Biochimica et Biophysica Acta, 469 (1977) 202—210

BBA 77787

EFFECTS OF WHEAT GERM AGGLUTININ ON MEMBRANE TRANSPORT

ELLEN LI and STUART KORNFELD

Division of Hematology-Oncology, Departments of Medicine and Biochemistry, Washington University School of Medicine, St. Louis, Mo. 63110 (U.S.A.)

(Received January 26th, 1977)

Summary

(1) Low concentrations of wheat germ agglutinin are cytotoxic toward several tissue culture lines, including Chinese hamster ovary cells, Swiss 3T3 cells, mouse L cells and baby hamster kidney cells. The LD_{50} ranged from 1 to 5 μg wheat germ agglutinin per ml. Similar concentrations of the lectin inhibited the transport of the non-utilizable amino acids α-aminoisobutyric acid and cycloleucine and inhibited the uptake of thymidine. In contrast, 2-deoxy-D-glucose uptake was not altered and colchicine uptake was enhanced.

(2) The inhibition of α-aminoisobutyric acid uptake occurred within minutes after lectin addition and was maximal by 1 h. Maximal inhibition ranged from 50 to 70% of control values. Studies of the kinetics of the uptake demonstrated that wheat germ agglutinin decreased the V of the uptake by 70% without affecting the apparent K_m. Ovomucoid, a haptene inhibitor of wheat germ agglutinin-binding to cell surface receptors, prevented the wheat germ agglutinin-induced inhibition of α-aminoisobutyric acid transport. Three other lectins (Concanavalin A, *Phaseolus vulgaris* E-phytohemagglutinin and L-phytohemagglutinin) inhibited the uptake by 20% or less at doses up to 50 μg/ml.

(3) We propose that the cytotoxicity of wheat germ agglutinin probably results in part, if not totally, from membrane alterations which impair multiple membrane transport systems.

Introduction

During the course of studies on the selection of lectin-resistant Chinese hamster ovary cells, we observed that wheat germ agglutinin is extremely toxic toward this cell line [1]. Similar findings have been reported by Stanley, et. al. [2]. The mechanism of this toxicity has not been explained. Recently two reports [3,4] have appeared which demonstrate that wheat germ agglutin inhibits amino acid transport in mouse fibroblasts and in human peripheral blood lymphocytes, suggesting that wheat germ agglutinin may exert its toxic effects

by altering plasma membrane function. For that reason, we have studied the effect of wheat germ agglutinin on a variety of transport systems, using Chinese hamster ovary cells and three other tissue culture cell lines as target cells. Our results indicate that low doses of wheat germ agglutinin alter several transport systems in these cell lines.

Materials and Methods

Materials

Minimal essential medium α [5] was obtained from Flow Laboratories. Fetal calf serum, trypsin, penicillin and streptomycin were purchased from Grand Island Biological Company. Tisssue culture grade plastic petri dishes and T-flasks were products of Falcon and Lux. Tissue culture clusters of 24 (16 mm diameter) wells and 6 (35 mm diameter) wells, used for uptake studies, were products of Costar. α-[1-^{14}C]Aminoisobutyric acid was from Amersham/Searle Corporation. All other radioactive materials were purchased from New England Nuclear. Crystallized bovine serum albumin, ovomucoid, wheat germ, 2-deoxy-D-glucose, cycloleucine, α-aminoisobutyric acid and colchicine were obtained from Sigma Chemical Corporation.

Cells. Wild type and variant Chinese hamster ovary cells were grown in suspension culture and monolayer culture as described previously [1]. Baby hamster kidney cells and mouse L cells were obtained from Ms. Randi Leavitt of this institution and were grown in monolayer cultures as described for Chinese hamster ovary cells. Swiss 3T3 cells were obtained from Mr. Brock Whittenberger of this institution and were plated for short term cultures as described below from confluent T-flasks grown in Dulbecco's modified Eagles medium supplemented with 10% calf serum as described previously [6].

For uptake studies 16 mm diameter wells were plated with $2 \cdot 10^5$ cells and 35 mm diameter wells were plated with 10^6 cells 24 h prior to use.

Lectins. Wheat germ agglutinin was purified by affinity chromatography on ovomucoid-sepharose [7]. The wheat germ agglutinin was further fractionated on SP-Sephadex and QAE-Sephadex as described by Rice and Etzler [8]. Concanavalin A was purchased from Miles-Yeda, Ltd. *Phaseolus vulgaris* E-phytohemagglutinin and L-phytohemagglutinin were prepared as described [9,10].

Uptake assays. Confluent or nearly confluent monolayer cultures in 16 mm diameter wells were washed twice with minimal essential medium α. The cells were incubated with 0.48 ml of medium supplemented with 0.1% bovine serum albumin and other additions described in the text, at 37°C in a 5% CO_2- 95% air atmosphere. After the desired period of incubation, 20 μl of either α-[1-^{14}C]aminoisobutyric acid (1 μCi/well) or α-[*methyl*-^{3}H]aminoisobutyric acid (2 μCi/well) were added and the cells were incubated at 37°C for an additional 5 min. As a rule, the α-aminoisobutyric acid was used at a final concentration of 0.2 mM. The incubation medium was then removed and the cells were washed rapidly three times with cold phosphate buffered saline. The cells were lysed in 0.2 ml of 10% Triton X-100. The lysates were transferred to scintillation vials in 1 ml of water and counted in 10 ml of scintillation cocktail (3a70, Research Products International Corporation) in a liquid scintillation spectrometer.

Uptake of 1-[*carboxy*-^{14}C]aminocyclopentane-1-carboxylic acid (cycloleucine) at 1 μCi/well, [*methyl*-^{3}H]thymidine at 1 μCi/well, 2-[^{3}H(G)]deoxy-D-glucose at 0.5 μCi/well and [ring C, *methoxy*-^{3}H]colchicine at 6 μCi/well was assayed as described for uptake of α-aminoisobutyric acid, except that colchicine uptake was measured using 35 mm diameter wells with 0.7 ml of incubation medium for up to 30 min.

In all of these assays, uptake was linear with time over the period examined and was expressed on the basis of per unit cell protein. Cell protein per well was measured by the method of Lowry et al. [11] after solubilizing the cells in a 5% SDS, Lowry A solution. Bovine serum albumin was used as the standard. All values are an average of duplicate or triplicate determinations.

Results

Wheat germ agglutinin cytotoxicity. Previously we have shown that wheat germ agglutinin is toxic to Chinese hamster ovary cells [1]. Wheat germ agglutinin was also quite toxic to baby hamster kidney cells, mouse L cells and Swiss 3T3 cells as reflected by the reduced plating efficiencies of these cell lines when grown in the presence of lectin. Shown in Fig. 1 is the effect of various concentrations of wheat germ agglutinin on the plating efficiency of

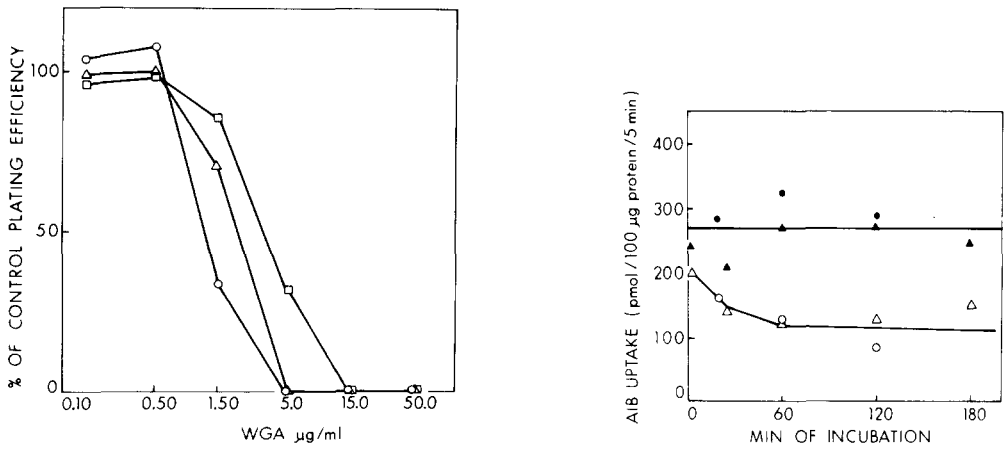

Fig. 1. The effect of wheat germ agglutinin on the plating efficiency of Chinese hamster ovary cells, mouse L cells and baby hamster kidney cells. Plastic petri dishes (35 mm diameter) were plated with 3—4 · 10^2 cells. After a 4 h incubation at 37°C, the medium was replaced with 3 ml of fresh medium containing various concentrations of wheat germ agglutinin. On day 5, the colonies were stained with 3% methylene blue/10% formaldehyde and counted. Control plating efficiencies were approximately 50%. The values represent the average of duplicate plates. WGA, wheat germ agglutinin; ○———○, Chinese hamster ovary cells; △———△, mouse L cells; □———□, baby hamster kidney cells. The LD50 for Chinese hamster ovary cells varied between 1.0 and 5.0 μg/ml in separate experiments.

Fig. 2. The initial rate of α-aminoisobutyric acid uptake by Chinese hamster ovary cells after varying periods of incubation with and without 20 μg/ml of wheat germ agglutinin. After the indicated incubation periods, 0.2 mM α-[1-^{14}C]aminoisobutyric acid (final concentration) was added for 5 min. The cultures were washed and lysed for counting as described in Materials and Methods. The open symbols indicate cultures with added wheat germ agglutinin and the filled symbols indicate controls. The groups of ○●, △▲ indicate two experiments done on different days.

Chinese hamster ovary cells, baby hamster kidney cells and mouse L cells. Swiss 3T3 cells did not form colonies that are readily visualized, but at 50 μg/ml wheat germ agglutinin no cells could be detected on the plate.

Four chromatographically distinct forms of wheat germ agglutinin have been described by Rice and Etzler [8]. Three of these forms (wheat germ agglutinin I, IIa and III) have been isolated and tested for cytotoxicity. All three were toxic to Chinese hamster ovary cells at doses similar to those found for the unfractionated wheat germ agglutinin. Plating efficiencies were reduced 80% by all three forms at concentrations between 1.5 and 5.0 μg/ml. The fourth chromatographically distinct form (wheat germ agglutinin IIb) described by Rice and Etzler [8], was not detected in this preparation. In the experiments described below, the unfractionated wheat germ agglutinin was used.

The effect of wheat germ agglutinin on α-aminoisobutyric acid uptake by Chinese hamster ovary cells. Wheat germ agglutinin, when added to the incubation medium, caused a rapid decrease in the initial rate of α-aminioisobutyric acid uptake (Fig. 2). The maximal inhibition ranged from 50% to 70% and was reached after 1 h of preincubation with 20 μg/ml of wheat germ agglutinin.

The effect of wheat germ agglutinin concentration on initial rates of α-aminoisobutyric acid uptake is shown in Fig. 3. The concentration of wheat germ agglutinin at which half-maximal inhibition of α-aminoisobutyric acid uptake occurred, 1 μg/ml, correlates with the concentration at which the plating efficiency is reduced 50%. Cell agglutination was observed at lectin concentrations of 5 μg/ml and greater. Ovomucoid, a haptene inhibitor of wheat

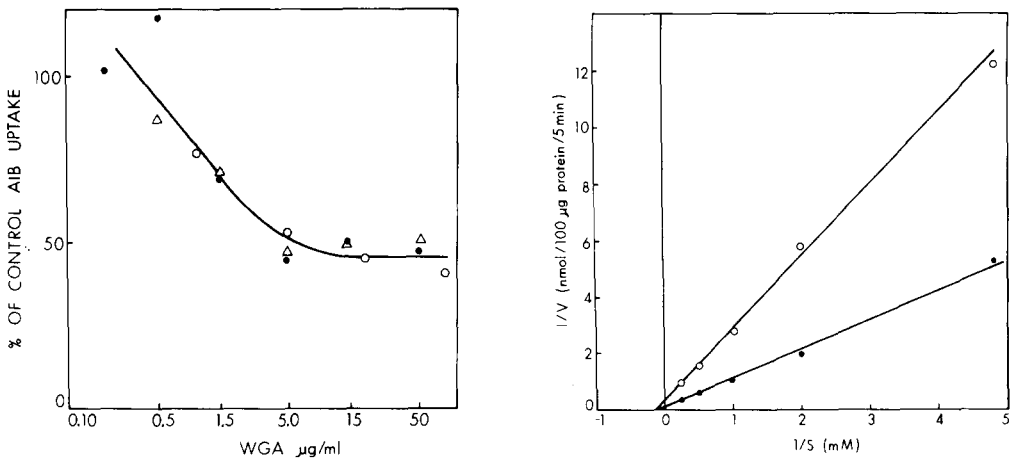

Fig. 3. The initial rate of α-aminoisobutyric acid (AIB) uptake by Chinese hamster ovary cells after incubation with varying concentrations of wheat germ agglutinin (WGA). Cultures were incubated with wheat germ agglutinin for 2 h. 0.2 mM (final concentration) of either α-[1-^{14}C]aminoisobutyric acid or α-[*methyl*-^{3}H]aminoisobutyric acid was then added for 5 min. Results are expressed as the percentage of control cultures. ●, △, ○ indicate three experiments done on different days.

Fig. 4. Uptake of α-aminoisobutyric acid by control and wheat germ agglutinin-inhibited Chinese hamster ovary cells as a function of α-aminoisobutyric acid concentration. Cells were incubated 2 h with or without 20 μg/ml of wheat germ agglutinin. The cells were then exposed to varying concentrations of α-[*methyl*-^{3}H]aminoisobutyric acid for 5 min. Results are shown in the form of a double reciprocal plot and are from one of two similar experiments. ●, control; ○, treated with wheat germ agglutinin.

germ agglutinin-binding to cells, had no effect on α-aminoisobutyric acid uptake when added to the medium at a concentration of 750 μg/ml, but this amount of ovomucoid blocked and also reversed the wheat germ agglutinin-induced inhibition for wheat germ agglutinin-concentrations up to 10 μg/ml (Table I).

A kinetic analysis of α-aminoisobutyric acid transport in the presence of wheat germ agglutinin is shown in Fig. 4. Wheat germ agglutinin decreased the apparent V from a control value of 10 nmol/100 μg protein per 5 min to 3 nmol/100 μg protein per 5 min, without affecting the apparent K_m of 7 mM. This apparent K_m is greater than that reported by Sander and Pardee [12], probably because of the presence of competing amino acids in the incubation medium. When the incubation medium was replaced by phosphate buffered saline (pH 7.5) containing 0.1% glucose and 0.1% bovine serum albumin, uptake of α-aminoisobutyric acid was increased 3—5-fold and was inhibited 43% after a 20 min preincubation with 10 μg/ml of wheat germ agglutinin. However, phosphate buffered saline was not used routinely due to the large fluctuations in uptake observed from experiment to experiment. The observations on the relative effect of wheat germ agglutinin on the kinetics of α-aminoisobutyric acid uptake should not be affected by the presence of competing amino acids.

Intracellular α-aminoisobutyric acid was released from the cells at a lower rate in the presence of wheat germ agglutinin, demonstrating that the observed decrease in the rate of uptake could not be due to an increased rate of efflux. Cells preloaded with α-[*methyl*-^{3}H]aminoisobutyric acid and then washed free of extracellular label, released 40% of the intracellular α-aminoisobutyric acid in 15 min when incubated in the presence of 20 μg/ml of wheat germ agglutinin compared to 60% released in control cultures.

Preincubation of Chinese hamster ovary cells with 1 mM ouabain, an inhibitor of the sodium ion pump, rapidly reduced α-aminoisobutyric acid uptake. Uptake was inhibited 27% after a 2 min preincubation and was reduced 60% after 2 h at 37°C, suggesting that uptake can be inhibited by interfering with

TABLE I

THE EFFECT OF OVOMUCOID ON α-AMINOISOBUTYRIC ACID UPTAKE IN CHINESE HAMSTER OVARY CELLS

Cells were preincubated at 37°C for 1 h and 3 h with and without 10 μg/ml of wheat germ agglutinin (WGA) prior to measuring the uptake of α-[*methyl*-^{3}H]aminoisobutyric acid (0.2 mM final concentration) as described in Materials and Methods. Ovomucoid (750 μg/ml final concentration) was added at the times indicated below during the preincubation. The values listed are the average of duplicate determinations.

Experimental conditions	α-Aminoisobutyric acid uptake (pmol/100 μg protein per 5 min)	
	1 h Preincubation	3 h Preincubation
Control, no ovomucoid	270 ± 30 (S.D.)	245 ± 15
Control, 0′ ovomucoid	260 ± 10	225 ± 2
WGA treated, no ovomucoid	150 ± 2	110 ± 3
WGA treated, 0′ ovomucoid	225 ± 3	225 ± 5
WGA treated, 1 h ovomucoid	—	200 ± 5

the sodium ion pump. However, wheat germ agglutinin at concentrations up to 20 μg/ml had no effect on (Na^+, K^+)-ATPase (ATP phosphohydrolase EC 3.6.1.3) activity assayed by the method of Schimmel et al. [13] in membranes prepared from Chinese hamster ovary cells. Preincubation of the cells with both wheat germ agglutinin and 1 mM ouabain for 2 h decreased uptake 82%. These results indicate that wheat germ agglutinin does not inhibit α-aminoisobutyric acid uptake by inhibiting the sodium ion pump.

The effect of other lectins on α-aminoisobutyric uptake. Three other lectins (concanavalin A, *P. vulgaris* E-phytohemagglutinin and L-phytohemagglutinin) inhibited α-aminoisobutyric acid uptake by Chinese hamster ovary cells by 20% or less when tested at doses up to 50 μg/ml (Table II). At these concentrations, the two lectins of *P. vulgaris* also caused marked agglutination of the cells whereas concanavalin A did not.

Uptake of other compounds by Chinese hamster ovary cells. The effect of wheat germ agglutinin on the initial rates of uptake of 2-deoxy-D-glucose, thymidine, cycloleucine and colchicine by Chinese hamster ovary cells is shown in Table III. The absolute amount of uptake varied from day to day, particularly in the case of colchicine, but the relative effects of wheat germ agglutinin were reproducible. Both thymidine uptake and cycloleucine uptake were reduced in wheat germ agglutinin-treated cells, while colchicine uptake was increased and 2-deoxy-D-glucose uptake was not affected. Similar results have been obtained in the case of thymidine uptake when trichloroacetic acid soluble counts have been measured.

The effect of wheat germ agglutinin on α-aminoisobutyric acid uptake in other cell lines. Clones 1021, 13, and 15B are variants of Chinese hamster ovary cells which bind less wheat germ agglutinin and are resistant to wheat germ agglutinin cytotoxicity relative to the wild type cells [1,14]. As shown in Table IV, these three clones are also resistant to the inhibitory effects of wheat germ agglutinin on α-aminoisobutyric acid uptake. The initial rate of uptake (expressed on a per unit cell protein basis) in control cultures of these clones, particularly clone 13 and clone 15B, was less than the rate in control cultures of wild type cells.

Wheat germ agglutinin also inhibited α-aminoisobutyric acid uptake by

TABLE II

THE EFFECT OF OTHER LECTINS ON α-AMINOISOBUTYRIC ACID UPTAKE BY CHINESE HAMSTER OVARY CELLS

The cells were preincubated 2 h at 37°C with 10 μg/ml and 50 μg/ml of the indicated lectins and then pulsed 5 min with 0.2 mM of α-[*methyl*-^{3}H]aminoisobutyric acid as described in Materials and Methods.

Lectin	Concentration (μg/ml)	α-Aminoisobutyric acid uptake * (% of control)
Concanavalin A	10	89 ± 3 (S.D.)
	50	79 ± 5
P. Vulgaris E-phytohemagglutinin	10	85 ± 7
	50	81 ± 7
P. Vulgaris L-phytohemagglutinin	10	99 ± 2
	50	94 ± 5

TABLE III

THE EFFECT OF WHEAT GERM AGGLUTININ ON UPTAKE OF OTHER SUBSTRATES BY CHINESE HAMSTER OVARY CELLS

The cells were preincubated 2 h with or without 20 μg/ml of wheat germ agglutinin and pulsed with the indicated substrate as described in Materials and Methods.

Substrate	Concentration	Initial rate of uptake (pmol/100 μg protein per 5 min) ±S.D.	
		Control	Wheat germ agglutinin-treated
Cycloleucine	0.6 mM	1060 ± 70	410 ± 80
	2.0	2600 ± 120	660 ± 60
Thymidine	0.25 mM *	0.012 ± 0.001	0.006 ± 0.001
	0.25 *	0.020 ± 0.002	0.011 ± 0.001
2-deoxy-D-glucose	0.2 mM	110 ± 3	120 ± 1
	2.0	840 ± 30	820 ± 90
	10.0	4080 ± 200	3860 ± 400
Colchicine	0.5 μM *	0.030 ± 0.002	0.090 ± 0.013
	0.5 *	0.080 ± 0.001	0.204 ± 0.010
	0.5 *	0.190 ± 0.030	0.350 ± 0.030

* Separate experiments.
** The medium used in these experiments contains 0.1% glucose.

mouse L cells and baby hamster kidney cells, as shown in Table IV. In the absence of fetal calf serum, α-aminoisobutyric acid uptake by Swiss 3T3 cells was sharply reduced. α-Aminoisobutyric acid uptake by Swiss 3T3 cells in the presence of 10% fetal calf serum was reduced 30% after 2 h of preincubation with 50 μg/ml of wheat germ agglutinin.

TABLE IV

THE EFFECT OF WHEAT GERM AGGLUTININ ON α-AMINOISOBUTYRIC ACID UPTAKE IN OTHER CELL LINES

The cells were preincubated 2 h with the indicated concentrations of wheat germ agglutinin and assayed for uptake of α-[*methyl*-^{3}H]aminoisobutyric acid (0.2 mM final concentration) as described in Materials and Methods. The number in parenthesis indicates the number of separate experiments.

Cell	α-Aminoisobutyric acid uptake % of control * ±S.D.		
	Wheat germ agglutinin concentration (μg/ml)		
	1.5	5.0	15
Wild type Chinese hamster ovary cells (CHO)	75 ± 2(2)	48 ± 2(3)	45 ± 5(20)
CHO, clone 1021		108 ± 2(2)	108 ± 4(2)
CHO, clone 13		103 ± 7(2)	98 ± 7(2)
CHO, clone 15B		111	75
Baby hamster kidney cells	50 ± 10(2)	58 ± 15(2)	52 ± 10(2)
Mouse L cells	75 ± 7(2)	58 ± 5(2)	49 ± 1(2)

* Control uptake values ± S.D. (pmol/100μg protein per 5 min) are as follows, wild type Chinese hamster ovary cells, 260 ± 20 (4), CHO clone 1021, 190 ± 40 (2); CHO clone 13, 160 ± 30 (2); CHO clone 15B, 115; baby hamster kidney cells, 200 ± 28 (2); mouse L cells, 460 ± 100 (2).

Discussion

Inhibition of α-aminoisobutyric acid uptake by wheat germ agglutinin has been previously reported by Isselbacher [3] using mouse and hamster fibroblast lines and by Greene, et. al. [4] using human peripheral blood lymphocytes. The present experiments demonstrate that low concentrations of wheat germ agglutinin perturb several membrane functions in addition to the transport system which mediates α-aminoisobutyric acid uptake, as reflected by the altered uptake of thymidine, cycloleucine and colchicine. The finding that the uptake of 2-deoxy-D-glucose is not altered demonstrates that wheat germ agglutinin does not affect all membrane functions, but that it exerts a selective effect on different transport systems.

In contrast to the decreased uptake of α-aminoisobutyric acid, thymidine and cycloleucine, colchicine uptake is stimulated by wheat germ agglutinin. Ling and coworkers [15] have shown that a number of metabolic inhibitors (cyanide, azide and dinitrophenol) rapidly stimulate the uptake of colchicine and other drugs by Chinese hamster ovary cells without irreversibly damaging the cells, suggesting that modulation of drug permeability in these cells is an energy-dependent process. These observations have been confirmed by us. It is of interest that wheat germ agglutinin has a similar effect on colchicine uptake.

The molecular basis for the inhibiting effect of wheat germ agglutinin on α-aminoisobutyric acid uptake is not presently understood. The fact that the haptene ovomucoid both prevented and reversed the inhibiting effect indicates that wheat germ agglutinin must interact selectively with cell surface binding sites in order to exert its effects on membrane function and that it is not immediately cytotoxic. The production of marked cell agglutination with little or no effect on uptake by the two *P. vulgaris* lectins indicates that cell agglutination cannot explain the inhibition observed with wheat germ agglutinin. The failure of wheat germ agglutinin to inhibit membrane (Na^+, K^+)-ATPase argues against the lectin inhibiting uptake by interfering with the sodium ion pump. Sander and Pardee have reported a reduction in the rate of α-aminoisobutyric acid uptake (expressed on a per unit cell protein basis) associated with M and early G_1 periods in synchronized populations of Chinese hamster ovary cells and mouse L cells with a doubling of the transport rate later in G_1 and with the specific transport activity remaining at the elevated level during the rest of the cell cycle. The apparent K_m does not change during the cell cycle. Preliminary results suggest that uptake of uridine and thymidine follow a similar pattern [12]. Although the effect of wheat germ agglutinin on α-aminoisobutyric acid uptake is too rapid to be explained by the arrest of cells in a certain phase of the cell division cycle, there may be a relationship between the proposed membrane perturbation induced by wheat germ agglutinin and changes in the cell surface during M and early G_1.

Attempts to study the effects of wheat germ agglutinin on α-aminoisobutyric acid uptake in membrane vesicles isolated from Chinese hamster ovary cells are currently in progress. Preliminary results suggest that wheat germ agglutinin does inhibit sodium ion gradient dependent α-aminoisobutyric acid uptake.

These experiments also demonstrate that low concentrations of wheat germ agglutinin are cytotoxic to many tissue culture cell lines, including baby ham-

ster kidney cells, mouse L cells and Swiss 3T3 cells as well as Chinese hamster ovary cells. Of note is the finding that there is a good correlation between the amount of the lectin required to induce cytotoxicity and the amount required to inhibit α-aminoisobutyric acid uptake in these cell lines. Maximal effects on both the plating efficiency and on transport properties were seen at lectin concentrations far below the concentrations required to saturate wheat germ agglutinin binding-sites on Chinese hamster ovary cells [1]. These effects also occur at concentrations of the lectin which do not induce gross cell agglutination. In addition, variant cells which are resistant to the cytotoxic effects of wheat germ agglutinin do not demonstrate inhibition of α-aminoisobutyric acid uptake. Based on these findings, we propose that the cytotoxicity of wheat germ agglutinin probably results in part, if not totally, from membrane alterations which impair multiple membrane transport systems. This mechanism of cytotoxicity is very different from that of the toxic lectins ricin and abrin, which enzymatically inactivate the 60 S ribosomal subunit, thus inhibiting protein synthesis [16,17].

Acknowledgments

This work was supported in part by Grant R01 CA 08759 from the U.S.P.H.S. Ms. Ellen Li is a recipient of the Mr. and Mrs. Spencer T. Olin Fellowship for Women in Science.

References

1 Briles, E.B., Li, E. and Kornfeld, S. (1977) J. Biol. Chem. 252, 1107—1116
2 Stanley, P., Caillibot, V. and Siminovitch, L. (1975) Cell 6, 121—128
3 Isselbacher, K.J. (1972) Proc. Natl. Acad. Sci. U.S. 69, 585—589
4 Greene, W., Parker, C.M. and Parker, C.W. (1976) J. Biol. Chem. 251, 4017—4025
5 Stewart, C.C. (1973) J. Reticuloendothel. Soc. 14, 332—349
6 Todaro, G.J., Green, M. and Goldberg, B.D. (1964) Proc. Natl. Acad. Sci. U.S. 51, 66
7 Marchesi, V.T. (1972) Methods Enzymol. 28, 354—356
8 Rice, H. and Etzler, M.E. (1975) Biochemistry 14, 4093—4099
9 Kornfeld, S., Rogers, J. and Gregory, W. (1971) J. Biol. Chem. 246, 6581—6586
10 Kornfeld, R. and Kornfeld, S. (1970) J. Biol. Chem. 245, 2536—2545
11 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265—275
12 Sander, G. and Pardee, A.B. (1972) J. Cell. Physiol. 80, 267—272
13 Schimmel, S.D., Kent, C., Bischoff, R. and Vagelos, P.R. (1973) Proc. Natl. Acad. Sci. U.S. 70, 3195—3199
14 Gottlieb, C., Skinner, A. and Kornfeld, S. (1974) Proc. Natl. Acad. Sci. U.S. 71, 1078—1082
15 See, Y.P., Carlsen, S.A., Till, J.E. and Ling, V. (1974) Biochim. Biophys. Acta 373, 242—252
16 Sperti, S., Montanaro, L., Mattioli, A. and Stirpe, F. (1973) Biochem. J. 136, 813—815
17 Benson, S., Olsnes, S., Pihl, A., Skorve, J. and Abraham, A.K. (1975) Eur. J. Biochem. 59, 573—580