COMPARATIVE STUDY OF SOME STRUCTURAL AND FUNCTIONAL INDICES OF THE LIVER AND ADRENAL CORTEX IN PHYSIOLOGICALLY NORMAL MICE OF DIFFERENT GENETIC LINES

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Interlinear differences with respect to morphometric characteristics of the subcellular organization of the hepatocytes and also to indices of adrenocortical function and rate of metabolism of steroid hormones in the liver were found in adult male CBA and C57BL mice. Steroid hormone production by the adrenals and the rate of their metabolism in the liver were found to be inversely proportional in the animals of the two lines. The pattern of structural and functional indices of the adrenal cortex and liver may perhaps be responsible for differences in the responses of the liver of animals of these two strains to pathogenic factors.

KEY WORDS: interlinear differences; liver; adrenals; steroid hormones.

The liver and adrenals function as a single system in the formation of responses to external factors. The unique character of the response of this system is evidently largely determined by the initial morphological and functional state of its organs and the characteristics of its regulatory interrelations, which are themselves largely determined by the genotype.

Young male CBA and C57BL mice have been shown to differ in the ultrastructural organization of their hepatocytes under normal conditions [6, 7]. Young and adult animals of these two lines also differ essentially in the response of the liver to CCl_4 and to partial hepatectomy [1, 4, 6-8].

The object of this investigation was to study to what extent the special ultrastructural features of the hepatocytes of young mice of the above strains are also characteristic of the adult animal. Adrenocortical function and the rate of metabolism of steroid hormones in the liver of adult animals also were investigated on the grounds that possible interlinear differences with respect to these indices could be responsible for differences in the response of their liver to experimental factors.

EXPERIMENTAL METHOD

Male CBA and C57BL mice aged 2 months and kept under identical conditions were used. The liver was taken from five animals of each strain for electron microscopy. Samples of liver were fixed in OsO_4 and embedded in Epon. A twin-grid closed test system was used for morphometry of the electron-microscopic images of the hepatocytes in accordance with published recommendations [14]. The rate of metabolism of steroid hormones in the liver was estimated from their disappearance from 1 ml medium containing 150 mM KCl, 10 mM Tris-HCl, pH 7.4, 1 mM NADPH, and 10 μg corticosterone or testosterone, after addition of 0.25 ml of 20% nucleus-free liver homogenate in 150 mM KCl, pH 7.4, and incubation for 20 min at 37°C. The reaction under these conditions was linear. The reaction was stopped with 0.5 ml 0.2 N H_2SO_4. Adrenal function was judged from corticosteroid production and the reaction for ACTH in vitro. The adrenals were removed and cut into four parts at 0-4°C. The glands from each animal were preincubated in 2 ml Krebs–Ringer–phosphate buffer, pH 7.4, with 0.2% glucose in an atmosphere of oxygen at 37°C for 45 min. The medium was then changed and the glands incubated under the same conditions for 1 h. After repeated change of medium the glands were incubated for a further hour with ACTH (1 i.u./ml). The concentration of 11-hydroxycorticosteroids (11-HCS) in the incubation media of the adrenals and of corticosterone and testosterone in the incubation media of the liver was determined by fluorometric methods [2, 3] on the Spekol spectrofluorometer (East Germany). Pro-

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TABLE 1. Relative Volume and Surface Area of Cytoplasmic Structures of Hepatocytes of Intact CBA and C57BL Mice (M ± m)

Parameter studied	Structure	C57BL mice	CBA mice
Vv	Mitochondria	28,0±1,70	26,4±1,10
Vv	Granular endoplasmic reticulum	17,80±0,97	17,3±0,90
Vv	Lysosomes	1,20±0,14	0,85±0,10*
Vv	Microsomes	1,40±0,10	1,10±0,09*
Vv	Lipids	3,80±0,91	2,50±0,50
Vv	Glycogen	6,80±1,00	14,2±1,60*
Sv	Mitochondria	1,25±0,07	1,74±0,06*
Sv	Granular endoplasmic reticulum	4,07±0,25	4,53±0,27

Legend: 1. Vv) Volume of cytoplasmic structure expressed in % of volume of cytoplasm; Sv) surface area of membranes of cytoplasmic structure per unit volume of cytoplasm (μ^2/μ^3). 2. Here and in Table 3, asterisk denotes $P < 0.05$ compared with C57BL mice.

TABLE 2. Density of Distribution of Ultrastructures in Hepatocytes of Intact CBA and C57BL Mice per μ^3 of Cytoplasm (M ± m)

Structure	C57BL mice	CBA mice
Free ribosomes	3724,0±279,2	4657,6±220,3 ($P<0,01$)
Attached ribosomes	4487,2±257,4	5236,1±263,6 ($P<0,05$)
Mitochondria	0,27±0,03	0,41±0,03 ($P<0,001$)

TABLE 3. Adrenocortical Function and Rate of Metabolism of Exogenous Corticosterone and Testosterone by Nucleus-Free Liver Homogenates of Intact CBA and C57BL Mice (M ± m)

Line of mice	Indices of adrenocortical function: 11-HCS production, μg/mg protein/h		Indices of liver function: rate of corticosterone metabolism, μg/mg protein/h	Indices of liver function: rate of testosterone metabolism, μg/mg protein/h
	without ACTH	with ATCH		
C57BL	5,52±1,25 (4)	37,04±10,59 (4)	1,20±0,10 (5)	1,61±0,37 (6)
CBA	1,91±0,49* (4)	6,48±0,39* (4)	2,13±0,21* (6)	3,06±0,52* (6)

Legend. Number of animals shown in parentheses.

tein in the homogenates of liver and adrenals was determined by Lowry's method [10]. Differences between the mean values compared were taken to be significant at the $P < 0.05$ level (Student's criterion).

EXPERIMENTAL RESULTS

Analysis of the morphometric measurements revealed interlinear differences in these characteristics of several cytoplasmic structures of the hepatocytes in the mice of the two lines (Table 1). Comparison of

these data with the results of previous investigations of hepatocyte ultrastructure in intact young animals of the same lines [6, 7] revealed changes in the indices of subcellular organization of their hepatocytes which were evidently connected with the increase in age of the animals. Meanwhile some features distinguishing the subcellular organization of the parenchymatous cells of the young animals, reflecting the higher functional capacity of the hepatocytes of CBA mice than of young C57BL mice, also were found to apply to the adult animals. The number of attached and free ribosomes in the hepatocytes of the two strains of mice, which can be regarded as reflecting the intensity of protein synthesis in the cell, were most demonstrative in this respect. For instance, the higher content of ribosomes in the hepatocytes of CBA mice than of C57BL mice probably reflects the higher level of protein synthesis in the parenchymatous cells of the liver of the former strain, as is confirmed by the biochemical data [13]. The results of morphometry of the mitochondria (Table 1) may also definitely reflect the higher functional capacity of the hepatocytes of CBA than of C57BL mice. For equal relative volumes of mitochondria, their number and their surface area per unit volume of cytoplasm were greater for CBA mice. The level of biosynthesis of corticosteroids in the adrenals was considerably higher in the C57BL mice, especially in the presence of ACTH (Table 3). This reflects the considerable potential capacity of the adrenals of C57BL mice under conditions of stress.

One of the general manifestations of the action of corticosteroids on tissue is their ability to depress cell division, especially division of hepatocytes. Under the conditions of stress, after partial hepatectomy in young and adult C57BL mice very high mortality and much lower mitotic activity of the hepatocytes was observed than in CBA mice [1, 4]. Together with high figures for adrenal function in C57BL mice, the lower potential capacity of their hepatocytes for protein synthesis may also perhaps be one of the reasons why restoration of the weight of the liver in these animals is delayed and their mortality is high after partial hepatectomy. In C57BL mice, parallel with the high potential capacity of their adrenals, low ability of their liver tissue to metabolize corticosterone and testosterone was observed. A low level of adrenal function and a high rate of metabolism of steroid hormones in the liver were found in the CBA mice (Table 2). These differences in the pattern of hormonal homeostasis reflect differences in hormone–tissue relations in normal animals of these lines. The reciprocal relations between the intensity of steroid hormone metabolism in the liver and the potential functional capacity of the adrenals in CBA and C57BL mice can be regarded as a manifestation of the action of autoregulatory tissue mechanisms stimulating their response to hormonal influences. Observations showing a decrease in the number of receptors of steroid hormones during prolonged administration of these substances in high doses [5] also suggest similar mechanisms.

The patterns of structural and functional indices and of functional interrelations of the liver and adrenals found in the mice of these two lines could be of great importance in determining the outcome of pathological processes associated with exposure to various harmful factors. For instance, CBA mice in particular, characterized by higher activity of metabolism of steroid hormones and higher ability of their liver to synthesize proteins, as well as by a lower level of production of steroid hormones than C57BL mice, are distinguished by much lower resistance to induction of hepatomas by CCl_4 and 9,10-dimethyl-1,2-benzanthracene [8, 9]. The oncogenic action of these substances is largely determined by the formation of their active metabolites in the course of their "detoxication" in the liver [11, 12]. Their low range of metabolism of steroid hormones are resistant to induction of hepatomas, but during acute CCl_4 poisoning the parenchyma of their liver is more severely damaged than that of CBA mice [6, 7].

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