ADRENERGIC RECEPTORS IN THE LIVER PARENCHYMA OF CHILDREN WITH CHRONIC HEPATITIS

T. Ya. Kondratenko, N. V. Kuzina, I. V. Zakharova, A. F. Leont'ev, D. D. Pashkevich, V. M. Senyakovich, A. E. Aleksandrov, and S. A. Klochkov

UDC 616.37-002.2-053.2-092-07

KEY WORDS: liver parenchyma; chronic hepatitis; adrenergic control.

Adrenergic receptors of the liver parenchyma, located on the outer surface of hepatocytes, mediate the physiological effects of neurotransmitters, hormones, and other biologically active substances, and at the same time regulate the function of the liver [7, 8, 15, 17]. According to data obtained by several workers, adrenergic control of liver metabolism is disturbed in some pathological states [11, 18, 19].

Currently the role of receptors in the development of liver pathology, and in particular, of β- and α-adrenergic systems in cirrhosis, in various forms of portal hypertension, and in hepatomas, is being intensively studied in several laboratories [2, 6, 12, 16, 20]. However, receptors of the liver parenchyma in children have virtually not been studied. In the present investigation we used ^{3}H-prazocin (^{3}H-PRZ), an α_1-adrenergic receptor antagonist, to identify α_1-adrenergic receptors, and the β-adrenergic radioligand ^{3}H-dihydroalprenolol (^{3}H-DHA) to analyze β-adrenergic receptors of the hepatic parenchyma in children.

The aim of this investigation was to compare the β- and α_1-adrenergic receptors of the liver without any damage to the parenchyma and in children with chronic hepatitis.

EXPERIMENTAL METHOD

Samples were obtained during biopsy operations on the liver by the marginal resection method, on children aged from 2 to 14 years with the extrahepatic form of portal hypertension (EHPH) without damage to the parenchyma (control group, n = 7) and with damage to the parenchyma (a group of patients with chronic hepatitis, n = 6).

The diagnosis was made on the basis of morphological and electron-microscopic analysis, taking account of clinical-biochemical, virologic, and immunologic data.

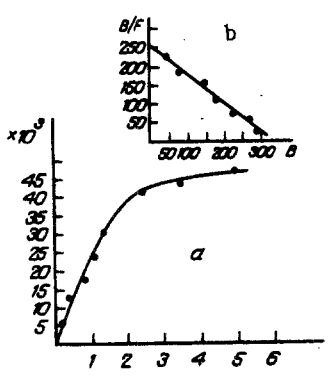
Samples after removal were quickly frozen in liquid nitrogen and kept at −70°C.

The membranes of the liver were isolated by the method described in [3]. The tissue was homogenized in buffer containing 0.25 M sucrose, 1 mM EDTA, and 10 mM Tris-HCl, pH 8.0, in a Polytron homogenizer (USA). The homogenate was centrifuged ("Beckman" I2-21, USA) for 20 min at 2000*g* and the resulting supernatant was centrifuged for 60 min at 32,000*g*. The residue was resuspended in 40 ml buffer containing 50 mM Tris-HCl, pH 7.4 and 10 mM $MgCl_2$, and centrifuged under the same conditions. The final residue was suspended in 10 ml buffer and kept at −70°C until required for analysis of binding.

Analysis of binding of ^{3}H-DHA with β-adrenoreceptors was carried out by incubating membrane proteins (100 μg) in a total volume of 500 μl with 50 mM Tris-HCl, pH 7.4, 10 mM $MgCl_2$, and 0.1-5 nM ^{3}H-DHA (70 Ci/mmole, Amersham, England) for 20 min at 30°C. Free and membrane-bound ligand was separated by filtration of the samples on GF/C filters ("Whatman," England). The filters were washed with cold buffer (4°C; 3 times, 3 ml each time), dried, and counted

Research Center for Molecular Diagnosis, Ministry of Health of the USSR, Moscow Research Institute of Pediatrics, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR M. Ya. Studenikin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 113, No. 2, pp. 127-129, February, 1992. Original article submitted July 17, 1991.

 0007-4888/92/0002-0158$12.50



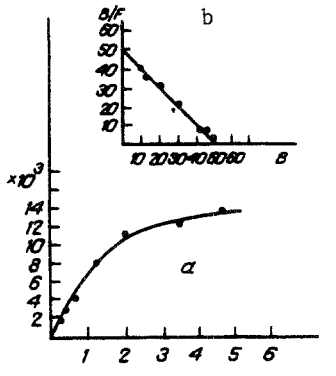


Fig. 1 Fig. 2

Fig. 1. Specific binding of ^{3}H-DHA by membranes of the hepatic parenchyma in children (control group). a) Specific binding of ^{3}H-DHA; abscissa: concentration of ligand (nM), ordinate: specific binding of ^{3}H-DHA (in cpm/mg protein); b) Scatchard plot, abscissa: B) bound ^{3}H-DHA (fmoles/mg protein), ordinate: B/F) ratio of bound to free ^{3}H-DHA (fmoles/mg/nanomole).

Fig. 2. Specific binding of ^{3}H-DHA by membranes of hepatic parenchyma in children (chronic hepatitis group). a) Specific binding of ^{3}H-DHA, abscissa: concentration of ligand (nM), ordinate: specific binding of ^{3}H-DHA (in cpm/mg protein); b) Scatchard plot, ordinate B/F) ratio of bound to free ^{3}H-DHA (fmoles/mg/nanomole), abscissa: B) bound ^{3}H-DHA (fmoles/mg protein).

on a "RackBeta" liquid radiospectrometer ("LKB," Sweden), with a counting efficiency of 40%. Specific binding of ^{3}H-DHA was determined as the difference between total binding in the absence of 1-alprenolol ("Sigma," USA) and nonspecific binding in the presence of 10 μM alprenolol.

Analysis of binding of ^{3}H-PRZ membrane proteins (200 μg) were incubated in total volume of 1000 μl with 50 mM Tris-HCl, pH 7.4, 10 mM $MgCl_2$, and 0.05-8 nM ^{3}H-PRZ (85 Ci/mmole, "Amersham," England) for 20 min at 25°C. Specific binding of ^{3}H-PRZ was determined as the difference between binding in the presence and absence of 10 μM prazocin ("Sigma," USA).

The protein concentration was determined by Bradford's method [1], using bovine serum albumin as the standard. Parameters of receptor binding (K_d – dissociation constant, and B_{max} – maximal number of binding sites) was carried out by Scatchard plot [14].

EXPERIMENTAL RESULTS

Binding of ^{3}H-DHA by membranes of the hepatic parenchyma was of saturable and high-affinity type both in control samples and in material from patients with chronic hepatitis (Figs. 1 and 2). Nonspecific binding of ^{3}H-DHA in the presence of 10 μM alprenolol in the control samples amounted to 25% of total binding with the same concentrations of ligand (0.1-5 nM); the parameters of binding, determined by Scatchard plot, were as follows: K_d = 1.2 ± 0.5 nM, B_{max} = 261.2 ± 50 fmoles/mg protein; mean values ±SEM.

Binding of the α_1-adrenergic radioligand ^{3}H-PRZ by membranes of the hepatic parenchyma also was of the saturable and high-affinity type both in the control group and in the chronic hepatitis group. Scatchard plot analysis revealed the following binding parameters in the control samples: K_d = 0.6 ± 0.12 nM, B_{max} = 92.8 ± 8.0 fmoles/mg protein (Table 1). The results are in agreement with data in the literature for binding of ^{3}H-DHA and ^{3}H-PRZ in the rabbit and bovine liver [19].

TABLE 1. Characteristics of Adrenergic Receptors of Hepatic Parenchyma in Children

	Control (n = 7)	Chronic hepatitis (n = 6)
β-Adrenoreceptors (^{3}H-DNA)		
B_{max}(fmoles/mg)	261,7±50	68,5±18,8
K_d (nM)	1,2±0,5	0,9±0,15
α_1-Adrenoreceptors (^{3}H-PRZ)		
B_{max}(fmoles/mg)	92,8±8,0	195,0±22,0
K_d (nM)	0,6±0,12	0,8±0,15

The affinity of binding of ^{3}H-DHA by membranes of the hepatic parenchyma in material from chronic hepatitis was virtually indistinguishable from the control, whereas the number of binding sites was considerably reduced (Table 1). The parameters of binding of ^{3}H-PRZ in chronic hepatitis did not differ significantly from the control (Table 1).

Changes in adrenergic receptors have been demonstrated both on rat liver cells after partial hepatectomy [5], hepatoma cells [16], and human peripheral blood mononuclears in a severe form of cirrhosis accompanied by ascites [9].

In the present investigation changes in β- and α_1-adrenergic receptors in the hepatic parenchyma were found in children with chronic hepatitis. It was shown that the concentration of β-adrenergic receptors falls significantly in the hepatic parenchyma in chronic hepatitis compared with the normal control. A significant decrease in the concentration of β-adrenergic receptors may perhaps be a specific indicator of a chronic parenchymatous lesion of the liver, reflecting the depressed compensatory properties of the hepatocyte: according to the observations of Huerta–Bahena and Sandnes et al., the β-adrenoreceptor level in the hepatocytes of the regenerating rat liver is increased [10, 13]. The very small increase which we found in the level of α_1-adrenergic receptors in the hepatic parenchyma in chronic hepatitis may perhaps be linked with their involvement in the regulation of hepatocyte growth during regeneration of the liver: according to some observations, blocking α_1-adrenoreceptors with prazocin causes depression of regeneration [4].

The results thus suggest an important role for adrenergic receptors in the pathogenesis of chronic parenchymatous liver disease in children and in the modulation of regenerative processes in the liver.

LITERATURE CITED

1. M. M. Bradford, Analyt. Biochem., **72**, 248 (1976).
2. C. N. D'Arville, M. Le, T. M. Kloppel, et al., J. Hepatol., **9**, 6 (1989).
3. M. P. G. Crom, M. J. Van Haperen, P. Pucheis, et al., Int. J. Biochem., **21**, No. 6, 649 (1989).
4. J. L. Cruise, S. J. Knechtle, R. R. Bollinger, et al., Hepatology, **7**, 1189 (1987).
5. J. L. Cruise, S. J. Muga, Lee Yi, et al., J. Cell Physiol., **140**, No. 2, 195 (1989).
6. S. A. Cummings, A. S. Kaumann, and R. J. Groszmann, Hepatology, **8**, No. 5, 1112 (1988).
7. J. H. Exton, Molec. Cell. Endocrinol., **23**, 233 (1981).
8. J. H. Exton, Am. J. Physiol., **248**, E633 (1985).
9. A. L. Gerbes, J. Remien, and D. Jungst, Lancet, **1**, 1409 (1986).
10. J. Huerta-Bahena, R. Villalobos-Molina, and A. Garcia-Sainz, Biochem. Biophys. Acta, **763**, 112 (1983).
11. G. Kunos, Trends. Pharmacol. Sci., **5**, 380 (1984).
12. R. Mastai, J. Bosch, M. Navasa, et al., J. Hepatol., **4**, 72 (1987).
13. D. Sandnes, T. E. Sand, G. Sager, et al., Exp. Cell Res., **165**, 117 (1986).
14. G. Scatchard, Ann. New York Acad. Sci., **51**, 660 (1949).
15. P. H. Schmelck and J. Hanoune, Molec. Cell. Biochem., **33**, 35 (1980).
16. F. Senae, K. A. Miyamoto, and R. R. Koshiura, Cancer Lett., **39**, 267 (1988).
17. G. L. Stiles, M. G. Caron, and R. J. Lefkowitz, Physiol. Rev., **64**, 661 (1984).
18. H. Storm, C. Van Hardeveld, and A. H. Kassenaar, Biochim. Biophys. Acta, **798**, 350 (1984).
19. S. J. Sulakhe, V. B. Pulga, and S. Tran, Molec. Cell. Biochem., **83**, 81 (1988).
20. D. Valla, C. Gaudin, P. Geoifroy, et al., J. Hepatol., **4**, 86 (1987).