INFLUENCE OF LECTINS ON THE BINDING OF ^{125}I-LABELED EGF TO HUMAN FIBROBLASTS

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SUMMARY

Lectins that interact with mannose (concanavalin A), galactose (ricin, abrin), or N-acetylglucosamine (wheat germ agglutinin) block ^{125}I-labeled EGF binding to the surface of cultured human fibroblasts at 37° or 5°. Lectins specific for fucose or N-acetylgalactosamine, soybean agglutinin or gorse lectin, respectively, do not interfere with growth factor binding. The inhibition of ^{125}I-labeled EGF binding by concanavalin A at 37° or 5° could be reversed rapidly by the addition of α-methyl mannoside. The results suggest that the fibroblast membrane receptor for EGF is, or is closely associated with, a glycoprotein or glycolipid that contains mannose, galactose and N-acetylglucosamine residues.

INTRODUCTION

Epidermal growth factor is a low molecular weight polypeptide (M.W. approximately 6000) that has been isolated from the submaxillary gland of the male mouse and from human urine (1,2). EGF is a potent mitogen for epidermal and epithelial cells in vivo (3) and in cell culture stimulates the proliferation of a number of different cell types (4). The interaction of ^{125}I-labeled EGF with the cell surface of cultured human fibroblasts has demonstrated the presence of specific receptors (5,6). Further studies have shown that cell-bound ^{125}I-labeled EGF is internalized and degraded (7). Although recent work has identified by photo-affinity labelling a putative EGF receptor in 3T3 cells (8), little is known about the nature of the receptor for this growth factor. Lectins represent a class of reagents which have been intensively investigated due to their reactivity with the carbohydrate moieties of mammalian cell membranes. Among their various effects on membrane functions, lectins have been reported

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abbreviations: EGF, epidermal growth factor; Con A, concanavalin A; WGA, wheat germ agglutinin

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to inhibit the binding of ^{125}I-labeled insulin (9) and ^{125}I-labeled prolactin (10) to membrane receptors. This report describes the influence of different lectins on the activity of the EGF receptor in cultured human fibroblasts.

METHODS AND MATERIALS

EGF was isolated from the submaxillary gland of the male mouse as described by Savage and Cohen (11) and labeled with $^{125}I^-$ as previously described (7). Human foreskin fibroblasts were grown in Dulbecco's Modified Eagle Medium (GIBCO) plus 10% calf serum (Flow Laboratories) as described elsewhere (7). Abrin and WGA were purchased from Cal Biochem, succinyl Con A from L'Industrie Biologique Francaise, all other lectins were obtained from SIGMA. Na^{125}I was purchased from New England Nuclear.

The binding of ^{125}I-labeled EGF to human fibroblasts was carried out essentially as described elsewhere (6,7). In brief, confluent monolayers of fibroblasts in 60 mm Falcon culture dishes were washed twice with warm Hanks' Balanced Salt Solution and a small volume of binding medium (Dulbecco's Modified Eagle Medium plus 0.1% bovine serum albumin) added. For experiments conducted at 37° the final volume of binding medium was 1.5 ml, for experiments conducted at 4° the final volume was adjusted to 2.0 ml. A saturating level of ^{125}I-labeled EGF (10 ng/ml) was added to the binding medium and the cells were incubated for 1 hr. at 37° or 1½ hr. at 4°. Then the monolayers of cells were washed 8 times with a total of 13 ml of cold Hanks' Balanced Salt Solution containing 0.1% bovine serum albumin to remove unbound radioactivity. The cells were solubilized in 0.5N NaOH and the amount of radioactivity determined in a Nuclear Chicago spectrometer. Nonspecific binding was determined by adding a vast excess of unlabeled EGF (10 μg/ml) to the binding medium. All results are expressed in terms of specific ^{125}I-labeled EGF binding.

RESULTS AND DISCUSSION

The data presented in Fig. 1 show the effect of increasing concentrations of Con A or WGA which interact mainly with mannose or N-acetylglucosamine, respectively, on the binding of ^{125}I-labeled EGF to monolayer cultures of human fibroblasts at 37°. The binding of ^{125}I-labeled EGF to human fibroblasts was inhibited by 50% when the cells were preincubated with 11 μg/ml Con A or 68 μg/ml WGA. At the highest concentration of lectins tested (100 μg/ml), binding was inhibited 82% and 62% by Con A and WGA, respectively. Collateral experiments carried out with 10 μg/ml Con A in Dulbecco's Modified Eagle Medium which contains glucose (25 mM) or phosphate buffered saline resulted in similar levels of inhibition of ^{125}I-labeled EGF binding, 56% and 61%, respectively. This result indicates that the presence of glucose in the binding medium did not effect the ability of Con A to block the binding reaction.

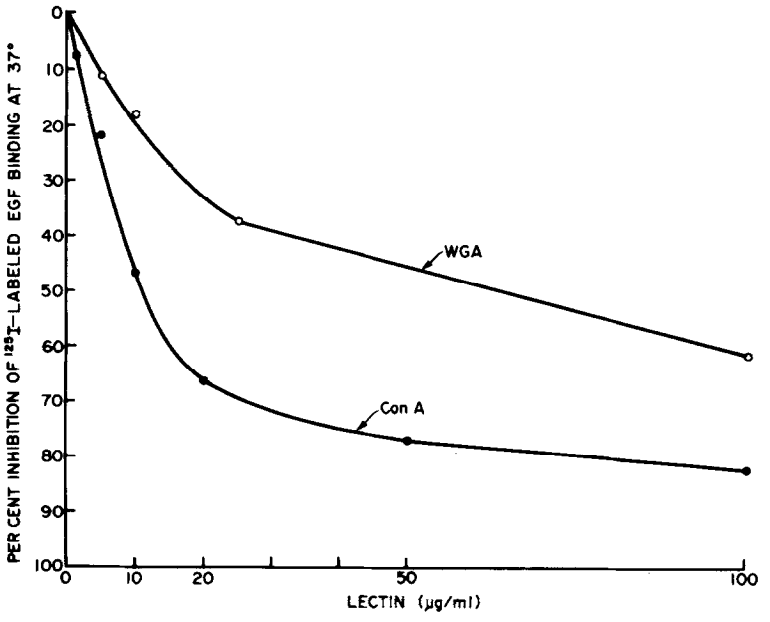

Fig. 1. Inhibition of ^{125}I-labeled EGF binding to human fibroblasts by Con A and WGA at 37°. Monolayer cultures of human fibroblasts were incubated for 1 hr. at 37° in the presence of indicated amounts of Con A or WGA. After the 1 hr. incubation, ^{125}I-labeled EGF (24,386 cpm/ng) was added to each dish at a final concentration of 10 ng/ml. The cultures were incubated for an additional one hour at 37° and the cell-bound radioactivity determined, as described under "Methods and Materials".

When Con A is incubated with cells at 37°, a fraction of cell-bound lectin is internalized by endocytosis (7). Also, human fibroblasts have approximately 200-fold more binding sites for Con A than for ^{125}I-labeled EGF (6,12). In view of these facts, internalization of membrane segments in response to Con A binding might reduce the number of EGF binding sites without the lectin actually interacting with the growth factor receptor. Therefore, an experiment similar to that described in Fig. 1 was performed at 4° to minimize endocytosis. The results, shown in Fig. 2, demonstrate that at 4° Con A and WGA remain effective inhibitors of ^{125}I-labeled EGF binding. Under these conditions the binding was inhibited by 50% in the presence of 17 µg/ml Con A or 85 µg/ml WGA. Maximal inhibition of ^{125}I-labeled EGF binding by Con A (70%) or WGA (52%) was reached in the presence of 100 µg/ml of either lectin. Higher concentrations (500 µg/ml)

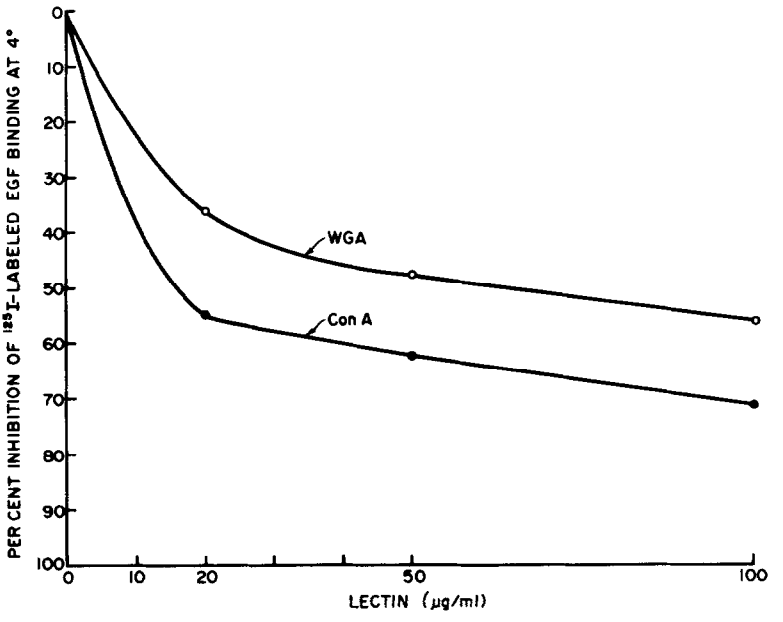

Fig. 2. Inhibition of ^{125}I-labeled EGF binding to human fibroblasts at 4°. Monolayer cultures of human fibroblasts were incubated for 1½ hr. at 4° in the presence of indicated amounts of Con A or WGA. After the 1½ hr. incubation, ^{125}I-labeled EGF (47,573 cpm/ng) was added to each dish at a final concentration of 10 ng/ml. The cultures were incubated for an additional 1½ hr. at 4° and the cell-bound radioactivity determined, as described under "Methods and Materials".

of either Con A or WGA did not increase the level of inhibition (data not shown).

The data presented in Table I show the effects of lectins other than Con A or WGA on the binding of ^{125}I-labeled EGF. The toxic lectins abrin and ricin, which interact with galactose residues, were approximately as effective as Con A in blocking ^{125}I-EGF binding. The toxic effect of abrin and ricin on cells is produced by the rapid inhibition of protein synthesis following the internalization of cell-bound lectin (13). Since internalization of cell-bound abrin or ricin does not occur at low temperatures (14), we have tested the ability of these lectins to effect ^{125}I-labeled EGF binding at 4°. Preincubation of human fibroblasts at 4° for 1½ hrs in the presence of abrin, ricin$_I$, ricin$_{II}$, or Con A (final concentration of 100 µg/ml for each lectin) inhibited the subsequent binding of the growth factor by 61%, 51%, 43%, and 76%, respectively. Therefore, the

TABLE I

Effect of Lectins on ^{125}I-labeled EGF Binding at 37°

Preincubation Additions		% Inhibition of ^{125}I-labeled EGF Binding
Abrin (Jequirity bean toxin)	5 µg/ml	43
	20 µg/ml	76
	100 µg/ml	82
Castor bean agglutinin Type I (Ricin I)	5 µg/ml	40
	20 µg/ml	52
	100 µg/ml	75
Castor bean agglutinin Type II (Ricin II)	5 µg/ml	34
	20 µg/ml	48
	100 µg/ml	55
Succinyl Con A	100 µg/ml	24
	500 µg/ml	55
Lens culinaris agglutinin	100 µg/ml	31
	500 µg/ml	54
Soybean agglutinin Type VI	100 µg/ml	0
	500 µg/ml	4
Gorse (Ulex europeus) agglutinin	100 µg/ml	2
	500 µg/ml	20

Monolayers of human fibroblasts were incubated at 37° for 1 hr. in the presence of the indicated lectin. Following the 1 hr. incubation, ^{125}I-labeled EGF (21,777 cpm/ng) was added to each dish at a final concentration of 10 ng/ml. The cultures were incubated for an additional 1 hr. at 37° and the cell-bound radioactivity determined, as described under "Methods and Materials".

effects of abrin and ricin on ^{125}I-labeled EGF binding are apparently due to their interaction with the cell surface and not to effects on cell metabolism. The results in Table I also indicate that succinyl Con A and the Lens culinaris agglutinin, both of which are divalent and interact with mannose residues, have a significant effect on ^{125}I-labeled EGF binding, but are less potent than the tetravalent Con A. These differences do not necessarily suggest that cross-linking of the lectin binding sites is required for the inhibition of growth factor binding. The differences may result from other aspects of lectin reac-

TABLE II

Reversibility of Con A Inhibition of ^{125}I-labeled EGF Binding

	Preincubation Additions	Binding Incubation: ^{125}I-labeled EGF plus	^{125}I-labeled EGF Bound per Dish
Exp. 1 (37°)	none	none	2004
	Con A	none	578
	Con A + α-methyl mannoside	none	1821
Exp. 2 (37°)	none	none	3394
	none	α-methyl mannoside	3501
	Con A	none	946
	Con A	α-methyl mannoside	2697
Exp. 3 (4°)	none	none	1793
	none	α-methyl mannoside	1738
	Con A	none	376
	Con A	α-methyl mannoside	922

Monolayer cultures were incubated for 1 hr. at 37° (Exp. 1 and 2) or 1½ hr at 4° (Exp.3) with the reagents listed under preincubation additions. After this preincubation, ^{125}I-labeled EGF (final concentration 10 ng/ml) was added to each dish with or without the simultaneous addition of α-methyl mannoside as indicated under binding incubation. The cultures were incubated for an additional 1 hr. at 37° (Exp. 1 and 2) or 1½ hr. at 4° (Exp. 3) and the cell-bound radioactivity determined as described under "Methods and Materials". The final concentrations were Con A 100 μg/ml, and α-methyl mannoside 100 mM. The specific activity of the ^{125}I-labeled EGF was 21,584 cpm/ng for Exp. 1; 26,222 cpm/ng for Exp. 2; and 30,293 cpm/ng in Exp. 3.

tivity, e.g. the association constant of the Lens culinaris agglutinin for mannose is approximately 100-fold less than that of Con A (16). The data in Table I show that the lectins from soybean or gorse that interact with N-acetylgalacto-

samine or fucose, respectively, do not have an appreciable effect on the binding reaction.

Additional experiments were carried out to investigate the nature of the inhibition of ^{125}I-labeled EGF binding produced by Con A. Most of the effects of Con A are prevented or reversed by the addition of α-methyl mannoside, a competitive inhibitor of Con A binding to biological material. The following experiments were performed to determine whether α-methyl mannoside could prevent and/or reverse the Con A inhibition of ^{125}I-labeled EGF binding. The results shown in Table II, Exp. I demonstrate that when α-methyl mannoside is added at the same time as Con A, the lectin does not inhibit ^{125}I-labeled EGF binding. The reversibility of the Con A inhibition of ^{125}I-labeled EGF binding is shown by the data in Exp. 2 and 3 of Table II. In these experiments the cells were preincubated with Con A at either 37° (Exp. 2) or 4° (Exp. 3)--conditions which reduced the subsequent binding of ^{125}I-labeled EGF to 27% or 21% of the control values, respectively. If, following the preincubation with Con A, α-methyl mannoside was added to the cells at the same time as ^{125}I-labeled EGF, binding of the growth factor was increased to 79% or 52% of the control values at 37° or 4°, respectively. Additional experiments have shown that α-methyl mannoside is the most effective saccharide of those tested for reversing the Con A inhibition of ^{125}I-labeled EGF binding. Alpha-methyl glucoside (100 mM) was approximately 60% as effective as α-methyl mannoside (100 mM). None of the following saccharides at concentrations of 100 mM reversed the inhibition of ^{125}I-labeled EGF binding produced by Con A: galactose, glucosamine, glucose, N-acetylglucosamine, fucose, lactose, melobiose.

The interaction of Con A with insulin receptors in fat cells mimics certain effects on cellular metabolism, such as increased glucose oxidation, that are typical responses to insulin binding (9,17). We have tested the ability of Con A to provoke a cellular response that is normally generated by the interaction of EGF with human fibroblasts--the stimulation of DNA synthesis as judged by increased incorporation of ^{3}H-thymidine (15). Con A at concentrations of 1-30 μg/

ml did not increase DNA synthesis either in the absence or presence of EGF (data not shown).

Since Con A, WGA, ricin, and abrin block the binding of ^{125}I-labeled EGF to human fibroblasts at either 37° or 4° and, at least in the case of Con A, the inhibition of binding is readily reversed, the most straightforward interpretation of the data is that the fibroblast receptor for EGF is either a glycoprotein or a glycolipid which contains mannose, N-acetylglucosamine, and galactose residues. An alternative and more complex explanation of our results is that the membrane receptor for EGF is physically very closely associated with another membrane macromolecule which contains the binding sites for lectins. Although we cannot discriminate between these two models at present, we consider the former to be more plausible.

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