EFFECT OF HYDROCORTISONE ON THE LIVER CYTOCHROME P-450 SYSTEM AND INTENSITY OF FOOD ANAPHYLAXIS IN GUINEA PIGS

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The concept of the linked function of humoral and cellular factors of the immunologic system and of cytochrome P-450-dependent mono-oxygenases of the liver in the maintenance of chemical homeostasis was first put forward in 1977 [3], and later confirmed and developed [4, 5].

In 1982 reciprocal relations between the intensity of food anaphylaxis in guinea pigs and the state of the liver cytochrome P-450 were demonstrated experimentally for the first time [6, 7].

The aim of the present investigation was to study the effect of the widely used anti-allergic agent hydrocortisone, an endogenous substrate for cytochrome P-450-dependent liver mono-oxygenases, on the development of food anaphylaxis was studied in guinea pigs.

EXPERIMENTAL METHOD

Experiments were carried out on 300 noninbred male guinea pigs weighing 250-300 g kept on the standard animal house diet. Food sensitization and anaphylaxis to hen's egg protein were induced as described previously [8]. The intensity of the manifestations of anaphylaxis was estimated on the basis of the lethal effect and the anaphylactic index [15].

After 2 weeks the state of the liver cytochrome P-450 system was determined in control and sensitized animals. The microsomal fraction was isolated from the animals' liver by differential centrifugation [9]. The content of cytochromes b_5 and P-450 was determined by the method in [14] on a "Specord" spectrophotometer. The content of microsomal protein was measured by a modified Lowry's method [12]. The rate of p-hydroxylation of aniline and of demethylation of aminopyrine [2] also were determined in the microsomal fraction. The state of the liver cytochrome P-450 system in the intact animal also was estimated from the duration of hexobarbital sleep. Hexobarbital was injected intraperitoneally in a dose of 30 mg/kg. To assess the effect of hydrocortisone on the liver cytochrome P-450 system the hormone was injected in the form of a suspension into the animals once daily for 3 days in doses of 0.1, 0.25, 1.0, 2.5, 10.0, and 25.0 mg/kg. Control animals received an intraperitoneal injection of an equal volume of 0.85% sodium chloride. To study the effect of hydrocortisone on the intensity of manifestations of food anaphylaxis, the hormone was injected by the scheme mentioned above in doses of 0.1, 0.25, 1.5, and 25.0 mg/kg into sensitized animals over a period of 3 days before intravenous injection of the reacting dose of antigen. The results were subjected to statistical analysis [1].

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TABLE 1. Effect of Hydrocortisone on Liver Cytochrome P-450 System in Intact and Sensitized Animals

Exptl. conditions	Dose of hydrocortisone, mg/kg	Cont. of microsomal fraction of liver: of cytochrome P-450	Cont. of microsomal fraction of liver: of cytochrome b_5	Enzyme activity: aminopyrene N-demethylase	Enzyme activity: aniline p-hydroxylase
		% relative to intact animals			
Hydrocortisone	0,10	98,0±13,3	101,9±7,4	110,2±14,5	118,0±14,6
	0,25	81,9±9,6	92,4±7,5	105,5±12,9	92,6±8,6
	1,0	98,5±16,6	98,9±3,9	104,6±8,0	118,6±13,8
	2,5	76,9±5,4***	89,4±5,9*	86,5±5,1**	80,7±7,00**
	10,0	72,5±6,2**	95,9±4,3	89,6±5,0	81,4±3,6***
	25,0	66,3±7,2***	89,9±6,0*	67,4±10,3***	78,2±8,6**
Sensitization	—	86,7±3,2**	105,6±9,3	84,7±2,2**	65,8±2,3***
Sensitization + hydrocortisone	0,10	74,7±4,1**	100,0±1,6	85,2±2,4**	64,6±5,4**
	2,5	87,1±1,8**	103,5±2,1	85,0±1,6**	83,0±2,8**

Legend. Mean values of 3-5 experiments, in each of which three or four animals were used, are given. In each experiment an individual control group of desensitized animals was used. The content of cytochromes P-450 and b_5 in the control groups was 1.22 ± 0.17 and 0.85 ± 0.06 nmole/mg protein respectively, and activities of aminopyrene N-demethylase and aniline p-hydroxylase were 9.55 ± 0.11 and 0.93 ± 0.08 nmole/min/mg protein respectively. *P < 0.05, **P < 0.01, ***P < 0.001 compared with control.

TABLE 2. Effect of Hydrocortisone on Duration of Hexobarbital Sleep in Guinea Pigs

Exptl. conditions	Dose of hydrocortisone, mg/kg	Dose of hexobarbital, mg/kg	No. of animals	Duration of hexobarbital sleep, min: 0	1—20	21—40	41—60	mean	P
Control	—	30	30	9	15	6	—	13,9	—
Hydrocortisone	2,5		30	7	9	11	3	20,1	Sen <0,05

TABLE 3. Dependence of Manifestations of Food Anaphylaxis on Duration of Hexobarbital Sleep

Group of animals	No. of animals	Dose of hexobarbital, mg/kg	Mean duration of hexobarbital sleep, min	Exptl. conditions	Criteria of anaphylaxis: No. of fatal cases, abs.	No. of fatal cases, %	anaphylactic index
1-	14	30	0	Sensitization	7	50	2,60
2-	21		46,0	»	20	95,2	3,88
				»	P<0,001		

EXPERIMENTAL RESULTS

The experiments showed that an increase in the doses of hydrocortisone injected was accompanied by a decrease in the concentrations of cytochromes P-450 and b_5 and weakening of mono-oxygenase activity (Table 1).

Doses of hydrocortisone between 0.1 and 2.5 mg/kg had no unambiguous action on parameters of the liver cytochrome P-450 system, changes in which under these conditions were fluctuating in character, evidently because of variability of individual sensitivity of the animals to small doses of the hormone. Injection of hydrocortisone in a dose of 2.5 mg/kg or more was followed by inhibition of the liver cytochrome P-450 system and by a decrease both in the hemoprotein content and in mono-oxygenase activity. Injections of hydrocortisone led to more marked inhibition of mono-oxygenase activity and to a greater decrease in the content of cytochrome P-450 in sensitized animals.

TABLE 4. Effect of Hydrocortison on Intensity of Manifestation of Food Anaphylaxis

Exptl. conditions	Dose of hydrocortisone, mg/kg	No. of animals	Criteria of anaphylaxis		
			No. of fatal cases		anaphylactic index
			abs.	%	
Sensitization	—	45	14	31,1	2,27
Sensitization + hydrocortisone	0,10	29	11	37,9	2,33
	0,25	34	17	50,0*	2,62
	2,50	26	14	53,9*	3,12
	25,0	35	19	54,3**	2,84

Legend. Injection of reacting dose of antigen into intact animals did not cause the development of anaphylaxis. *P < 0.05, **P < 0.01 compared with sensitization.

The effect of hydrocortisone on the liver cytochrome P-450 system in the intact animal was determined in animals by measuring changes in the duration of hexobarbital sleep after injection of the hormone.

The data in Table 2 show that a dose of hydrocortisone of 2.5 mg/kg, which lowers the concentrations of both forms of cytochrome and mono-oxygenase activity in the liver, significantly increased the duration of hexobarbital sleep.

To study the effect of the duration of hexobarbital sleep on the intensity of anaphylactic reactions in the two groups of animals, differing sharply in the duration of hexobarbital sleep, food anaphylaxis was induced (Table 3). The number of fatal cases of anaphylactic shock in the group of animals with long hexobarbital sleep (46.4 min) was almost twice as high as the corresponding parameter in the group of animals resistant to the action of hexobarbital.

Hexobarbital sleep, evidence of depressed activity of the liver cytochrome P-450 system, thus reliably reflects increased sensitivity of animals to anaphylactic shock.

After determination of the character of the action of hydrocortisone on the liver cytochrome P-450 system, an attempt was made to estimate the direct effect of the hormone on the intensity of manifestations of food anaphylaxis (Table 4).

It was shown that injection of hydrocortisone into sensitized animals in a dose of 0.25 mg/kg or more in the course of 3 days before injection of the reacting dose of antigen, significantly increased the severity of the anaphylactic reaction. The results of this investigation thus demonstrate inhibition of the liver cytochrome P-450 system of guinea pigs by hydrocortisone, and this is accompanied by a decrease in resistance of the animals to food anaphylaxis.

The facts thus demonstrated are an important contribution to the analysis of the causes of absence of an anaphylactic effect of hydrocortisone in guinea pigs [13]. The increase in severity of anaphylaxis under the influence of this hormone may be due to a certain extent to weakening of the histaminolytic activity of the liver of sensitized guinea pigs receiving glucocorticoid derivatives [11]. This last state of affairs may be interpreted as one cause of the appearance of chronic forms of allergy [10, 11]. That is why analysis of the state of the liver cytochrome P-450 systems is important both for the study of mechanisms of hypersensitivity reactions and also for the planning of new approaches to the treatment of allergic diseases.

LITERATURE CITED

1. E. V. Gubler, Computerized Methods of Analysis and Diagnosis of Pathological Processes [in Russian], Leningrad (1978).
2. I. I. Karuzina and A. I. Archakov, in: Modern Methods in Biochemistry [in Russian], Moscow (1977), pp. 49-62.

3. I. E. Kovalev, Khim.-farm. Zh., No. 12, 3 (1977).
4. I. E. Kovalev, Khim.-farm, Zh., No. 11, 11 (1980).
5. I. E. Kovalev and O. Yu. Polevaya, Antibodies Against Physiologically Active Compounds [in Russian], Moscow (1981).
6. I. E. Kovalev, L. A. Piruzyan, V. A. Shaternikov, et al., Dokl. Akad. Nauk SSSR, 266, 247 (1982).
7. I. E. Kovalev, T. G. Khlopushina, I. N. Marokko, et al., Patol. Fiziol., No. 4, 22 (1983).
8. V. A. Shaternikov, I. N. Marokko, N. N. Pyatnitskii, et al., Vopr. Pitan., No. 2, 27 (1982).
9. J. Ahokas, O. Pelkonen, and N. Kärki, Cancer Res., 37, 3737 (1977).
10. K. Chang, D. Miklich, H. Chai, et al., Clin. Allergy, 12, 369 (1982).
11. K. Dave and K. Sachdev, Br. J. Pharmacol., 30, 224 (1967).
12. E. Hartree, Anal. Biochem., 48, 422 (1972).
13. R. Hicks, J. Pharm. Pharmacol., 21, 202 (1969).
14. T. Omura and R. Sato, J. Biol. Chem., 239, 2379 (1964).
15. W. Weigle, C. G. Cochrane, and F. Dixon, J. Immunol., 85, 469 (1960).