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α_1-ADRENERGIC RECEPTORS IN THE LIVER PARENCHYMA IN CHILDREN: CHANGES ASSOCIATED WITH CIRRHOSIS

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

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An important role is nowadays ascribed to adrenergic systems in the development of several liver diseases [7, 9-12]. β- and α-adrenergic receptors and their agents are involved in the pathogenesis of severe forms of cirrhosis of the liver, hepatomas, and certain forms of portal hypertension [6, 8, 13, 15, 16]. However, receptors of the liver parenchyma in children have virtually not been studied. In preliminary experiments a very small increase was found in the number of α_1-adrenergic receptors in the liver parenchyma of children with chronic hepatitis, possibly due to the participation of these receptors in the regulation of hepatocyte growth during regeneration of the liver [4, 5].

The aim of this investigation was to study α_1-adrenergic receptors of the liver parenchyma in children with cirrhosis and to establish the role of these receptors in the development of parenchymatous liver damage.

EXPERIMENTAL METHOD

Specimens were obtained during surgical biopsy of the liver by the marginal resection method, performed on children aged from 2 to 14 years with an extrahepatic form of portal hypertension (EHPH) without parenchymatous damage (control group, n = 7) and with parenchymatous damage (group of patients with cirrhosis, n = 8).

The diagnosis was based on morphological and electron-microscopic analysis, together with consideration of clinical, biochemical, virological, and immunological data. After removal the specimens were quickly frozen in liquid nitrogen and stored at −70°C. The liver membranes were isolated by the method in [3]. The tissue was homogenized in buffer containing 0.25 M sucrose, 1 mM EDTA, 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 of buffer containing 50 mM Tris-HCl, pH

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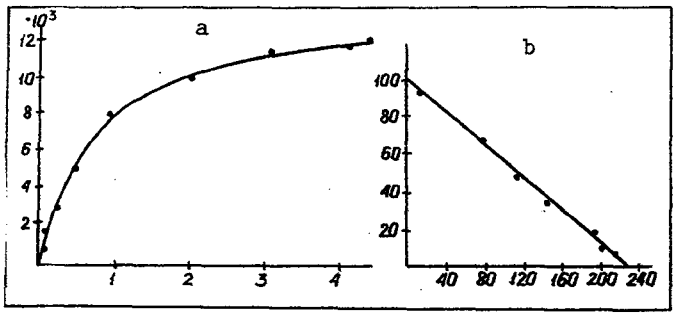

Fig. 1. Specific binding of ^{3}H-PRZ by membranes of liver parenchyma in children (group with cirrhosis): a) specific binding of ^{3}H-PRZ. Abscissa, concentration of ligand (in nM); ordinate, specific binding of ^{3}H-PRZ (in cpm/mg protein); b) Scatchard plot. Abscissa, B) bound ^{3}H-PRZ (in fmoles/mg protein); ordinate: B/F – ratio of bound ^{3}H-PRZ to free (in fmoles/mg/nM).

7.4, 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 binding analysis. Binding of the β-adrenergic radioligand ^{3}H-dihydroalprenolol (^{3}H-DHA) by membranes of the liver parenchyma was analyzed by incubation of 100 μg protein in a total volume of 500 μl with 50 mM Tris-HCl, pH 7.4, 10 mM $MgCl_2$, 0.1-5 nM 3H-DHA (70 Ci/mmole, "Amersham," England) for 20 min at 30°C. Free and membrane-bound ligand were separated by filtration on GF/C filters ("Whatman," England). The filters were washed with cold buffer (4°C, three times, 3 ml each time), dried, and counted on a "Rack-Beta" liquid radiospectrometer (LKB, Sweden), with 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 the α_1-Adrenergic Ligand ^{3}H-Prazosin (^{3}H-PRZ). Membrane proteins (200 μg) were incubated in a total volume of 1000 μl with 50 mM Tris-HCl, pH 7.4, 10 mM $MgCl_2$, 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 in the absence of 10 μM prazosin (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) were determined on a Scatchard plot [14].

EXPERIMENTAL RESULTS

The study of the adrenergic systems in different forms of cirrhosis and, in particular, of β_2-adrenoreceptors of human peripheral blood mononuclears in a severe form of cirrhosis, has been undertaken in several laboratories [2, 9, 12, 17]. However, adrenergic receptors of the liver in children with different forms of parenchymatous damage (chronic hepatitis, cirrhosis) have virtually not been studied. In the present investigation we studied a α_1-and β-adrenergic receptors of the liver parenchyma in children with cirrhosis.

Binding of ^{3}H-PRZ by membranes of the liver parenchyma in cirrhosis was saturating, and characterized by high affinity (Fig. 1). The high-affinity region of binding of ^{3}H-PRZ was characterized by the following parameters, determined on a Scatchard plot: $K_d = 1.5 \pm 0.4$ nM, $B_{max} = 254.1 \pm 28.4$ fmoles/mg protein (mean values ± standard error of the mean). The affinity of ^{3}H-PRZ binding in cirrhosis was virtually the same as in the control, but the number of binding sites was increased much more than in chronic hepatitis (Table 1).

According to some workers [4, 5] α_1-adrenoreceptors take part in regulating growth of the hepatocytes during regeneration of the liver. For instance, it was shown that blocking α_1-adrenergic receptors by prazosin causes reduction of regeneration [4]. The significant increase in the concentration of α_1-adrenergic receptors which we found in the liver parenchyma in cirrhosis may perhaps be connected with stimulation of regeneration of necrotic tissue, possessing enhanced compensatory properties, which are much weaker in chronic hepatitis.

TABLE 1. Characteristics of Binding of ^{3}H-PRZ in Liver Parenchyma of Children

Binding parameters	Control	Chronic hepatitis	Cirrhosis
B_{max}, fmoles/mg protein	92,8±8,0	195,0±22,0	254,1±28,4
K_d, nM	0,6±0,12	0,8±0,15	1,5±0,4

TABLE 2. Characteristics of Binding of ^{3}H-DHA in Liver Parenchyma of Children

Binding parameters	Control	Chronic hepatitis	Cirrhosis
B_{max}, fmoles/mg protein	261,7±50,0	68,5±18,8	216,2±36,0
K_d, nM	1,2±0,5	0,9±0,15	1,3±0,27

Binding of the β-adrenergic ligand ^{3}H-DHA in the liver parenchyma in cirrhosis also was saturating and characterized by high affinity; the binding parameters of ^{3}H-DHA were virtually indistinguishable from the control, unlike the value of B_{max}, which was significantly lower in chronic hepatitis (Table 2).

The present study thus shows that the number of α_1-adrenergic receptors in the liver parenchyma of children is much greater in cirrhosis than in the control, whereas the number of β-adrenergic receptors is virtually unchanged.

These results may be evidence that α_1-adrenergic receptors play an important role in the development of cirrhosis of the liver in children, and reflect the degree of parenchymatous damage of the liver and its compensatory properties.

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