Tissue Differences in Insulin Receptors

Acute Changes in Insulin Binding Characteristics Induced by Wheat Germ Agglutinin

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SUMMARY

The plant lectin, wheat germ agglutinin (WGA), produced differential effects on insulin binding by insulin receptors from rat adipocytes, rat liver, human placenta, and human monocytes. Treatment of adipocyte membranes with WGA (1 μg/ml) markedly enhanced insulin binding (500% of control, 100%) conducted with 5×10^{-10} M ^{125}I-insulin. The lectin's effect on insulin binding by liver membranes was significantly less (260% of control) and required a larger WGA concentration (5 μg/ml) to produce the stimulation. Insulin binding by placental membranes was increased only slightly (140%) by WGA, and monocytes failed to respond. The rate of insulin dissociation from the membrane preparations was decreased by lectin treatment. This effect was most prominent with adipocyte membranes, followed next by liver membranes, whereas only a slight inhibition was found with placental membranes. Scatchard analysis of the adipocyte receptor binding data indicated that WGA treatment completely linearizes the normally curvilinear plot to one high-affinity component (Kd of 0.22 nM) and decreases total insulin binding capacity by 60%. Similar effects were found with liver membranes, except that the extent of the changes was not as dramatic. WGA treatment did not linearize the Scatchard plot determined for placental membranes. Adipocyte and liver receptors solubilized by Triton X-100 responded to WGA, although the response was less in the former (350% above control, 100%) and greater in the latter (500%). Solubilization of placental receptors did not improve their response to the lectin. These results suggest differences in the structure of the insulin receptors from different tissues. Furthermore, the effects of WGA on adipocyte and liver receptors indicate the potential usefulness of this lectin in studies of acute changes in the the insulin binding properties of these insulin target tissues. **DIABETES *30*:196–202, March 1981.**

Insulin receptors from many different tissues and species have been studied extensively by insulin binding analysis.[1–4] Based on results of insulin binding studies, it was suggested that the binding site of the receptor has been highly conserved through evolution.[4] Unfortunately, conventional insulin binding studies do not provide reliable information of the non-insulin-binding regions on the receptor. Although little is known about these regions, they may be important for the proper function of the receptor, e.g., they may help control insulin binding affinity and participate in the coupling of the receptor to effector systems.

Technical problems in receptor purification prevent a direct inspection of non-insulin-binding regions on receptors from different tissues. Therefore, in the present report, an indirect technique employing the plant lectin, wheat germ agglutinin (WGA), was used to probe for differences and similarities in insulin receptors from adipose tissue, liver, placenta, and monocytes. We have recently shown that WGA increases the insulin sensitivity of rat adipocytes by acutely enhancing the insulin binding affinity of the insulin receptor.[5] This "response" of the receptor to the lectin was used in the present study to compare the different receptor populations to the highly "sensitive" fat cell receptor.* The results indicate the existence of differences among the insulin receptors from the various tissues. Furthermore, a consideration of the lectin's effects on insulin binding by adipocyte and liver receptors suggests a possible modification of currently discussed models that attempt to explain curvilinear Scatchard plots of insulin binding data.

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* The use of the terms response and sensitivity are done for convenience in describing the results. They are not meant to imply that the WGA-mediated increase in insulin binding is a physiologic attribute of the insulin receptor.

MATERIALS AND METHODS

Materials. Crude collagenase (type I) was supplied by Worthington Biochemical Co. Bovine albumin fraction V was obtained from Reheis Chemical Co. Silicone oil was purchased from Arthur H. Thomas. WGA and Dextran T-500 were obtained from Pharmacia, and J. T. Baker supplied Triton X-100. Crystalline porcine insulin was a gift of Eli Lilly. New England Nuclear supplied carrier-free Na ^{125}I (high concentration). Polyethylene glycol was supplied by Matheson, Coleman, and Bell. Bovine gamma globulin (fraction II) was purchased from Calbiochem. Ficoll was purchased from Sigma Co. and Hypaque was obtained from Winthrop. n-Butyl phthalate was purchased from Fisher Scientific Co..

Monocyte separation. Human monocytes were obtained from four normal male volunteers (ages 25–37). Heparinized blood (120 ml) was diluted 1:1 (v/v) with saline buffered with 0.8 mM Na_2HPO_4, pH 7.4. Aliquots of 20 ml were layered over 10-ml aliquots of a Ficoll-Hypaque gradient (density 1.078) and centrifuged at 18°C for 30 min at 500 × *g*.[6] The mononuclear cell fraction was removed from the gradient and subjected to further centrifugation for 10 min at 400 × *g*. The cell pellet was washed with a 25 mM Tris, 1% (w/v) albumin buffer, pH 7.6, that contained 1 mM EDTA, 9 mM glucose, 5 mM $MgSO_4$, 5 mM KCl, and 120 mM NaCl. Mononuclear cells were resuspended in this buffer and the percentage of monocytes was determined by cell size methodology.[7]

Isolation of membrane fractions. Fat cell plasma membranes were prepared from adipocytes isolated from fat pads of male Sprague-Dawley rats (150–200 g) by collagenase treatment.[8] The method of Jarett[9] was used to obtain the plasma membranes from the isolated cells. Liver plasma membranes from these rats were prepared by the two-phase polymer methods described by Lesko et al.[10] Crude microsomal membranes from fresh human placenta were isolated as outlined by Maturo et al.[11] Fat cell "ghost" membranes were prepared by a slight modification[12] of the method reported by Rodbell.[13]

Preparation of soluble insulin receptor. The insulin binding activity was solubilized from the membrane preparations by treatment with 1% (v/v) Triton X-100 as previously described.[14] Remaining particulate material was removed by centrifugation at 150,000 × *g* for 90 min at 4°C.

Insulin binding studies. Insulin binding by the various membranes was conducted in Krebs-Ringer phosphate buffer, pH 7.4, containing 0.1 g% bovine albumin. The binding assays were carried out for the indicated time and temperature with the indicated concentrations of ^{125}I-insulin. Insulin degradation under these conditions was less than 10% of the total ^{125}I-insulin added as measured by the TCA precipitation method,[15] and was not altered by WGA. The membranes were removed from the incubation mixture by the centrifugation method of Rodbell et al.[16] and the amount of bound ^{125}I-insulin determined. Specific binding was defined as previously described using 5 μg/ml native insulin to estimate nonspecific binding of ^{125}I-insulin.[14] Nonspecific binding was less than 10% of the total amount of bound hormone, and treatment with WGA did not significantly alter this insulin binding component.

Insulin binding by the soluble receptor preparations was conducted at 4°C for 16 h in the Krebs-Ringer phosphate buffer that contained 0.05% Triton X-100 (v/v).[14] The polyethylene glycol precipitation method was used to separate bound hormone from free ^{125}I-insulin.[14] Specific binding was determined as outlined above for membranes.

Monocyte insulin binding was conducted in the Tris-albumin buffer used in the cell isolation procedure. Monocytes (1×10^6) were incubated with 5×10^{-10} M ^{125}I-insulin in the presence and absence of native insulin (1×10^{-6} M) for 3 h at 20°C. Following the incubation, the mixture (0.35 ml) was transferred to microfuge tubes that contained 75 μl of n-butyl phthalate and centrifuged for 3 min in a Beckman microfuge. The tips containing the cell pellets were removed from the microfuge tubes and the radioactivity was determined.

Protein determination. Protein was measured by the method of Miller,[17] using bovine serum albumin standards.

RESULTS

The effects of various concentrations of WGA on insulin binding by membranes from adipocytes, liver, and placenta are shown in Figure 1. It is evident that the different membrane preparations respond to the plant lectin. However, fat cell membranes are the most sensitive, both in the degree of stimulation (fivefold enhancement) and in the amount of WGA required to elicit the maximum effect (1 μg/ml). Liver and placental membranes required a larger lectin concentration (5 μg/ml) to maximally stimulate insulin binding. The magnitude of the response was much different, however, between these two preparations; liver membranes were more responsive than the placental membranes, although neither preparation responded as well as the adipocyte membranes. These differences in response were not an artifact of the membrane concentration used in the insulin binding studies. Decreasing the amount of membranes by one half or doubling the amount did not alter any aspect of the response to WGA by the three preparations (data not shown). Also, these differences do not appear to result from the different methods used in the preparation of the three membrane fractions. For example, isolation of liver membranes by the method used to prepare placental membranes did not alter the characteristics of the liver membrane response to WGA (data not shown).

Table 1 illustrates the results obtained with another human tissue, circulating monocytes. As shown, WGA failed to stimulate insulin binding by these cells. At high concentrations of WGA (5 μg/ml or greater), microscopic examina-

FIGURE 1. Effects of WGA on specific insulin binding by adipocyte (○-○), liver (●--●), and placenta (△—△) membranes. The membranes were incubated for 60 min at 21°C with the indicated concentrations of WGA and 5×10^{-10} M ^{125}I-insulin. Specific insulin binding was determined as described in METHODS and expressed as a percent of control (100). The results are the mean ± SEM of three experiments.

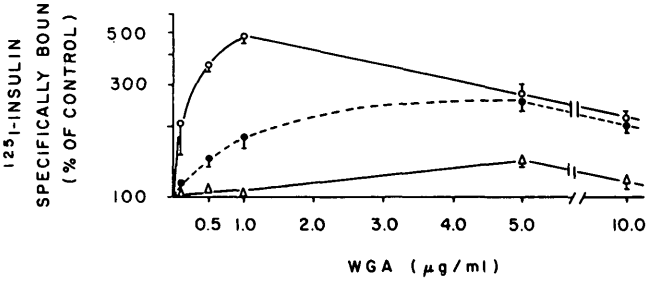


TABLE 1
Effects of WGA on insulin binding by human monocytes

Subjects	Specifically bound ^{125}I-insulin (% of control) at WGA concentrations of: 0	0.1 μg/ml	0.5 μg/ml	1.0 μg/ml	3.0 μg/ml
A	100 (5.0)*	85	73	79	—
B	100 (5.4)	88	95	86	—
C	100 (6.0)	85	92	104	61
D	100 (7.2)	69	129	69	51
Mean ± SE	(5.9 ± 0.5)	82 ± 4	97 ± 12	84 ± 7	—

* The values in the parentheses represent the amount (fmol × 10^1) of ^{125}I-insulin specificially bound to 1 × 10^6 monocytes. The conditions for measuring insulin binding are given in METHODS.

tion indicated marked cellular destruction (data not shown). However, with WGA concentrations of 3 μg/ml or less, the cells appeared intact, although no insulin binding response to the lectin was observed. Thus the results presented in Figure 1 and Table 1 indicate a marked difference in the various receptor populations from rat adipocytes, rat liver, human placenta, and human monocytes.

These differences were further studied in the membrane preparations by examining the effect of WGA on the dissociation of insulin from the receptor (Figure 2). WGA treatment of these preparations resulted in a decrease in the insulin dissociation rate which was initiated by the addition of excess native insulin. As expected, the three preparations displayed a differential sensitivity to this lectin-mediated effect. The inhibition of dissociation was most prominent with adipocyte membranes, followed by liver membranes, and was only slightly present in the placental preparation. This ranking of membrane sensitivity corresponds to the order of WGA responsiveness indicated in Figure 1.

The possibility that WGA alters the phenomenon termed negative cooperativity first reported by De Meyts et al.[18] was studied in a second series of experiments with fat cell membranes (Figure 3). Insulin dissociation was initiated by rapid suspension of the membranes in a large volume of buffer in the absence or presence of native insulin. As shown, the presence of insulin caused an increase in the rate of radiolabeled insulin dissociation from control membranes. WGA treatment of the membranes during the insulin binding phase of the experiment markedly reduced the rate of insulin dissociation even though the lectin was not present in the dissociation buffer. Moreover, in contrast to the

FIGURE 2. Insulin dissociation in the presence (○---○) and absence (●-●) of WGA. Liver, adipocyte, and placental membrane preparations were preincubated with optimal concentrations of WGA (5, 1, and 5 μg/ml, respectively) and ^{125}I-insulin (5 × 10^{-10} M) for 30 min at 21°C. Dissociation was initiated (0 time) by the addition of native insulin (1 × 10^{-6} M). The membranes were isolated from the incubations at the indicated times by centrifugation for 3 min (see METHODS). Nonspecific insulin binding was corrected by parallel studies in which native insulin (1 × 10^{-6} M) was included in the preincubation phase.

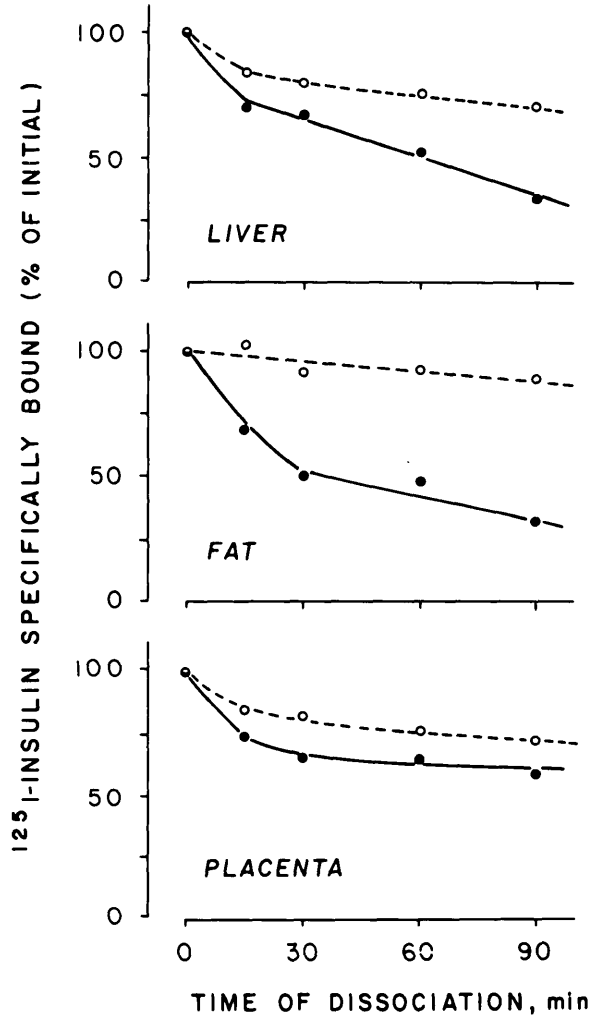
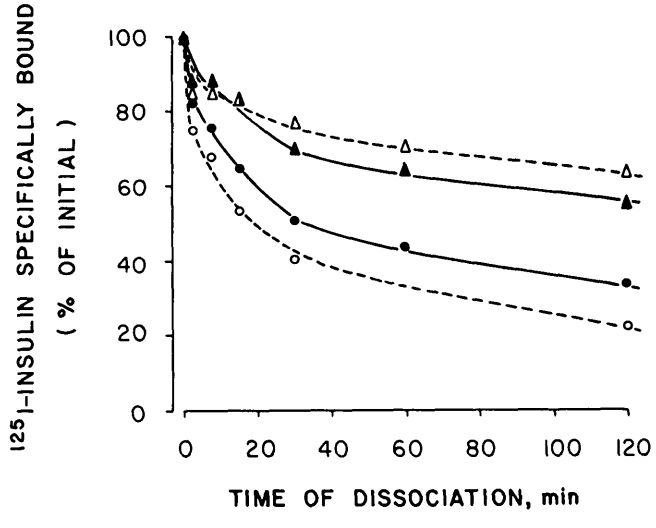


FIGURE 3. Effect of WGA on "negative cooperativity." Adipocyte membranes (650 μg protein for each system) were incubated for 45 min at 21°C with 5 × 10^{-10} M ^{125}I-insulin in the presence (△,▲) or absence (○,●) of 1 μg/ml WGA. The membranes were then subjected to centrifugation (10,000 × g for 3 min at 4°C), and at time 0 the membrane pellet for each treatment group was resuspended in 30 ml of a Krebs-Ringer phosphate buffer that contained 100 ng/ml insulin (○, △) or no hormone (●, ▲). As measured by ^{14}C-sucrose, this maneuver diluted the resident incubation medium in the membrane pellets by 1800-fold or more. The temperature of the buffer was maintained at 15°C during the dissociation phase of the experiment. At the indicated times, a 2-ml aliquot was removed from each treatment group and added to a millipore filtration manifold fitted with 25-mm Gelman membrane filters (GA-6 Metricel, 0.45 μm) under vacuum. Filtration was complete within 10 s and the filters were washed twice by 5 ml of an ice-cold buffer. Nonspecific binding of radiolabeled insulin was estimated by parallel experiments in which 5 μg/ml of native hormone was included in the incubation with the labeled hormone. The results have been corrected for nonspecific insulin binding at each time point.



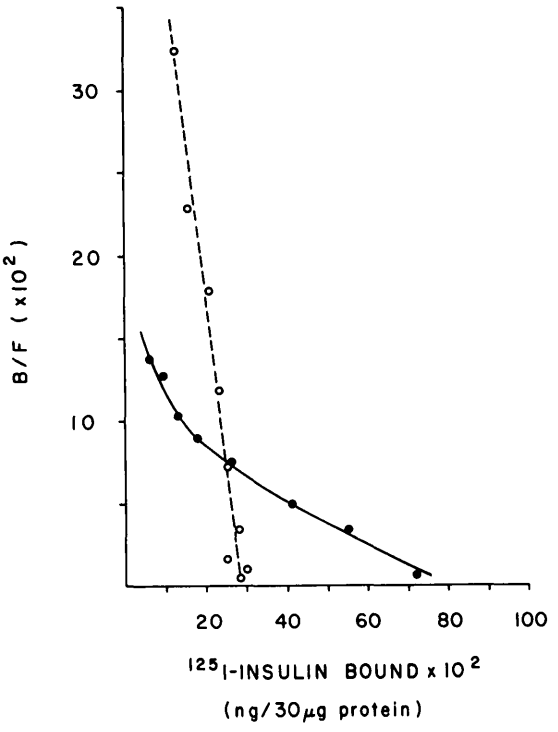


FIGURE 4. Scatchard plots of insulin binding to adipocyte membranes in the presence (O---O) and absence (●-●) of 1 μg/ml WGA. Insulin binding studies were conducted as described in METHODS using a 16-h incubation period at 4°C. The results are representative of three separate experiments.

findings with untreated membranes, the presence of native insulin during dissociation failed to increase the off rate of ^{125}I-labeled hormone. Instead, its presence tended to further inhibit the rate of dissociation over that caused by WGA alone. Although the cause of this effect is unknown, it has also been demonstrated in isolated fat cells as well as in fat cell membranes (J. N. Livingston, unpublished observations). Therefore, these findings indicate that pretreatment of adipocyte membranes with the lectin markedly alters the process underlying "negative cooperativity."

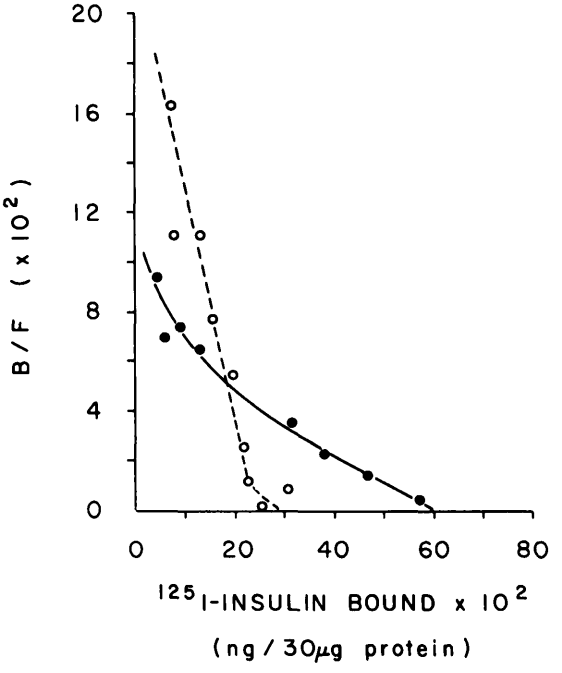
The effects of WGA on the insulin receptor number and binding affinity for these membranes are shown in Scatchard plots (Figures 4–6). Treatment with 1 μg/ml WGA produced striking changes in the shape of the Scatchard plot for adipocyte membranes (Figure 4). The lectin caused a complete linearization of the normally curvilinear plot and decreased the maximum binding capacity to less than half of the normal capacity. Apparently, a loss of low-affinity binding sites was responsible for the decrease in the insulin binding capacity. It is difficult, however, to compare the curvilinear Scatchard plot for untreated membranes with the linear plot and determine whether the other lectin-induced changes arise from an increase in the number of high-affinity sites or from an increase in their insulin binding affinity. Although the dissociation studies indicate an overall increase in the insulin binding "affinity" of the membranes, this effect could be produced by an increase in the affinity of the high-affinity sites or by the increase in the proportion of high-affinity sites over the low-affinity sites.

These findings were unexpected, as previous insulin binding studies with intact adipocytes showed only an increase in the overall receptor binding affinity following WGA treatment.[5] There was no indication of a reduction in total binding capacity nor was the Scatchard plot completely linearized. The different results may be explained in part by the different conditions required for adipocyte insulin binding studies, and by the possibility of insulin internalization into the intact cell[19–21] with its consequent artifactual effects on Scatchard analysis of insulin binding data. Furthermore, the concentration of WGA used in the previous experiment did not produce maximal enhancement of insulin binding.

The lectin produced effects in liver membranes similar to those found with adipocyte membranes. The Scatchard plot was almost completely linearized into one high-affinity component and a decrease in the total insulin binding capacity was observed (Figure 5). Low-affinity binding was essentially absent, but the degree of enhancement of insulin binding by the lectin at low insulin concentrations was not as great as that found in the adipocyte membranes. In contrast to the findings with adipocyte and liver membranes, WGA treatment of placental membranes failed to linearize the Scatchard plot and only a small reduction in the total insulin binding capacity was observed (Figure 6). A small increase in the amount of insulin bound at low insulin concentrations was elicited by the lectin, which agrees with the small lectin effect on insulin dissociation.

The effects of WGA on the fat cell receptor was also produced by treating the adipocytes with the lectin before isolating the membranes (Figure 7). In these studies, fat cell "ghosts" were used in the insulin binding studies since they can be rapidly prepared from the intact cells. As shown, treatment of intact adipocytes with WGA before preparation of the membrane fraction resulted in linearization of the Scatchard plot of insulin binding data. The addition of the plant lectin to the membranes from WGA-treated cells failed to further increase the insulin binding affinity or to further

FIGURE 5. Scatchard plots of insulin binding to liver membranes in the presence (O---O) and absence (●-●) of 5 μg/ml WGA. The conditions are the same as outlined in Figure 4.



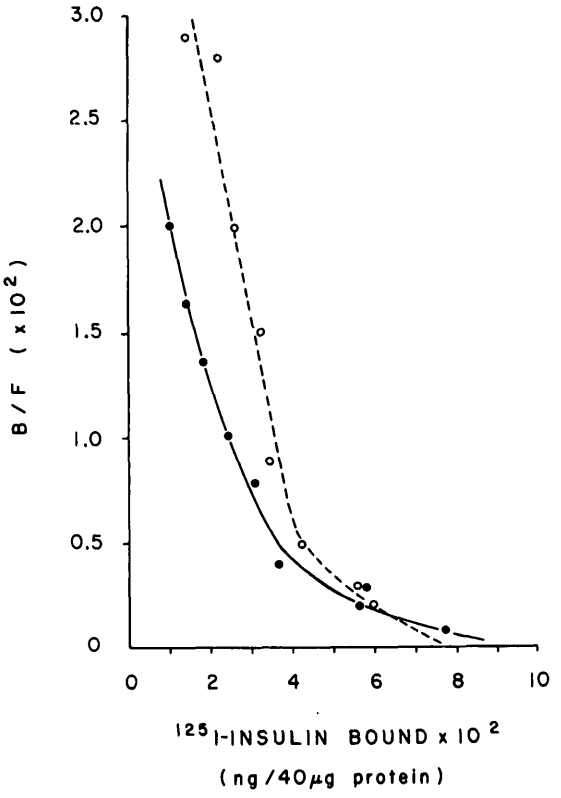
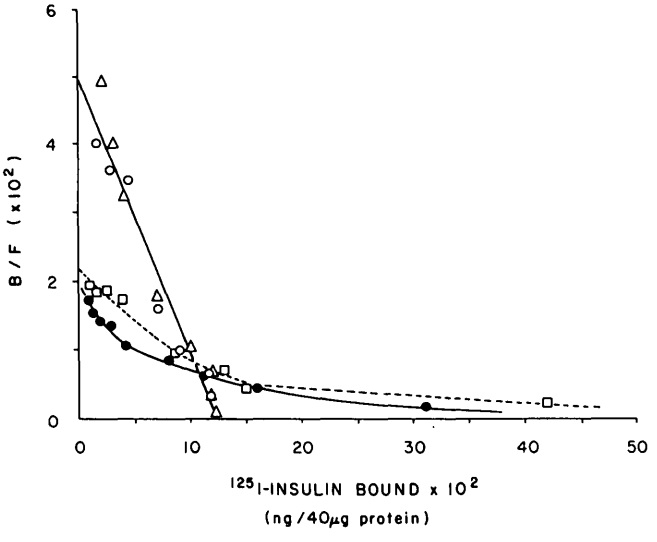


FIGURE 6. Scatchard plots of insulin binding to placental membranes in the presence (○---○) and absence (●-●) of 5 μg/ml WGA. See Figure 4 for an outline of the incubation conditions.

alter the insulin binding capacity, indicating that the changes produced during lectin treatment of the cells was fully retained during the preparation of the membranes. These effects, however, were reversed by the inclusion in the binding assay of ovomucoid, a glycoprotein that has been used previously to remove WGA bound to adipocytes.[5] Thus, the changes in the insulin binding appear to result from the presence on the cell membranes of WGA, which reportedly dissociates very slowly at 4°C.[22] Furthermore, the ability of ovomucoid and n-acetyl D-glucosamine (data not shown) to reverse the lectin-induced changes indicates that the alterations in the insulin-binding are the result of WGA-cell surface association(s) and are not caused by "permanent" changes in either the insulin receptor or the fat cell membrane.

The insulin receptors were solubilized from the three membrane preparations and the effect of WGA on their insulin binding activities was examined (Figure 8). The soluble receptors responded to the lectin; however, some differences were evident between the soluble preparations and their membrane-bound counterparts. For example, the adipocyte receptor in the soluble form was not as responsive to WGA as the membrane-bound receptor (compare Figure 8 with Figure 1). Conversely, the soluble liver receptor was more responsive to the lectin than the membrane form, both in the degree of stimulation and in the amount of lectin required to produce maximal binding activity. For the soluble placental receptor, there was only a small WGA enhancement of insulin binding activity, which was less than the first produced in the membrane preparation. As in the membrane preparation, a WGA concentration of 10 μg/ml caused modest (liver) to marked (placenta and adipocyte) inhibition of insulin binding from the peak binding levels.

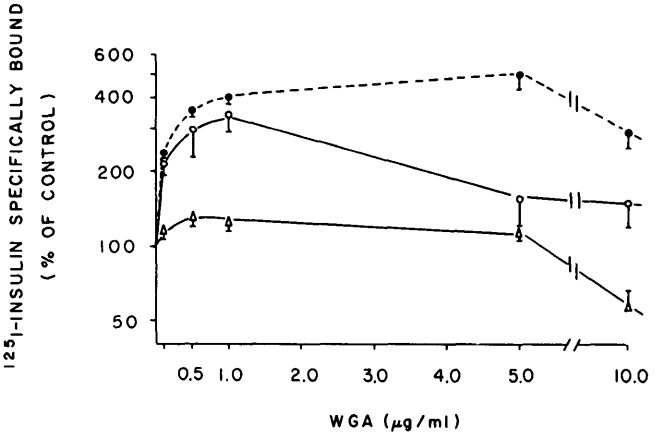
FIGURE 7. Scatchard plots of insulin binding data from "ghosts" prepared from control and WGA-treated adipocytes. Fat cells were incubated in the presence (○,△,□) or absence (●) of 1 μg/ml WGA for 30 min at 37°C. Adipocyte ghost membranes were prepared from these cells as indicated in METHODS. To ghost from WGA-treated cells (○), ovomucoid (5 mg/ml) (□) or WGA (1 μg/ml) (△) was added during the insulin binding assay. The insulin binding studies were conducted under the conditions described for Figure 4.



DISCUSSION

We[5] and others[23,24] have previously shown that WGA increases insulin binding by intact rat adipocytes in an acute and completely reversible manner. In the present study, this effect of the lectin was used to probe for differences among various insulin receptor populations. Rat adipocyte membranes were chosen as a standard to compare the receptors from rat liver, human placenta, and human monocytes. The latter two tissues were used because they represent the only readily available sources of human insulin receptors; more-

FIGURE 8. Effects of WGA on insulin binding by receptors solubilized from adipocyte (○-○), liver (●---●), placental (△-△) membranes. The incubation conditions and binding assay are described in METHODS; the concentration of ^{125}I-insulin was 5×10^{-10} M. The results are the mean ± SEM of three separate experiments.



over, circulating monocytes have been extensively used in studies of insulin-resistant conditions.[25] Rat liver membranes were studied since liver, along with adipose tissue, represents a well-established insulin target organ.[26]

WGA did not enhance insulin binding equally among the four tissues. Adipocyte membranes were the most sensitive to the lectin as judged by any of the methods used to examine insulin-receptor interaction, i.e., WGA dose-response characteristics, Scatchard analysis of insulin binding data, and the rate of ^{125}I-insulin dissociation. The effect of WGA on liver membranes was not as great as the lectin's effect on adipocyte membranes; however, their response was much greater than the WGA effect on placental membranes. Although the response in placental membranes was quite small, it was significant and highly reproducible. Only human monocytes completely failed to demonstrate any significant increase in insulin binding following WGA treatment. This lack of effect was not caused by an inability of WGA to associate with monocytes, since high lectin concentrations caused cellular agglutination and eventual lysis. Because of the lack of response and the difficulties involved with isolating sufficient monocyte membranes, further studies using this tissue were not conducted.

The cause of the differential responses among the three membrane preparations resides either in subtle differences in receptor structure or in differences in the membrane environment that contribute to or inhibit the WGA effect. Solubilization of the receptors by detergent treatment was an attempt at removing the receptor from the influences of other membrane constituents. This maneuver did not potentiate the response of the placental receptor to WGA; in fact, the effect was diminished by receptor solubilization. In contrast, soluble liver receptors were more responsive to the lectin than its counterpart, the membrane-bound receptor. A change was also noted for the fat cell receptor, which, like the placental receptor, was less sensitive in the soluble form to WGA.

Cuatrecasas[24] has also compared the ability of WGA to enhance insulin binding by solubilized and membrane-bound receptors from liver and fat. He concluded that solubilized receptors responded in nearly the same fashion to WGA as the receptors in the membrane preparations. In these experiments, solubilization of liver receptors increased their response to the plant lectin, as it did in the present study. However, in contrast to our findings, Cuatrecasas reported that solubilization of adipocyte receptors also increased the enhancement of insulin binding found after lectin treatment. The cause of this difference is not readily evident, although different conditions for measuring insulin binding activity were used by Cuatrecasas, including a preincubation step with WGA and the insulin receptor before the insulin binding studies were conducted.

The studies with the soluble receptors do not completely rule out an influence of some membrane component on the WGA effect, since the detergent solubilizes a variety of proteins and lipids besides the insulin receptor. There is also the possibility that Triton X-100 treatment introduces artifacts that alter the WGA effect. However, these results suggest that structural differences exist among the different receptor populations, especially since removal of the placental receptor from its membrane environment did not overcome its relative insensitivity to the plant lectin. The possibility of receptor heterogeneity among various tissues agrees with the results of two studies that have used antibodies against the insulin receptor to examine structural similarities of different receptor populations.[4,27] In both studies differences in antibody reactivity to receptors from mammalian tissues were reported. Also, Maturo and Hollenberg[28] described differential effects when insulin receptors from adipocytes and transformed fibroblasts were subjected to a variety of chemical and enzymatic treatments.

Although these findings and those of the present study suggest differences in receptor structure, the demonstration of virtually identical insulin binding properties in all insulin receptors examined supports conservation of the insulin binding site on the receptor.[4] However, even though the binding site may be conserved, our results suggest that it differs among different receptor populations with regard to the changes elicited by perturbation of receptor carbohydrates following binding of WGA.

The studies with WGA in adipocyte and liver membranes are relevant to other aspects of the insulin receptor. The WGA-induced linearization of the Scatchard plot to one high-affinity component with a marked reduction in the low-affinity binding capacity has not been described previously for insulin receptors under any conditions. These dramatic effects of the lectin may offer insight into such phenomena as curvilinear Scatchard plots, negative cooperativity, and the acute changes in receptor affinity that occur under physiologic conditions.[29]

Although the molecular events underlying the lectin-induced effects are not yet understood, certain fundamental properties are evident from the present results. For example, the experiments with fat cell "ghosts" indicate that WGA treatment of intact adipocytes will produce the same changes in insulin binding as lectin treatment of the adipocyte plasma membrane (Figure 7). These experiments also demonstrate that the alterations in binding are easily reversed by simply removing the bound lectin. Thus, the action of the lectin involves a direct interaction of WGA with the insulin receptor or with another membrane component(s).

The studies with insulin dissociation indicate that WGA abolishes "negative cooperativity" (Figure 3). A mechanism for WGA action based on this effect could explain the increase in insulin binding at low insulin concentrations. However, it is also possible that the effect on cooperativity is secondary to a more primary event. Furthermore, an alteration mediated simply by this mechanism would not explain the decrease in the insulin binding capacity if the present model for negative cooperativity is correct.[30] This latter effect of WGA treatment is difficult to interpret in light of current models of the insulin receptor, since none can provide for a rapid (dynamic) change in the number of binding sites.[30–34] Although it is possible that this lectin-mediated effect has no physiologic counterpart, one should consider the possibility that the number of binding sites can be acutely modulated and is a part of normal receptor physiology, like the acute modulation of binding affinity.

In summary, these studies show that insulin receptors from different tissues and species are not equivalent when probed with WGA. Furthermore, the results demonstrate the potential use of WGA in studies of acute changes in the insulin binding properties of target cells.

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