INCREASED α_2-ADRENORECEPTOR DENSITY IN PLATELETS OF SUBJECTS WITH HYPO-α-CHOLESTEROLEMIA

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Blood vessels of animals with experimental hypercholesterolemia have increased ability to contract in response to catecholamines [2], and this has been explained by an increase in the density of their α-adrenergic receptors [7, 8]. The density of adrenoreceptors in cells of different tissues has been shown to depend on the activity of adenylate cyclase coupled with the receptor membrane (by the feedback principle), whose activity increases with elevation of the cholesterol (CHS) level or the CHS/phospholipid ratio in cell membranes [6, 15].

The CHS concentration in cells and their membranes is largely determined by two opposite processes: entry of CHS into the cell chiefly in the composition of low-density lipoproteins (LDL), and its elimination by high-density lipoproteins (HDL). A low plasma level of CHS of HDL (HDL-CHS) in man (hypo-α-cholesterolemia – HAC) reflects insufficiency of the function of elimination of tissue CHS from the body [11].

The blood platelets are a readily available and widely used material with which to study adrenergic receptors in various pathological states [3, 4]. However, data on the regulatory action of cell membrane lipids on adenylate cyclase activity and on the α_2-adrenoreceptor level in platelets are contradictory [4, 10].

The aim of the investigation was to study functional characteristics of the adrenoreceptor system of the cells in HAC, which is a known risk factor of ischemic heart disease (IHD). For this purpose, a comparative study was undertaken of the density of α_2-adrenoreceptors and their affinity for the antagonist yohimbine in platelets of individuals with various plasma HDL-CHS levels.

EXPERIMENTAL METHOD

The investigation was conducted on a group of individuals with the diagnosis of presumptive IHD. Altogether 32 men and 1 woman were studied: IHD was diagnosed in 13 subjects, and in 20 the diagnosis of IHD was rejected (on the basis of the results of coronary angiography). In the group with HAC (8 patients with IHD and 3 subjects without IHD) there were some with HDL-CHS levels ≤ 36 mg/dl – the criterion of HAC for Moscow men aged 40-49 years [14]. The control group (with normo-α-cholesterolemia; 10 patients with IHD and 12 subjects without IHD) was formed from individuals with HDL-CHS values > 36 mg/dl; the mean age of subjects of the control group was 42.1 ± 4.1 years, and of the subjects with HAC 45.3 ± 3.0 years. No patients with diabetes or other endocrine diseases were tested.

Blood for analysis of lipids was taken in the fasting state from the cubital vein into tubes containing EDTA (1 mg/ml). Plasma levels of CHS, triglycerides (TG), and HDL-CHS were determined by the use of enzyme kits from WAKO (Japan), on a Centrifichem automatic analyzer (England). CHS of LDL (LDL-CHS) were calculated by the formula:

$$\text{LDL-CHS} = \text{CHS}_{\text{tot}} - (\text{HDL-CHS} + \text{TG}/5)$$ [1].

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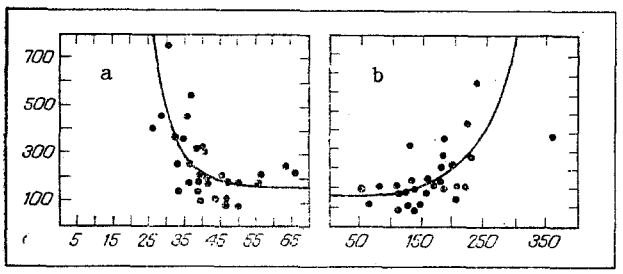

Fig. 1. Correlation between number of α_2-adrenoreceptors in platelets and plasma levels of HDL-CHS (a) and LDL-CHS (b) of group of subjects tested. Ordinate, density of receptors (in fmoles ^{3}H-yohimbine/mg protein). Abscissa: a) HDL-CHS concentration, b) LDL-CHS concentration (in mg/dl).

TABLE 1. Blood Plasma Lipids and Parameters of ^{3}H-Yohimbine Binding by Platelets of Subjects with Normo-α-Cholesterolemia and HAC (M ± m)

Group of subjects	Total CHS, mg/dl	HDL-CHS, mg/dl	LDL-CHS, mg/dl	TG, mg/dl	Binding of ^{3}H-yohimbine, fmoles/mg protein	K_d
Normo-α-cholesterolemia (22)	224±9,6	47±1,8	141±11,0	110±8,3	210±26,9	3,6±0,6
Hypo-α-cholesterolemia (11)	256±21,0	33,5±1,1***	200±20,0*	117±17,0	378±54,0**	4,2±0,6

Legend. *p < 0.05, **p < 0.01, ***p < 0.001 compared with normo-α-cholesterolemia.

Platelets were isolated from 60 ml blood taken from the cubital vein into tubes containing 3.2% sodium citrate solution (final ratio 8:1), by the usual methods [12]. The final protein concentration in the membrane fraction was 0.7-1.5 mg/ml. Protein was measured by Lowry's method [5]. To determine the density of α_2-adrenoreceptors, ^{3}H-yohimbine (85-95 Ci/mmole, Amersham) was used as the ligand. Incubation of the membrane suspension with ^{3}H-yohimbine (1-16 nM) and subsequent separation of the unbound ligand on Whatman GF/C filters were carried out as described in [12]. Nonspecific binding was determined in parallel tests with an excess (10 μM) of phentolamine. The number of specific binding sites (density of α_2-adrenoreceptors) and the dissociation constant of ^{3}H-yohimbine (K_d) were determined by Scatchard's method [9].

EXPERIMENTAL RESULTS

In accordance with the conditions of selection of individuals with HAC the mean plasma HDL-CHS level in this group was lower than in the control group (Table 1). The mean values of the plasma CHS and TG concentrations did not differ in the two groups compared. Since we know that a low level of HDL-CHS is accompanied by a raised LDL-CHS concentration [13], the value of LDL-CHS also was calculated for all subjects. In the group with HAC (Table 1) the mean LDL-CHS concentration in the blood plasma was higher than in the group with normo-α-lipoproteinemia.

Analysis of the parameters of specific ^{3}H-yohimbine binding in the two groups showed a larger number of specific binding sites of the labeled ligand (α_2-adrenoreceptors), but with identical affinity for the ligand (K_d) in the group with HAC (Table 1). Because of differences in the plasma lipid spectra of the two groups, correlation could be postulated between the number of α_2-adrenoreceptors in the platelets and the plasma levels of HDL-CHS or LDL-CHS. The character of the presumed correlation was analyzed statistically for the whole group of subjects. It was found that for both HDL-CHS and LDL-CHS the character of their correlation with the receptor density was described more accurately by a non-linear, than a linear, regression function (Fig. 1A, B). Correlation between the number of α_2-adrenoreceptors (y) and the HDL-CHS concentration (x) was best described by the parabolic function $y = A + Be^{-\beta(x-x_0)}$ (Fig. 1; Table 2), in which x_0 was taken to be the minimal value of HDL-CHS, namely 20 mg/dl, found in the male population of Moscow. The character of correlation between the number of α_2-adrenoreceptors (y) and the plasma HDL-CHS level (x) was described by the function $y = A + Be^{\beta x}$ (Fig. 1; Table 2). As the data in

TABLE 2. Statistical Analysis of Correlation between Number of α_2-Adrenoreceptors in Platelets and Plasma HDL-CHS and LDL-CHS Levels

Correlation studied	Character of correlation (function)	Coefficients of variation of parameters of function, %		
		A	B	β
Number of receptors/HDL-CHS	$y = A + Be^{\beta x}$ or $y = 145{,}70 + 3{,}88e^{0{,}02x}$ $r = -0{,}71$*	19,4	114,0	26,2
Number of receptors/LDL-CHS	$y = A + Be^{-\beta(x-x_0)}$ or $y = 156{,}20 + 164{,}00e^{-0{,}16(x-20)}$ $r = 0{,}73$*	15,5	65,5	30,0

Legend. HDL-CHS or LDL-CHS (mg/dl) = x. Number of receptors (fmoles of bound ^{3}H-yohimbine/mg platelet membrane protein) = y. *$p < 0.001$.

Fig. 1 show, there was a tendency for the number of binding sites of ^{3}H-yohimbine in the platelets to increase with a fall in the HDL-CHS level (≤ 40 mg/dl) or with a rise in the LDL-CHS level (≥ 220 mg/dl) in the blood plasma.

The results suggest that the increase in the number of α_2-adrenoreceptors found in the platelets of subjects with HAC may be due to the character of the plasma lipid spectrum. The possibility that plasma lipids may affect the density of adrenergic receptors was demonstrated, in principle, on animals kept on a hypercholesterol diet: an increase in the plasma CHS concentration led to an increase in the number of α-adrenoreceptors in the rabbit aorta [7] and to a decrease in the number of α-adrenoreceptors in the rat heart [6].

Activity of adenylate cyclase, coupled with adrenergic receptors, is regulated, as was stated above, by the lipid composition of the membrane [6, 15]. This suggests that the mechanism of the phenomenon now discovered is linked with regulation of the CHS level or the CHS/phospholipid ratio in the platelet membrane by the blood plasma lipoproteins. In fact, changes in concentration of HDL (HDL-CHS) and (LDL-CHS) have opposite effects on the density of α_2-receptors in the platelets. Since we know that HDL are acceptors and LDL are donors of the cellular CHS, and taking the above-mentioned suggestion into consideration, the opposite action of these lipoproteins on the cell adrenoreceptor density becomes understandable. We found no significant correlation between the number of α_2-adrenoreceptors in the platelets and the total plasma CHS concentration of the subjects studied. Possibly the ratio of HDL to LDL is a more important factor in regulation of the α-receptor level than the total plasma CHS concentration.

When the possible mechanisms of regulation of the α-receptor level by lipoproteins is discussed, the results of a study [4] showing that an increase or decrease in the CHS level in platelets during their incubation for 5 h with a dispersion of phospholipids did not lead to any change in the number of specific binding sites of the α-adrenergic antagonist ^{3}H-dihydroergocryptine, must also be noted. An exhaustive explanation of this fact is impossible, but it could be connected with the insufficient duration of the experiment for changes in the cell receptor density to be manifested. This suggestion is confirmed by the fact that a change in the number of α- and β-adrenoreceptors in animal tissues was observed after a long period (over 3 months) on a hypercholesterol diet [6, 7].

The ability of platelets to aggregate is a factor largely determining thrombus formation and progression of atherosclerotic lesions in blood vessels, which lie at the basis of the development of IHD. An increase in the density of α_2-adrenoreceptors may perhaps increase the likelihood of platelet aggregation and thrombus formation in response to the action of catecholamines (adrenalin) and this may be an additional cause of the more frequent development of atherosclerosis and IHD in subjects with hypo-α-cholesterolemia.

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ANTIPYROGENIC PROPERTIES OF OXYTOCIN

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Several humoral factors of endogenous origin are known which can influence the development of the febrile reaction and, in particular, can weaken it. These include interleukin 1 inhibitor, found in the brain of patients with fever, and which is also considered to block the action of endogenous pyrogen [9], a mediator of fever, and vasopressin [8]. Ability to depress the body temperature of normal animals has been shown to be a property of steroids [7], ACTH, and melanocyte-stimulating hormone [5]. Despite progress in the study of the antipyrogenic properties of hormones, the effect of other substances with hormonal activity on the origin and development of fever is not yet clear.

The aim of this investigation was to study the effect of oxytocin on the course of experimental fever.

EXPERIMENTAL METHOD

Noninbred rabbits weighing 2.5-3.0 kg were used. Fever was induced by intravenous injection of pyrogenal, a lipopolysaccharide from Salmonella typhi (N. F. Gamaleya Research Institute of Epidemiology and Microbiology) in a dose of 5 MPD†/kg or endogenous pyrogen (EP) in a dose of 1.2 ml/kg. EP was obtained in a culture of human peripheral blood monoclears isolated in a ficoll-verigrafin solution [4]. The cells were incubated in a concentration of 5 million/ml for 2 h at 37°C with heat-inactivated Staph. epidermidis microbial particles (in a 1:40 ratio). After incubation, the cells were washed twice and cultured in in a concentration of 5 million/ml in RPMI-1640 medium (Serva), supplemented with L-glutamine (2 mM), penicillin (100 units/ml), and streptomycin (100 μg/ml), for 18 h at 37°C in an atmosphere containing 7.5% CO_2 in air. The cells were precipitated by centrifuging (400 g, 15 min), and the supernatant liquid was used as the source of EP. Oxytocin (Serva, Gedeon Richter) was introduced by intravenous drip in an apyrogenic solution of 0.87% sodium chloride at a rate of 10-12 ml/h in a dose of 0.4 or 4 μg/kg in 1h, or intramuscularly in a dose of 0.2 μg/kg at 0.5 h intervals. The animals of the control group were infused with 0.87% saline solution. The influence of oxytocin on EP formation was studied in a culture of

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†Maximal permissible dose.

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