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Insulin-Like Activity of Concanavalin A and Wheat Germ Agglutinin—Direct Interactions with Insulin Receptors

(glucose transport/lipolysis/adenylate cyclase/affinity chromatography/lymphocyte transformation/growth factors)

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ABSTRACT **Concanavalin A and wheat germ agglutinin are as effective as insulin in enhancing the rate of glucose transport and in inhibiting epinephrine-stimulated lipolysis in isolated adipocytes. These lectins, also like insulin, inhibit basal as well as epinephrine-stimulated adenylate cyclase activity of membranes obtained from homogenates of fat cells. Low concentrations of wheat germ agglutinin enhance the specific binding of insulin to receptors of fat cells and liver membranes. Higher concentrations of this plant lectin, as well as of concanavalin A, competitively displace the binding of insulin to receptors in these tissues. These effects are equally apparent in insulin-binding proteins solubilized from membranes, indicating that the plant lectins interact directly with insulin receptors. All of the effects observed with the plant lectins are reversed by simple sugars that bind specifically to these plant proteins. Agarose derivatives of the plant lectins effectively adsorb solubilized insulin-binding proteins, and these can be eluted with buffers containing specific simple sugars. The possible implications of these findings to certain biological properties (mitogenicity) of these lectins and to the mechanism of action of other growth-promoting substances are considered.**

Concanavalin A and wheat germ agglutinin are plant proteins that can bind to specific carbohydrate determinants on the surface of mammalian cells, and they can agglutinate various normal and neoplastic animal cells. In addition concanavalin A can, by unknown mechanisms, stimulate mitosis and blastogenic transformation of lymphocytes (1), inhibit phagocytosis by polymorphonuclear leukocytes (2), and prevent lymphocyte cap and patch formation induced by anti-immunoglobulin (3). In this report, we demonstrate that very low concentrations of these plant lectins have profound insulin-like effects on metabolic processes of isolated fat cells, and that they can interact directly with the cell surface receptors for insulin in these cells. These observations may be of importance in understanding the basis of the biological properties (e.g., lymphocyte transformation) of these lectins, and they may also provide a better understanding of the mechanism of action of insulin.

METHODS

Isolated fat cells were prepared from 80–140 g Sprague–Dawley rats (4). The procedures for the preparation of liver (5, 6) and fat cell membranes (7), and for measurement of the specific binding of [^{125}I]insulin to cell (8) and to membrane (7) receptors have been described. Insulin receptor proteins were solubilized from liver membranes with Triton X-100 (6). Glycerol in the incubation medium was determined by the method of Ryley (9). Adenylate cyclase activity was determined by a modification (10) of the technique of Pohl *et al.* (11). Fat cell membranes (10) were freshly prepared for each experiment by homogenization (Polytron), centrifugation, and suspension of the pellet in 50 mM Tris·HCl (pH 7.6); adenylate cyclase assays were begun within 10 min. Concanavalin A (three-times crystallized) was from Miles. Wheat germ agglutinin, a gift from Dr. V. Marchesi, was purified by affinity chromatography and was homogeneous on Na dodecylsulfate disc gel electrophoresis (12).

Agarose derivatives of the lectins were prepared by the CNBr procedure (13), or by reaction with activated *N*-hydroxysuccinimide esters of diaminodipropylaminosuccinyl-agarose (14). 40 ml of Sepharose 4B was activated with 6 g of CNBr and reacted with 60 ml of ice-cold 0.1 M sodium phosphate buffer (pH 7.4) containing 500 mg of concanavalin A and 0.1 M α-methyl-D-mannopyranoside. After 16 hr at 4°, 2 g of glycine was added and the incubation was continued for 8 hr at 24°. This adsorbent contained 5.5 mg of protein per ml of gel. Wheat germ agglutinin (1.4 mg/ml) was similarly coupled to activated agarose in the presence of 0.1 M *N*-acetyl-D-glucosamine; 1.1 mg of protein was coupled per ml of gel. ^{125}I-labeled plant lectins were used during the coupling procedures. By phase-contrast microscopy, the lectin–beads became heavily coated with erythrocytes when these were mixed at 24° for 1–2 hr. The cells could be rapidly desorbed with 50 mM *N*-acetyl-D-glucosamine.

RESULTS

Effects on glucose transport

Concanavalin A and wheat germ agglutinin are very effective in enhancing the rate of [^{14}C]glucose oxidation in isolated fat cells (Fig. 1). The maximal effects are similar to those that can be achieved with insulin. The concentration required for the half-maximal effect is about 20 nM for concanavalin A (molecular weight: 100,000) and about 4 nM for wheat germ agglutinin (molecular weight: 25,000). These effects result from interactions of the lectins with high-affinity binding sites that represent only a fraction of the total number of lectin-binding sites on the fat cells, since direct binding studies with iodinated lectins demonstrate that saturation requires concentrations greater than 0.1 mg/ml (manuscript in preparation). Fat cells do not agglutinate with either lectin under various conditions studied.

The increased rates of glucose oxidation induced by concanavalin A and by wheat germ agglutinin can be completely and selectively abolished by addition of the specific sugars

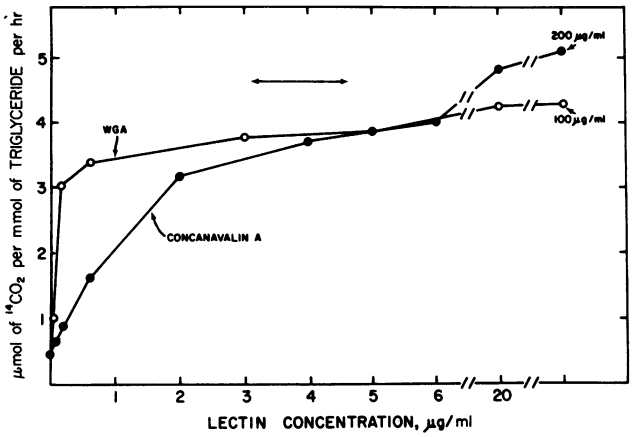

FIG. 1. Effect of concanavalin A and of wheat germ agglutinin (WGA) on the conversion of [^{14}C]glucose to $^{14}CO_2$ by isolated fat cells. Fat cells were incubated for 2 hr at 37° in 1.25 ml of Krebs–Ringer bicarbonate buffer containing 1% (w/v) albumin, and 0.2 mM [^{14}C]glucose (6.4 Ci/mol). The maximal insulin (0.2 munit/ml) response is indicated by the *arrow*.

α-methyl-D-mannopyranoside or *N*-acetyl-D-glucosamine (Table 1). Since, as will be described below, wheat germ agglutinin at low concentrations increases the binding of iodoinsulin to fat cells, the possibility exists that the lectins could be exerting their effect by facilitating effects of endogenous insulin possibly retained on the surface of the fat cells. The inability of a large excess of insulin antiserum to alter the metabolic effects of the cells in the presence or absence of the lectins (Table 1) makes this possibility unlikely.

The effects on glucose oxidation of suboptimal concentrations of the lectins can be further increased by addition of insulin (Table 2). However, the combined effect of insulin and the plant lectins does not surpass the maximal effect that can be achieved by either agent alone. Furthermore, simultaneous addition of both plant lectins in the absence or presence of insulin does not result in greater effects than can be achieved by any one of these three compounds. Tryptic digestion of fat cells, a process that decreases the apparent affinity of insulin for its biological receptor (15), also results in an apparent fall in the binding affinity of wheat germ agglutinin for the sites responsible for enhanced glucose transport (unpublished results). The data suggest that the two plant lectins may be acting by a similar mechanism, and that this mechanism may resemble that by which insulin normally exerts its effects.

TABLE 1. *Reversal by specific sugars of plant lectin stimulation of glucose oxidation by fat cells*

Additions	Production of $^{14}CO_2$*
None	4,400 ± 200
Insulin (0.2 munits/ml)	34,200 ± 1,900
α-Methyl-D-mannopyranoside (25 mM)	4,500 ± 400
N-Acetyl-D-glucosamine (25 mM)	4,800 ± 300
Insulin + α-methyl-D-mannopyranoside + *N*-acetyl-D-glucosamine	32,200 ± 1,100
Concanavalin A (5 μg/ml)	38,700 ± 2,100
Concanavalin A + α-methyl-D-mannopyranoside	4,600 ± 500
Concanavalin A + *N*-acetyl-D-glucosamine	36,800 ± 800
Wheat germ agglutinin (2 μg/ml)	33,000 ± 1,800
Wheat germ agglutinin + *N*-acetyl-D-glucosamine	5,400 ± 200
Wheat germ agglutinin + α-methyl-D-mannopyranoside	33,300 ± 900
Insulin antiserum (1:250 dilution)	4,300 ± 300
Insulin + insulin antiserum	4,200 ± 300
Concanavalin A + insulin antiserum	38,500 ± 2,200
Wheat germ agglutinin + insulin antiserum	34,100 ± 2,000

The conditions are as described in Fig. 1. The fat cell concentration is about 2 × 10^4 cells per ml.

* cpm per 2 hr; average value ±SEM of three replications.

TABLE 2. *Combined effects of insulin, wheat germ agglutinin, and concanavalin A on glucose oxidation by isolated fat cells*

Addition	Production of $^{14}CO_2$*	
	No insulin	+ Insulin (0.2 munit/ml)
None	4,100 ± 200	45,300 ± 600
Wheat germ agglutinin		
0.15 μg/ml	29,000 ± 400	45,300 ± 700
2.1 μg/ml	39,100 ± 500	46,500 ± 700
10 μg/ml	44,700 ± 700	45,900 ± 600
Concanavalin A		
0.2 μg/ml	9,100 ± 300	44,500 ± 700
2.4 μg/ml	47,300 ± 1,100	45,200 ± 900
50 μg/ml	49,100 ± 1,000	48,300 ± 800

* See Table 1.

Effects on lipolysis

Concanavalin A and wheat germ agglutinin are also as effective as insulin in reversing epinephrine-induced lipolysis in fat cells (Table 3). This antilipolytic property, which does not

TABLE 3. *Suppression by wheat germ agglutinin and concanavalin A of epinephrine-stimulated lipolysis in fat cells*

Additions	Glycerol released*
None	6.8 ± 0.5
Epinephrine, 0.16 μg/ml	24.0 ± 1.1
Epinephrine, 0.16 μg/ml +	
Wheat germ agglutinin, 0.11 μg/ml	20.5 ± 0.8
Wheat germ agglutinin, 0.33 μg/ml	14.0 ± 0.6
Wheat germ agglutinin, 1.0 μg/ml	10.3 ± 0.7
Wheat germ agglutinin, 5.0 μg/ml	6.4 ± 0.5
Concanavalin A, 2 μg/ml	25.7 ± 1.4
Concanavalin A, 6 μg/ml	20.1 ± 0.8
Concanavalin A, 30 μg/ml	10.9 ± 0.7
Concanavalin A, 100 μg/ml	8.8 ± 0.9
Insulin, 20 μunits/ml	6.3 ± 0.5
Insulin, 5 μunits/ml	12.9 ± 0.6

Fat cells (3 × 10^4 cells per ml) were incubated for 2 hr at 37° in Krebs–Ringer bicarbonate buffer containing 3% (w/v) albumin.

* μmol of glycerol released per mmol of triglyceride; average value ±SEM of three replications.

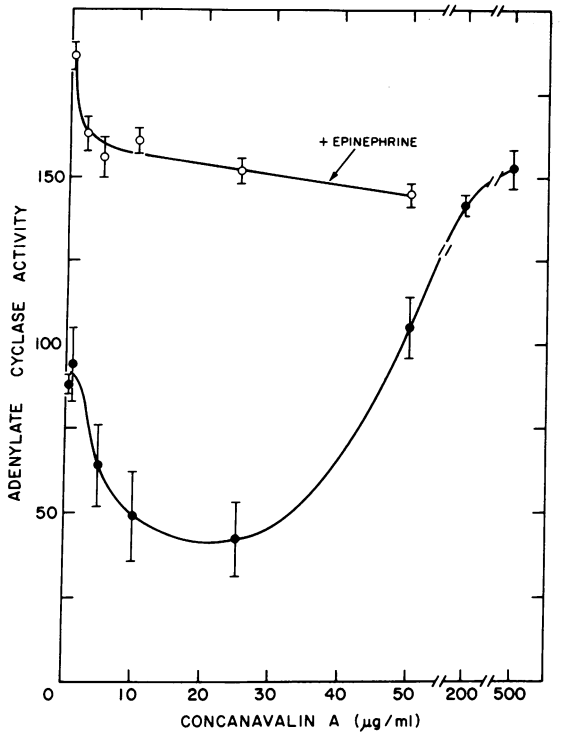

FIG. 2. Effect of concanavalin A on the basal and the epinephrine (1 μM)-stimulated adenylate cyclase activities of fat cell membranes. The incubation mixture (0.1 ml) contained 50 mM Tris·HCl (pH 7.6), 7 mM $MgCl_2$, 1 mM EDTA, 2.5 mM aminophylline, 0.1% (w/v) albumin, 3 mM [α-^{32}P]ATP (1.5 μCi), and about 100 μg of membrane protein; 5 mM phosphoenolpyruvate and 60 μg/ml of pyruvate kinase were used to regenerate ATP. After 10 min at 30°, the tubes were placed in boiling water for 3 min. A recovery mixture (0.5 ml) containing [^{3}H]cyclic AMP was added to each sample. Cyclic AMP was isolated on a column containing 1 g of alumina (16) that was eluted with 2.5 ml of 50 mM Tris·HCl (pH 7.6). Activity is expressed as pmol of cyclic AMP produced per min per mg of protein (average of triplicates ±SEM).

depend on the presence of glucose in the medium, is not mediated by effects on membrane transport. The plant lectins are effective in concentrations as low as those that demonstrably activate glucose transport. The antilipolytic properties are also completely reversed by the specific simple sugars.

Inhibition of adenylate cyclase activity

Physiological concentrations of insulin can inhibit adenylate cyclase activity in isolated liver and fat cell membranes (10) and in fat cell ghosts (17). This effect of insulin is also mimicked by low concentrations of the plant lectins. Concanavalin A, in the concentration range of 5–50 μg/ml, effectively inhibits adenylate cyclase activity in the absence or presence of epinephrine (Fig. 2). Concentrations of concanavalin A greater than 50 μg/ml markedly reverse the inhibition of basal enzyme activity and cause stimulation of this enzyme.

Wheat germ agglutinin (2–50 μg/ml) is also quite effective in decreasing the basal and the epinephrine-stimulated activities of adenylate cyclase (Fig. 3). The paradoxical effects on basal enzyme activity observed with concanavalin A are not apparent with this lectin.

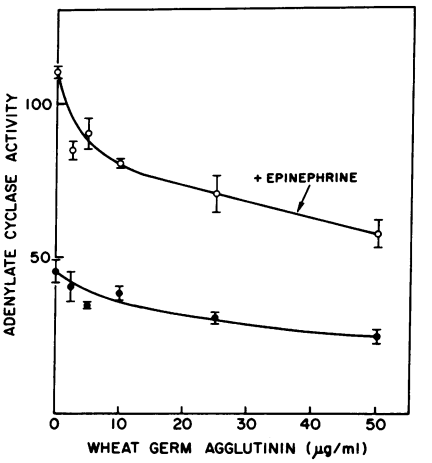

FIG. 3. Effect of wheat germ agglutinin on adenylate cyclase activity of fat cell membranes (as in Fig. 2).

Direct interaction of plant lectins with the insulin receptor

Very low concentrations of wheat germ agglutinin markedly enhance the specific binding of [^{125}I]insulin to fat cells and to liver membranes, whereas higher concentrations inhibit the binding of insulin to these tissues (Table 4). These effects are completely reversed by the simple sugar, *N*-acetyl-D-glucosamine. The increased binding of insulin results from an enhancement in the rate of lectin–cell association, and is not the result of unmasking of new insulin receptors (manuscript in preparation). The inhibition of insulin-binding, on the other hand, results from binding of the lectin to a chemically distinct site on the insulin receptor macromolecule; insulin and wheat germ agglutinin bind competitively at this site.

TABLE 4. *Effect of wheat germ agglutinin and concanavalin A on the specific binding of insulin to isolated fat cells and liver membranes*

Addition	Specific [^{125}I]insulin bound*	
	Fat cells	Liver membranes
None	6,500 ± 300	16,200 ± 900
Wheat germ agglutinin		
1 μg/ml	16,100 ± 650	
4 μg/ml		30,700 ± 1,050
16 μg/ml	3,100 ± 270	
40 μg/ml		19,300 ± 700
Concanavalin A		
5 μg/ml	2,900 ± 210	
40 μg/ml	1,200 ± 80	13,100 ± 400
500 μg/ml		5,700 ± 200

Fat cell (10^6 cells per ml) and liver membrane (0.6 mg of protein per ml) suspensions (0.2 ml) were incubated in Krebs–Ringer bicarbonate–0.1% albumin for 60 min at 24° with the plant lectins. [^{125}I]Insulin (1.8×10^5 cpm for fat cells and 1.2×10^5 cpm for membranes) was added to each sample, and the specific binding of insulin was determined after incubation for 50 min at 24°. All of the effects of wheat germ agglutinin and concanavalin A are completely reversible if 80 mM *N*-acetyl-D-glucosamine or α-methyl-D-mannopyranoside, respectively, are included in the incubation medium.

* cpm bound, average value ±SEM of three replicates.

TABLE 5. *Inhibition by plant lectins of insulin-binding to solubilized insulin receptors of liver membranes*

Addition	Specific binding of [^{125}I]insulin (cpm)
None	4,500 ± 300
Wheat germ agglutinin	
1 μg/ml	9,400 ± 400
50 μg/ml	2,900 ± 100
Concanavalin A	
200 μg/ml	3,000 ± 200

Liver membrane proteins (30 μg in 0.2 ml of Krebs–Ringer bicarbonate buffer) were incubated with plant lectins at 24° for 50 min before the addition of [^{125}I]insulin (7 × 10^4 cpm). Specific binding was determined (6, 18) after incubation at 24° for 40 min.

Concanavalin A can also competitively displace insulin from its receptor in fat cells and in liver membranes (Table 4). This plant lectin does not exhibit enhancement of insulin-binding at any concentration. Compared to wheat germ agglutinin, higher concentrations of concanavalin A are required to produce similar effects. The effects of concanavalin A are reversed by α-methyl-D-mannopyranoside.

All of the effects of wheat germ agglutinin and of concanavalin A that are described above for the binding of insulin to cells or to membranes are equally apparent when the binding of insulin to solubilized insulin-receptor structures from membranes (6, 18) is examined (Table 5). It is thus evident that the perturbations of insulin-binding result from direct interactions of the plant lectins with insulin-receptor structures.

Chromatography of insulin receptors on lectin–agarose affinity columns

The ability of the plant lectins to bind to the isolated insulin receptor (Table 5) suggested that insoluble derivatives of these plant lectins might be useful adsorbents for the purification of insulin receptor structures by affinity chromatography. Agarose derivatives of these plant proteins are indeed capable of adsorbing quite strongly the insulin-binding proteins isolated from liver membranes (Fig. 4). The columns can be washed extensively without desorption, and elution in high yield is achieved selectively with specific simple sugars. In the presence of the sugar no adsorption occurs and the insulin-binding activity is recovered quantitatively.

Concanavalin A and wheat germ agglutinin bind quite well to the insulin-binding protein in the presence of 0.2% Triton X-100, which is used in the chromatography buffers. Fortunately, most of the lectin-binding glycoproteins of the membrane bind very weakly, or not at all, in the presence of this detergent. Thus, very small quantities of protein adsorb to these affinity columns, and it is possible to achieve substantial (3000-fold) purification of the insulin-binding protein by these procedures. Some advantages of these adsorbents, compared to insulin–agarose derivatives (5), include the ease of elution, the high capacity for the binding protein, and the avoidance of possible contamination by insulin.

DISCUSSION

Since concanavalin A and wheat germ agglutinin can at low concentrations bind directly to the insulin receptor of liver and fat cell membranes, it is possible that the potent insulin-like activity of these plant proteins is explicable in terms of lectin–insulin receptor interactions. The existence of such interactions is consistent with earlier evidence (7) suggesting that the insulin receptor structures may be glycoproteins. It will be of interest to examine in detail some of the apparent similarities in the tertiary structures of insulin and concanavalin A (ref. 19 and D. C. Hodgkin, personal communication).

It is tempting to speculate that at least some of the biological effects of the plant lectins, such as their mitogenicity toward lymphocytes, might be related to the ability of these proteins to interact with insulin receptors or to otherwise initiate insulin-like biochemical events. The ability of concanavalin A to induce transformation of mature circulating lymphocytes despite the apparent lack of insulin receptors in these cells (20) does not necessarily negate this possibility. Mature lymphocytes may possess absorptive or "incomplete" insulin receptors that are nevertheless capable of being activated by unnatural and less biologically specific stimuli (plant lectins) than insulin. There is evidence for the existence of "incomplete" insulin receptors in immature mammary gland cells (21). If the initiation of insulin-like biochemical processes is significantly related to the processes of transformation in lymphocytes, there are apparent advantages for the absence of insulin receptors in normal, circulating lymphocytes. It is interesting that circulating human leukemic lymphocytes do possess substantial receptors for insulin (18).

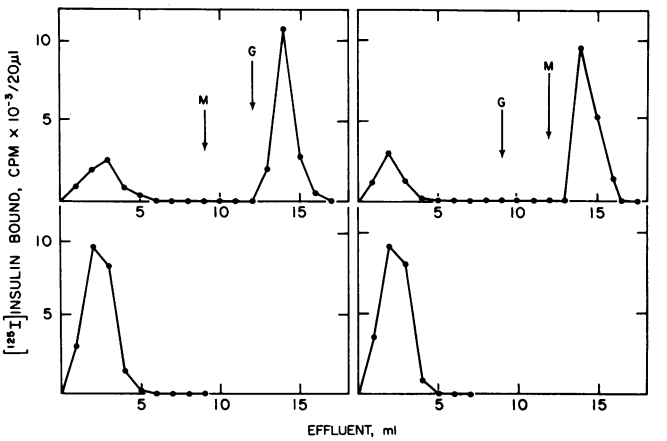

FIG. 4. Affinity chromatography of detergent-solubilized (5, 18) insulin receptors of liver cell membranes on columns containing wheat germ agglutinin–agarose (*left*) and concanavalin A–agarose (*right*). The columns (1 ml, Pasteur pipettes) were equilibrated at 24° for 3 hr with Krebs–Ringer bicarbonate buffer containing 0.1% (v/v) Triton X-100. 2 ml of membrane extract (14 mg of protein per ml) was applied (at 24°) to each column, which was eluted with 0.1 M sodium bicarbonate buffer (pH 8.4) containing 0.1% (v/v) Triton X-100, 0.1% (w/v) albumin, and either 0.3 M α-methyl-D-mannopyranoside (*M*) or 0.3 M *N*-acetyl-D-glucosamine (*G*); the column flow was stopped for 3 hr after addition of the eluting buffer. In control columns (*bottom*), the specific sugar was added to the crude sample before chromatography. The protein content of the pooled material of the void volume was the same as that of the control column, indicating that only a very small fraction of the total protein had actually adsorbed. The binding protein was purified about 3000-fold, and the recovery of binding activity was greater than 90%.

Whether the insulin-like effects of these plant lectins result from direct perturbations of insulin receptors or closely related structures, or whether they result from other cell surface interactions capable of initiating basically similar effects, the possibility exists that the biochemical basis of the mitogenicity of various lectins may be directly related to these insulin-like or "pleiotypic" (22) effects. Furthermore, these considerations may be applicable to other growth-promoting hormones or factors, and even possibly to certain carcinogens. It is well known that insulin has major growth-promoting properties in various cells in culture, and that serum contains essential growth-promoting properties that can be replaced by insulin under some circumstances. It has recently been demonstrated that somatomedin ("sulfation factor", "thymidine factor"), the presumed mediator of growth hormone action, has very potent insulin-like properties in various target tissues, and can interact directly with insulin receptors of fat cells, liver membranes, and chondrocytes (23). Somatomedin also inhibits adenylate cyclase activity in membrane preparations of these tissues (manuscript in preparation). It is possible that the potential for growth or transformation in normal cells is governed by the specific composition of a special class of receptors on the cell membrane that, once stimulated, initiate similar kinds of biochemical events. Such discriminatory controls might be bypassed by compounds, such as the plant lectins, that interact with abortive receptors, or perhaps directly with the structures that are ordinarily coupled to the receptors. It will be interesting to determine if other mitogenic substances (phytohemagglutinins, x-rays, viruses, antigens, etc.) can initiate insulin-like responses in cells.

It is worthwhile considering the possibility that inhibition of adenylate cyclase activity, which may be fundamental to the actions of insulin (10, 17, 24), may be the common biochemical basis for the insulin-like properties of various substances. Concentrations of concanavalin A that cause toxicity in lymphocytes correspond to those that paradoxically stimulate adenylate cyclase activity (Fig. 2). It is notable, furthermore, that compounds that elevate lymphocyte cyclic AMP concentrations inhibit lymphocyte transformation (25), that lymphocytes exposed for 24 hr to phytohemagglutinin have decreased levels of cyclic AMP (25), and that certain phytohemagglutinins inhibit platelet adenylate cyclase activity (26). These considerations are especially pertinent in view of the recent demonstration of important relationships between cyclic AMP and cell growth (27).

Various compounds (polyamines, organomercurials, polyene antibiotics, ouabain), as well as very mild enzymic digestion of cells, are known to enhance glucose transport or inhibit lipolysis in fat cells (28). Although these insulin-like effects do not appear to be modulated by perturbations of insulin receptors, there is good evidence that at least some of these compounds are capable of inhibiting adenylate cyclase activity in liver and fat cell membranes in a manner similar to that observed with insulin (manuscript in preparation).

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