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Insulin Receptor Antiserum and Plant Lectins Mimic the Direct Effects of Insulin on Nuclear Envelope Phosphorylation

Abstract. *Insulin directly inhibits protein phosphorylation in isolated rat liver nuclear envelopes. In the present studies, an antiserum to insulin receptor as well as the plant lectins concanavalin A and phytohemagglutinin mimicked insulin action in isolated nuclear envelopes. These studies suggest that insulin and agents that mimic it may directly regulate nuclear functions.*

Insulin has multiple effects on target cells, including regulation of membrane transport, enzyme activation, and RNA levels (*1*). Many of these effects appear to be mediated by changes in phosphorylation and dephosphorylation reactions (*2*) that can be demonstrated in isolated subcellular fractions (*3*). However, the exact site (or sites) of insulin action is unknown.

Table 1. Combined effects of insulin, antiserum to insulin receptors, and Con A on ^{32}P incorporation into rat liver nuclear envelopes. Values are the mean ± standard error of three separate experiments.

Addition	^{32}P incorporated (percent of control)
Insulin ($10^{-11}M$)	64 ± 0.6
Antibody to receptors (1:10,000)	66 ± 0.4
Con A (10 μg/ml)	68 ± 1.8
Insulin ($10^{-11}M$) plus antibody to receptors (1:10,000)	71 ± 1.6
Insulin ($10^{-11}M$) plus Con A (10 μg/ml)	70 ± 1.9

Since insulin is internalized by target cells (*4*) and since nuclear envelopes have specific binding sites for insulin (*5, 6*), it was thought that insulin regulates RNA levels by directly interacting with the nucleus. Recently, three direct effects of insulin on nuclei and nuclear envelopes were demonstrated. First, Schumm and Webb reported that insulin increases the efflux of messenger RNA (mRNA) from isolated nuclei (*7*). This effect was confirmed both in our laboratory (*8*) and the laboratory of Agutter (*9*). Second, we showed that insulin activates nuclear envelope nucleoside triphosphatase (NTPase) (*10*), an enzyme located at or near the nuclear pore complex. Since NTPase controls the transport of mRNA through the nuclear pore complex and into the cytoplasm (*11*), this finding suggests that the regulation of NTPase may be one mechanism whereby insulin controls mRNA metabolism. Third, we showed that insulin decreases ^{32}P incorporation into nuclear envelope proteins, including those proteins at the nuclear pore complex which presumably contain the NTPase (*12*). This effect of insulin,

however, was not seen with other cellular membranes, including the plasma membrane (*12*). In view of the concept that phosphorylation and dephosphorylation of the nuclear envelope NTPase may control the activity of insulin (*13*), we thought that regulation of nuclear envelope phosphorylation by insulin might trigger a series of events leading to enhanced mRNA transport.

Two major groups of substances, plant lectins and antibodies to the insulin receptor, can mimic many actions of insulin in the intact cell (*1*). These agents presumably bind to the plasma membrane insulin receptor, generate the same signal (or signals) as insulin (*14*), and are then internalized (*15*). It is not known, however, whether these insulin-like agents can act directly on nuclei. We therefore examined the direct effects of plant lectins and an antiserum to the insulin receptor on ^{32}P incorporation into isolated nuclear envelopes.

When insulin was added directly to nuclear envelopes prepared from liver of fasted rats, a detectable effect on ^{32}P incorporation occurred between 10^{-13} and $10^{-12}M$, a maximum effect occurred at $10^{-11}M$, and at higher concentrations the hormone effect was diminished (Fig. 1). A similar dose response curve was seen previously with nuclear envelopes prepared from liver of diabetic rats (*12*). In nuclear envelopes we (*5*) and others (*6*) have observed a major insulin binding site with a dissociation constant of approximately 5 n*M*. Since both the stimulatory and inhibitory effects of insulin on nuclear envelopes occur at concentrations far below this dissociation constant, it is likely that both effects are mediated by this binding site. Moreover, biphasic dose-response curves of insulin have been observed for insulin in other broken cell systems (*3, 7, 14, 16*).

Next, we examined the effects of an antiserum (B2) to the insulin receptor obtained from a patient with insulin resistance and acanthosis nigricans (*17*). This antiserum mimics both the early effects of insulin, such as enhanced glucose transport, and the late effects of insulin, such as enhanced enzyme synthesis (*17*). This latter effect is believed to be mediated in part via increased RNA synthesis (*1, 17*). Like insulin, this antiserum inhibited ^{32}P incorporation into nuclear envelope proteins in a biphasic manner (Fig. 1). A detectable effect was seen at a 1:20,000 dilution and a maximum effect at 1:10,000. These concentrations are well within the range of antibody concentrations known to mimic insulin's effects on target cells (*17*). In contrast, normal human serum over the same concentrations was without effect.

We then studied two mitogenic plant lectins, concanavalin A (Con A) and phytohemagglutinin (PHA). Both lectins, like insulin and antiserum to the insulin receptor, inhibited ^{32}P incorporation into nuclear envelope proteins in a biphasic manner (Fig. 1). In contrast, the nonmitogenic lectin wheat germ agglutinin was without effect (*18*).

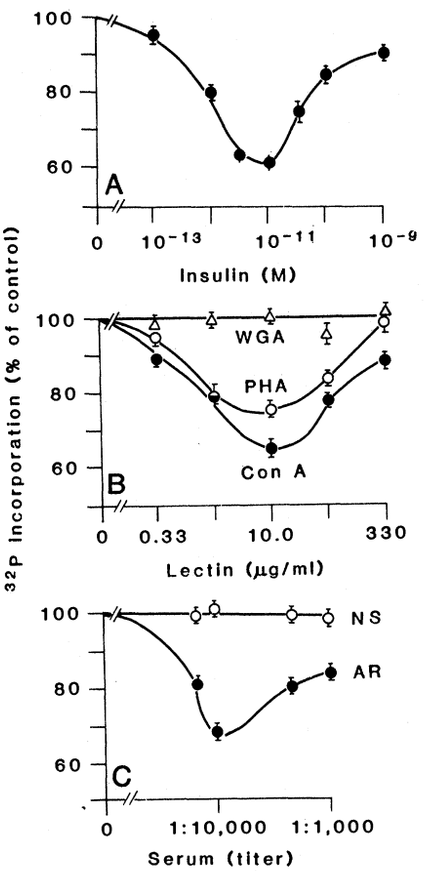

Fig. 1. Inhibition of ^{32}P incorporation into nuclear envelope proteins by insulin, Con A, and PHA. Female Sprague-Dawley rats, 140 to 160 g (Simonsen Laboratories), were fasted for 18 hours and killed. Isolated nuclei were prepared by the method of Blobel and Potter (*26*). Purified nuclear membranes were then prepared by the method of Monneron *et al.* (*27*) except that glycerol was omitted during the gradient centrifugation step. Incorporation of ^{32}P was measured by incubating 50 to 100 μg of nuclear membrane protein in 100 μl of 25 m*M* KCl, 5 m*M* $MgCl_2$, and 50 m*M* tris-HCl, *p*H 7.4. The membranes were incubated for 10 minutes at 4°C in the presence of insulin and other agents. The mixture was then warmed to 30°C and exposed for 30 seconds to 20 μ*M* [γ-^{32}P]ATP. The reaction was terminated by adding 4 ml of 5 percent trichloroacetic acid, 1.5 percent sodium pyrophosphate, and 1 percent monobasic sodium phosphate at 4°C. The samples were then processed by the method of Lam and Kasper (*28*). (A) Effect of insulin. (B) Effect of Con A, wheat germ agglutinin (*WGA*), and PHA. (C) Effect of antiserum to insulin receptor (*AR*) and normal human serum (*NS*).

To test the mode of action of the agents that mimicked insulin, we added them at maximum effective concentrations to nuclear envelopes to which $10^{-11}M$ insulin was also added (Table 1). The effects of the agents on ^{32}P incorporation were no greater than the effect of insulin itself. When a stimulating concentration of insulin was added together with inhibitory concentrations of the agents, the effect of insulin was inhibited. These data suggested, therefore, that the agents acted through the same pathway as insulin. Insulin has been shown to inhibit the phosphorylation of several types of nuclear envelope proteins, in particular those of the nuclear pore complex (*12*). Our studies suggest that plant lectins and antibody to the insulin receptor have similar effects.

Antiserum to the insulin receptor and plant lectins can mimic many of the biological effects of insulin in several systems (*17, 19*). The antiserum to the receptor interacts at or near the insulin binding site of the plasma membrane insulin receptor (*17, 19*), generates transmembrane signals (*14, 17*), and is internalized (*15*); then, like other antibodies, it may bind to the nucleus (*20*). Although this antibody blocks insulin binding to the plasma membrane insulin receptor, it does not block insulin binding to the nuclear envelope (*21*). This observation is in concert with data demonstrating that the binding sites for insulin on nuclear envelopes and plasma membranes have different characteristics (*5, 6*). However, since we have now observed that this antiserum has insulin-like effects in nuclear envelopes, it seems likely that it binds to a different site from insulin in the nuclear envelope but still generates the same biological effects. Similar types of antisera, which do not block insulin binding but mimic insulin action, have been produced to the plasma membrane insulin receptors (*17, 19, 22*).

Plant lectins, in addition to their mitogenic and metabolic effects, mimic insulin actions in many cell types by directly interacting with the glycoprotein residues of the insulin receptor (*23*) and then generating the same transmembrane signal as insulin (*14*). Lectins, after binding to cell surface receptors, are patched, capped, endocytosed (*15*) and may interact with intracellular organelles. For example, glycoproteins that bind Con A have been identified in rat liver nuclear envelopes (*24*). We have now demonstrated that Con A and PHA can mimic

insulin action in isolated nuclear envelopes. In contrast, the nonmitogenic lectin wheat germ agglutinin is ineffective in this system. Our data therefore support those of Beachy *et al.* (*14*) showing that Con A and PHA, but not wheat germ agglutinin, stimulate the production of a mediator substance in lymphocytes that activates mitochondrial pyruvate dehydrogenase.

It has been suggested that the binding of insulin to the plasma membrane receptor may be sufficient to initiate all of insulin's actions (*25*). However, since agents that mimic insulin are internalized and act on the nuclear envelope, it is also possible that insulin and agents that mimic it have direct effects in the cell interior. After internalization these agents may bind to the nuclear envelope insulin receptor, inhibit nuclear envelope phosphorylation, and thus initiate a series of events leading to enhanced mRNA transport from the nucleus into the cytoplasm.

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Calcium-Dependent Stress Maintenance Without Myosin Phosphorylation in Skinned Smooth Muscle

Abstract. *Stress development depended on calcium-stimulated myosin phosphorylation in an arterial smooth muscle preparation in which the concentration of calcium was controlled. However, developed stress was maintained at a concentration of calcium that did not support phosphorylation. These results, in conjunction with other evidence, suggest that the interaction of two regulatory mechanisms with different calcium sensitivities regulate both stress and the rate and energetics of contraction.*

Biochemical studies of contractile proteins isolated from vertebrate smooth muscle have provided strong evidence that phosphorylation of the 20,000-dalton myosin light chain (LC 20) by myosin light chain kinase enables cross-bridges to attach to the thin filament and cycle (*1*). The hypothesis that cross-bridge phosphorylation is necessary and sufficient to regulate contraction is strongly supported by the dependence of force development on phosphorylation in smooth muscle in which the cell membranes are disrupted ("skinned") so that the concentration of calcium can be controlled in contractions produced by exogenous Mg^{2+} and adenosine triphosphate (ATP) (*2*).

Murphy and co-workers (*3*) found that cross-bridge phosphorylation preceded contraction after stimulation of intact smooth muscle. However, Ca^{2+}-dependent steady-state stress was maintained after phosphorylation decreased during sustained contractions. The steady-state stress was associated with low cross-bridge cycling rates, as shown by isotonic shortening velocities, and was termed "latch" (*3*). Further studies led to our hypothesis that a second Ca^{2+}-dependent regulatory mechanism controls the formation of attached noncycling or slowly cycling cross-bridges characteristic of latching and that this system has a higher sensitivity to Ca^{2+} than does myosin light chain kinase and phosphorylation (*4*).

The apparent absence of a latch state in skinned tissues (*2*) may reflect loss or inactivation of a Ca^{2+}-dependent regulatory mechanism producing latching. Alternatively, myosin phosphorylation may be required for Ca^{2+} binding to a second regulatory site. We report Ca^{2+}-dependent stress maintenance at concentrations of Ca^{2+} below those required for myosin phosphorylation in skinned smooth muscle. Mild skinning procedures (*5*) were briefly applied to tissues whose contractile capacity had been determined. The preparation of thin (338 ± 18 μm) strips from the tunica media of swine carotid arteries, determination of their optimum length (L_0) for isometric stress generation (S) in response to K^+ depolarization, and measurement of phosphorylation after quick freezing have been described (*4, 6*). The tissues were treated for 60 minutes at 22°C with a solution containing 5 m*M* EGTA, 20 m*M* imidazole (*p*H 6.7), 50 m*M* potassium acetate, 0.5 m*M* dithio-