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Differences in organizational structure of insulin receptor on rat adipocyte and liver plasma membranes: Role of disulfide bonds

(dithiothreitol/oxidized glutathione/insulin binding/*N*-ethylmaleimide/Scatchard analysis)

JOHN B. SCHWEITZER, ROBERT M. SMITH, AND LEONARD JARETT*

Departments of Pathology and Medicine, Division of Laboratory Medicine, Washington University School of Medicine, St. Louis, Missouri 63110

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ABSTRACT **Binding of ^{125}I-labeled insulin to rat liver and adipocyte plasma membranes has been investigated after treatment of the membranes with agents that modify disulfide bonds or sulfhydryl groups. Dithiothreitol, a disulfide-reducing agent, produced a bimodal response in adipocyte plasma membranes with dose-dependent increases in binding occurring over the range of 0–1 mM dithiothreitol; 5 mM dithiothreitol produced decreased binding. Insulin binding reached its maximal increase at 1 mM and was 3 times control values. Scatchard analysis of the 1 mM dithiothreitol effect revealed a straight line plot indicative of one class of sites with a K_a of 1.0×10^8 M^{-1} which is intermediate between the two K_as obtained from the curvilinear Scatchard plot of control membranes. There was a 20-fold increase in the number of intermediate-affinity receptors compared to high-affinity receptors. The increased ^{125}I-labeled insulin binding after dithiothreitol treatment was reversed by oxidized glutathione in a dose-dependent manner. Interposition of treatment with *N*-ethylmaleimide, an alkylating agent, prevented oxidized glutathione from reversing the dithiothreitol effect. Reduced glutathione produced the same effect as dithiothreitol. Liver plasma membranes treated with up to 1 mM dithiothreitol exhibited a maximum increase in insulin binding of 20% compared to control. Dithiothreitol at 5 mM decreased insulin binding below that of control membranes. The results indicate that the dithiothreitol effect on insulin binding to adipocyte plasma membranes is due to disruption of disulfide bonds, and that the structural organization of the insulin receptor on the plasma membranes is different for liver and for adipose tissue. The data imply that the insulin receptors on the plasma membrane of adipocytes possess at least two functionally distinct subclasses of disulfide bond but liver insulin receptors do not.**

Numerous hormone-responsive systems exist in which receptor binding assays have indicated that disulfide bonds or sulfhydryl groups in the receptor have an important role in ligand binding. Treatment of the β_1-adrenergic receptor in turkey erythrocytes with dithiothreitol, a potent reducing agent that cleaves disulfide bonds, produced decreased specific binding of agonist by decreasing the number of high-affinity binding sites (1). This effect was reversible by treatment involving reoxidation of the newly formed sulfhydryl groups and could be made irreversible by treating with the alkylating agent *N*-ethylmaleimide (MalNEt) after dithiothreitol treatment. The same types of manipulation of the central nervous system nicotinic acetylcholine receptor indicated that cleavage of disulfide groups produced decreased agonist binding by decreasing the receptor affinity rather than the number of sites (2). Dithiothreitol produced a dose-dependent decrease in ^{125}I-labeled TSH binding to thyroid membranes, apparently by decreasing the receptor affinity, whereas the oxidizing agent diamide increased receptor affinity (3). Neither agent changed total receptor number in the thyroid membrane system. Recently reported morphological data indicate that the clustering of occupied enkephalin receptors in neuroblastoma cells was prevented by treatment with any of several reducing or alkylating agents, including dithiothreitol, MalNEt, and iodoacetate (4).

The insulin receptor has been shown to contain disulfide bonds. Using polyacrylamide gel electrophoresis, Pilch and Czech (5) obtained data showing that the insulin receptor in rat liver and adipocyte plasma membranes was a complex of M_r ≈300,000 in the absence of reducing agents (5). Dithiothreitol treatment reduced the complex to a major component of M_r 125,000 and a minor component of M_r 225,000. Jacobs *et al.* (6) obtained similar results in rat liver and human placenta membranes suggesting a total M_r of the receptor complex of 310,000. After treatment with 10 mM dithiothreitol, two subunits were produced: a M_r 135,000 molecule that contained the insulin-binding region and a M_r 45,000 molecule that may be a glycoprotein. These two studies indicate that the receptor may be composed of at least two different subunits linked by disulfide bonds. Jarett and Smith have shown that insulin receptors on the rat adipocyte plasma membrane occur in natural groups (7) which can be disrupted by treatment with cytochalasin B, but not by cytochalasin D (8), the former being a thiol reagent (9).

These observations motivated the present study of the specific binding of ^{125}I-labeled insulin (^{125}I-insulin) to its receptor. It was found that the use of various chemical agents that modify disulfide bonds or sulfhydryl groups produces large changes in specific insulin binding to the adipocyte insulin receptor. Similar manipulations of liver plasma membranes had minimal effect on ^{125}I-insulin binding. These data suggest that disulfide bonds are important for insulin receptor interactions in adipocyte plasma membranes prior even to insulin binding, but disulfide bonds do not play the same role in liver plasma membranes.

MATERIALS AND METHODS

Male Sprague–Dawley rats (120 g) were obtained from Eldridge Laboratory Animals (Barnhardt, MO). Collagenase, bovine serum albumin (fraction V), dithiothreitol, MalNEt, and both oxidized (GSSG) and reduced glutathione were purchased from Sigma. The lots of collagenase and albumin were chosen as described (10). Porcine insulin was a gift from R. Chance (Eli Lilly). Carrier-free Na^{125}I was purchased from Amersham and Enzymobeads were from Bio-Rad.

Preparation of Adipocyte Plasma Membranes. Adipocytes were isolated from rat epididymal fat pads as described (11) and plasma membranes were prepared as described by McKeel and Jarett, except for omission of EDTA from the fractionation

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Abbreviations: MalNEt, *N*-ethylmaleimide; GSSG, oxidized glutathione.

* To whom reprint requests should be addressed at: Department of Pathology and Laboratory Medicine, Hospital University of Pennsylvania, 3400 Spruce Street, Philadelphia, PA 19104.

medium (12, 13). The plasma membrane fractions were routinely frozen in dry ice/ethanol, stored at −70°C, and used within 1 week after preparation.

Preparation of Rat Liver Plasma Membranes. The liver of a decapitated rat was perfused with isotonic saline prior to its removal. The liver was then finely minced and homogenized in 0.25 M sucrose/10 mM Tris·HCl, pH 7.4/1 mM EDTA. The homogenate was centrifuged at 20,000 × *g* for 20 min, and the resulting pellet was resuspended in the same buffer. The supernatant of a 1000 × *g* centrifugation was centrifuged at 20,000 × *g*, yielding a pellet containing primarily mitochondria and plasma membranes. This pellet was resuspended and layered on a Ficoll/sucrose gradient. The gradients were centrifuged, and the plasma membrane band was removed, diluted with buffer, and pelleted by centrifugation. Approximately 25 mg of plasma membrane was recovered from 8 g of liver tissue. Marker enzyme studies showed that the plasma membrane preparations had less than 10% mitochondria and approximately 20% endoplasmic reticulum; there was a 6- to 8-fold enrichment in plasma membrane marker enzymes. Liver plasma membranes were frozen and stored in the same manner as adipocyte plasma membranes.

Preparation of ^{125}I-Insulin. Iodination was carried out by a lactoperoxidase method, using the Enzymobead radioiodination kit, to a specific activity of 120–180 μCi/μg (1 Ci = 3.7 × 10^{10} becquerels). The radiolabeled insulin was then purified over a Sephadex G-50 column followed by a cellulose (Whatman) column. The resulting ^{125}I-insulin contained an average of 0.5 atom of ^{125}I per insulin molecule and was >98% precipitable by trichloroacetic acid. Such preparations have been demonstrated to be predominately monoiodinated and to retain full biological activity (14, 15). The specific activity of the insulin was 900–1200 cpm/fmol.

Protocol for ^{125}I-Insulin Binding Studies. Membranes were thawed, resuspended with gentle homogenization, divided into equal portions, and treated with various reducing, alkylating, or oxidizing agents (as described in the legends to figures or tables) in sequential incubations for 10 min at 37°C. Between incubations the membranes were pelleted in a Microfuge (Eppendorf, 5412) for 1 min, the supernatant was aspirated, and the membranes were resuspended with fresh Krebs–Ringer phosphate buffer (pH 7.4) for use in further incubations or in the binding assay. All tubes including controls underwent the same number of incubations and centrifugations in each experiment. ^{125}I-Insulin binding assays were carried out in a total volume of 0.1 ml, plasma membrane content was 100–150 μg of protein per incubation tube, and the final bovine serum albumin concentration was adjusted to 1%.

All binding assays were carried out at 24°C and were terminated at 20 min for adipocyte plasma membranes or 30 min for liver plasma membranes by transferring 70 μl of the incubation mixture to a 0.4-ml Microfuge tube containing 0.3 ml of 0.01 M Na phosphate/0.25 M sucrose, pH 7.4, and centrifuging. After centrifugation, the bottom of the tube containing the pellet was cut off, blotted dry, and assayed for radioactivity. Under the conditions described, ^{125}I-insulin binding was at equilibrium. Degradation of free ^{125}I-insulin over the entire time course of the binding assay was <8% for control membranes of either liver or adipose tissue, as judged by trichloroacetic acid precipitability. Treatment of either type of membrane with 1 mM dithiothreitol produced a small increment in degradation to a maximum of 12% (data not shown). In all studies, unless indicated otherwise, the concentration of ^{125}I-insulin per tube was 723 pM, and total ^{125}I-insulin binding was assayed in quadruplicate.

Nonspecific binding was assayed in duplicate and was defined as the mean radioactivity associated with the membrane pellet derived from tubes incubated under the same conditions as the total binding tubes except for the addition of native insulin at 25 μg/ml. This number was then subtracted from each of the four total binding values to arrive at specific ^{125}I-insulin binding. Nonspecific binding was 10% or less of total binding in all experiments.

Graphic analysis, by the method of Scatchard (16), was performed on data obtained by incubating a fixed concentration of ^{125}I-insulin, 145 pM, with increasing concentrations of native insulin over the range of 0 to 542 nM. After nonspecific binding was subtracted, a graph was made of bound ^{125}I-insulin/total ^{125}I-insulin versus total insulin; a curve of best fit was obtained, and the results were used for a Scatchard type plot described (17). An alternative method of analysis involved incubation of increasing amounts of ^{125}I-insulin, measurement of ^{125}I-insulin bound, and plotting bound/free versus bound after the appropriate calculations were performed. After all experiments, residual plasma membranes for each experimental condition were assayed for protein concentration by the method of Lowry *et al.* (18).

RESULTS

Effect of Dithiothreitol on ^{125}I-Insulin Binding to Adipocyte Plasma Membranes. Dithiothreitol at 1 mM produced a maximal stimulation of specific insulin binding which consistently amounted to at least a 3-fold increase in ^{125}I-insulin binding to the plasma membranes compared to control conditions. The response to dithiothreitol was dose dependent starting at 0.1 mM with optimal stimulation occurring at 1 mM; 5 mM dithiothreitol caused binding to drop below that of control (Fig. 1). The decrease in specific insulin binding at 5 mM dithiothreitol could not be attributed to carryover of dithiothreitol into the insulin binding experiments because the result was unchanged when an additional cycle of resuspension of the plasma membranes with fresh buffer, centrifugation, and aspiration of the supernatant was interposed between the dithiothreitol treatment and the insulin binding assay (data not shown).

To determine the nature of the 1 mM dithiothreitol effect on ^{125}I-insulin binding, data was analyzed by a Scatchard plot (Fig. 2). Control membranes exhibited a curvilinear Scatchard plot with a high-affinity binding constant (K_a) of 1.2 × 10^9 M^{-1}

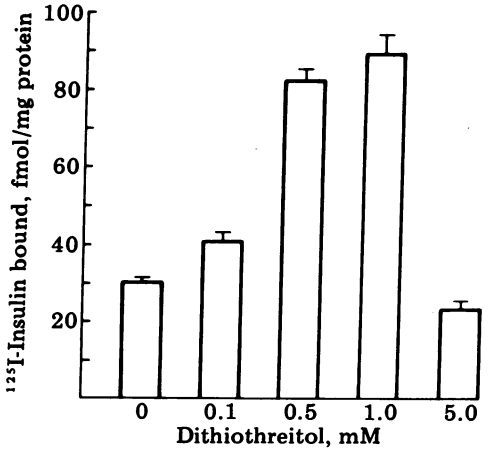

FIG. 1. Effect of dithiothreitol treatment of adipocyte plasma membranes on specific ^{125}I-insulin binding. Adipocyte plasma membranes were divided into five aliquots and treated for 10 min at 37°C with the concentration of dithiothreitol shown. Membranes were then centrifuged for 1 min, the supernatant was aspirated, and the pellet was resuspended for use in the insulin binding assay. Values shown are the means (±SEM) of quadruplicate determinations of specific ^{125}I-insulin binding.

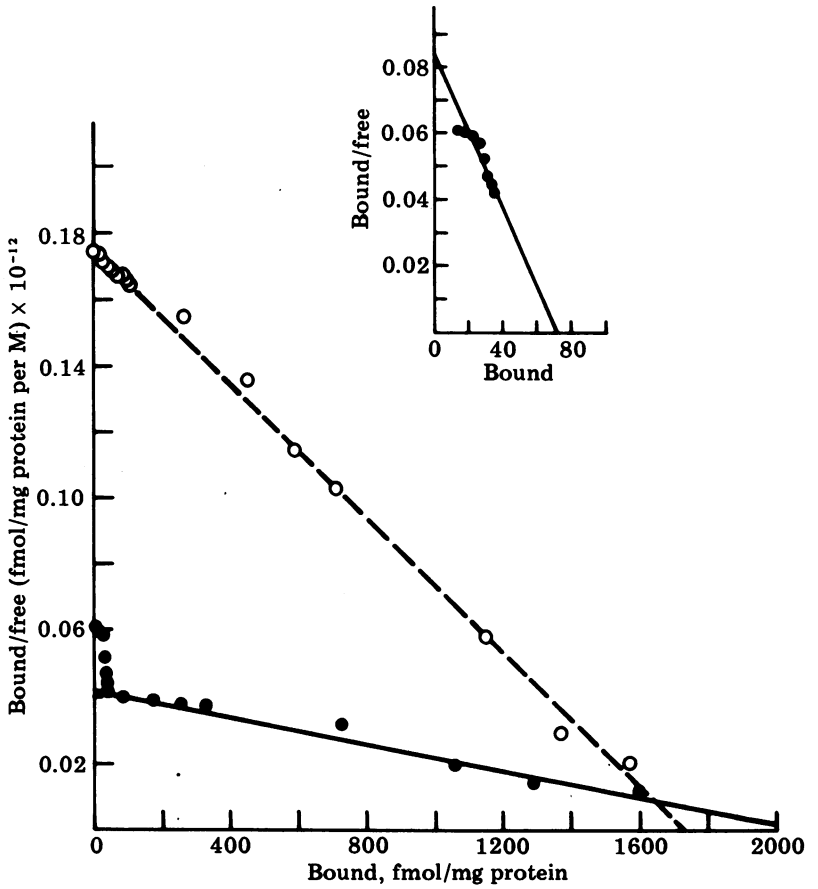

FIG. 2. Scatchard analysis of the dithiothreitol effect on specific ^{125}I-insulin binding. Adipocyte plasma membranes were divided into equal aliquots and treated for 10 min at 37°C with buffer (●) or 1.0 mM dithiothreitol (○) prior to being prepared for use in the binding assay. Membranes were incubated with 145 pM ^{125}I-insulin and concentrations of native insulin ranging from 0 to 542 nM. (*Inset*) Expanded scale showing high-affinity region of control membranes. Dithiothreitol-treated membranes produce a straight-line Scatchard plot with $K_a = 1.0 \times 10^8$ M^{-1}; the control membranes exhibit the classical curvilinear Scatchard plot with high-affinity $K_a = 1.2$ and 10^9 M^{-1} and low-affinity $K_a = 2.0 \times 10^7$ M^{-1}.

and a low-affinity binding constant of 2.0×10^7 M^{-1}. The Scatchard plot of ^{125}I-insulin binding to dithiothreitol-treated membranes was a straight line, indicating one class of binding sites, with a binding constant of 1.0×10^8 M^{-1}, intermediate between the two binding constants derived from control curves. As indicated by the closeness of the intercepts along the horizontal axis, the total number of receptors was relatively unaffected by dithiothreitol.

Fig. 2 was derived from the experimental protocol in which a fixed amount of radiolabeled insulin was incubated with increasing amounts of unlabeled insulin, but the same result has been obtained by incubating with increasing amounts of ^{125}I-insulin as described in *Materials and Methods* (data not shown). Increased specific binding of ^{125}I-insulin to plasma membranes could be produced by reduced glutathione, although the magnitude of the increase was not as great and required higher concentrations to achieve an effect (data not shown). This is consistent with the much higher reducing efficiency of the bifunctional agent dithiothreitol relative to agents that have only one sulfhydryl group (19).

Effects of GSSG and MalNEt on the Increased Binding Produced by Dithiothreitol. GSSG, an oxidizing agent, reversed the effect of 0.5 mM dithiothreitol on specific ^{125}I-insulin binding in a dose-dependent fashion with nearly complete reversal occurring at a concentration of 200 mM (Fig. 3). GSSG alone had little, if any, effect on ^{125}I-insulin binding (data not shown).

Interposition of an incubation with the alkylating agent MalNEt between the dithiothreitol treatment and the GSSG treatment maintained specific insulin binding at levels closely approaching those produced by dithiothreitol treatment alone (Fig. 4). The MalNEt effect was dose-dependent with the maximum occurring at 5 mM MalNEt. Dithiothreitol treatment followed by MalNEt treatment caused no significant difference in binding to occur compared to dithiothreitol treatment alone (data not shown).

Effect of Dithiothreitol and GSSG Treatment of Liver Plasma Membrane. Over the same range of dithiothreitol concentrations as used in the adipocyte plasma membrane experiments, specific ^{125}I-insulin binding to liver plasma membranes showed a maximal increase of only 20% over control values (Table 1). In other experiments this stimulation of binding has varied between 10% and 20%. Scatchard analysis of insulin binding data after 1 mM dithiothreitol treatment of liver plasma membranes demonstrated a curvilinear shape, as did control membranes (data not shown). Treatment of liver plasma membranes with a wide range of GSSG concentrations

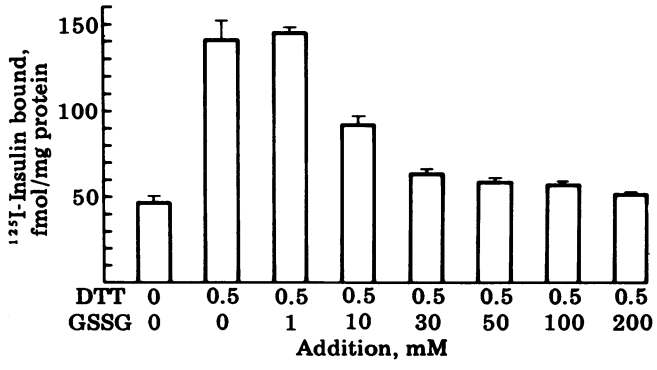

FIG. 3. Reversal of the effect of dithiothreitol (DTT) on specific ^{125}I-insulin binding by GSSG. Adipocyte plasma membranes were treated for 10 min at 37°C with the concentration of dithiothreitol shown and centrifuged, the supernatant was aspirated, and the membranes were resuspended for a second 10-min treatment at 37°C with the concentration of GSSG shown. Membranes were then prepared for the binding assay. Values shown are the means (±SEM) of quadruplicate determinations of specific ^{125}I-insulin binding.

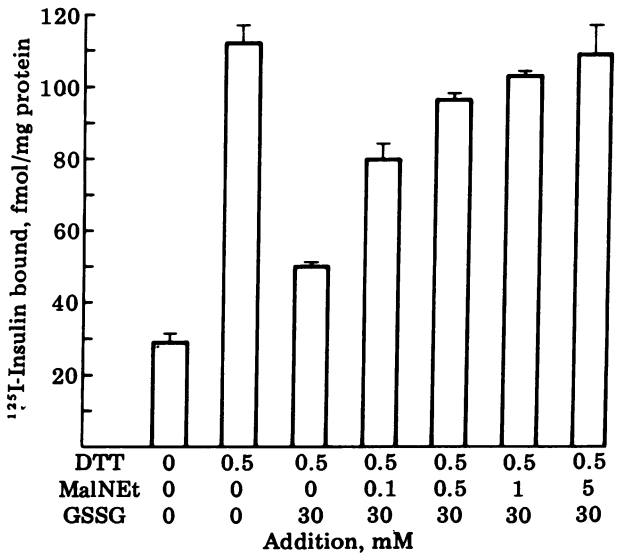

FIG. 4. MalNEt prevents the GSSG reversal of the effect of dithiothreitol on specific ^{125}I-insulin binding. Adipocyte membranes were treated with dithiothreitol, MalNEt, and GSSG in the concentrations shown in sequential 10-min treatments at 37°C, separated by washes as described in the legend to Fig. 3. Values shown are the means (±SEM) of quadruplicate determinations of specific ^{125}I-insulin binding.

produced no significant differences in ^{125}I-insulin binding from control membranes (Table 1). As was the case with adipocyte plasma membranes, binding dropped below control values when liver plasma membranes were treated with 5 mM dithiothreitol.

DISCUSSION

The present study has investigated the role that disulfide and sulfhydryl groups play in insulin binding to highly purified adipocyte and liver plasma membranes. Treatment of adipocyte plasma membranes with various concentrations of dithiothreitol, a potent reductant of disulfide linkages, produced a bimodal response. Concentrations of dithiothreitol up to and including 1 mM increased specific ^{125}I-insulin binding, whereas at 5 mM it decreased specific ^{125}I-insulin binding. The maximal increase in specific insulin binding was at least 3-fold and occurred when membranes were treated with 1 mM dithiothreitol. Several observations are evidence that this dose-dependent increase in insulin binding was specifically the result of cleavage of disulfide bonds. First, the dithiothreitol effect was reversible with GSSG, a finding that is consistent with reformation of disulfide bonds. Second, treatment with alkylating levels of MalNEt immediately after dithiothreitol treatment prevented GSSG from reversing the dithiothreitol effect on specific insulin binding, implying that MalNEt permanently blocked the sulfhydryl groups formed by dithiothreitol treatment. Finally, dithiothreitol's effect was reproduced by utilizing reduced glutathione, another reducing agent known to be capable of breaking disulfide bonds.

Table 1. Effect of dithiothreitol or GSSG on specific ^{125}I-insulin binding to liver plasma membranes

Dithiothreitol		GSSG	
Conc., mM	^{125}I-Insulin bound, fmol/mg protein	Conc., mM	^{125}I-Insulin bound, fmol/mg protein
0	57.8 ± 0.9	0	57.3 ± 2.0
0.1	57.8 ± 1.5	1.0	58.8 ± 2.8
0.5	65.0 ± 2.5	5.0	62.9 ± 3.0
1.0	68.0 ± 0.5	20	58.5 ± 2.8
5.0	45.5 ± 1.5	50	58.1 ± 4.0
		100	52.5 ± 0.7

Liver plasma membranes were treated for 10 min at 37°C with the concentration of dithiothreitol or GSSG shown. Membranes were then centrifuged for 1 min, the supernatant was aspirated, and the pellet was resuspended for use in the insulin binding assay. Values represent the mean (±SEM) of quadruplicate determinations.

Specific insulin binding to liver plasma membranes was increased to only 20% at 1 mM dithiothreitol before dropping below control values at 5 mM dithiothreitol. The difference observed in insulin binding after 1 mM dithiothreitol treatment in adipocyte membranes compared to liver plasma membranes strongly implies that the structural organization of insulin receptors in the plasma membranes of the two tissues is different, at least with respect to disulfide bonds. Shechter *et al.* (20) have reported observations which support the concept of tissue-specific differences in structural organization of insulin receptors on plasma membranes. They found that coincubating physiological concentrations of ^{125}I-insulin with anti-insulin antibody had little or no effect on specific ^{125}I-insulin binding to adipocytes but markedly enhanced specific ^{125}I-insulin binding to liver plasma membranes and fibroblasts.

Further analysis of the effect of 1 mM dithiothreitol on ^{125}I-insulin binding to adipocyte plasma membranes was carried out by using the method of Scatchard (16). Control membranes exhibited the standard curvilinear plot that has been widely reported in many tissues (21–25). The high-affinity portion of the control curve yielded a K_a of 1.2×10^9 M^{-1}, and the low-affinity portion of the curve, from 100 fmol/mg of protein bound out to 2000 fmol/mg, yielded a K_a of 2×10^7 M^{-1}. This curve is almost identical to that found by Pilch and Czech (5) with adipocyte plasma membranes. The Scatchard plot of the data from dithiothreitol-treated adipocyte membranes yielded a straight line between 0 and 2000 fmol/mg of protein bound with a calculated K_a of 1×10^8 M^{-1}. The increased specific binding observed with 1 mM dithiothreitol can be explained by comparing the number of high-affinity sites in the control curve to the number of intermediate-affinity sites with dithiothreitol treatment, as judged by intercepts on the bound axis. Although dithiothreitol treatment decreased affinity slightly more than 1 order of magnitude, compared to control high-affinity sites, the number of intermediate-affinity sites associated with the dithiothreitol-treated plasma membranes was at least 20 times greater.

The typical curvilinear Scatchard plot of insulin binding has been the subject of many interpretations. The plot indicates that insulin receptors do not function as a single population of independent receptors. DeMeyts *et al.* (21) have explained the curvilinear plot on the basis of negative cooperativity with a homogeneous population of interacting receptors in which increasing fractional occupancy of the receptor decreased the average affinity of the remaining unoccupied receptors. This concept has been challenged (26). Furthermore, several groups have suggested that at least two types of insulin-binding sites exist. The data of Olefsky and Chang (27, 28) indicated that there are at least two functionally distinct binding sites. Pilch and Czech (5) hypothesize that in fat cells there is a single high-affinity receptor with a K_d of 3×10^{-9} M and multiple low-affinity receptors with variable dissociation constants. Krupp and Livingston (29, 30) separated two insulin-binding species from fat and liver plasma membranes by polyacrylamide gel electrophoresis and found that one species with the binding characteristics of whole plasma membranes could be converted into the other species with lower affinity by the addition of insulin. Maturo and Hollenberg (31) also obtained two insulin binding entities from solubilized liver membranes. The smaller of these proteins was converted to the larger upon the addition of a glycoprotein fraction from liver plasma mem-

branes. The addition of the glycoprotein also conferred the binding characteristics of the intact liver plasma membrane on the molecule.

Whatever interpretation of the curvilinear Scatchard plot is correct, 1 mM dithiothreitol treatment of adipocyte plasma membranes produced a classical linear Scatchard plot, indicative of one class of sites with homogeneous affinity. Because liver plasma membranes continue to exhibit a curvilinear Scatchard plot after treatment with 1 mM dithiothreitol, we suggest that the phenomenon of simplifying the Scatchard analysis of insulin binding from a curvilinear plot to a straight line by reducing disulfide bonds is limited to certain tissues and possibly solely to adipocytes. Jarett and Smith (7) have reported the existence of naturally occurring clusters of up to at least six insulin receptors prior to insulin binding. An attractive hypothesis would be that disulfide bonds are necessary for the maintenance of these groups and that dispersal of the cluster occurs with dithiothreitol treatment. The receptor clusters would then represent the physiologically relevant high-affinity receptor but, for steric or other reasons, the individual receptors in the cluster cannot all be occupied with insulin molecules with the same high affinity. Once these groups are dispersed with dithiothreitol, however, all receptors could be occupied and exhibit the same affinity which might partially account for the increase in numbers of receptors of intermediate affinity that the Scatchard analysis showed. Morphological studies utilizing ferritin-insulin visualization of insulin receptors by electron microscopy should provide additional data consistent with this hypothesis. Preliminary studies of ferritin-insulin binding to liver plasma membranes indicate a predominance of single receptors (unpublished observations), a finding that correlates with the absence of an effect of 1 mM dithiothreitol on specific insulin binding.

Shechter *et al.* (20) reported data showing conversion of the curvilinear Scatchard plot in liver and fibroblasts to a homogeneous straight line plot with high affinity after coincubation of physiological concentrations of insulin with bivalent anti-insulin IgG. However, monovalent IgG could not produce this effect. Bivalent anti-insulin IgG produced no effect on insulin binding to adipocyte plasma membranes. They interpreted their results as evidence that crosslinking of occupied insulin receptors caused them to behave homogeneously as high-affinity sites. They suggested that adipocytes did not respond in the same way because the insulin receptors already exist in an aggregated state, as shown by Jarett and Smith (7). The implication of their data and the present data is that, although all tissues may exhibit a curvilinear Scatchard plot of insulin binding in the native state, the organization of receptors that produces that plot may be quite dissimilar.

The possibility that liver plasma membranes exist with insulin receptors in a reduced state led to the investigation of whether GSSG might affect specific insulin binding in liver by virtue of its ability to form disulfide bonds. However, no GSSG effect could be found at concentrations up to 100 mM. This does not rule out the possibility that, under the appropriate conditions, disulfide bond or sulfhydryl group status is important in determining how insulin binds to its receptor on liver plasma membrane.

In both liver and fat adipocyte plasma membranes, 5 mM dithiothreitol treatment produced decreased specific insulin binding. Based on the data of Pilch and Czech (5) and Jacobs *et al.* (6) which showed that the receptor complex in rat fat and liver membranes and human placenta membranes is dissociated into subunits with 10 mM dithiothreitol, the present data imply that 5 mM dithiothreitol approaches the threshold for affecting a disulfide bond necessary for the integrity of the basic insulin receptor. The bimodal effect of dithiothreitol treatment on adipocyte plasma membranes strongly implies the existence of at least two functionally distinct subclasses of disulfide bond in the organization of insulin receptors on the adipocyte plasma membranes whereas only one type exists in liver membranes.

Elucidation of the role played by disulfide bonds or sulfhydryl groups in insulin receptor structure and function will require both biochemical and morphological study of different tissues. Tissue-specific receptor organization on the plasma membrane could affect both the type and the degree of hormonal response to insulin. Manipulations of disulfide bond or sulfhydryl group status of the insulin receptor may produce differences in the biological activity of insulin on a particular tissue.

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