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Effect of Insulin, Epinephrine, Hydrocortisone and ACTH on the Catheptic Activity of Rat Spleen

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In our experiments under *in vivo* conditions we followed the response of spleen on injected insulin, epinephrine, hydrocortisone and ACTH.

Insulin caused a decrease in spleen weight, a decrease of protein nitrogen and an increase of catheptic activity 30 min. after application. Afterwards catheptic activity was significantly above control value in 60 min. (expressed in E. U./g. tissue) although decreasing tendency was observed. 30 and 60 min. after epinephrine application spleen weight and protein nitrogen were increased whereas catheptic activity remained under the control value (expressed in E. U./mg.N). After 3 hrs the spleen weight was reduced (reduced protein nitrogen) whereas catheptic activity was significantly increased, specially if expressed in E. U./g. tissue. Hydrocortisone caused a decrease in spleen weight and protein nitrogen. Catheptic activity was significantly above control value 30 min. and 3 hrs after application, if expressed in enzyme unit per gram tissue. Two hours after ACTH administration protein nitrogen was increased, whereas catheptic activity was under the control value (expressed in E. U./mg. N).

INTRODUCTION

Biochemical and physiological investigations are very often affected by specific and non-specific neuro-humoral regulations in an organism as part of a general adaptation syndrome described by Selye[1].

Even if the measurements of catheptic activity are carried out very carefully the results are considerably scattered. This can be ascribed to the effect of stress. The observed changes in activity can be explained also by the environmental changes (temperature, humidity etc.). Kipnis[2] found that cortical steroids are essential for the catabolic component of normal tissue protein turnover.

Scope of the present work was to investigate the effect of some hormones on activity of cathepsins in rat spleen. In order to find out the possible effect of hormones on catheptic activity they were used in unphysiologically high doses and the changes were studied in early time intervals after application.

MATERIAL AND METHODS

120 female Wistar rats, weighing 160—200 g. were used; they were given corn, milk and vitamins enriched food. Animals were starved 24 hrs before the experiment. All experiments were performed in the same season of the year to exclude the effect of temperature, humidity *etc.* Just before the experiment animals were brought to the laboratory and injected intraperitoneally a single dose in amount of 1 ml./rat of a following 4 hormones:

Insulin (Galenika, Yugoslavia 40 I. U./ml). Original sample was diluted to 0.2 I. U./ml. using 0.9% NaCl.

Epinephrine U. S. P. (Nutritional Biochemical Co., Cleveland, Ohio, USA) was dissolved in equimolar amount of HCl. The stock solution was 10^{-2}M, prepared fresh each time and diluted to 10^{-3}M with 0.9% NaCl. The correction of *p*H was unnecessary because the epinephrine solution had the *p*H of the physiological solution.

Hydrocortisone acetate (Galenika, Yugoslavia, 2% suspension) contained 25 mg./ml. of hydrocortisone acetate.

Adrenocorticotropic hormone — ACTH (Galenika, Yugoslavia). The preparation containing 25 I. U. was dissolved in 2 ml. of physiological solution.

Control rats were injected *i. p.* the same volume (1 ml.) of physiological solution and sacrificed in the same time as animals treated with hormones.

Animals were anesthetized with chloroform and the organs isolated 30 and 60 min., 2 and 3 hrs after application of hormones or physiological solution. The organs were blotted, weighed and kept at 0° C throughout the further procedure.

Five percent spleen homogenates were prepared in 0,2% NaCl solution in Potter-Elvehjem teflon pestle homogenizer. No attempt was made to homogenize the samples totally. Homogenates were acidified to *p*H 3.5 with 6.35 *N* H_2SO_4 and centrifuged at 12,000 rpm for 20 min. (355,000 gmin) in refrigerated Sorvall RC-2 centrifuge.

Cathepsin activity was measured in the supernatant by the Anson method at *p*H 3.5 using 2% hemoglobin as substrate. Nitrogen was determined in the supernatant by the modified Kjeldahl method[3].

RESULTS

Table I show the spleen weight and nitrogen content in different times after treatments. 30 min. after insulin application the weight decreased by 10% and soluble nitrogen by 30%, 30 min. later (60 min. after application) these values remained unchanged. Spleen weight and nitrogen content after 2 and 3 hrs gradually increased compared to the value at 30 min. Three hrs after application protein nitrogen was 20% and spleen weight 5% lower than control values.

Within the first hour after epinephrine application spleen weight and soluble protein nitrogen were considerably but not significantly increased followed by a 30% decrease after 3 hrs.

Hydrocortisone caused a decrease in spleen weight and protein nitrogen already 30 min. after injection. Decreased spleen weight (by 20%) was observed still within next 3 hrs. Protein nitrogen was diminished after 3 hrs, while it was increased 2 hrs after hydrocortisone administration.

Spleen weight was increased by 26% 60 min. after the injection of ACTH but it was slightly under control value after 2 and 3 hrs. Protein nitrogen was within the range of controls during the first hour, increased by 14% and decreased by 10% after 2 and 3 hrs, respectively.

Table II shows the effect of injected substances on catheptic activity in spleen supernatant.

Insulin caused an increase of the catheptic activity expressed per mg. of nitrogen by 65% within the first hour. The activity was still above the control value after 2 and 3 hrs. Activity expressed per g. of wet tissue was higher than in control after 60 min. and 2 hrs, whereas it was lower 3 hrs after insulin application.

One hour after epinephrine was injected catheptic activity was decreased but it was increased significantly after 2 and 3 hrs. The catheptic activity when expressed per g. of wet tissue was less pronounced.

TABLE I

Weight and Nitrogen Content of the Rat Spleen

Treatments	No. exp.	Time intervals after application			
		30 min.	60 min.	2 hrs	3 hrs
Spleen weight					
Control	24		0.75 ± 0.04		
Insulin	24	0.67 ± 0.04	0.67 ± 0.06	0.71 ± 0.06	0.72 ± 0.05
Epinephrine	24	0.88 ± 0.07	0.89 ± 0.09	0.62 ± 0.03*	0.54 ± 0.03***
Hydrocortisone	24	0.71 ± 0.02	0.59 ± 0.03**	0.61 ± 0.03**	0.60 ± 0.06**
ACTH	24	0.84 ± 0.05	0.94 ± 0.13	0.71 ± 0.06	0.73 ± 0.06
mg.N/g. tissue					
Control			13.7 ± 0.4		
Insulin		9.9 ± 0.7***	10.3 ± 0.3***	11.1 ± 0.7***	11.3 ± 0.9***
Epinephrine		17.3 ± 1.8	16.6 ± 1.2	11.9 ± 0.9*	9.5 ± 0.9***
Hydrocortisone		11.9 ± 0.5**	11.8 ± 0.5***	14.5 ± 0.7	12.5 ± 0.5
ACTH		14.2 ± 0.6	13.7 ± 1.0	15.7 ± 0.8**	12.4 ± 0.7

Mean values ± S. E., ***$p < 0.01$, **$p < 0.02$, *$p < 0.05$, $p > 0.05$ is not significant.

TABLE II

Catheptic Activity of Spleen Supernatant

Treatments	Time intervals after application			
	30 min.	60 min.	2 hrs	3 hrs
E. U./mg. N				
Control		1.02 ± 0.05		
Insulin	1.71 ± 0.19	1.66 ± 0.08***	1.42 ± 0.13***	1.19 ± 0.13
Epinephrine	0.75 ± 0.06***	0.83 ± 0.09**	1.27 ± 0.14	2.08 ± 0.21
Hydrocortisone	1.41 ± 0.10	1.33 ± 0.07***	1.06 ± 0.05	1.33 ± 0.13***
ACTH	0.95 ± 0.13	0.94 ± 0.04	0.82 ± 0.07**	0.89 ± 0.07
E. U./g. tissue				
Control		14.0 ± 0.6		
Insulin	15.2 ± 1.1	16.1 ± 1.3*	15.9 ± 1.2	13.0 ± 0.3
Epinephrine	12.7 ± 0.6	13.2 ± 0.7	14.2 ± 1.3	19.1 ± 0.8***
Hydrocortisone	16.7 ± 0.6***	15.6 ± 0.8	15.2 ± 0.3	16.3 ± 1.1**
ACTH	13.3 ± 1.3	12.9 ± 1.0	12.9 ± 1.3	12.3 ± 0.9

Mean values ± S. E., ***$p < 0.01$, **$p < 0.02$, *$p < 0.05$, $p > 0.05$ is not significant.

Proteolytic activity was increased by 35% 30 and 60 min. after hydrocortisone application. 2 hrs after treatment activity was within the control range and was increased by 30% after 3 hrs. Again the changes in activity expressed per g. of wet tissue were less pronounced.

ACTH caused a decrease in the catheptic activity 2 hrs after application. Activity was 14% under the control value if expressed in E. U./mg.. N.

DISCUSSION

Studies concerning the mutual connection between lymphatic tissue and adrenocortical steroids were carried out by several authors *in vivo*[4-7] and *in vitro*[7-10]. Due to some different function spleen can be considered as an exemption when compared with lymph nodes and thymus. It has been well documented that adrenocortical steroids are essential for the catabolic component of normal tissue protein turnover. This protein catabolic effect is greatly enhanced when an animal is exposed to pharmacological doses of corticosteroids with resultant loss of body weight, muscle wasting, impaired growth, osteoporosis and lympholysis. The net result is a strikingly negative nitrogen balance[2,11-14].

Our experiments show that a high single dose of insulin and hydrocortisone caused an immediate mobilization of the catheptic activity, a decrease of spleen weight and a decrease in protein nitrogen of the spleen. Two hours after insulin administration it seems that highly activated cathepsins at the beginning have a tendency toward normalization. The changes in spleen proteolytic activity after hydrocortisone administration show a two phase response. This can be ascribed to the direct effect of injected hydrocortisone and its metabolites formed later in the spleen[15].

In our experiments epinephrine showed the most pronounced effect on catheptic activity. The protein catabolism 3 hrs after administration was increased considerably. This can be explained by increased catheptic activity, decreased spleen weight and nitrogen content.

Our experiments showed that insulin, epinephrine and hydrocortisone in relatively high doses had immediate effect on catheptic activity in spleen. However, from the obtained results we cannot explain the mechanism of action.

Many authors have shown that some physical, chemical or physiological factors can induce changes in the cell composition of the spleen. Thus intact cells either leave the spleen or they accumulate in it. The scope of our work was not to compare morphological changes with direct biochemical effects of applied hormones. However, a possibility exists that such high doses of hormones can also cause morphological changes. We only wanted to find out the *in vivo* effect of mentioned hormones on metabolic processes on the molecular level. For this reason we followed the changes in short time intervals after the application massive doses of hormones. In our opinion we can conclude that some of the tested hormones have a direct effect on the catheptic activity in spleen.

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IZVOD

Uticaj insulina, epinefrina, hidrokortizona i ACTH na katepsinski aktivitet u slezeni štakora

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U našim eksperimentima pratili smo odgovor slezene na *in vivo* injiciran insulin, epinefrine, hidrokortizon i ACTH.

Insulin prouzrokuje smanjenje težine slezene i smanjenje proteinskog dušika, a katepsinski aktivitet povećan je već 30 minuta posle aplikacije. Premda je katepsinski aktivitet znatno povećan iznad kontrolne vrednosti u 60. minuti (izražen u E. U./g. tkiva) zapažena je u kasnijim satima tendencija ka kontrolnoj vrijednosti. 30 i 60 minuta nakon aplikacije epinefrina težina slezene i proteinski dušik su povećani a katepsinski aktivitet ostaje ispod kontrolne vrijednosti (izražen u E. U./mg. N). Poslije 3 sata težina slezene je reducirana (smanjen je proteinski dušik), a katepsinski aktivitet izražen u jedinicama na gram tkiva je znatno povećan. Hidrokortizon prouzrokuje smanjenje težine slezene i proteinskog dušika. Katepsinski aktivitet znatno je povećan iznad kontrolne vrijednosti 30 min. i 3 sata nakon aplikacije ako ga izrazimo u E. U./g. tkiva. 2 sata nakon aplikacije ACTH proteinski dušik se povećava međutim, katepsinski aktivitet je ispod kontrolne vrijednosti ako ga izrazimo u jedinicama na mg. dušika.

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