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Significance of Adenylate Cyclase System of the Liver in Its Chronic Diseases

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 124, No. 4, pp. 450-453, April, 1998
Original article submitted April 16, 1997

The activity of adenylate cyclase in the hepatocyte plasma membranes, content of cAMP, and cAMP/cGMP ratio in the liver and blood plasma are decreased in patients with chronic liver diseases (fatty dystrophy, chronic hepatitis, and cirrhosis). This decrease depends on the disease severity and is most pronounced in cirrhosis. The sensitivity of liver adenylate cyclase to insulin and glucagon is changed. It is concluded that disorders in the adenylate cyclase system are an important molecular mechanism in the pathogenesis of chronic liver diseases.

Key Words: *adenylate cyclase; cyclic nucleotides; chronic diseases of the liver*

The significance of the adenylate cyclase system for functional activity of the liver in health and disease has not been evaluated. Cyclic nucleotides, primarily cyclic adenosine monophosphate (cAMP), actively participate in biochemical processes regulated by the liver: regulation of cell metabolism, realization of hormone effects on lipid and carbohydrate metabolism, protein production, and modification of cell growth and differentiation [8,10]. There are some contradictory reports about changes in the activity of membrane-bound cAMP adenylate cyclase (AC) and the levels of cyclic nucleotides in the liver during chronic diseases in rats [11]. Patients with chronic diseases of the liver develop metabolic and hormonal disorders whose biochemical mechanisms are still unknown.

Our purpose was to investigate AC system of the liver in man in health and chronic disease and to elucidate the role of this system in the disease development.

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MATERIALS AND METHODS

A total of 125 patients with chronic diseases of the liver (fatty dystrophy, chronic hepatitis, and cirrhosis of different etiology and severity) were examined. The diagnosis was made on the basis of comprehensive examinations including general clinical biochemical, morphological, x-ray, and ultrasonic studies. Control group consisted of 12 subjects without gastrointestinal disease or liver involvement, according to morphological studies. Adenylate cyclase (EC 4.6.1.1) activity in plasma membranes of liver cells was assayed by the radioisotope method [4]. The incubation medium contained 50 mM Tris-HCl (pH 7.5), 30°C, 5 mM $MgCl_2$, 0.5 mM cAMP, 0.5 mM isobutylmethylxanthine, 0.1 mM ATP (0.5 μCi ^{32}P-ATP), 20 mM creatine phosphate, 0.2 mg/ml creatine kinase, 0.1 mM GTP. The reaction was triggered by adding 10-50 mg protein. The duration of incubation was 20 min at 30°C. cAMP and cGMP in liver homogenates and blood plasma were radioimmunoassayed

0007-4888/98/0004-0399$20.00

Table 1. AC Activity in the Liver of Patients with Chronic Diseases (pmole/mg Protein/min, *M±m*)

Group of patients	AC activity
Control	18.60±0.70
Fatty dystrophy of the liver	13.90±0.96
Chronic virus hepatitis(HBsAg⁺)	12.82±0.83*
Cirrhosis of the liver:	
primary biliary	4.94±0.58**
virus (HBsAg⁺)	4.51±0.69**
alcoholic	4.07±0.25**

Note. Here and in Table 2: *p<0.05, **p<0.01 *vs.* the control.

using standard kits (Amersham). Total protein content in liver homogenates and hepatocyte plasma membranes was measured by Lowry's method. Radioactivity was determined in a Mark-II scintillation counter. Results were statistically processed using Student's t test.

RESULTS

Normally (in the absence of essential morphological changes, control group), AC activity in hepatocyte plasma membranes is rather high (Table 1). Its absolute values coincide with results of others [15]. The data presented in Table 1 indicate a significant decrease of AC activity in the majority (72%) of patients with chronic diseases, the degree of this decrease depending mainly on the severity of disease, being most pronounced in cirrhosis, particularly of viral and alcoholic etiology. On the other hand, in some patients, mainly with chronic moderate hepatitis of unknown origin, AC activity did not change (in 10%) or slightly increased (in 12%). The content of cAMP was decreased in the liver and plasma of examined patients (Table 2).

Liver and plasma levels of cAMP were increased in about 25% patients with fatty dystrophy. This is probably characteristic of the initial stage of liver involvement and is in line with the data on increased cAMP content in the liver in acute experimental diseases in rats [16].

The decrease in plasma cAMP was the greatest in viral (HBsAg⁺) and alcoholic cirrhosis of the liver during exacerbation: 16.3±0.4 and 14.7±1.32 pmole/ml (p<0.05) *vs.* 24.3±0.95 pmole/ml in the control.

The decrease in cAMP level in the majority of patients was probably due to the drop in AC activity. A decrease in plasma cAMP content in chronic involvement of the liver reflects similar changes in liver tissue. A probable explanation of disorders in the production and content of cAMP is changed count of cells producing these substances because of damage to pathologically changed liver (development of fibrosis, necrosis, etc.). The mechanisms of a decrease in the activity of AC system in hepatocyte plasma membranes and in cAMP production in, for instance, chronic alcoholic intoxication include shifts in the efficacy of b-adrenergic receptors [13], redistribution of regulating GTP-bound proteins and inhibition of cAMP-related signals [9], and changed ATP level [12].

The content of cGMP in the liver and blood plasma increased from 30% in chronic hepatitis to 50% in cirrhosis, this increase being statistically insignificant because of individual differences (5-15 pmole/ml in the plasma). Association between changes in cAMP and cGMP production and the disease severity brought us to conclusion about the significance of cyclic nucleotides in the pathogenesis of chronic diseases of the liver. The cAMP/cGMP ratio in the liver and plasma was decreased (Table 2), which reflects disorders in the activity of liver cyclase systems in disease. The decrease in this ratio depends on the activity of the pathologic process and is most expressed in cirrhosis, which recommends this coefficient for specifying the diagnosis and assessing the severity of liver involvement.

One of the main factors affecting the activity of AC are hormones, including the pancreatic, whose effects on the liver are mediated by AC: in health insulin inhibits AC and glucagon stimulates it [1]. *In vitro* studies demonstrated that in the absence of morphological changes in the liver insulin (10^{-6} M) inhibits AC activity by 50% (Fig. 1). In chronic hepatitis, the sensitivity of the enzyme to inhibitory effect of insulin was retained: its level decreased 2.3 times. On the other hand, in primary biliary cirrhosis of the liver, the activity of AC changed negligibly under the action of insulin: the enzyme reaction to

Table 2. Content of Cyclic Nucleotides in Liver Tissue in Chronic Diseases (pmole/mg protein, *M±m*)

Group of patients	CAMP	CGMP	cAMP/cGMP
Control	13.65±0.89	8.08±0.89	1.69±0.42
Fatty dystrophy of the liver	8.93±0.54*	9.81±0.96	0.91±0.20
Chronic virus hepatitis (HBsAg⁺)	8.32±0.45*	10.49±0.61	0.79±0.16
Virus cirrhosis of the liver (HBsAg⁺)	7.02±0.32**	11.63±1.76	0.60±0.12

this hormone was markedly reduced. Unlike insulin, glucagon (10^{-6} M) activated AC in normal liver, the degree of this activation being lower (by 50%) in chronic active hepatitis and the lowest in cirrhosis.

Thus, we have detected changes in the sensitivity of liver AC to hormones in chronic liver diseases, which are probably caused by disorders of receptor reactions as a result of damage to hepatocyte membranes and changes in their lipid layer. These findings prove that desensitization occurs in this disease, consisting in decreased hepatocyte AC capacity to respond to hormones, which eventually leads to a decrease in liver cAMP level. In accordance with modern concepts, desensitization is an important process in regulation of AC activity [5-7].

Our results demonstrate disorders in AC system of the liver, which may be an important component of molecular mechanisms of liver diseases. Presumably, not only the relationship between hormone receptors and nucleotide-binding protein of the enzyme is disordered, but the patterns of reaction between the regulatory and catalytic components of AC complex are changed [3]. These data and our previous report about normalization of cAMP and cGMP content in the plasma of patients with chronic hepatitis during drug therapy are in good correlation with the protective effects of synthetic cAMP derivatives in the liver [14]. The parallelism between changes in AC system and degree of liver involvement and the development of hormonal and metabolic disorders point to important role of AC system in the pathogenesis of chronic liver diseases.

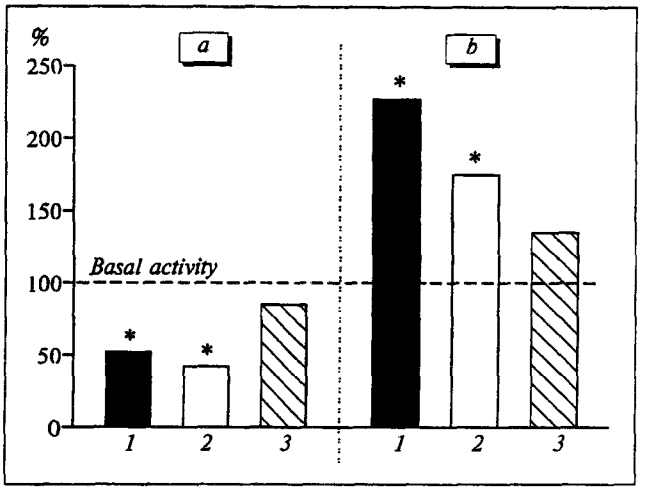

Fig. 1. Activity of adenylate cyclase in the liver of patients with chronic diseases during incubation with insulin (*a*) and glucagon (*b*). *1*) control; *2*) chronic viral hepatitis (HBsAg⁺); *3*) primary biliary cirrhosis of the liver. Ordinate: adenylate cyclase activity, % of basal activity. *$p<0.05$ *vs.* basal activity.

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