Effects of Glucocorticoids on the Alkaline Phosphomonoesterase Isoforms

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Creation of a glucocorticoid depot in mice by daily subcutaneous injections of prednisolone in a dose of 30 mg/kg for a week or by a single injection of kenalog in a dose of 2 mg/kg modifies the activity of alkaline phosphomonoesterase differently in different organs. Polyacrylamide gel electrophoresis showed inhibition of the enzyme fractions in some tissues and the appearance of additional isoforms in others.

Key Words: *glucocorticoids; alkaline phosphomonoesterase; isoforms*

Glucocorticoids (GC) regulate metabolism and play an important role in homeostasis [3]. The effects of endogenous and exogenous GC on the organism depend on the activities of various enzymatic systems. Alkaline phosphomonoesterases (AP, CP 3.1.3.1) expressed virtually in all animal tissues differently react to hypo- and hypercortisolism [7,8].

AP are phosphohydrolases with a low substrate specificity. They catalyze the transfer of phosphoric acid from various phosphomonoesters and participate in the phosphate transport, ossification, glycogen utilization, and regulation of many biochemical processes [5]. Three structural genes coding for AP have been identified: tissue nonspecific gene responsible for the production of AP in the liver, lungs, kidneys, bone, and other organs and tissues, intestinal gene, and placental gene.

High variability of GC levels in adaptive and pathological processes and wide clinical application of GC prompted us to study the effect of GC on the response of AP isoforms in some organs.

MATERIALS AND METHODS

Experiments were carried out on adult male CBA mice weighing 18-20 g. In order to create a depot and maintain a stable high level of GC, the mice were subcutaneously injected with prednisolone (Gedeon Richter) in a dose of 30 mg/kg every day for 7 days. One day after the last injection the animals were decapitated. In the other series the mice were subcutaneously injected with the long-acting GC kenalog (Berlin Chemie) in a single dose of 2 mg/kg [2]. Normal saline was injected instead of hormones to the control mice. After decapitation, homogenates were prepared from the liver, kidneys, lungs, bone tissue (tail vertebrae), and intestine in 0.05 M Tris-HCl buffer (pH 7.2), extracted in the presence of Triton X-100 at 4°C for 12-18 h, and centrifuged at 6000 rpm for 20 min. AP activity in the resultant supernatants was determined by the method [10] based on measurements of intensity of the fluorescence of α-naphthol formed after hydrolysis of α-naphthylphosphate (pH 9.6). AP activity was expressed in mg α-naphthol/mg tissue during 15 min. AP isoforms were detected by vertical electrophoresis in polyacrylamide gel in 225×130×2 mm plates at 18-20°C [6,7].

Polyacrylamide gel was prepared as follows: $T=(a+b)/m\times100\%$, $C=b/(a+b)\times100\%$, where a is the amount of acrylamide, g; b amount of bisacrylamide, g: and m is volume of buffer, ml.

The upper separating gel: $T=3.85\%$, $C=2.60\%$, riboflavin 5 μg/ml. The lower separating gel: $T=10.395\%$, $C=2.59\%$, ammonium persulfate 0.175 mg/ml. Working buffer: 0.008 M Tris titered with

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boric acid to pH 7.0. The upper gel (2.5 cm) was layered over solidified lower gel (9 cm). Bromophenol blue (1.5 ml 0.001% water solution) was added to the upper electrode solution. Tissue extracts and blood sera were mixed with an equal volume of 0.5 M sucrose in the working buffer diluted 5 times, and the gel (20 μl) was then added. The electrophoresis protocol was as follows: 12 mA during the first hour and then 25 mA till the moment when the bromophenol blue band was 7 cm below the borderline between the upper and lower gels.

After electrophoresis, the activity of AP was evaluated by the azocoupling method [1]. The gel was incubated in a mixture of naphthol-AS-TR-phosphate (3.7 mM), dimethylphormamide (5 vol.%), strong blue (1 mg/ml), $MgCl_2$ (0.1 mM) in 0.1 M Tris-HCl buffer (pH 9.0) for 30 min at 37°C in the dark in a bath with a shaker, washed in distilled water, and left overnight in a fresh incubation mixture. The gel was fixed in the ethyl alcohol:distilled water:glacial acetic acid system (5:10.5:1.5). Protein mobility (R_f) was calculated by bromophenol blue. The distance passed by the proteins and the stain was measured starting from the beginning of the lower gel.

RESULTS

Under the effect of kenalog a GC depot was formed during 5 days [2]. Hypercortisolism involved changes in AP activity in various organs (Table 1). Daily injections of prednisolone for one week cause greater changes in the enzyme activity: a 6-fold increase in the kidneys, 50% in the liver, 30% in the intestine, and 20% in the bones. Prednisolone induced no significant changes in the activity of AP in the lungs. Kenalog increased the enzyme activity in the lungs (150%), kidneys (100%), and liver (30%) and decreased it in the intestine (20%) and bones (10%). Organs and tissues possess different sensitivity to GC; moreover, the reactions of bone tissue and intestine to the studied drugs were opposite, which is important for clinical practice.

Glucocorticoids modified the levels and activities of individual isoforms, which depended on the drug injected. In the liver of experimental and control mice AP activity was observed in the band corresponding to the intermediate fractions: R_f= 0.227-0.238 in the control, R_f=0.232 after kenalog, and R_f=0.227 after prednisolone (Table 2). Experimental mice had one or two accessory bands among the fast fractions of AP: R_f=0.261-0.268 and Rf= 0.352 after kenalog and R_f=0.265 after prednisolone. On the electropherograms of the kidneys of control animals AP activity was located at the beginning of the upper gel (S-1). It was weaker at the interface between the upper and lower gels and stronger at the R_f=0.30-0.152 domain; in addition, a pronounced AP activity band was observed among the intermediate forms R_f=0.220-0.230. AP activity was absent in the starting area in kenalog-treated mice, while in prednisolone-treated mice there was a wide band of AP activity in the "slow" domain (R_f=0.-0.152). AP activity in the intermediate domain R_f= 0.218-0.225 was observed in the kidneys of all experimental animals. In the lungs of control and experimental animals, AP activity was observed in the intermediate forms (one or two bands). The electrophoretic spectrum of bone AP in control mice included slow fractions at the beginning of the upper and lower gels and intermediate forms of AP (R_f= 0.225-0.246). After injection of prednisolone, the activity of AP in the slow forms disappeared. In the control, the activity of intestinal AP was registered in slow (R_f=0-0.024), intermediate (R_f= 0.218-0.230), and fast forms (R_f=0.320-0.397). The slow AP fraction was not observed in some animals injected with prednisolone and in all mice injected with kenalog (Table 2).

In the blood AP was represented mainly by the forms controlled by tissue-nonspecific AP gene characteristic of the liver, kidneys, lungs, and bones. The additional fraction of AP rapid forms was not observed in all experimental mice.

Thus, different AP isoforms in the studied organs and tissues differently react to GC: the activities of

TABLE 1. AP Activity in Mouse Organs after Injection of GC (*M±m*)

Organs	Control, 0.85% NaCl (*n*=5)	Kenalog, 2 mg/kg once (*n*=13)	Control, 0.85% NaCl (*n*=7)	Prednisolone, 30 mg/kg daily for 1 week (*n*=12)
Liver	0.3±0.03	0.4±0.04*	0.4±0.04	0.6±0.02*
Kidneys	2.6±0.95	5.1±0.97	1.5±0.56	9.1±0.74*
Lungs	0.2±0.02	0.5±0.18	0.2±0.02	0.2±0.12
Bone	11.3±1.66	10.2±1.71	11.2±2.75	14.8±2.09
Intestine	9.8±1.36	7.3±1.19	13.1±3.4	17.9±2.43

Note. Experiments with hormone injections (prednisolone and kenalog) were carried out at different time, and therefore each group had individual control. *p<0.05 *vs.* control.

TABLE 2. Prevalent Electrophoretic AP Isoforms in Organs and Blood Sera of Mice after GC Injection

Organ, isoforms		Electrophoretic mobility (R_f)						
		control, 0.85% NaCl			kenalog, 2 mg/kg once		prednisolone, 30 mg/kg daily for 1 week	
		1	2	3	4	5	6	7
Liver	M	0.238	0.227	0.232	0.232	0.232	0.227	0.227
	F-1				0.268	0.261	0.265	0.265
	F-2				0.352			
Kidneys	S-1	0	0	0	0	0	0	0
	O	0-0.048	0	0				
	S-2	0.048-0.111	0.030-0.152	0.042-0.100	0.042-0.127	0.042-0.127	0-0.152	0-0.152
	M	0.230	0.220	0.225	0.225	0.218	0.220	0.220
Lungs	M	0.214	0.212		0.190	0.183	0.212	
			0.235	0.232	0.225	0.225	0.235	0.220
Bone	S-1	0	0	0	0	0	0	0
	O-S-2	0-0.127	0	0		0		
	M	0.246	0.242	0.225	0.225	0.225	0.220	0.220
Intestine	S-1	0	0	0	0	0	0	0
	O-S-2	0-0.024	0	0				0
	M	0.230	0.227	0.218	0.190	0.197	0.212	0.212
	F-2	0.397	0.356	0.352	0.352	0.352	0.348	0.348
Blood	M	0.238	0.159-0.227	0.225	0.155-0.232	0.225	0.227	0.227
	F-1				0.254	0.254	0.258	0.258

Note. 1-7) value for individual mice. AP activity in the upper gel (S-1), at the interface between the upper and lower gels (0); S-2) slow AP forms (R_f=0-0.159); M) medium (R_f=0.150-0.250); F-1) fast (R_f=0.250-0.300); and F-2) very fast forms (R_f>0.300).

the fractions present in the control decreased or increased; moreover, some fractions could be completely inhibited, and new fractions with other mobility appeared. The selective effect of steroids on different enzyme isoforms was demonstrated in many studies. Hydrocortisone activated some isoforms of malate dehydrogenase [9] and tyrosine aminotransferase [3,4] in the rat liver, testosterone activated hexokinase in the rat prostate [11], etc.

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