Lectins Mimic Insulin in the Induction of Tyrosine Aminotransferase

Abstract. *Various lectins were found to induce tyrosine aminotransferase in H-35 rat hepatoma cells grown in monolayer culture. Wheat germ agglutinin gave a maximal induction of tyrosine aminotransferase 6 hours after its addition. The induction time course was similar to that elicited by insulin. Fourteen micrograms of wheat germ agglutinin per milliliter gave half-maximal enzyme induction and 50 micrograms per milliliter gave the maximal response. The induction of tyrosine aminotransferase by wheat germ agglutinin was additive with the induction by either dexamethasone or dibutyryl adenosine 3',5'-monophosphate, but was not additive with the tyrosine aminotransferase induction by insulin. Wheat germ agglutinin also mimicked insulin in the inhibition of cellular protein degradation in the absence of serum. The insulin-like effects of lectins should be considered in lectin-mediated manipulations such as agglutination.*

Tyrosine aminotransferase (TAT) is a liver-specific enzyme that catalyzes the first step of tyrosine catabolism. The induction of rat liver TAT by hydrocortisone was first described by Lin and Knox in 1957 (*1*). It has since been shown that TAT is inducible in vivo by glucocorticoids, insulin, and agents that raise the intracellular level of adenosine 3',5'-monophosphate (cyclic AMP) (*2*). The Reuber H-35 rat hepatoma, first adapted to tissue culture by Pitot *et al.* (*3*), has proved to be a good model system in which to study the hormonal regulation of TAT, showing inducibility by glucocorticoids, insulin, and dibutyryl cyclic AMP (*4*). Although the precise mechanisms by which cyclic AMP and insulin increase TAT activity remain to be elucidated, both agents as well as glucocorticoids have been shown to act by specifically increasing the rate of TAT synthesis in H-35 cells (*4*). In the course of our study on the mechanisms by which cyclic AMP induces TAT, we attempted to use a red blood cell ghost–mediated microinjection procedure (*5*) to deliver purified catalytic subunits of cyclic AMP–dependent protein kinase into intact H-35 hepatoma cells. Lectin by itself, an agent used to facilitate this microinjection procedure (*6*), was found to enhance TAT activity. We therefore commenced a study on the regulation of TAT activity by lectins. In this report, we present results on the time course, dose response, and possible mechanisms of TAT induction by the lectin wheat germ agglutinin (WGA).

When 50 μg of WGA per milliliter was added to H-35 cells, TAT-specific activity increased approximately sixfold in 6 hours and then declined, approaching the basal level by 24 hours (Fig. 1A). The time course of the WGA-mediated induction of TAT was similar to that of TAT induction by insulin in H-35 cells as reported by Reel *et al.* (*4*) and our own unpublished observations. If WGA was removed after 1 hour of incubation and then replaced with fresh medium, TAT induction was equal in magnitude to the induction seen with continuous lectin treatment and followed a similar time course (Fig. 1A). Incubations with WGA for as short a time as 15 minutes also yielded a persistent induction of TAT 5 hours later (data not shown). The induction of TAT by the continuous presence of WGA was abolished by addition of the competing monosaccharide *N*-acetyl-D-glucosamine (50 m*M*). The dose response of TAT induction by a 1-hour incubation with various concentrations of WGA is shown in Fig. 1B. The maximal induction was achieved with WGA at 50 μg/ml, and the concentration that gave half-maximal TAT induction was approximately 14 μg/ml.

Various other lectins were tested to see if they also induced TAT. The E and L forms of phytohemagglutinin, soybean agglutinin, *Lens culnaris* agglutinin B, and concanavalin A (Con A) were all found to induce TAT at the 50 μg/ml dosage when incubated with H-35 cells for 1 hour, followed by a 4-hour incubation period in lectin-free medium (data not shown). Of the lectins tested, only ricin I showed no consistent induction of TAT, which may be attributed to the cytotoxic effect of this lectin, as evidenced by the shedding of cells from the monolayer. One previous report stated that high concentrations of Con A (200 μg/ml) rapidly and reversibly decreased TAT activity in FU-5-5 cells, a clone derived from H-35, grown in monolayer culture (*7*). Under our conditions we were unable to detect this inhibitory effect of Con A at doses up to 200 μg/ml.

In order to determine whether lectins were acting by a mechanism similar to any of the known inducers of TAT, we studied the induction of TAT by increasing concentrations of dibutyryl cyclic AMP, dexamethasone, and insulin in the absence and presence of WGA (50 μg/ml). As shown in Fig. 2, TAT induction by both dibutyryl cyclic AMP and dexamethasone was additive to the induction

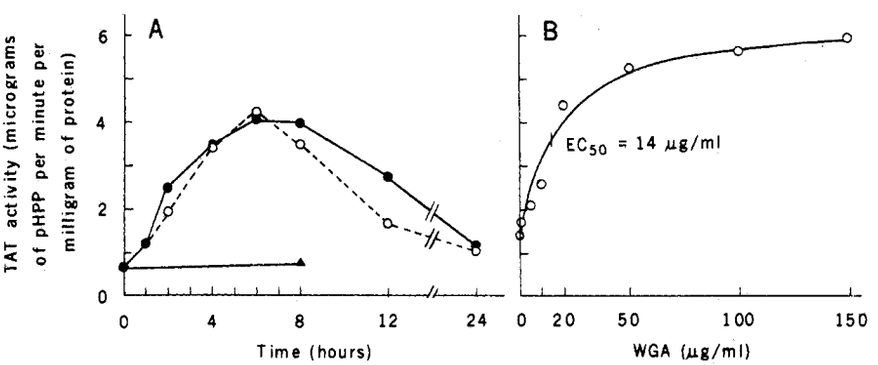

Fig. 1. Time course and dose response of WGA induction of TAT. The H-35 cells were grown as monolayers in 60-mm dishes at 37°C (5 percent CO_2) in Eagle's modified minimal essential medium (MEM) with 7.5 percent newborn calf serum and 2.5 percent fetal calf serum supplemented with penicillin (50 U/ml) and streptomycin (50 μg/ml). When the culture reached approximately 80 percent confluency, the medium was replaced overnight with serum-free MEM. Wheat germ agglutinin was then added in serum-free MEM and at predetermined time intervals cells were harvested in 0.7 ml of 50 m*M* sodium phosphate (*p*H. 7.4), 0.1 m*M* EDTA, 5 m*M* dithiothreitol, and 100 μ*M* pyridoxal phosphate. Cells were broken by freezing and thawing and homogenized by passage through a 20-gauge needle. Tyrosine aminotransferase activity of the homogenate was determined spectrophotometrically by a modification of the method of Diamondstone (*17*), using *p*-hydroxyphenylpyruvate (*pHPP*) as the standard. Protein was determined by the fluorescamine reaction (*18*), with bovine serum albumin as the standard. Each value represents the mean of duplicate determinations. (A) Time course of TAT induction by WGA (50 μg/ml) in MEM, either present continuously (●) or removed after the first hour and replaced with fresh MEM (○); WGA (50 μg/ml) plus 50 m*M* *N*-acetyl-D-glucosamine, present continuously (▲). (B) Dose response. The H-35 cells were treated with various concentrations of WGA for 1 hour, and replaced with fresh MEM for an additional 4 hours before processing; EC_{50}, effective concentration that gives half-maximal response.

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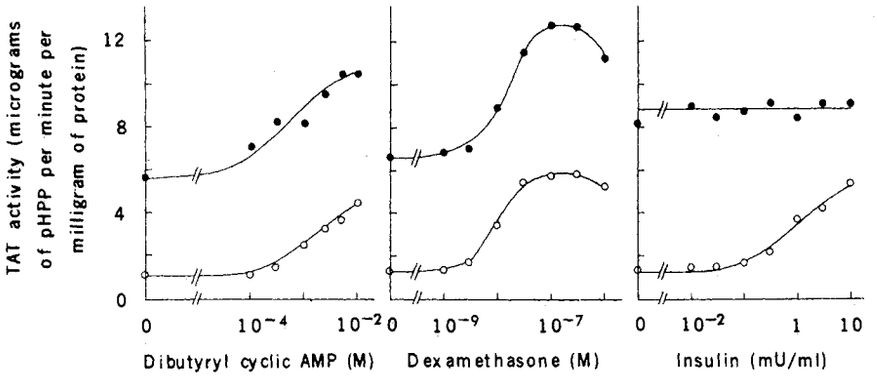

Fig. 2. Additivity of TAT induction by WGA with increasing concentrations of dibutyryl cyclic AMP, dexamethasone, and insulin. Wheat germ agglutinin (50 μg/ml) was present (●) or absent (○) continuously for 6 hours. Dibutyryl cyclic AMP was added during the last 3 hours of the incubation. Dexamethasone and insulin were present during the entire 6-hour incubation period. Culture and assay conditions were as described in the legend to Fig. 1.

by 50 μg of WGA per milliliter, while insulin did not further enhance TAT activity in the presence of WGA. We noted, however, that the absolute increase in TAT activity achieved by this concentration of WGA was greater than that achieved by the highest concentration of insulin tested (Fig. 2).

The nonadditive effect of WGA and insulin on TAT activity suggested that WGA may increase TAT activity by means of an insulin-like mechanism. We investigated another known effect of insulin on H-35 cells to see if WGA mimicked insulin in responses other than TAT induction. Under nutritional stepdown caused by the removal of serum, the rate of proteolysis of stable cellular proteins increases and insulin can inhibit this increase (*8*). Table 1 shows that a 1-hour treatment with WGA (50 μg/ml) retarded protein degradation under serum-free conditions. This result was qualitatively similar to that obtained with insulin.

Other insulin-like effects of lectins have been described previously: Con A and WGA have insulin-like effects on amino acid transport and lipolysis in adipocytes (*9*); furthermore, Con A mimics insulin, when added to adipocyte plasma membranes, in the generation of a second messenger capable of activating mitochondrial pyruvate dehydrogenase (*10*).

Wheat germ agglutinin at 1 μl/ml enhances insulin binding to adipocytes and liver cell membranes, but higher concentrations compete with insulin binding (*11*). This biphasic effect of WGA on insulin binding has also been confirmed in Zajdela hepatoma cells (*12*); these results suggest that lectins may be interacting directly with plasma membrane insulin receptors. The binding of lectins to the insulin receptor may not always be competitive with insulin binding (*13*). Many, but not all, immobilized lectins directly bound solubilized insulin receptors from human placenta (*14*). Those lectins that do not directly bind the insulin receptor may still activate the insulin receptor by its simultaneous clustering, which occurs when the lectin binding proteins are cross-linked. This type of clustering of heterologous membrane proteins has been demonstrated in lymphocytes treated with antibodies directed against their surface immunoglobulins (*15*).

Table 1. Effect of insulin and WGA on the degradation of stable cellular proteins in serum-deprived H-35 cells. Monolayers in 35-mm tissue culture dishes were labeled with [^{3}H]leucine (20 mCi/mmole) for 27 hours in leucine-free minimal essential medium (MEM) containing 10 percent fetal calf serum. The labeling medium was then aspirated and the cells were washed twice with MEM containing 2 m*M* leucine. The rapidly degrading class of proteins was allowed to decay for 12 hours in the presence of MEM containing 2 m*M* leucine and 10 percent fetal calf serum. The cells were again washed ($t = 0$) and incubated at 37°C for 150 minutes in serum-free MEM containing 2 m*M* leucine. Insulin was added at 1 mU/ml at $t = 0$. Wheat germ agglutinin, at 50 μg/ml, was added for the 1 hour immediately preceding the 150-minute decay period ($t = -1$ hour). The percent degradation of stable cellular proteins equals the amount of trichloroacetic acid–soluble radioactivity recovered in the medium during the 150-minute incubation period divided by the total radioactivity present in the monolayer plus the medium. Each value is the mean ± standard deviation for three separate plates. Levels of significance were determined by a two-tailed Student's *t*-test.

Treatment	Stable protein degraded in 150 minutes (%)
Control	2.65 ± 0.07
Insulin	1.69 ± 0.28*
WGA	2.08 ± 0.15*

*$P < .01$, compared to serum-free controls.

We have demonstrated that various lectins induced TAT in H-35 cells. The time course of TAT induction elicited by WGA was similar to that elicited by insulin; furthermore, its effect on TAT activity was not additive with insulin. Wheat germ agglutinin also mimicked the insulin effect in inhibiting cellular proteolysis under serum-free conditions. Since lectins have many uses for cell biologists, including agglutination of cells with other cells, with red blood cell ghosts, or with glycolipid-containing liposomes (*16*), and the study of cellular architecture, we caution against the use of lectins as manipulative tools without consideration of their insulin-like effects on enzyme induction and protein degradation.

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