Vol. 98, No. 2, 1981 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
January 30, 1981 Pages 367-373

INSULIN-LIKE EFFECTS OF WAX BEAN AGGLUTININ IN RAT ADIPOCYTES

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Received November 6,1980

SUMMARY: Wax bean agglutinin (WBA) was found to mimic the activities of insulin in mediating glucose oxidation and antilipolysis. In contrast, soybean and peanut agglutinins do not exert any of these activities. Unlike concanavalin A and wheat germ agglutinin that were reported previously to exhibit insulin-like activites, WBA neither enhances nor competes with the [^{125}I]insulin binding at relatively high concentrations. Moreover, mild trypsinization of adipocytes, a treatment which greatly diminishes the binding and bioactivity of insulin in fat cells, only slightly affects glucose oxidation induced by WBA. ED_{50} values for WBA mediated glucose oxidation and antilipolysis are 9.3 μg and 40.0 μg, respectively, compared with the nearly identical concentrations required for 50% of maximal effect of both glucose oxidation and antilipolysis, mediated by wheat germ agglutinin. The present studies suggest that these two activities may be triggered by WBA via surface glycoproteins that are distinct from the binding site of insulin.

INTRODUCTION

Several agents mimic insulin's action on various target tissues (1,2) and they can be classified into three main groups: (a) agents [such as vanadyl ions (3,4)], calcium ions and a calcium ionophore (5), or deprivation of extracellular K^+ [a condition known to increase endogenous ATP levels (6)]; (b) external modifiers of the plasma membrane, such as oxidants, sulfhydryl reagents, proteases, and phospholipases (2); and (c) agents, such as lectins, anti-receptor and anti-membrane antibodies, that interact with external plasma membrane determinants (2). The latter group may be more helpful in studying the very first events in hormone-cell interaction, since lectins and antibodies, like hormones, are believed to bind to cell surface determinants with high affinity in a non-covalent and reversible fashion.

Concanavalin A (Con A) and wheat germ agglutinin (WGA) mimic the actions of insulin in fat cells (7,8). In view of the glycoproteinous nature of the

0006-291X/81/020367-07$01.00/0

insulin receptor (9) and the ability of these lectins to compete with the binding of [^{125}I]insulin, their insulin-like actions are thought to be mediated by direct interaction with the insulin receptor (7,8).

In the present study we demonstrate that wax bean agglutinin (WBA), a lectin isolated from yellow wax bean (Phaseolus vulgaris), also mimics the effects of insulin. It does not, however, compete with insulin's binding to its receptor. We suggest that WBA exerts its insulin-like actions via membrane glyooproteins which are distinct from insulin binding sites. The possibility that a glycoprotein effector molecule is an integral part of the hormonal mediated system, and that WBA-mediated glucose oxidation and antilipolysis may be triggered via different glycoprotein receptors, is discussed.

MATERIALS AND METHODS

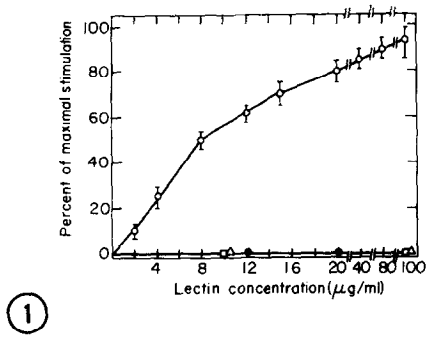
Porcine insulin was purchased from Eli Lilly Co. D[U-^{14}C]glucose (4-7 mCi/mol) was from New England Nuclear. Collagenase Type I (134 U/mg) was obtained from Worthington. Con A and WGA were from Miles-Yeda, Rehovot. WBA was prepared according to Sela et al. (10). Fat cells were isolated (11) from Sprague-Dawley or Wistar male rats (100-200 grams, from the Weizmann Institute Animal Breeding Center). Glucose oxidation was measured by conversion of [U-^{14}C]glucose to $^{14}CO_2$ using 0.2 mM glucose. Soybean and peanut agglutinins were from Dr. N. Sharon. Ovoinhibitor was a gift from Dr. A. Gertler, The Hebrew University. Preparation of [^{125}I]insulin and its binding to fat cells were according to reference 12.

Lipolysis was performed by incubating fat cell suspensions at 37°C for 3 hours together with isoproterenol (2×10^{-7} M) or adrenocorticotropic hormone (0.1 µg/ml), and with insulin or lectins as mentioned in the text. Aliquots from the medium were taken, bovine serum albumin was removed by trichloroacetic acid precipitation, and the glycerol content was determined by the Triglyceride C-37 Rapid-stat Kit described in the Pierce Catalogue, 1976.

The results are expressed as the mean average ± standard deviation of four experiments.

RESULTS

Effect of wax bean agglutinin on glucose oxidation. WBA normally produced 85-100% of the maximal activation of glucose oxidation induced by insulin. This effect was obtained at 0.2 mM glucose, a concentration at which the rate of oxidation is limited by the entry of glucose into the cell (13). As shown in figure 1, 50% stimulation is obtained at 8-12 µg/ml of WBA; fetuin, an efficient glycoprotein inhibitor of WBA, completely inhibited WBA-mediated



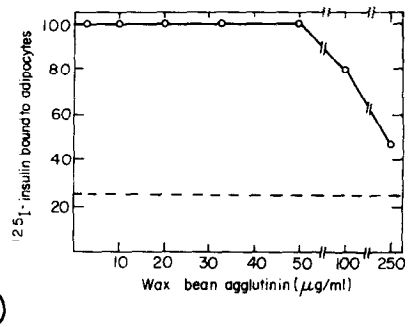


Figure 1 Effect of increasing concentration of various lectins on glucose oxidation. Suspensions of adipocytes (about $3x10^5$ cells in each assay) were incubated for 2 hrs at 37°C in the presence of various concentrations of WBA (o), and two concentrations of peanut agglutinin (□), soybean agglutinin (Δ) and WBA in the presence of fetuin (●). The conversion of glucose to CO_2 was determined. Final concentration of glucose in the assay was 0.2 mM. Results are expressed as percent of maximal stimulation as obtained with 2 mM insulin. Maximal glucose oxidation in the presence of insulin was 3.5 fold higher than in the absence of the hormone.

Figure 2 Binding of [^{125}I]insulin to adipocytes in the presence of WBA. Adipocyte suspensions (about $3x10^5$ cells/ml prepared from 120 gram male Wistar rats) and [^{125}I]insulin (1 ng/ml, 100,000 cpm/ng) were incubated in Krebs-Ringer-bicarbonate buffer (pH 7.4) containing 0.3% bovine serum albumin at 25°C for 1 hr in the presence of varying concentrations of WBA. Cells were then separated and counted for their radioactive content. Extent of binding was 2 fmoles of the labeled hormone per $3x10^5$ cells. Nonspecific binding (with 1 μM native insulin) is represented by dashes.

glucose oxidation at a concentration of 2 mg/ml; peanut agglutinin, a lectin specific for D-galactose-β(1→3)-N-acetyl-D-galactosamine (14) and soybean agglutinin, specific for N-acetyl-D-galactosamine-like saccharides, were completely ineffective in stimulating glucose oxidation at concentrations up to 100 μg/ml.

Effect of lectins on insulin binding. The binding of [^{125}I]insulin to fat cells was not affected by WBA up to a concentration of 50 μg/ml. At 100 μg/ml there was only 20% displacement, whereas 47% displacement was measured at 250 μg/ml (Fig. 2). Peanut and soybean agglutinins did not cause any displacement of [^{125}I]insulin up to a concentration of 100 μg/ml (not shown), whereas WGA, a lectin that mimics some of the major biological effects of insulin, enhanced the binding of [^{125}I]insulin 2-3 fold at low concentrations (1-2 μg/ml), and caused a 50% inhibition of insulin binding at 16 μg/ml (8).

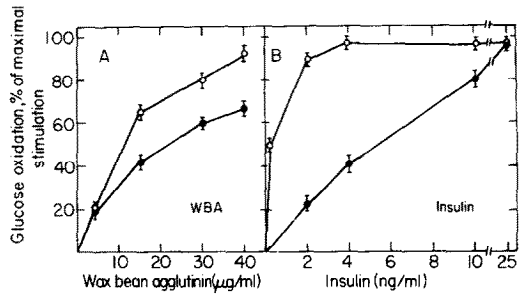


Figure 3 Effect of WBA and insulin on glucose oxidation in trypsinized fat cells. One ml samples of fat cell suspensions ($3x10^5$ cells/ml) were incubated in Krebs-Ringer-bicarbonate buffer (pH 7.4) containing 0.5% bovine serum albumin for 10 min at 37°C with trypsin (30 μg/ml). Ovoinhibitor (80 μg) was then added. After 15 min, varying concentrations of WBA (A) or insulin (B) were added to either untreated (o) or trypsinized (•) cells. The effect on glucose oxidation was then determined (conditions as described in Fig.1). Trypsinization did not affect basal activity of glucose oxidation.

Con A, however, linearly inhibited the binding of [^{125}I]insulin to adipocytes and 50% inhibition was obtained at 12 μg/ml of the lectin.

Insulin-like effects of WBA in trypsinized cells. Mild trypsinization (30 μg/ml trypsin, 10 min) of fat cells decreased insulin affinity for its receptor, resulting in higher ED_{50} values for insulin-mediated glucose oxidation (15). As shown in figure 3A, WBA-mediated glucose oxidation was only slightly affected by mild trypsinization of adipocytes, and the ED_{50} was increased two fold to about 22 μg/ml for trypsinized cells. With insulin, however, a 33-fold increase in the hormone concentration was required to produce 50% maximal activation of glucose oxidation in these cells (Fig. 3B and reference 15).

The antilipolytic action of wax bean agglutinin. WBA, like insulin, inhibits both isoproterenol and adrenocorticotropic hormone stimulated lipolysis (Fig. 4). WBA, however, was less efficient in inhibiting lipopolysis than in inducing glucose oxidation: the ED_{50} values for antilipolytic activity and for glucose oxidation mediated by WBA are 40.0 and 9.3 μg/ml, respectively, i.e. a ratio of 4.3. In the case of WGA, the ED_{50} values for antilipolysis and glucose oxidation are 0.92 and 0.70 μg/ml, respectively, i.e. a ratio of 1.3. The maximal antilipolytic effect of WBA, even at high concen-

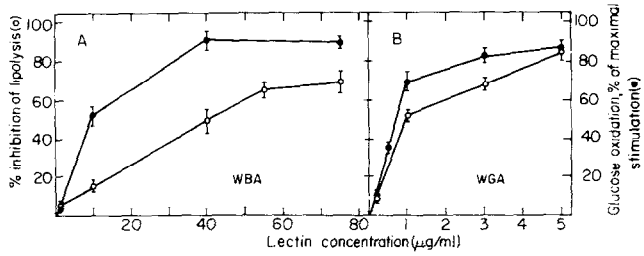


Figure 4 Glucose oxidation and inhibition of lipolysis by WBA (A) and WGA (B). The antilipolytic action (o) and the glucose oxidation activity (●) of both lectins were tested with the same preparation of adipocytes for 2 hrs at 37°C. Glucose oxidation was as described in Fig.1. The antilipolytic activity was measured in the presence of $2x10^{-7}$ M isoproterenol. Glycerol released to the medium was 60 and 200 nmol/ml in the absence and presence of isoproterenol, respectively. Insulin at 3 nM gave 85-95% inhibition of lipolysis.

trations, did not exceed 70% of the maximal effect of either insulin or WGA in this assay.

DISCUSSION

In the present study we provide data to suggest that WBA mimics two of the biological effects of insulin on rat adipocytes. These insulin-like effects seem to be exerted via membrane glycoproteins other than the insulin receptor, an interpretation based mainly on two observations: (a) WBA does not compete with the binding of [^{125}I]insulin within the concentration range needed to produce its effects; and (b) mild trypsinization of fat cells abrogrates only slightly glucose oxidation induced by WBA. Membrane components to which insulin binds appear to be extremely sensitive to tryptic digestion in cells from all target tissues, so that the effect of trypsin seems a reliable criterion to determine the relevance of insulin receptors to any given external stimulant. Lang et al. (16) have demonstrated that all four different molecular weight components from lymphocyte membrane that were bound by insulin, disappeared upon trypsinization at a concentration known to destroy insulin binding. Previously it was shown that mild trypsinization of fat cells reduced WGA-mediated glucose oxidation, but did not affect such activity induced by Con A (8), although the latter displaced [^{125}I]insulin from its receptor. WBA, however, does not compete with insulin binding and is only slightly

affected by trypsinization, and may thus induce its insulin-like reactions via glycoproteins not related to the insulin receptor. Furthermore, our data suggest that the two insulin-like activities may not be triggered by WBA via a common receptor on the plasma membrane. From figure 4 it is apparent that higher concentrations of WBA are required to inhibit lipolysis in fat cells than those needed to stimulate glucose oxidation, the ratio between the respective ED_{50} values being 4.3. However, when WGA was tested simultaneously under identical conditions, the measured ED_{50} values for both of the insulin-like activities are nearly identical, and the ratio is 1.3.

This and other studies suggest the existance of a glycoprotein effector which is a distinct glycoprotein entity accessible to lectins. Perturbation of this effector molecule either by a hormone-receptor complex to form a ternary hormone-receptor-effector complex, or by direct interaction with a lectin or antibody, could trigger bioresponses. The possible existance of a non-receptor glycoprotein that interacts with the insulin receptor has been raised in various reports. Maturo and Hollenberg (17) have suggested that the insulin receptor in liver membranes has a glycoprotein subunit which by itself does not bind insulin but alters the affinity of receptors. Jacobs et al. (18) have demonstrated that the soluble insulin receptors from both rat liver membrane and human placenta consist of a 135,000 molecular weight component that binds insulin, and of a 45,000 molecular weight component that does not. Antibodies prepared against enriched preparations of rat adipocytes' plasma membrane proteins were reported to mimic some of the activities of the hormone, although they do not compete with insulin in binding to fat cells (19).

The relation between the "natural" binding site of insulin and the receptor(s) for lectins that possess insulin-like activity, requires further elucidation. Wax bean agglutinin which may act via a unique class of surface glycoproteins, could serve as a useful probe in studying receptor parameters of insulin-like activities.

ACKNOWLEDGEMENTS

This work was supported in part by grants to Dr. H. R. Lindner by the Ford Foundation and Population Council, New York. B.S. holds the Bertram Blank Career Development Chair.

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