganization of the membrane structures leading to changes in the permeability of the membranes [4]. This concept has been used to explain the mechanism of action of steroid hormones and, in particular, their anti-inflammatory, catabolic, anticarcinogenic, etc., actions. The functional state of the lysosomal membranes of the target organs for glucocorticoids has not been adequately investigated.

In the investigation described below, free, total, and bound activity of the lysosomal enzymes of the thymus and spleen was studied in response to injection of various doses of hydrocortisone.

EXPERIMENTAL METHOD

Experiments were carried out on 80 male rabbits weighing 2-2.5 kg. Hydrocortisone was injected intraperitoneally in doses of 2.5, 5, and 10 mg/kg. Activity of the acid hydrolases (β-glucosidase, β-galactosidase, and hyaluronidase) was determined 30 min and 1, 4, 12, and 24 h after a single injection of the hormone. The free activity of each of the enzymes was studied in freshly prepared homogenates of thymus and spleen. Total activity was investigated in homogenates after complete destruction of the subcellular structures by the addition of the detergent Triton X-100 to the incubation medium. This gives the sum of the free and bound activities. The bound form of the enzymes was calculated as the difference between the total and free forms. Activity of β-glucosidase and β-galactosidase was determined by the method of Patel and Tappel [7, 8]. Hyaluronidase activity was studied by Dische's carbazole method [5]. Since Triton X-100 inactivated the hyaluronidase irreversibly, the activity discovered in the samples after incubation for 2 h was studied. As the authors and Aronson and Davidson [2] have shown, this time of hydrolysis can be used to determine the maximal hyaluronidase activity. Protein was determined in parallel tests on all samples by Lowry's method.

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TABLE 1. Effect of Hydrocortisone (10 mg/kg) on β-Glucosidase and β-Galactosidase Activity in Rabbit Thymus and Spleen

Enzymes studied	Enzyme activity (in ΔE/min/mg protein)	Control	Time after injection of hydrocortisone				
			30 min	1 h	4 h	12 h	24 h
			Thymus				
β-Glucosidase	Free	46,3±2,5	39,3±1,8	23,3±1,5*	36,8±1,7	83,6±4,7*	67±3,1
	Total	65,4±4,3	95,9±6,1	90,9±4,9	90,0±7,0	133,6±9,5	134±9,9
	Bound	19,1±1,9	56,0±3,9	67,6±4,8	54,0±2,3	50,0±4,0	67,5±5,3
	Free/total (in %)	70,7	40,9	25,6	40,8	62,6	49,8
			Spleen				
	Free	77,0±5,6	77,8±6,1*	56,1±4,4	62,5±6,7	119±10,1	102,3±9,4
	Total	202±12,6	219,9±15,0*	235±10,1	239±11,6	320±19,7	357±16,5
	Bound	125±7,0	142,1±9,1*	179±6,0*	176,5±5,3	201±10,4	254,7±10,2
	Free/total (in %)	38,1	35,4	23,9	26,2	37,8	28,5
			Thymus				
	Free	148±11,4	83,2±5,6	96,5±7,0	131±9,8*	170,5±7,9*	290±12,0
	Total	288±13,1	261±10,5	303±12,1*	340±14,5	381,2±13,1	445±16,5
	Bound	140±9,1	177,8±8,4	206,5±9,1	209±8,3	210,7±7,6	155±8,2*
	Free/total (in %)	66,8	31,9	31,8	38,5	44,7	65,1
			Spleen				
β-Galactosidase	Free	270,8±8,1	257,8±8,7	219±7,7	260±8,3*	276,2±9,6*	149,3±12,2
	Total	452±2,0	437±19,8	427±21,0*	480±20,6*	558±24,1	395±11,0
	Bound	182±6,1	179,2±5,3*	208±2,1	220±13,4	282±13,5	235±14,7
	Free/total (in %)	59,7	58,8	51,2	54,1	50,0	62,6

*P > 0.05.

EXPERIMENTAL RESULTS

Data for the effect of hydrocortisone on the intensity of the acid hydrolases in a dose of 10 mg/kg are given in Table 1. A decrease in the free β-glucosidase and β-galactosidase activity in the thymus was observed 30 min after injection of the hormone. The free β-glucosidase activity of the thymus after 1 h was 50% below, and of β-galactosidase 35% below normal. After 4 h it rose again. After 12 h the level of β-glucosidase activity was 80.5% above normal and that of β-galactosidase 15.2% above normal. After 24 h a tendency was observed for the free β-glucosidase activity to return to normal. The active form of β-galactosidase at the end of the experiment was at almost twice the normal level. The change in free β-glucosidase and β-galactosidase activity in the thymus was thus biphasic in character, and the rate of change of free activity as well as the amplitude of the change were much more marked in the case of β-glucosidase than of β-galactosidase. The increase in the total activity of both enzymes took place on account of a sharp rise in the membrane-bound (latent) form of the enzymes. For instance, after 30 min the bound β-glucosidase activity was increased more than threefold. After 1 h the increase in activity continued and it was at almost 3.5 times the normal level, but this was followed by a slight decrease in activity and by a further increase toward the end of the first day. Significant changes were observed in the ratio between the active and latent forms of the enzyme.

In the spleen the character of the changes in β-glucosidase and β-galactosidase activity was similar to that in the thymus, but the variations here were much smaller, because of the greater sensitivity of the thymus to hormonal influences and also because the spleen is not a completely lymphoid organ. By examining the character of the changes in β-glucosidase and β-galactosidase activity in the spleen, conclusions were drawn which were similar to those regarding the binding of these enzymes with the lysosomal membranes of the thymus.

Consequently, the increase in the strength of the bond between the enzymes and the lysosomal membrane structures inactivating them in the early stages of the experiment was thus the result of the action of hydrocortisone and it represents one of the possible mechanisms of the action of this hormone the cell. On the other hand, the increase in the strength of this bond prevented release of the enzymes from the cellular structures into the blood stream.

The change in the activity of these enzymes depended on the dose of hydrocortisone. Injection of the hormone in a dose 2.5 mg/kg did not lead to statistically significant changes, but in a dose of 5 mg/kg the changes observed in the activity of all fractions of β-glucosidase and β-galactosidase were less marked than after a dose of 10 mg/kg.

Determination of acid hyaluronidase in homogenates of the thymus showed that its activity at first rises appreciably, and then falls after 24 h. The change in hyaluronidase activity in the spleen follows a similar pattern. This rise in hyaluronidase activity can be presumed to lead to destruction of the cellular mucoproteins and mucopolysaccharides.

It can be concluded from these results that hydrocortisone regulates the functional state of the lysosomal membranes of the lymphoid organs.

LITERATURE CITED

1. P. V. Sergeev, R. D. Seifulla, and A. I. Maiskii, Molecular Aspects of the Action of Steroid Hormones [in Russian], Moscow (1971).
2. N. N. Aronson and E. A. Davidson, J. Biol. Chem., 240, 3222 (1965).
3. C. De Duve, in: Subcellular Particles, Ronald Press (1959), p. 128.
4. C. De Duve and R. Wattiaux, Ann. Rev. Physiol., 28, 435 (1966).
5. Z. Dische, J. Biol. Chem., 167, 189 (1947).
6. J. K. Grant, Essays in Biochemistry, Vol. 5, London and New York (1969), p. 1.
7. V. Patel and A. L. Tappel, Biochim. Biophys. Acta, 191, 86 (1969).
8. V. Patel and A. L. Tappel, Biochim. Biophys. Acta, 191, 653 (1969).
9. G. Weissman, Biochem. Pharmacol., 14, 525 (1965).
10. G. Weissman, H. Sessa, and S. Weissman, Biochem. Pharmacol., 15, 1537 (1966).