CORRELATION BETWEEN THE SEVERITY OF MANIFESTATIONS OF EXPERIMENTAL FOOD ANAPHYLAXIS AND THE CYTOCHROME P-450W/P-450L RATIO

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UDC 616-056.3-036.11-02:613.2/032.3-092:616.36.36-008.931:577.152.112

KEY WORDS: cytochromes P-450W and P450L; guinea pig liver microsomes; experimental food anaphylaxis

There is no doubt about the urgency of research into the metabolic bases of allergy, which are at the center of attention, are being intensively studied, but are still far from final solution" (A. D. Ado [1]). With an increase in the degree of environmental pollution at the present time there is a steady increase in the number and severity of allergic diseases [7]. One of the biological systems functionally linked with the development of the immediate- and delayed-type hypersensitivity reaction is the liver microsomal monooxygenase system [3, 4, 6], which included a family of isoforms of cytochrome P-450 [13]. This system metabolizes both exogenous chemical compounds and endogenous modulators of allergic reactivity, namely steroid hormones, prostaglandins, leukotrienes, and polyunsaturated fatty acids [13]. The writers showed previously that the whole family of isoforms of cytochrome P-450 can be divided into two groups depending on the orientation of their active centers in the microsomal membrane: into cytochromes P-450W with active centers facing the aqueous medium, and cytochromes P-450L with active centers facing the lipids of the membrane [5], and a method of quantitative measurement of these two groups of isozymes have been developed [8].

The aim of this investigation was to study correlation between the two groups of cytochrome P-450 isoforms in guinea pig liver microsomes, when modified as a result of preliminary treatment of animals with inducers, and the manifestation of experimental food anaphylaxis (EFA) in these animals.

EXPERIMENTAL METHOD

Noninbred male guinea pigs weighing 250-300 g were kept on the standard animal house diet. EFA was chosen as the model of immediate-type allergic reactions, for in such a model it is necessary that the allergen be introduced into the body by the natural route [2]. The demonstrativeness of EFA also is confirmed by the most suitable method of evaluation of the allergenicity of food proteins, developed on its basis [11]. EFA to hen ovalbumin (OVA) was induced as described previously [10]. Phenobarbital (PB) from "Serva" (West Germany), dissolved in physiological saline, and 3-methylcholanthrene (MCh, also from "Serva"), in 1% starch solution, were injected intraperitoneally into intact and sensitized animals daily for 3 days in doses of 50 and 20 mg/kg respectively. The reacting dose of OVA was injected into the animals 24 h after the last injection of the inducer. Microsomes were isolated from the liver by the method in [12]. The content of cytochromes b_5 and P-450 in microsomal preparations was determined in method in [15], the content of cytochromes P-450W and P-450L by the most suitable method [8], and the content of microsomal protein by a modified Lowry's method [14]. Activity of the microsomal monooxygenase system in vivo was estimated from the duration of hexobarbital-induced sleep. Hexobarbital was injected intraperitoneally in 0.14 M NaCl in a dose of 30 mg/kg. The experimental results were subjected to statistical analysis [9].

Research Center for Molecular Diagnosis, Ministry of Health of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. D. Ado.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 112, No. 8, pp. 200-202, August, 1991. Original article submitted October 10, 1990.

 0007-4888/91/0008-1180$12.50

TABLE 1. Effect of Food Sensitization by OVA and Injection of PB and MCh on Content of Microsomal Hemoproteins in Guinea Pig Liver (M ± m)

Group of animals	Content of microsomal cytochromes, nmoles/mg protein			
	b_5	P-450	P-450W	P-450W/P-450L
Control	0,86±0,05	0,89±0,08	0,44±0,04	0,98
Sensitization to OVA	0,80±0,06	0,70±0,04*	0,26±0,01*	0,59*
Injection of PB	1,25±0,16*	1,62±0,24*	0,82±0,1*	1,05
Injection of MCh	1,21±0,15*	1,42±0,19*	0,34±0,02*	0,32*

Legend. *$p < 0.01$.

TABLE 2. Changes in Severity of EFA to OVA under the Influence of PB and MCh

Group of animals	Criterion of severity of food anaphylaxis		
	mortality, per cent	convulsions, per cent	Anaphylactic index
Sensitization to OVA (n=32)	43,8	56,3	2,55
Sensitization by OVA PB (n = 24)	20,8*	33,3*	1,81*
Sensitization by OVA MCh (n=30)	66,7*	76,6*	3,28*

Legend. Injection of reacting dose of antigen into intact animals did not cause anaphylaxis to develop. *$p < 0.05$ compared with ordinary sensitization. n) number of animals.

EXPERIMENTAL RESULTS

The experimental results show that during food sensitization with OVA the total concentration of cytochrome P-450 fell by 21%, whereas both total and relative (P-450W/P-450L) quantity of cytochromes P-450W fell by 40% (Table 1). Preliminary treatment of the animals with inducers PB and MCh led to an increase in the total content of cytochrome P-450 by 82 and 60% respectively, but the quantity of cytochromes P-450W after induction by PB rose by 86%, whereas after induction by MCh it fell by 23%. The relative content of cytochromes P-450W after induction by PB and MCh was 1.05 and 0.32 respectively of the control values.

EFA to OVA in animals receiving PB (Table 2) was manifested significantly less strongly with respect to all parameters than the manifestations of the anaphylactic reaction during ordinary sensitization and, in particular, than in animals treated with MCh. In the latter, the criteria of severity of EFA significantly exceeded the corresponding values in the control animals by 1.5 times as regards mortality by 1.4 times as regards the number of convulsions, and by 1.3 times as regards the anaphylactic index.

Evaluation of the state of the monooxygenase system in vivo by the duration of hexobarbital sleep test enabled animals resistant to hexobarbital (with zero duration of hexobarbital sleep — ZHS) to be distinguished from those sensitive to hexobarbital (with long hexobarbital sleep — LHS), lasting on average 53.5 min). Animals with LHS were distinguished by a significantly higher (by 1.54 times) total content of cytochrome 2-450 (Table 3), but their relative content of cytochromes P-450W was 2.7 times lower than in animals with ZHS. Negative correlation was found between the duration of hexobarbital sleep in the animals and the relative content of cytochromes P-450W in the liver microsomes (Fig. 1 — Missing in Russian original). The highest content of cytochromes P-450W was observed in animals with ZHS. The severity of EFA in animals with LHS was significantly greater with a respect to all criteria than the severity of EFA in the animals with

TABLE 3. Intensity of Manifestations of EFA to OVA in Animals Differing in the Duration of Hexobarbital Sleep

Parameter	ZHS (1)	ZHS + PB (2)	ZHS + MCH (3)	LHS (4)	LHS + PB (5)	LHS + MCh
Number of animals	24	24	24	24	24	24
Cytochrome, nmoles/mg protein"b_5	0,62	0,87* (1)	0,77* (1)	0,86* (1)	0,89	1,04
P-450	0,78	1,04* (1)	1,25* (1)	1,20* (1)	1,55	1,49
P-450W	0,40	0,78* (1)	0,38	0,34	0,75* (4)	0,41
P-450W/P-450L	1,05	1,25* (1)	0,44* (1)	0,39* (1)	0,93* (4)	0,37
Animals sensitized with OVA						
Number of animals	53	20	28	36	22	28
Mortality, per cent	32,1	25,0	53,6* (1)	61,1* (1)	36,4*(4)	53,6
Convulsions, per cent	45,3	25,0* (1)	82,1* (1)	72,2* (1)	45,5* (4)	71,4
Anaphylactic	2,19	1,38* (1)	3,21* (1)	3,30* (1)	2,36* (4)	3,14

Legend. *$p < 0.005$; (1) and (4) — numbers of groups of animals relative to which the significance of differences was calculated.

ZHS. PB and MCh caused an increase in the total content of cytochrome P-450, which was more marked in the guinea pigs with ZHS (by 79.5 and 60.3% respectively) compared with animals with LHS (by 29.2 and 24.2%, respectively). The action of PB was characterized by a virtually equal increase in the total content of cytochromes P-450W in animals with ZHS and LHS (by 1.9 and 2.2 times respectively). However, under these circumstances the relative content of cytochromes P-450W in the group of guinea pigs with LHS was increased by 2.4 times, but in the group of animals with ZHS only by 1.2 times. Similar changes took place in the severity of food anaphylaxis under the influence of PB: in the group of animals with LHS more marked weakening of the anaphylactic reaction was observed with respect to all its parameters. Against the background of an increase in the total content of cytochrome P-450 in both groups MCh caused a reduction by 2.4 times in the relative content of cytochromes P-450W in the animals with ZHS, but did not affect this parameter in animals with LHS. The severity of EFA in the latter group of animals was unchanged by MCh, whereas in the group of guinea pigs with ZHS injection of MCh was accompanied by a marked increase in the severity of the anaphylactic reaction.

Thus the change in relative content of cytochromes P-450W taking place against a background of induction of PB and MCh is characterized by modulation of anaphylactic sensitivity in guinea pigs. Significant negative but nonlinear correlation was observed in this case between the increase in severity of the EFA manifestations and the decrease in the relative content of cytochromes R-450W (Fig. 2 — Missing in the Russian original).

Predominance of cytochromes P-450L with active centers facing the membrane lipids as a result of the action of inducers of the MCh type has now been confirmed experimentally [15]. The substrate specificity of isoforms of cytochrome P-450 (cytochromes P-450c + P-450d) induced by inducers of the MCh type, unlike cytochrome isoforms P-450b+e, induced by inducers of the PB type, is characterized by low catalytic activity relative to hexobarbital and by a high rate of metabolism of hydrophobic steroid hormones [13]. These data, together with the results of the present investigation, enable the increased anaphylactic sensitivity of guinea pigs with a low relative content of cytochromes P-450W (distinguished by LHS) to be interpreted perhaps as the result of increased metabolism of lipophilic glucocorticoids, which play a key role in the mechanism of the antiallergic resistance of the body. The results demonstrate the urgency of the study of correlation between the characteristics of organization of cytochrome P-450 isoforms in the microsomal monooxygenase complex and the biological resistance of the organism to unfavorable external environmental influences.

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