ROLE OF VALENCE AND THE CYTOSKELETON IN THE INSULIN-LIKE ACTIVITY OF CONCANAVALIN A

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1. Introduction

Plant lectins bind to glycoproteins and glycolipids of cell membranes and initiate a broad spectrum of biological and morphological responses [1–3]. Con A, a lectin derived from the jackbean, has been shown to bind glycoproteins with a high specificity for gluco- and mannopyranosides. This binding leads to cell agglutination, changes in mobility of immunoglobulin receptors on lymphocytes, inhibition of phagocytosis by polymorphonuclear leukocytes, and stimulation of DNA synthesis [3–6]. In addition, con A binding to adipocytes has been shown to mimic a variety of insulin effects [7–12]. Con A has 4 sugar binding sites, and studies using several chemical derivatives of con A have suggested that many of its biological activities on lymphocytes are dependent upon its multivalent binding [3,13–16]. Furthermore, studies with agents which disrupt microtubules have suggested that stimulation of some of these effects also requires an intact cytoskeletal system [6,17]. Here, we have defined the roles of ligand valence and an intact cytoskeleton in the insulin-like action of con A.

2. Materials and methods

2.1. *Materials*

Porcine insulin (lot 7GUHSL) was purchased from Elanco, bovine serum albumin (fraction V, lot N53309) from Armour, and crude collagenase (CLS45K137) from Worthington Biochemicals. Cytochalasins B and D and colchicine were purchased from Aldrich Chemicals, vincristine and vinblastine from Eli Lilly, and dinonylphthalate from Eastman Chemicals. ^{125}I-Labeled insulin (^{125}I-insulin) was prepared by a modification of the chloramine-T method at spec. act. 100–200 μCi/μg [18].

Con A and its derivatives were obtained from a variety of sources, and all lots provided consistent results. Con A itself was purchased from Miles Labs, Pharmacia Chemicals and Polysciences (Warrington PA). Succinyl-Con A was purchased from Polysciences, and was received as a kind gift from Dr G. Edelman (New York). Acetyl-Con A was also obtained from Dr Edelman. Monovalent con A produced by proteolytic digestion [15] was a generous gift of Dr J. R. Wands, Boston.

2.2. *Binding studies and glucose oxidation bioassay*

Isolated adipocytes were prepared from epididymal fat pads of 100–180 g Sprague-Dawley rats by collagenase digestion [18]. Unless otherwise noted, all studies of ^{125}I-insulin binding were performed in Krebs-Ringer bicarbonate buffer with albumin (pH 7.4) at 37°C as in [18]. 'Non-specific' binding, determined as that amount of tracer bound in the presence of a 10^6-fold excess of unlabeled insulin has been subtracted from all data to yield 'specific' binding. Glucose oxidation was studied by measuring the conversion of [U-^{14}C]glucose to $^{14}CO_2$ [18] with an incubation period of 60 min. All glucose oxidation assays were performed in triplicate.

3. Results

As reported in [7,8], con A stimulated glucose oxidation in isolated rat adipocytes (fig.1). This effect

Abbreviations: con A, concanavalin A; ATPase, adenosine triphosphatase

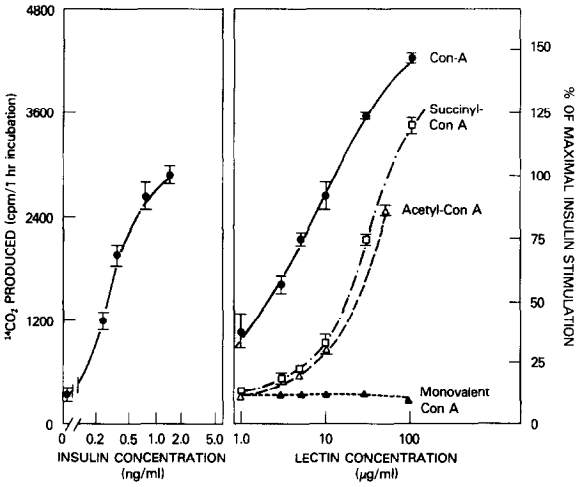

Fig.1. Effect of insulin, con A, and con A derivatives on glucose oxidation by isolated rat adipocytes. Isolated adipocytes were prepared and glucose oxidation studied as in section 2. The data shown represent the mean ± SEM for triplicate samples.

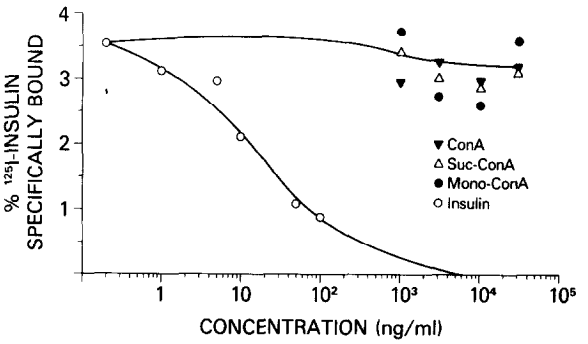

Fig.2. Effect of con A and its derivatives on ^{125}I-insulin binding to adipocytes. ^{125}I-Insulin binding to adipocytes was measured as in section 2. The unlabeled insulin and the con A derivatives were added at the indicated concentrations to the incubation buffer containing the ^{125}I-insulin (0.2 ng/ml), after which the suspension of adipocytes was added to give a final cell concentration of ~2 × 10^5/ml. Non-specific binding defined as the binding of ^{125}I-insulin observed in the 10 μg/ml has been subtracted from each point.

was observed at as low as 1 μg/ml, and increased thereafter in a dose-dependent manner, reaching a maximum at 100 μg/ml. In contrast to the mitogenic effect of con A [16], at no concentration of con A did this effect diminish. Compared to insulin, the normal hormonal stimulator of glucose metabolism in adipose tissue, con A was ~100-fold less potent on a molar basis. Maximal stimulation of glucose oxidation by con A, however, was consistently 50–60% higher than that produced by insulin (fig.1).

Native con A is a tetravalent tetramer [13]; to evaluate the role of valence, both bivalent and monovalent con A analogues were studied. The bivalent derivatives, succinyl- and acetyl-con A which differ greatly in charge [3,13], both stimulated glucose oxidation with equipotency, but were ~10% as active as native con A (fig.1). In contrast, monovalent con A was totally without effect, even at the highest concentration tested (100 μg/ml).

In an attempt to better understand the mechanism of con A stimulation of glucose metabolism, several additional experiments were performed. Since it has been suggested that the insulin-like effect of con A is due to binding to the insulin receptor [8], the effects of all derivatives on ^{125}I-insulin binding to adipocytes were examined. Under the conditions of our experiments which closely mimic those of the glucose oxidation assay, neither con A nor any of its derivatives produced systematic inhibition of ^{125}I-insulin binding, even at up to 30 μg/ml (fig.2). Thus, the difference in activity of the different con A derivatives cannot be explained by a difference in the ability of these to interact with the binding site of the insulin receptor.

A second possibility is that the interactions of the different ligands with the cytoskeletal system of the cell allowed for different biological potencies. A possible role of microtubules and microfilaments in the actions of both con A and insulin has been suggested by studies using the various agents which alter these structures [6,17,19]. Pretreatment of the adipocytes with colchicine, vincristine, and vinblastine, agents which alter microtubular function [20], at 10 μM, had no effect on basal, insulin-stimulated, or con A-stimulated glucose oxidation (table 1). As noted, cytochalasin B, an agent which disrupts microfilaments and interferes with actin polymerization [21], directly inhibits glucose transport and oxidation [22]. Despite this, stimulation by both con A and insulin was still observed, although the absolute rates of glucose metabolism were decreased at the higher concentration of drug (10 μg/ml). Cytochalasin D, which also disrupts microfilaments and has little direct effect on glucose transport [22], produced minimal inhibition of basal, insulin-stimulated or con A-stimulated glucose oxidation (table 1).

Table 1
Effects of anti-microtubule and anti-microfilament agents on insulin- and con A-stimulated glucose oxidation

Expt.	Addition	14 CO_2 produced (cpm/h incubation)			Stimulated/Basal	
		Basal	Insulin (1.0 ng/ml)	Con A (1 μg/ml)	Insulin	Con A
I	None	101 ± 8	745 ± 47	649 ± 28	7.4	6.4
	Colchicine 10 μM	98 ± 10	649 ± 41	686 ± 44	6.5	7.0
	Vinblastine 10 μM	92 ± 4	636 ± 63	593 ± 40	6.9	5.9
	Vincristine 10 μM	97 ± 5	670 ± 38	597 ± 7	6.9	6.2
II	None	295 ± 23	1732 ± 196	1581 ± 77	5.9	5.4
	Cytochalasin B					
	1 μg/ml	19 ± 2	143 ± 20	125 ± 10	7.5	6.6
	10 μg/ml	6 ± 3	14 ± 5	16 ± 2	2.3	2.7
	Cytochalasin D					
	1 μg/ml	240 ± 7	1445 ± 73	1441 ± 69	6.0	6.0
	10 μg/ml	189 ± 10	1377 ± 179	1290 ± 210	7.3	6.8

4. Discussion

Con A binds to cell surface glycoproteins, glycolipids and polysaccharides; and, depending on the cell type, this interaction produces a wide variety of biological and morphological responses [1–3]. The actions of con A have been most extensively studied in lymphocytes [3–6,16], however, con A also has been shown to bind to and/or have effects on many other tissues. In the adipocyte, con A has been found to mimic many of the actions of insulin including stimulation of glucose transport and metabolism, inhibition of lipolysis and adenylate cyclase, stimulation of lipogenesis and pyruvate dehydrogenase and stimulation of glycogen synthase and magnesium ATPase [7–12,24].

There is considerable evidence that the stimulation of lymphoid cells by con A requires cross-linkage of cell-surface glycoprotein receptors [6,16]. Native con A at pH 7 is a tetramer with 4 saccharide binding sites [3,13]. Derivatives which are dimeric and divalent can be prepared by both succinylation and acetylation [13], and monovalent derivatives have been prepared by either proteolytic treatment [15] or a combination of succinylation and photoaffinity labeling [13]. These derivatives retain their carbohydrate binding specificity but show a marked alteration in biological action on lymphocytes [3,13–15]. Thus, compared to native con A, the dimeric derivatives show weak agglutination properties, inability to cap glycoprotein receptors on cells, and failure to inhibit Ig receptor cap formation; mitogenic activity, however, is retained [14]. Monovalent con A preparations show variable properties depending on the method of preparation [13–15].

The cytoskeletal system also appears to play a role in a number of con A effects. Drugs which disrupt microtubules such as colchicine, vincristine and vinblastine have been shown to inhibit the mitogenic response to con A [6,16], as well as effects of succinyl-con A on glial cells in tissue culture [17]. In addition, intracellular membrane-associated actin is accumulated into patches located directly under the receptor patches when HeLa cells are treated with con A [23].

Here we have characterized the role of both receptor aggregation and the cytoskeleton in the insulin-like effect of con A. We find that, as with the mitogenic effect, the insulin-like effect of con A in adipocytes is dependent on ligand valence. Divalent derivatives of con A such as acetyl- and succinyl-con A are only ~10% as potent as native con A in this action, and monovalent con A is totally without effect. However, in contrast to observations on the mitogenic action, the insulin-like effect of con A is not blocked by any of the agents which disrupt microtubules, microfilaments or alter actin polymerization [20–22].

These data may have relevance not only to the mechanism of action of con A, but also to the mechanism of insulin action. It is generally believed that

con A exerts its insulin-like effect by binding to the insulin receptor [8]. Monovalent antibodies to the insulin receptor, which block insulin action, partially inhibit the insulin-like effect of con A [25]. Con A has also been shown to bind to solubilized insulin receptors and this property has proven useful for receptor purification [2,8]. The interaction of con A with the receptor, however, does not appear to occur at the insulin-binding site, since in most cases binding of ^{125}I-insulin is not reduced ([26]; this study]. De Meyts [26] has suggested that con A may bind to the site on the receptor important for negative cooperativity, since con A treatment has been shown to block the acceleration of dissociation of ^{125}I-insulin from its receptor produced by addition of unlabelled insulin (negative cooperativity).

Data have been presented suggesting that cross-linking or aggregation of insulin receptors is important in insulin action [22]. Thus, bivalent antibodies to the insulin receptor mimic insulin's metabolic effects, but are without insulin-like activity, and, in fact, block insulin action when monovalent [22,25]. Furthermore, on fibroblasts and lymphocytes, insulin receptors have been shown to patch and cap [27,28]. If con A is indeed acting through binding to the insulin receptor, it would not be surprising that monovalent con A would be inactive and the divalent derivatives less active than native con A. In [29] the divalent and monovalent con A derivatives exhibited a reduction in potency with respect to their abilities to inhibit negative cooperativity among insulin receptors. These data, then, further support a role for crosslinking in the action of con A and in the triggering of insulin receptors. This action appears to be independent of microtubules and microfilaments, but the possibility that some other intracellular mechanochemical protein is involved cannot be excluded.

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