EFFECT OF EXPERIMENTAL HYPERINSULINEMIA ON PLASMA MEMBRANE INSULIN RECEPTOR ACTIVITY IN ADIPOSE AND LIVER TISSUES OF RATS WITH DISTURBED LIVER INNERVATION

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The action of insulin in a target cell begins with binding of its molecule to insulin receptors (IR), located on the outer surface of the plasma membrane of the cell. Parameters reflecting IR activity (their number and affinity for the hormone) vary in certain physiological and pathological states. Recent research has shown that IR activity is influenced by a number of hormonal and nonhormonal factors, but the mechanisms of regulation of IR activity and the general principles of this regulation call for further study. The fact that changes in IR activity proceed in the same direction in different tissues during starvation [6, 9, 12], in rats with experimental diabetes [5, 9], and in mice and man in obesity [11] deserves attention. In our view this similarity of direction is due to participation of trophic innervation in the regulation of IR activity. Meanwhile, it has now been established that insulin itself regulates IR activity (desensitization). A decrease in the IR content in association with an increase in insulin concentration has been demonstrated both *in vivo* [7] and *in vitro* on cell cultures [8]. This raises doubts about the role of the autonomic nervous system in regulation of IR activity.

The object of the present investigation was to study the role of the autonomic innervation in the depression of IR activity in experimental hyperinsulinemia, for which purpose IR were studied in plasma membranes of adipose and liver tissue of intact rats and rats with disturbed innervation of the liver. Liver tissue was chosen for disturbance of its innervation because of the anatomical peculiarities of the liver, namely that it is innervated chiefly by nerve fibers which enter the organ through its hilus [2]. This means that it is possible to produce almost total denervation of the liver simply by dividing nerve plexuses in the region of the hilus.

EXPERIMENTAL METHOD

Male Wistar rats weighing 150-180 g were used. In the experiments of series I IR were studied in plasma membranes of adipose and liver tissues of intact rats: control and with hyperinsulinemia. Experimental hyperinsulinemia was produced by the scheme described previously [7, 15]. Crystalline insulin zinc suspension was injected daily into the rats subcutaneously, starting with a dose of 0.4 unit per rat, which was increased every 1-2 days by 1.0 unit. On the 11th day the dose was increased to 6.0 units, and this quantity was given for 3 days more. The animals were killed 24 h after the last injection. Control animals received the same volume of physiological saline.

In the experiments of series II the IR of the above-mentioned tissues were studied in rats with disturbed innervation of the liver [3]. One half of the rats received insulin by the scheme described above 5 days after the operation, the other half received physiological saline; each group consisted of six animals.

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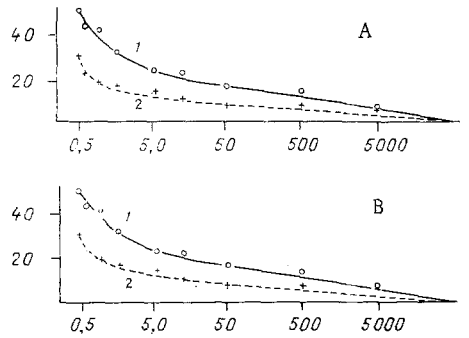

Fig. 1. Effect of hyperinsulinemia on specific binding of [^{125}I]insulin with plasma membranes of adipose (A) and liver (B) tissues in intact rats. 1) Control, 2) hyperinsulinemia. Abscissa, insulin concentration (in mg/ml); ordinate, specific binding (in % of total radioactivity).

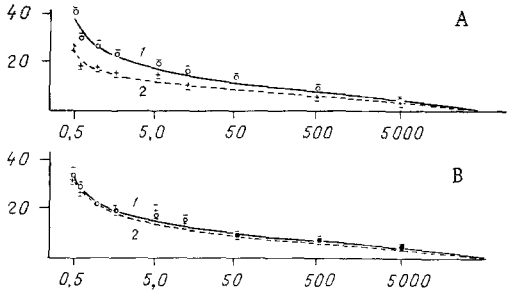

Fig. 2. Effect of hyperinsulinemia on specific binding of [^{125}I]insulin with plasma membranes of adipose (A) and liver (B) tissues in rats with disturbed innervation of the liver. Legend as in Fig. 1..

Plasma membranes were isolated from the liver [14] and adipose tissue [1] of the rats and were kept not more than 2 months at —20°C and thawed immediately before irradiation of the IR in saturation experiments under equilibrium conditions. Each sample (in two repetitions) contained 0.2 ng of [^{125}I]insulin (Poland, 3.0 IBq*/mg), 10 μg protein of adipose tissue membranes or 150 μg protein of liver tissue membranes, and crystalline unlabeled insulin in increasing concentrations (from 0 to 5000 mg/ml) in 0.4 ml 0.1 M Tris-HCl buffer, pH 7.4, with 1% bovine serum albumin and 1 mM $MgCl_2$. Nonspecific binding was determined with unlabeled insulin in a concentration of 50 μg/ml. The reaction was carried out at 30°C for 1 h. Free and bound [^{125}I]insulin were separated by millipore filters (Synpor, Czechslovakia), with a pore diameter of 0.6 μ. The radioactivity of the filters was determined on an automatic gamma-counter (Hungary).

EXPERIMENTAL RESULTS

Data on specific binding of [^{125}]insulin with plasma membranes of adipose tissue of control rats and rats with exogenous experimental hyperinsulinemia during an increase in the concentration of unlabeled insulin in the samples are given in Fig. 1A. This binding was reduced in animals with hyperinsulinemia. Analysis of the results obtained by the use of Scatchard plots (not shown) [13] indicates a decrease in the concentration of insulin binding

*As in Russian original. The letter "I" before "Bq" (Becquerel) is not identified — Translator.

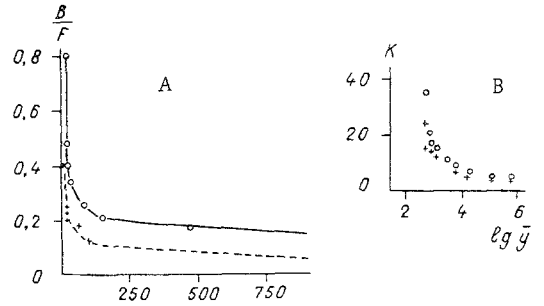

Fig. 3. Graphic analysis of data shown in Fig. 1A. A) Scatchard plot (dependence of B/F on B), B) method of Meyts and De Roth [reduced affinity (K = B/F) (R_0-B)] as a function of logarithm of occupancy $\bar{Y} = B/R_0$, B and F denote concentration of specifically bound and free insulin respectively. R_0) total number of binding sites.

sites from 35.5 to 24.0 nmoles/mg membrane protein. Graphic representation of dependence of reduced affinity on IR occupancy by the method of Meyts and De Roth [10] makes it possible to assess the affinity of free and hormone-occupied IR for insulin and the degree of negative cooperative interaction between IR, i.e., the degree of reduction of their affinity for insulin with an increase in the fraction of IR occupied by hormones. It follows from this graphic function plotted for the data in Fig. 1A (not shown) that the affinity of free ($\bar{K}_f$) and engaged ($\bar{K}_e$) IR is almost equally increased in hyperinsulinemia, whereas the degree of negative cooperative interaction between IR shows no significant change: The value of $\alpha = \bar{K}_f/\bar{K}_e$ was 0.241 and0.211, respectively in the control and in animals with hyperinsulinemia. A decrease in IR activity in the adipocytes of rats with exogenous hyperinsulinemia on account of their number, without any change in their affinity, has been found by other workers [7, 15]. The reason for the difference between these results and our own is not clear, but it may be differences in the experimental conditions (insulin preparation, weight of the animals, the use of membranes instead of whole adipocytes). Our own results showed a decrease in IR activity in liver membranes in hyperinsulinemia (Fig. 1B). Appropriate graphic analysis (not shown in this paper) revealed that this decrease was due to a decrease in the concentration of insulin binding sites (from 22.0 to 15.0 nmoles/mg membrane protein) and a decrease in affinity of IR for the hormone without any significant change in the degree of negative cooperative interaction between IR (the values of α were 0.175 and 0.193 in the experiment and control, respectively).

Experiments on animals with disturbed innervation of the liver showed that hyperinsulinemia in these animals reduced the specific binding of [^{125}I]insulin with IR of adipose tissue membranes: With all concentrations of unlabeled insulin, binding of the labeled hormone was significantly reduced in the experimental animals compared with the control (Fig. 2A). However, IR of the liver membranes of these animals did not respond to hyperinsulinemia by a reduction in insulin binding activity: Curves showing displacement of [^{125}I]insulin by increasing amounts of unlabeled insulin coincided in this case (Fig. 2B). Graphic analysis of the data in Fig. 2A by Scatchard's method (Fig. 3A) shows a reduction in the number of insulin binding sites in hyperinsulinemia (from 22.5 to 16.0 nmoles/mg membrane protein). Analysis of these same data as a Meyts and De Roth function (Fig. 3B) shows a decrease in the affinity of IR for insulin with no change in interaction between them of the negative cooperative type (the values of α were 0.200 in the experiment and 0.208 in the control). Consequently, the character of the decrease in insulin-binding activity of IR in plasma membranes of adipose tissue during hyperinsulinemia was the same in the intact rats and in rats with disturbed innervation of the liver, whereas IR in the liver with disturbed innervation do not react to hyperinsulinemia.

The results show that disturbance of the innervation of the liver modifies the response of IR in that organ to hyperinsulinemia. We know that denervation of the liver causes definite changes in its metabolism [4]. The results now obtained suggest that IR play a role in these changes. It is not clear whether the effect of the autonomic nervous system on IR is direct or indirect, but the fact that regulation of IR activity in a peripheral tissue is disturbed after disturbance of its innervation is interesting on its own account.

Changes in the parameters of IR under the influence of insulin in isolated cells and in blood cells (monocytes), which some recent research has demonstrated, are evidence that IR activity may also be regulated without the participation of the nervous system. However, regulation of IR expression at a higher level evidently does involve the nervous system, and this is responsible for the more effective regulation found in typical insulin target cells.

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