EFFECT OF EMOTIONAL–PAINFUL STRESS ON CYTOCHROME P-450 INDUCTION IN THE RAT LIVER

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Cytochrome P-450 is the principal protein of the microsomal monooxygenase system of the liver, which plays a central role in steroid metabolism, detoxication of xenobiotics, and activation of certain procarcinogens. The study of induction of this system will assist with the understanding of mechanisms involved in the regulation of activity of cytochrome P-450-dependent processes. The state of the microsomal monooxygenase system in the liver cells is known to depend on the age, sex, and nutrition of the animals and on other factors, and it is also under hormonal control. The effect of stress on the cytochrome P-450 content and on its induction in the liver cells has virtually never been studied.

In this communication we show that emotional–painful stress induces a marked decrease both in the basic pool of cytochrome P-450 and in the newly synthesized fraction; the effectiveness of the induction process, however, is unchanged.

EXPERIMENTAL METHOD

Stress in noninbred albino rats immobilized in the supine position was induced by stimulation of the hind limbs by an alternating electric current of 3 mA for 45 min, preceded by mobilization for 15 min. The group of animals in which cytochrome P-450 was induced received an intraperitoneal injection of phenobarbital (PB) in a dose of 80 mg/kg immediately after exposure to stress. The procedure was repeated for 3 days. Hypobaric hypoxia was created in a pressure chamber at an "altitude" of 5000 m for 35 days. PB was injected during the last 5 days. The animals were killed by decapitation under superficial ether anesthesia 24 h after the last injection of PB. The liver was washed out with 1% KCl solution, cooled to 4°C. The content of cytochrome P-450 in the liver homogenate was determined by the method in [7] in Matsubara's modification [5], with a coefficient of molar extinction of 104 $mM^{-1} \cdot cm^{-1}$. RNA and DNA were determined by Spirin's method [2] in Trudolyubova's modification [3], and protein by the biuret reaction. The thymus and adrenals were removed immediately after the liver and weighed freed from capsule. The results were subjected to statistical analysis by Student's method.

TABLE 1. Changes in Weight of Thymus and Adrenals (in mg) during Stress and Induction of Mono-oxygenase System (M ± σ)

Parameter	Control, n = 32	Stress, n = 6	PB, n = 6	PB + stress, n = 7
Weight of thymus	778±26 (100)	261±41* (34)	534±93* (69)	288±34* (37)
Weight of adrenal	31±1 (100)	51±5* (165)	44±4* (142)	52±9* (168)
Thymus / adrenal	25 (100)	5 (20)	12 (48)	5,5 (22)
RNA / DNA	1,06±0,14 (100)	1,19±0,18 (112)	1,21±0,26 (114)	1,30±0,36 (123)

Legend. Here and in Tables 2 and 3: *$p < 0.05$ compared with control; percentages shown between parentheses.

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TABLE 2. Effect of Stress on Induction of Cytochrome P-450 and Total Protein Content in Liver of Rats (M ± σ)

Parameter	Control, n = 32	Stress, n = 6	PB, n = 6	PB + stress, n = 7
Cytochrome P-450, nmoles·g^{-1} tissue	33,8±7,5	21,6±4,8*	70,1±9,6*	52,1±13,5*
	(100)	(64)	(208)	(154)
Total protein, mg·g^{-1} tissue	248±10	287±71	259±32	283±55
	(100)	(116)	(104)	(114)

TABLE 3. Induction of Cytochrome P-450 in Rat Liver during Prolonged Adaptation of Animals to Hypoxia (M ± σ)

Parameter	Control	Adaptation	PB	PB + adaptation
Cytochrome P-450, nmoles·g^{-1} tissue	33,0±6,6	28,2±7,6*	108,7±17,1*	77,4±14,6*
	(100)	(86)	(329)	(235)
n	29	36	11	9
Total protein, mg·g^{-1} tissue	262±54	269±38	347±16	324±13
	(100)	(103)	(132)	(124)
n	49	64	11	9

EXPERIMENTAL RESULTS

It will be clear from Table 1 that the development of emotional–painful stress in the animals was accompanied by a significant decrease in the weight of the thymus and an increase in weight of the adrenals. The body weight of the rats fell from 204 ± 18 to 187 ± 16 g. This trend of the parameters, typical of the development of stress [1], persisted also during administration of PB (Table 1).

The content of cytochrome P-450 doubled after induction (Table 2). The development of emotional–painful stress in the group of animals receiving PB led to lowering of the cytochrome P-450 level by 18 nmoles/g tissues, or 26% of the initial concentration. This fall may be connected both with inhibition of cytochrome synthesis de novo and with its breakdown. A similar action of stress also was observed in the group of animals not receiving PB, in which the cytochrome P-450 level fell by 12 nmoles/g, i.e., by 36% (Table 2). This suggests that this lowering of the enzyme level was not connected with inhibition of induction, more especially since it evidently took place against the background of activation of protein synthesis. An indirect indication of intensification of biosynthesis in the liver cells was the rise of the RNA level both during stress and during induction by PB (Table 1). It can be seen that during the combined action of both factors the effect increased additively (Table 1). This is in agreement with known data on activation of protein synthesis in mammalian cells during exposure to stress [4, 6]. It can thus be postulated that the fall of the cytochrome P-450 level against the background of activation of protein biosynthesis in stressed rats is due to triggering of processes responsible for degradation of the enzyme, and it may perhaps reflect a specific response of the liver cells to stress.

In connection with the facts described above it was decided to study the degree of induction of cytochrome P-450 during the action of other stressor factors. As one such factor we used keeping the animals for a long time under conditions of moderate oxygen deficiency, for under these circumstances changes in weight of the thymus and adrenals and in the parameters of protein metabolism (data not given) coincided qualitatively with the time course of emotional–painful stress. It follows from Table 3 that adaptation of animals receiving PB to hypoxia led to a fall of the cytochrome P-450 level by 31.3 nmoles/g tissue. Just as in the case of emotional–painful stress, it can be postulated that the lowering of the enzyme level was connected either with inhibition of its biosynthesis or activation of its degradation.

The intensity of induction did not differ significantly in the adapted and unadapted animals, in which it was 275 and 329% respectively. This fact indicates the absence of any effect of adaptation on the intensity of induction of cytochrome. Moreover, in the group of animals not receiving PB, adaptation to hypoxia led to a small but statistically significant lowering of the cytochrome P-450 level by almost 5 nmoles/g tissue (Table 3). These data suggest that in case of adaptation to oxygen deficiency, just as in the case of emotional–painful stress, the lowering of the cytochrome P-450 level is connected with degradation of the enzyme.

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