Interaction of Concanavalin A and Wheat Germ Agglutinin with the Insulin Receptor of Fat Cells and Liver*

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SUMMARY

Wheat germ agglutinin enhances the specific binding of insulin to isolated fat cells and to liver cell membranes at a concentration of about 1 μg per ml. Wheat germ agglutinin increases insulin binding by increasing the rate of insulin-receptor complex formation; the protein does not alter the rate of dissociation of the insulin-membrane complex or the total number of binding sites for insulin.

Higher concentrations of wheat germ agglutinin, as well as concanavalin A, block the binding of insulin to fat cells when the plant lectins are added to the cells before insulin. If the insulin-receptor complex is formed before addition of the plant lectin, only an increase in insulin binding with wheat germ agglutinin and virtually no effect of concanavalin A are demonstrable.

Studies using combinations of both plant lectins suggest that these proteins are binding to different regions of the insulin receptor and that some lectin molecules can bind to the cell in a way which is without effect on insulin binding unless the other lectin is also present.

Wheat germ agglutinin and concanavalin A modify insulin binding in membrane preparations in a manner similar to that described for intact fat cells. Similar alterations of insulin binding occur with soluble preparations of the insulin receptor of liver and fat cell membranes. These results indicate that the changes in the binding of insulin occur by direct binding of the plant lectins to the insulin receptor macromolecule. All of the effects of the plant lectins are reversed rapidly by adding simple sugars having selective specificity for the proteins, N-acetyl-D-glucosamine (for wheat germ agglutinin) or α-methyl-D-mannopyranoside (for concanavalin A).

Digestion of fat cells with trypsin, which results in a marked fall in the affinity of the membrane receptor for insulin, similarly reduces the apparent affinity of wheat germ agglutinin for the membrane sites with which the lectin interacts to enhance glucose oxidation in the fat cells. These studies suggest that the insulin-like biological activity of wheat germ agglutinin may result from direct interactions with the insulin receptor in a way which closely resembles the interaction with insulin. If concanavalin A stimulates glucose transport by interacting with the insulin receptor, such an interaction must be somewhat dissimilar from that of insulin since tryptic digestion of cells does not markedly affect the biological response to this protein.

These studies indicate that the insulin-binding macromolecules of liver and fat cell membranes are proteins of complex carbohydrate composition which have several chemically distinct sites capable of binding plant lectins in a manner which perturbs the insulin-receptor interaction.

Concanavalin A and wheat germ agglutinin are plant proteins which can bind to specific carbohydrate determinants on the surfaces of mammalian cells (1-10). These plant lectins can agglutinate various normal and neoplastic cells (11-19), and concanavalin A can stimulate mitosis and blastogenic transformation of lymphocytes (20-22), inhibit phagocytosis by polymorphonuclear leukocytes (23), and prevent lymphocyte cap and patch formation induced by anti-immunoglobulin (24). It has been shown recently that very low concentrations of concanavalin A and of wheat germ agglutinin can, like insulin, enhance the rate of glucose transport and inhibit lipolysis in isolated fat cells, and inhibit the activity of adenylate cyclase in isolated liver and fat cell membranes (25). The effects of digesting fat cells and membranes with proteases (26) and glycosidases (27, 28) on the biological responses of the cells to insulin, and on the specific binding of insulin to the cells and membranes, have suggested that the insulin receptor structures of these tissues may bear carbohydrate moieties.

The present studies describe the alterations caused by wheat germ agglutinin and concanavalin A on the specific interaction of insulin with adipose tissue cells, fat and liver cell membranes, and isolated insulin-binding proteins. The results support the view that the insulin receptor of these tissues is a glycoprotein which contains several distinct carbohydrate regions which are capable of interacting with the plant lectins in a manner which uniquely perturbs the insulin-receptor interaction.

MATERIALS AND METHODS

Crystalline pork zinc-insulin (24 units per mg) was purchased from Eli Lilly, Triton X-100 from Beckman, three times crystal-
lized concanavalin A from Miles, α-methyl-D-mannopyranoside from Calbiochem, N-acetyl-D-glucosamine from Sigma, and carrier-free Na[125I] in 0.1 M NaOH from Union Carbide. Wheat germ agglutinin was a gift from Dr. V. Marchesi, National Institutes of Health; it had been purified to homogeneity (sodium dodecyl sulfate disc gel electrophoresis) in one step from crude wheat germ lipase (Miles) by affinity chromatography (29).

Isolated fat cells were prepared from Sprague-Dawley rats (80 to 110 g) by the method of Rodbell (30). Conversion of [14C]glucose to [14CO2] was determined as described earlier (31). Crude fat cell membranes were prepared by homogenization and centrifugation of the isolated fat cells as described earlier (32). Liver membranes were prepared by homogenization and differential centrifugation in 0.25 M sucrose (33–35). Protein was determined by the method of Lowry et al. (36) after heating at 100° for 30 min in 1 M NaOH; bovine albumin was used as the standard.

The procedures used for the preparation of [125I]-insulin and for measuring the specific binding of iodoinsulin to cells and membranes have been described (26, 27, 32, 37). The nonspecific binding of [125I]-insulin was determined for every experimental point by including samples in which native insulin (5 µg per ml) was added to the tissue before [125I]-insulin. In all of the experiments (except those of Fig. 2) described in the present studies the concentration of [125I]-insulin (5 × 10−6 cpm per ml) used was approximately 5 × 10−10 M. Under these conditions the nonspecific binding of [125I]-insulin to fat cell membranes, and solubilized proteins is equal to 10 to 20% of the total radioactivity bound. The nonspecific binding of insulin in a given experiment is proportional to the concentration of [125I]-insulin in the medium (37) so that in the experiments described in Fig. 2 the nonspecific binding increased to about 30% of the total radioactivity bound with the highest [125I]-insulin concentration (5 × 10−6 cpm per ml). The plant lectins do not alter the nonspecific binding of [125I]-insulin to cells, membranes, or filters under any of the conditions used in the present studies. The specific activity of the [125I]-insulin preparations used in these studies varied between 900 and 1400 Ci per mmole.

The methods used to solubilize the membrane insulin-binding proteins with Triton X-100 and the assay used to detect soluble insulin-receptor complexes, which is based on the selective precipitation of the complex by polyethylene glycol, are presented elsewhere (33, 35, 38).

The range of molar concentration of the plant lectins which was used in the present experiments is described in Figs. 1 and 5; the molecular weight of wheat germ agglutinin was assumed to be 25,000 (6, 30) and that of concanavalin A 100,000 (3, 7, 40).

RESULTS

**Effect of Wheat Germ Agglutinin on Binding of Insulin**—Fat cells preincubated with very low concentrations (0.5 to 3 µg per ml) of wheat germ agglutinin show enhanced binding of iodoinsulin (Fig. 1). As the concentration of the plant protein is increased, progressive depression of insulin binding is observed so that with 80 to 100 µg of wheat germ agglutinin per ml there is almost complete suppression of insulin binding.

These effects of wheat germ agglutinin are altered markedly (Fig. 1) by the addition of N-acetyl-D-glucosamine, a simple sugar which binds specifically to this protein (41). As will be described shortly, the effects of wheat germ agglutinin on the binding of insulin to isolated liver and fat cell membranes are very similar to those observed in intact fat cells. However, the precise concentration of wheat germ agglutinin required to cause the specific changes in insulin binding described in Fig. 1 vary depending on the concentration of membrane or fat cells utilized. This is not surprising since with the low concentrations of wheat germ agglutinin which are usually used in these experiments the lectin is nearly quantitatively bound to the cells or membranes (42). It is important, however, that the quantitative effects of wheat germ agglutinin on insulin binding. It has been established independently (42) that wheat germ agglutinin binding to fat cells or membranes reaches equilibrium in 30 to 40 min with all concentrations of the plant lectin used in these experiments. Wheat germ agglutinin does not modify the nonspecific binding (51) of [125I]-insulin.

The ability of wheat germ agglutinin to enhance, and at higher concentrations to depress, insulin binding is completely reversible since addition of 0.1 M N-acetyl-D-glucosamine to the cells after they have been preincubated for 1 hour with the plant lectin leads rapidly to a reversal of both types of modification of insulin binding. The decrease in the binding of insulin to fat cells in the presence of high concentrations of wheat germ agglutinin does not result from an interaction of the plant lectin with insulin in the medium since similar effects are obtained if the cells preincubated with the lectin are washed before [125I]-insulin binding is determined.

It has been shown (27) that certain manipulations of fat or liver membranes, such as phospholipase digestion, high NaCl concentrations, and addition of lipid-active compounds, result in a large increase in the specific binding of insulin. This effect...
results from an apparent unmasking of insulin-binding structures which is not accompanied by detectable changes in the affinity of these structures for insulin (27). To determine whether the increased binding of insulin caused by low concentrations of wheat germ agglutinin was also a reflection of unmasking of new binding sites the effect of varying the concentration of 125I-insulin was studied (Fig. 2). Whereas wheat germ agglutinin causes a 300% increase in insulin binding when very low concentrations (about 2 × 10⁻¹⁶ M) of iodoinsulin are used, the binding is increased by only 25% when the concentration of insulin is increased to about 2 × 10⁻¹⁵ M and by 10% when it is increased to 5 × 10⁻¹⁰ M. It is apparent that wheat germ agglutinin does not cause exposure of new receptor structures but rather increases the apparent affinity of the receptor for insulin.

The apparent increase in the insulin-receptor affinity appears to result entirely from an increase in the rate of association of the complex (Fig. 3). Wheat germ agglutinin does not appear to alter the rate of dissociation (at 35°C) of the insulin-membrane complex (Fig. 3).

In the experiments described above the fat cells or membranes were preincubated with wheat germ agglutinin for a sufficiently long period of time (50 to 60 min) to permit equilibrium binding of the plant lectin (42). The effects of wheat germ agglutinin on insulin binding under these circumstances (Fig. 1) suggest that at high concentrations the plant lectin is acting quite differently, possibly acting to directly block the access of the hormone to its binding site. Experiments were therefore performed in which the fat cells were incubated with 125I-insulin before introduction of wheat germ agglutinin (Fig. 4). In these conditions the enhancement of insulin binding is more pronounced and persists over a much broader range of concentration of the plant lectin. Furthermore, when 125I-insulin is added to the cells before addition of wheat germ agglutinin it is very difficult to detect a substantial fall in insulin binding. It is also of interest that the enhancement of insulin binding is still readily demonstrable if the fat cells are preincubated with wheat germ agglutinin and washed before testing for binding of 125I-insulin.

**Effect of Concanavalin A on Binding of Insulin**—Concanavalin A can markedly inhibit the binding of insulin to fat cells provided the lectin is preincubated with the cells for a time which is sufficiently long to achieve binding equilibrium of the lectin (Fig. 5). In contrast to wheat germ agglutinin, concanavalin A does not cause an increase in insulin binding. The effects of concanavalin A can be completely blocked by adding the specific simple sugar (4, 12), a-methyl-D-mannopyranoside. Although in these experiments (Fig. 5) the plant lectin was preincubated with the sugar, it is also possible to observe a reversal of the fall in insulin binding if the sugar is added in high concentration after preincu-
cell membranes as they do in intact fat cells (Table I). Wheat germ agglutinin and concanavalin A have essentially the same effects on the binding of insulin to isolated fat and liver W.  Gonzalez et al. Fig. 5. Effect of concanavalin A on the specific binding of insulin to isolated fat cells in the absence (○) and presence (○) of α-methyl-α-mannopyranoside. The experiments were performed essentially as described in Fig. 1. The fat cells from five rats were suspended in 1 ml of Krebs-Ringer-bicarbonate buffer containing 0.1% (w/v) albumin. After incubating the cells (0.2 ml containing about 3 X 10^6 cells) at 24°C for 60 min with the indicated concentration of concanavalin A, 125I-insulin (1.8 X 10^5 cpm; 4 X 10^{-10} M) was added and the incubation was continued for another 50 min (24°C). In some cases concanavalin A was preincubated with 0.5 M α-methyl mannopyranoside (α-M). In these cases the final sugar concentration in the cell suspension was 50 mM; concentrations of the sugar as high as 0.1 M did not alter insulin binding. Under the conditions described here the binding of concanavalin A to fat cells approaches equilibrium (42).

Effect of the cell surface (Fig. 6). Concentrations of concanavalin A which alone cause little or no suppression of insulin binding can significantly increase the inhibition of insulin binding when combined with concentrations of wheat germ agglutinin which alone either cause marked depression of binding, or which cause an increase in binding, or which are virtually without effect. Thus, with relatively low concentrations of the plant lectins there is little interference of binding of one lectin for the other. Furthermore, the inhibitory effect on insulin binding by one lectin is enhanced by the presence of the other lectin. Direct studies of the binding of these two plant lectins to fat cells reveal that in the concentrations used here they bind quite independently to chemically different sites (42).

Combined Effects of Concanavalin A and Wheat Germ Agglutinin—Studies in which fat cells were incubated with both concanavalin A and wheat germ agglutinin suggest that although both plant lectins can block the specific binding of insulin to fat cells, they appear to do this by binding to different carbohydrate domains on the cell surface (Fig. 6). Concentrations of concanavalin A which alone cause little or no suppression of insulin binding can significantly increase the inhibition of insulin binding when combined with concentrations of wheat germ agglutinin which alone either cause marked depression of binding, or which cause an increase in binding, or which are virtually without effect. The amount of lectin relative to the glucose concentration is very important in determining the exact inhibition of insulin binding in a given experiment since under the conditions used in the present studies a very substantial proportion of the lectin in the medium is bound to the cells (42). The effects of the plant lectins on insulin binding are very reproducible (within 10 to 20%) between different experiments if the concentrations of the reactants are carefully controlled.

Effect of Plant Lectins on Binding of Insulin to Membranes—Wheat germ agglutinin and concanavalin A have essentially the same effects on the binding of insulin to isolated fat and liver cell membranes as they do in intact fat cells (Table I). Wheat germ agglutinin at low concentrations increases the binding of insulin, whereas a suppression of binding is observed with higher concentrations of the lectin. In these particular experiments (Table I) higher concentrations of wheat germ agglutinin are required to observe the effects on liver membranes compared to fat cell membranes partly because the concentration of protein in the former is much greater and since a large proportion of the lectin present in the medium is bound to the membranes under these conditions (42). Concanavalin A causes an inhibition of insulin binding to both types of membrane (Table I).
TABLE I

Effect of wheat germ agglutinin and concanavalin A on specific binding of insulin to liver and fat cell membranes

Fat and liver cell membranes were incubated for 50 min at 24° in 0.2 ml of Krebs-Ringer-bicarbonate buffer containing 0.1% (w/v) albumin and the plant lectin at the indicated concentration. 125I-Insulin (1.1 × 10^6 cpm; 1.8 × 10^{-10} M) was added, and specific binding was determined after incubating for 50 min at 24°. The liver and fat cell membrane concentrations were 680 and 41 µg of protein per ml, respectively.

<table>
<thead>
<tr>
<th>Addition</th>
<th>Specific 125I-insulin bounda (cpm/ml)</th>
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<tbody>
<tr>
<td></td>
<td>Fat membranes</td>
</tr>
<tr>
<td>None</td>
<td>830 ± 60</td>
</tr>
<tr>
<td>Wheat germ agglutinin</td>
<td></td>
</tr>
<tr>
<td>0.3</td>
<td>1,500 ± 110</td>
</tr>
<tr>
<td>1.2</td>
<td>2,010 ± 160</td>
</tr>
<tr>
<td>5</td>
<td>820 ± 40</td>
</tr>
<tr>
<td>20</td>
<td>590 ± 30</td>
</tr>
<tr>
<td>80</td>
<td>510 ± 40</td>
</tr>
<tr>
<td>300</td>
<td>400 ± 20</td>
</tr>
<tr>
<td>Concanavalin A</td>
<td></td>
</tr>
<tr>
<td>2.5</td>
<td>860 ± 40</td>
</tr>
<tr>
<td>40</td>
<td>760 ± 50</td>
</tr>
<tr>
<td>160</td>
<td>440 ± 20</td>
</tr>
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<td>600</td>
<td></td>
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</table>

a Counts per min; average ± standard error of the mean of three replications.

TABLE II

Effect of wheat germ agglutinin and concanavalin A on binding of insulin to solubilized receptor of liver and fat cell membranes

Liver and fat cell membranes were extracted (35, 38) with 1% (v/v) Triton X-100 and centrifuged for 45 min at 300,000 × g. The liver (210 µg of protein per ml) and fat (20 µg of protein per ml) supernatants were incubated for 50 min at 24° in 0.2 ml of Krebs-Ringer-bicarbonate buffer containing 0.1% (w/v) albumin, 0.4% (v/v) Triton X-100, and the indicated lectin. 125I-Insulin (1.4 × 10^6 cpm; 2.7 × 10^{-10} M) was then added, and the samples were incubated for 50 min at 24°. Specific insulin binding was determined by the polystyrene glycol assay (35, 38).

<table>
<thead>
<tr>
<th>Addition</th>
<th>Specific 125I-insulin bound</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Fat</td>
</tr>
<tr>
<td>None</td>
<td>1,030</td>
</tr>
<tr>
<td>Wheat germ agglutinin</td>
<td></td>
</tr>
<tr>
<td>0.3</td>
<td>2,800</td>
</tr>
<tr>
<td>1.2</td>
<td>4,800</td>
</tr>
<tr>
<td>5</td>
<td>5,250</td>
</tr>
<tr>
<td>20</td>
<td>3,200</td>
</tr>
<tr>
<td>80</td>
<td>880</td>
</tr>
<tr>
<td>300</td>
<td>250</td>
</tr>
<tr>
<td>Concanavalin A</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>810</td>
</tr>
<tr>
<td>500</td>
<td>620</td>
</tr>
</tbody>
</table>

Receptors—From the above studies it is not possible to ascertain if any or all of the effects of wheat germ agglutinin or concanavalin A result from direct binding or interaction of these proteins with the insulin-binding structures themselves or whether the effects result from binding to neighboring or even distant structures which in turn modify insulin binding through steric factors or through generalized conformational alterations of the membrane. For this reason the effects of the plant lectins were examined on detergent-solubilized membrane preparations (Table II) in which the insulin-binding protein behaves as a discrete and unique molecular species (having a molecular weight of about 300,000) by a variety of physical criteria (33, 35, 38). It is quite clear that the effects of wheat germ agglutinin and concanavalin A on the binding of insulin to the isolated receptors (Table II) are nearly the same as those described for intact fat cells and fat and liver cell membranes. Thus, all of the observed effects appear to result from direct interaction between the plant proteins and the insulin-binding macromolecules.

Consequences of Tryptic Digestion of Fat Cells—It has been established that digestion of isolated fat cells with trypsin selectively compromises the ability of these cells to respond to insulin (25, 43, 44) as well as to bind iodoinsulin specifically (26, 45). It has been demonstrated further that the principal effect of trypsin digestion of fat cells is to decrease the affinity of the membrane receptor for insulin (20); much higher concentrations of insulin are required to achieve maximal biological responses, and there is a corresponding fall in the affinity of the insulin-cell complex. Studies were performed to determine whether the ability of fat cells to respond to the plant lectins is also affected by digesting the cells with trypsin (Table III).

As described earlier (26), trypsin-treated fat cells can oxidize glucose in response to insulin as well as the native cells provided that a sufficiently high concentration of the hormone is present (Table III). As with insulin, higher concentrations of wheat germ agglutinin are required to stimulate glucose oxidation, but the maximal response is not affected. These results suggest that tryptic digestion of cells may cause a fall in the affinity of some specific membrane component for wheat germ agglutinin. The apparent effect on the affinity of the cell for wheat germ agglutinin, however, does not appear to be as marked as the effect on the affinity of the cells for insulin. In contrast to the observations with insulin and wheat germ agglutinin, concanavalin A stimulates glucose oxidation equally well in normal and trypsin-treated cells.

DISCUSSION

Concanavalin A and wheat germ agglutinin appear to be useful probes for studying the surface topography of various cells since they bind quite strongly to exposed carbohydrate components of the external surface of the cytoplasmic membrane (1-10, 19, 39, 46). The present studies support the results of earlier experiments which suggested that the insulin-binding structures of cell membranes may be glycoprotein in nature (25-28).

There appear to be several chemically distinct carbohydrate sites on the membrane which are capable of binding those plant lectins in a way which alters insulin binding. The enhancement of insulin binding caused by low concentrations of wheat germ agglutinin is probably the result of binding of the lectin to a site which is different from that which predominates when higher concentrations of the plant protein are used. Binding to the latter sites, which appear to have lower affinity for wheat germ agglutinin, effectively blocks the binding of insulin to the receptor. If insulin is already bound to the cell membrane the predominant effect of wheat germ agglutinin, even at high concentrations, is to enhance insulin binding.

Another plant lectin binding site capable of modifying insulin binding is that occupied by concanavalin A. The effect of this
**Table III**

<table>
<thead>
<tr>
<th>Addition</th>
<th>Conversion of $^3$H-glucose to $^14$CO₂$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Undigested cells</td>
</tr>
<tr>
<td>None</td>
<td>12,300 ± 400</td>
</tr>
<tr>
<td>Insulin$^b$</td>
<td>38,600 ± 1,800</td>
</tr>
<tr>
<td>1.5 µg/ml</td>
<td>51,300 ± 2,200</td>
</tr>
<tr>
<td>3.0 µg/ml</td>
<td>51,600 ± 2,700</td>
</tr>
<tr>
<td>6.0 µg/ml</td>
<td>51,100 ± 1,400</td>
</tr>
<tr>
<td>Wheat germ agglutinin</td>
<td>19,100 ± 100</td>
</tr>
<tr>
<td>0.8 µg/ml</td>
<td>19,200 ± 200</td>
</tr>
<tr>
<td>1.5 µg/ml</td>
<td>45,800 ± 1,000</td>
</tr>
</tbody>
</table>

$a$ Counts per min of $^14$CO₂ produced in 2 hours (31); average value ± S.E.M. of three replications.

$b$ Twenty-four units per mg.

Isolated fat cells were incubated at 37° for 15 min in Krebs-Ringer-bicarbonate buffer containing 1% (w/v) albumin and, where indicated, 0.1 mg per ml of trypsin. After addition of soybean trypsin inhibitor (0.2 mg per ml) samples were incubated at 37° for 60 min with 0.2 mM $^3$H-glucose (6 µCi per ml) and the indicated addition. As described previously (28), digestion of fat cells with trypsin leads primarily to a fall in the affinity of the cells for insulin. The earlier observations (26) that more drastic digestion with trypsin leads to a more severe and perhaps different type of effect was confirmed in the present studies since recovery of the insulin response in fat cells treated with 0.5 mg of trypsin per ml was not observable even with 50 units of insulin per ml.

The heterogenous nature of the binding sites for these proteins (42) has not been possible to determine the exact nature of the inhibition of insulin binding by applying conventional kinetic analyses.

The heterogenous nature of the binding sites for these proteins (42) has not been possible to determine the exact nature of the inhibition of insulin binding by applying conventional kinetic analyses. All of the effects of wheat germ agglutinin and concanavalin A on insulin-binding described above appear to result from the direct binding of these proteins to the insulin-receptor complex since the same effects can be reproduced on the solubilized receptor as are observed in the intact cells and membranes. This view is strengthened by the observation that insoluble agarose derivatives of these plant lectins can effectively adsorb the isolated insulin receptor protein in the presence of nonionic detergents (25). Columns containing wheat germ agglutinin- or concanavalin A-agarose selectively remove the soluble insulin-binding proteins from crude mixtures of membrane proteins. The insulin receptor macromolecules can be eluted from these affinity columns with buffers which contain the specific simple sugar. The insulin-binding protein can be purified at least 3000-fold by these procedures.

It is unlikely that the effects of the plant lectins on insulin binding can be explained by agglutination of cells since no discernible agglutination can be detected by careful microscopic examination, since the same effects persist in the solubilized membrane preparations, and since significant effects are detected with very low concentrations (10⁻⁶ M) of these proteins. Cross-linking of adjacent cell surface proteins by the plant lectins may contribute to some of the effects observed on insulin binding. However, the fact that lectin binding is reversed extremely rapidly by specific simple sugars suggests that most if not all of the lectin molecules are binding to the cell surface by a single site (42).

Wheat germ agglutinin and concanavalin A can mimic the effects of insulin on glucose transport and on lipolytic processes in fat cells (25). Furthermore, both plant lectins, like insulin (34), can inhibit at low concentrations the activity of adenylate cyclase in isolated liver and fat cells (25). The maximal effects of the plant lectins on these processes are the same as those obtained with insulin, and the concentrations required to observe these effects are relatively low. Maximal effects occur with 1 to 2 µg of wheat germ agglutinin per ml (25), a concentration which does not substantially block insulin binding but which is optimal for enhancing insulin binding to fat cells (Fig. 1). It is tempting to speculate that the insulin-like activity of the plant lectins results from their ability to bind and therefore directly perturb the insulin receptor. A variety of compounds, such as polyamines (47), organomercurials (48), polyanionic antibiotics (49), ouabain (50), and various enzymic digestions (51–53), have been reported to produce insulin-like activity in isolated fat cells. The mechanism by which these agents exert their insulin-like effects, however, may differ from that of the plant lectins since none of them is capable of altering insulin binding, at least in the range of concentration which is effective in biological experiments (27, 32). It will be of interest to compare the crystallographic structure of insulin with those of concanavalin A and wheat germ agglutinin when these become available, especially since similarities in the structures of insulin and concanavalin A are already apparent (54, 55).

It is not possible at present to definitely ascribe the insulin-like behavior of these plant lectins to their unique ability to directly interact with the insulin receptor. The ability of trypsin digestion of cells to modify the response of the cells to

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$^1$ P. Cuatrecasas, unpublished observations.

$^2$ D. C. Hodgkin, personal communication.
wheat germ agglutinin in a manner so similar to the modification of the insulin response (Table III) suggests that insulin-receptor interactions may be involved in the action of this plant lectin. The fact that the effects of trypsin digestion are less marked for wheat germ agglutinin than they are for insulin, and that there appears to be no effect at all for concanavalin A, does not constitute evidence against the possibility that these plant lectins modulate insulin-like effects by direct interaction with the insulin receptor. It is known that under these conditions trypsin does not destroy the receptor but only alters the apparent strength with which it can bind insulin (26). In these conditions the ability of the cell to respond to a given number of bound insulin molecules is unaltered. The cells are still capable of binding the maximal number of insulin molecules, and the maximal biological response to insulin is unimpaired. It is possible, therefore, that trypsin modifies in different ways the ability of the insulin receptor to bind, and therefore to respond, to insulin, wheat germ agglutinin, and concanavalin A.

It is of interest that the maximal effects of wheat germ agglutinin on enhancing insulin binding and on initiating insulin-like effects by direct interaction with the insulin receptor, and concomitantly a 50% decrease in the ability of the cell to respond to a given number of bound insulin molecules is unaltered. The cells are still capable of binding the maximal number of insulin molecules, and the maximal biological response to insulin is unimpaired. It is possible, therefore, that trypsin modifies in different ways the ability of the insulin receptor to bind, and therefore to respond, to insulin, wheat germ agglutinin, and concanavalin A.

Acknowledgments—The valuable technical assistance of Miss Lily Wu is gratefully acknowledged.

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