Insulin Receptors: Differences in Structural Organization on Adipocyte and Liver Plasma Membranes

Abstract. *Comparison was made of the distribution of the insulin receptor sites on adipocyte and liver plasma membranes by using ferritin-insulin. Two-thirds of the occupied insulin receptors on adipocytes occurred in groups of two or more whereas up to two-thirds of the receptors on liver occurred as single receptors. Ferritin-insulin did not cause aggregation of the receptor sites in either tissue. The naturally occurring groups of receptors on adipocyte membranes may play a role in the greater sensitivity of adipocytes to insulin.*

Assessment of biochemical and morphologic data indicates that insulin receptors on plasma membranes exhibit tissue-specific differences in structural organization. Shechter *et al.* (*1*) found that bivalent antibody [immunoglobulin G (IgG) fraction] to insulin, when added together with insulin to liver plasma membrane or cultured fibroblast preparations, caused a tenfold increase in specific insulin binding. When the data for such binding were subjected to Scatchard analysis, the usual curvilinear plot for both fibroblasts and liver plasma membranes appeared as a straight line. The bivalent antibody to insulin had no effect on insulin binding to adipocyte insulin receptors. In contrast, monovalent antibody (IgG fraction) to insulin had no effect on liver membranes or fibroblasts. The possible cross-linking or aggregation of insulin receptors by the antibody to insulin in liver tissue and fibroblasts was inferred by Shechter *et al.* to be important in converting the insulin receptor to the high-affinity state, whereas adipocyte insulin receptors were unaffected by externally induced cross-linking.

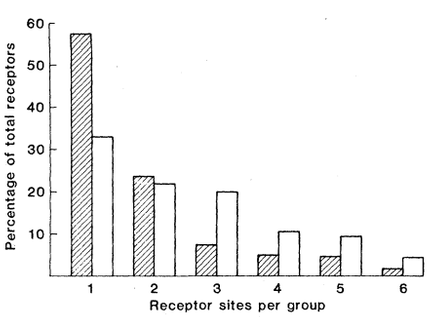

Fig. 1. The distribution of insulin receptors on adipocyte (open bars) and liver (shaded bars) plasma membranes. Freshly prepared liver or adipocyte plasma membranes were incubated with 7.2 n*M* Fm-I for 20 minutes at 24°C, in modified Krebs-Ringer phosphate (KRP) buffer, *p*H 7.4, with 3 percent bovine serum albumin in a total volume of 270 μl. The incubation was terminated by the addition of 2 ml of cold KRP buffer, and the membranes were centrifuged at 4°C for 20 minutes at 10,000*g*. The pellets were fixed with 2 percent glutaraldehyde in 0.1*M* sodium cacodylate and further processed for electron microscopy as described in the text. At least 300 ferritin particles were categorized per experimental condition.

We showed previously (*2*) that treatment of adipocyte plasma membranes with 1 m*M* dithiothreitol (DTT) causes a threefold increase in specific insulin binding and a concomitant change in the Scatchard analysis of insulin binding data from a curvilinear plot to a straight line. This effect was specifically due to cleavage of a disulfide bond by DTT. Similar treatment of liver plasma membranes produced only small increments in insulin binding, and these were not associated with conversion of the curvilinear Scatchard plot to linearity. By using a biologically active monomeric ferritin-insulin (Fm-I) preparation to visualize insulin receptor locations with the electron microscope, Jarett and Smith demonstrated (*3*) that approximately two-thirds of insulin receptors on adipocytes naturally exist in groups of two or more receptors prior to insulin binding. Shechter *et al.* (*1*) suggested that the reason for the failure of bivalent antibody to insulin to increase insulin binding or insulin action may be a consequence of the preexistence of these groups of insulin receptors. An ultrastructural study (*4*) of the distribution of insulin receptors on human placental syncytial trophoblast with Fm-I demonstrated that small groups of receptor sites were restricted to the distal portion of the microvillous projections, a result that differs markedly from the adipocyte data.

In the present study we made a quantitative comparison of the ultrastructural organization of insulin receptors on liver and adipocyte plasma membranes in an attempt to determine whether structural differences in the organization of insulin receptors might explain the biochemical studies. In contrast to our previous studies on adipocytes (*3*), we found that most of the insulin receptors on liver plasma membranes are solitary. In addition, occupancy of insulin receptors by Fm-I did not cause receptor aggregation on either adipocytes (*3*) or liver plasma membranes.

Adipocyte and liver plasma membranes were prepared by a modification of the method of McKeel and Jarett (*5*). The morphological procedures were described previously (*3*). The Fm-I, which was prepared by covalently linking insulin to ferritin with glutaraldehyde, was purified by column chromatography in Bio-Gel A (1.5*M*) and shown to be equal in biological and immunological activity. The Fm-I was incubated with plasma membranes as described in the legend to Table 1, diluted, and centrifuged at 10,000*g*. The pellets were fixed with 2 percent glutaraldehyde in 0.1*M* sodium cacodylate buffer, washed, postfixed in osmium tetroxide, embedded in Spurr resin, sectioned, and stained with uranyl acetate. Photographs were taken at random of areas containing adequate plasma membranes without regard to the presence or absence of ferritin particles in the field. Final magnification was 150,000. At least 300 ferritin particles were categorized per experimental condition.

Figure 1 shows that the distribution of insulin receptors on adipocyte plasma membranes was comparable to that reported for intact adipocytes (*3*), with two-thirds of the receptors occurring in

Table 1. Effect of fixation of liver plasma membranes on insulin receptor distribution. Control membranes were taken immediately from the fractionation procedure and incubated with Fm-I. Membranes to be fixed before being assayed with Fm-I were resuspended in 1 percent glutaraldehyde for 30 minutes, diluted with 100 m*M* tris-HCl, *p*H 7.4, to inactivate free glutaraldehyde and incubated for an additional 30 minutes. These membranes were then centrifuged at 10,000*g* for 15 minutes at 4°C, and the pellet was resuspended in Krebs-Ringer phosphate (KRP) buffer with gentle homogenization. Both the control and the glutaraldehyde-fixed plasma membranes were incubated with Fm-I at a final concentration of 7.2 n*M* at 37°C for 30 minutes. The incubation was carried out in a volume of 270 μl with 3 percent bovine serum albumin present in a modified KRP buffer, *p*H 7.4. The incubation was terminated by the addition of 2 ml of KRP buffer and centrifugation at 10,000*g* for 15 minutes at 4°C. The pellet was then fixed in 2 percent glutaraldehyde in 0.1*M* sodium cacodylate for 1 hour prior to being processed for electron microscopy as described in the text. Results shown are the average of two experiments.

Receptor sites per group	Average percentage of total receptors	
	Control	Fixed
1	65.2	63.6
2	19.4	23.8
3	8.9	6.8
4	3.2	1.4
5	2.8	3.6
6	0.8	0.5

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close proximity to one or more additional receptors, that is, within 200 to 400 Å. On liver plasma membranes, however, most of the receptors occurred singly or in a few groups of three or more. To determine whether these few groups of receptor sites were a result of ligand-induced aggregation, we examined liver plasma membranes that were fixed with 1 percent glutaraldehyde, washed, and subsequently incubated with Fm-I. Table 1 shows that the distribution of Fm-I was identical on both the control and prefixed membranes. When the multivalent ligand ferritin–concanavalin A (F-Con A) was incubated with fresh liver plasma membranes, large aggregates of ferritin particles were observed. Fixation of the liver membranes with glutaraldehyde totally blocked ligand-induced aggregation by the F-Con A (data not shown). Similar findings with both Fm-I and F-Con A were reported for adipocyte plasma membranes (*3*). The ability of glutaraldehyde to prevent the ligand-induced aggregation of F-Con A receptor sites and the data showing no difference in Fm-I distribution on control or prefixed membranes indicate that Fm-I binding to the insulin receptor did not cause aggregation of insulin receptors in either tissue.

The morphological data in Fig. 1 constitute proof of a major difference in the ultrastructural organization of the insulin receptor on adipocyte and liver plasma membranes. The adipocyte insulin receptors occur primarily in natural groups prior to insulin binding. However, most of the insulin receptors on liver plasma membranes occur singly. Bergeron *et al.* (*6*), using electron microscope radioautography, reached a similar conclusion, stating that there was no preferential localization of receptors over the hepatocyte plasmalemma to which insulin had access, and that there was no obvious clustering of grains. The morphological difference in the distribution of insulin receptor sites on adipocyte and liver plasma membranes are consistent with the biochemical differences reported for [^{125}I]insulin binding by Shechter *et al.* (*1*) and Schweitzer *et al.* (*2*).

Cross-linking or aggregation of the insulin receptor has been suggested as an important part of the initiation of insulin's action (*7*). This conclusion was based on data demonstrating the insulin-like properties of bivalent antibodies to the insulin receptor or other membrane components and the inability of monovalent forms of these antibodies to mimic the action of insulin. Morphological data indicate that Fm-I does not cause aggregation of insulin receptors at the ultrastructural level in adipocytes, liver, and placenta, even though the Fm-I is biologically active (*3*). This suggests that if insulin, as a monovalent ligand, causes a cross-linking to occur that helps elicit a biological response it must be by a different mechanism than aggregation of receptors. We propose a different model for insulin's action at the receptor level. Perhaps the natural presence of groups of insulin receptor sites on the adipocyte increases the probability of a cross-linking reaction that may be necessary for or may intensify insulin action. This could help explain the greater insulin sensitivity of adipocytes compared to liver where the receptor sites are not grouped. Such grouping of receptor sites on adipocytes might allow the insulin occupancy of one site to activate adjacent sites. This molecular cross-linking might occur between the disulfide bonds of insulin and the sulfhydryl groups in the insulin receptor.

Schweitzer *et al.* (*2*) have shown that the adipocyte membrane has a greater number of accessible disulfide bonds than liver and that conversion of these to sulfhydryl groups increases insulin binding. Recent data (*8*) show that opiate receptor function can be modulated through an oxidation-reduction mechanism; thus the oxidation of thiol groups by cupric ions mimics the action of morphine and those ions even compete for binding. Since several oxidizing agents, such as H_2O_2, vitamin K, and diamide, mimic insulin activity (*9*), bivalent antibodies may mimic the action of insulin through a similar oxidation-reduction reaction between the disulfide bonds of the antibody and sulfhydryl groups of the receptor, and not because of aggregation. The role of disulfide bonds and sulfhydryl groups in the initiation of insulin action must be substantiated by further experiments.

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Gyrate Atrophy of the Choroid and Retina: Improved Visual Function Following Reduction of Plasma Ornithine by Diet

Abstract. *In a patient with gyrate atrophy of the choroid and retina, an arginine-deficient diet has reduced plasma ornithine concentration fivefold during the past 20 months. Subjective improvement in her visual function was noted approximately 15 months after institution of the diet. This has been documented by improvements in the electroretinogram, dark-adaptation, and color vision. The improvement involves rod and, to a lesser extent, cone function. The results, although preliminary and limited to a single patient, suggest that reduction of plasma ornithine with a low arginine diet is beneficial in this disease.*

It is generally accepted that no therapy has been successful in the treatment of degeneration of the choroid and retina. This group of diseases is characterized by inexorably progressive loss of visual function leading to eventual blindness. The purpose of this report is to document the sustained improvement in visual function in a patient with one form of chorioretinal degeneration, gyrate atrophy.

Gyrate atrophy (GA) of the choroid and retina is an autosomal recessive chorioretinal degeneration that ultimately results in severe visual deficits expressed by high myopia, progressive concentric constriction of the visual field, cataracts, pathologically reduced dark-adaptation, extinguished electroretinogram (ERG), and abnormal electrooculogram (EOG) (*1*).

In 1973, Simell and Takki reported that GA patients have 10- to 15-fold increases in plasma ornithine concentra-

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