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CHANGES IN β-ADRENORECEPTOR DENSITY IN INTACT LYMPHOCYTES OF HYPERTENSIVE SUBJECTS

T. L. Krasnikova, V. A. Radyukhin, O. B. Il'inskii, Academician I. K. Shkhvatsabaya*, E. V. Parfenova, and Yu. I. Suvorov

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Increased activity of the sympathicoadrenal system is considered to be a trigger mechanism of essential hypertension [4]. The influence of the sympathicoadrenal system of the hemodynamics is exerted through the action of adrenergic mediators and hormones on α- and β-receptors of cells of target organs (heart, blood vessels). More recently, during a study of receptor characteristics of cells of different organs, human peripheral blood lymphocytes have been extensively used as a cell model to reflect the state of the receptors of these cells.

In the case of essential hypertension determination of receptor characteristics of lymphocytes is very important for our understanding of the cellular mechanisms controlling sensitivity to the action of catecholamines and it is of undoubted practical concern.

The aim of this investigation was to determine the density of β-adrenoreceptors of intact lymphocytes from patients with stable essential hypertension and to compare it with that of normal subjects.

EXPERIMENTAL METHOD

A group of patients with stage IIB of essential hypertension (according to the classification in [1]), consisting of 10 men aged from 32 to 44 years, whose diastolic blood pressure (BP) was at or above 110 mm Hg, was chosen for the investigation. For 2 weeks before the tests the patients received no hypotensive drugs including β-blockers. The control group consisted of eight normal men aged from 35 to 42 years (BP below 140/90 mm Hg). Lymphocytes were isolated from heparinized venous blood by centrifugation in a Ficoll–Verografin density gradient [3]. The lymphocyte fraction was washed with phosphate buffer and medium 199 with 0.03% solution of human serum albumin, the erythrocytes were hemolyzed, and the lymphocytes were finally freed from platelets and cell fragments by washing on a column with buffer con-

*Academy of Medical Sciences of the USSR.

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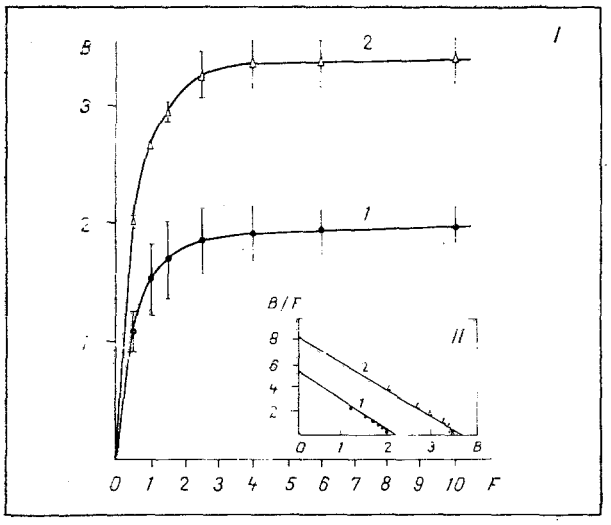

Fig. 1. Specific binding of [^{3}H]-DNA within intact lymphocytes under normal conditions (1) and in essential hypertension (2). I) Equilibrium binding with intact lymphocytes as a function of concentration of ligands ($\bar{x} \pm S_m$; data for three experiments). Abscissa (F): concentration of free [^{3}H]-DHA (in nM); ordinate (B): concentration of bound [^{3}H]-DHA (in fmoles/10^6 cells); II) Scatchard plot analysis. Abscissa (B) concentration of bound [^{3}H]-DHA (in fmoles/10^6 cells); ordinate (B/F) ratio of concentration of bound to free [^{3}H]-DHA. Maximal number of binding sites per 10^6 cells (B_{max}) in normal subjects 2.10 fmoles, number of receptors N = 1264, K_d = 0.39. In hypertension B_{max} = 3.69 fmoles, N = 2220, K_d = 0.39.

taining sorbitol. The content of mononuclear lymphocytes was about 90% and more than 95% of cells did not stain with trypan blue. The cells were kept at 4°C until required for analysis. Density of β-adrenoreceptors was determined in a suspension of intact cells [13] with [^{3}H]dihydroalprenolol ([^{3}H]-DHA, 60 Ci/mmole, from Amersham Corporation, England) in medium 199 (20 mM HEPES), pH 7.40, in seven different concentrations of [^{3}H]-DHA: from 0.5 to 10 mM. Nonspecific binding was determined in the presence of 2×10^{-6} M L-alprenolol (from Sigma, USA). Total and nonspecific binding were carried out with the addition of 10^{-4} M phentolamine (from Ciba Geigy, Switzerland), which considerably reduces nonspecific incorporation of [^{3}H]-DHA into cell lysosomes [13]. If the [^{3}H]-DHA concentration was less than or equal to 2 nM nonspecific binding was between 25 and 50%. The cells (2.5×10^6-4.5×10^6 per sample) were incubated with [^{3}H]-DHA for 15 min at 37°C, the reaction was stopped with 2 ml of medium 199 (4°C), and the cell suspension was quickly filtered on "Whatman" GF/c filters and then washed with 10 ml of medium 199 (4°C). The filters were counted in 5 ml of Bray's mixture on a Rack-Beta 1215 scintillation counter (LKB, Sweden). Specific binding was determined as the difference between total and nonspecific binding, and the density of the receptors (B_{max}) and dissociation constant (K_d) were determined on Scatchard plots. The results were subjected to statistical analysis ($P \leq 0.05$).

EXPERIMENTAL RESULTS

Specific binding of [^{3}H]-DHA with intact lymphocytes under the above conditions was characterized by saturation and high affinity (Fig. 1). Linearity on Scatchard plots indicated a homogeneous receptor population (r = 0.97 under both normal and hypertensive conditions). The lymphocyte receptor density, as shown by the results of analysis was 2.41 ± 0.29 fmoles/10^6 cells normally, equivalent to N = 1461 ± 175 receptors per cell; the hypertension B_{max} = 3.45 ± 0.35 fmoles/10^6 cells, N = 2076 ± 208; in normal subjects K_d varied from 0.31 to 1.67 mM, in hypertension from 0.30 to 1.88 mM (the variability of K_d was perhaps due to individual differences between subjects).

The results are evidence of a statistically significant increase (by 42%) in β-adrenoreceptor density of intact lymphocytes in essential hypertension. When the present investigation began no study of this problem had been published. Only recently two publications

with different results appeared, describing analysis of receptor binding on membrane preparations [7, 11]. Destruction of the cells and purification of the membrane fractions were accompanied by changes in receptor characteristics [13], and for that reason undertaking investigations with membrane preparations does not correspond sufficiently closely to physiological conditions. In the work of Doyle et al. [7] and Landmann et al. [11] considerable variation in the number of lymphocyte β-receptors was observed under normal conditions and in hypertension; whereas Doyle et al. [7] found no difference between normal and hypertension, Landmann et al. [11] observed a small increase in receptor density in essential hypertension. The considerable scatter and contradictory nature of the data were due in all probability to the use of membrane fractions and to the fact that the investigations were conducted on persons of both sexes and over a wise range of ages.

The increase in β-adrenoreceptor density of the lymphocytes in essential hypertension at first glance contradicts the current view on the character of changes in β-receptors in this disease [4]. The decrease in receptor density postulated in hypertension and aging on cells of blood vessels and the heart has not yet been demonstrated except in experiments on animals with the experimental genetic form of arterial hypertesnion (spontaneously hypertensive rats) [12]. Positive correlation has not been found between changes in β-receptor density on lymphocytes and the decrease in sensitivity to adrenergic agents in hypertension and aging. In the presence of reduced sensitivity through adrenergic influences, receptor density either was unchanged [2, 7] or was actually increased [10, 11] compared with normal. The only exception is one work [15] which arouses serious misgivings on technical grounds.

The absence of positive correlation can evidently be attributed to the fact that desensitization of target cells did not take place on account of a decrease in β-receptor density. This hypothesis is supported by the decrease in adenylate cyclase sensitivity of human lymphocytes [8] and rat heart [9] during aging. Density of cardiac β-receptors of old rats is about 48% higher than in young animals; stimulation of adenylate cyclase by isoproterenol and activation by guanyl nucleotide in old animals are lower by 34 and 38% respectively [9], i.e., desensitization does not occur because of loss of β-receptors.

Possibly in essential hypertension also the decrease in sensitivity to the action of β-adrenoreceptor agonists and antagonists is due not to a decrease in β-receptor density, but to changes in other components of the adenylate cyclase system.

The decrease in the number of β-adrenoreceptors in the heart and blood vessels of spontaneously hypertensive rats [12] suggests the existence of different mechanisms of desensitization in forms of hypertension so different in their etiology as genetic hypertension in animals and essential hypertension in man.

Only by studying the characteristics of the receptor system as a whole (receptor — adenylate cyclase — regulatory protein) of both cells of the cardiovascular system and lymphocytes will it be possible to decode the mechanisms of onset of pathology and cellular levels.

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