Proc. Natl. Acad. Sci. USA
Vol. 80, pp. 1189–1193, March 1983
Biochemistry

Insulin regulation of protein phosphorylation in isolated rat liver nuclear envelopes: Potential relationship to mRNA metabolism

(nucleoside triphosphatase/mRNA transport/protein phosphatase)

FRANCESCO PURRELLO[a,b,c], DANIEL B. BURNHAM[b], AND IRA D. GOLDFINE[a,b,d,e]

[a]Cell Biology Research Laboratory, Harold Brunn Institute, Mount Zion Hospital and Medical Center, San Francisco, California 94120; and Departments of [d]Medicine and [b]Physiology, University of California, San Francisco, California 94143

Communicated by Van Rensselaer Potter, November 19, 1982

ABSTRACT **The direct addition of insulin to highly purified nuclear envelopes prepared from the livers of diabetic rats resulted in a decrease in the incorporation of ^{32}P into trichloroacetic acid-precipitable proteins. Autoradiography of ^{32}P-labeled envelopes, solubilized in sodium dodecyl sulfate and subjected to electrophoresis, revealed that insulin decreased the phosphorylation of all major protein bands. Insulin produced detectable effects at concentrations between 0.1 and 1 pM, maximal effects at 10 pM, and progressively diminished effects at higher concentrations. Two insulin analogs, desdipeptide proinsulin and desoctapeptide insulin, had approximately 10% and 1%, respectively, the activity of native insulin. When nuclear envelopes were first phosphorylated with [γ-^{32}P]ATP and insulin was then added with an excess of unlabeled ATP, dephosphorylation was enhanced, suggesting that insulin was regulating nuclear envelope phosphatase activity. The direct addition of insulin to isolated rat liver nuclei in the presence of ATP stimulated the release of previously ^{14}C-labeled trichloroacetic acid-precipitable mRNA-like material, and the direct addition of insulin to nuclear envelopes stimulated the activity of nucleoside triphosphatase, the enzyme that participates in mRNA nucleocytoplasmic transport. Moreover, the dose–response curves for these functions mirrored insulin's inhibition of nuclear envelope phosphorylation. These data suggest, therefore, a mechanism whereby insulin directly inhibits the phosphorylation of the nuclear envelope, leading in turn to the regulation of mRNA metabolism.**

Insulin has numerous effects on target cells, including regulation of membrane transport, enzyme activities, and DNA and RNA metabolism (1, 2). There is evidence that insulin, like other hormones, regulates a number of cellular functions by phosphorylation and dephosphorylation reactions (3–6). Moreover, effects of insulin on phosphorylation and dephosphorylation have recently been observed in broken cell preparations (7–11).

We and others have demonstrated specific high-affinity binding sites for insulin on nuclei and nuclear envelopes of liver and other tissues (12–17). Moreover, morphological studies have suggested that insulin can enter intact cells and interact with the nuclear envelope (18–20). However, the exact role of insulin in nuclear functions is unknown. There are a number of reports demonstrating that insulin increases mRNA levels in liver and other tissues (21–29). Further, it has recently been reported that the direct addition of insulin to isolated nuclei stimulates the release of mRNA (30). These observations suggest, therefore, that one action of insulin on the nucleus is to regulate mRNA metabolism.

Several lines of evidence indicate that transport of mRNA from the nucleus is mediated by a specific enzyme of the nuclear envelope, the nuclear envelope nucleoside triphosphatase (NTPase; EC 3.6.1.15) (31–33) that is located at or near the nuclear pore complex (31–34). Recently, we have demonstrated that the direct addition of picomolar insulin to isolated rat liver nuclear envelopes stimulates NTPase activity (35), suggesting that insulin may regulate mRNA metabolism by means of this enzyme. Several groups have reported the presence of protein kinase and phosphatase activity in isolated nuclear envelopes (36–38), and it has been proposed that these enzymes may have a regulatory role in nucleocytoplasmic transport (36). Moreover, it has been suggested that phosphorylation reactions regulate nuclear envelope NTPase activity (39). Accordingly, in the present study we have investigated whether insulin has direct effects on the phosphorylation of nuclear envelopes prepared from rat liver.

EXPERIMENTAL PROCEDURES

Materials. The following were purchased: female Sprague–Dawley rats, 140–160 g, from Simonsen Laboratories (Gilroy, CA); porcine insulin from Elanco Products (Indianapolis, IN); [γ-^{32}P]ATP at a specific activity of 18–26 Ci/mmol (1 Ci = 3.7 $\times$ 10^{10} Bq) from Amersham; ^{35}S-labeled adenosine 5′-[γ-thio]-triphosphate (ATP[γ-^{35}S]) at a specific activity of 62 Ci/mmol and [6-^{14}C]orotic acid at a specific activity of 40–60 mCi/mmol from New England Nuclear; and ATP, highly purified sucrose (RNase free), spermidine, and streptozotocin from Sigma. Insulin analogues were gifts of R. Chance and F. Carpenter.

Preparation of Nuclei, Nuclear Envelopes, and Plasma Membranes. Female Sprague–Dawley rats, 140–160 g, were made diabetic with streptozotocin at 75 mg/kg (35). Purified rat liver nuclei were isolated by the method of Blobel and Potter (40), and nuclear envelopes were then prepared from these nuclei by the method of Monneron *et al.* (41), except that glycerol was omitted during the gradient centrifugation step. Electron microscopy of the preparation revealed sheets of bilayered membranes with pore complexes. Purified plasma membranes were isolated by the method of Ray (42).

Measurement of the Efflux of mRNA-like Material. Efflux of mRNA-like material from isolated nuclei was studied by the method of Agutter (43, 44). Rats were injected intravenously with 10 μCi of [^{14}C]orotic acid 30 min before sacrifice, and liver nuclei were prepared and adjusted to 0.2–0.4 mg of protein per ml (4–8 $\times$ 10^6 nuclei per ml) in 250 μl of buffer with 0.25 M sucrose/5.0 mM spermidine/25 mM KCl/5.0 mM $MgCl_2$/0.5

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviations: NTPase, nucleoside triphosphatase; ATP[γ-^{35}S], ^{35}S-labeled adenosine 5′-[γ-thio]triphosphate.

[c]Visiting scientist from the Istituto di Endocrinologia dell'Universitá di Catania, Catania, Italy.

[e]To whom reprint requests should be addressed at: Cell Biology Laboratory, Harold Brunn Institute for Medical Research, Mount Zion Hospital and Medical Center, P.O. Box 7921, San Francisco, CA 94120.

mM $CaCl_2$/0.3 mM $MnCl_2$/10 mM Tris·HCl, pH 7.4.[f] This mixture was incubated with or without insulin for 10 min at 4°C. Then ATP was added to 2.5 mM and efflux was measured for 10 min at 30°C. The nuclei were pelleted at 1,500 × *g* for 10 min at 4°C, and 250 μl of 50% trichloroacetic acid were added to the supernatant. The pellet obtained was hydrolyzed with 200 μl of 0.1 M NaOH, diluted with 0.9 ml of water, and added to 10 ml of Liquiscint (National Diagnostics, Somerville, NJ). When control studies were performed in the absence of ATP, the efflux of radioactive material was markedly reduced and insulin was without effect. Because mRNA efflux requires the presence of ATP (44) the data suggested that insulin was acting on the release of the mRNA-like materials.

NTPase Assay. NTPase was assayed as described (35). The release of ^{32}P from [γ-^{32}P]ATP was determined after absorption of the nucleotide to charcoal (33).

Phosphorylation Assay. The reaction mixture contained purified nuclear envelopes (50–100 μg of protein), 25 mM KCl, 5 mM $MgCl_2$, and 50 mM Tris·HCl at pH 7.4 in a final volume of 100 μl. The reaction tubes were preincubated for 10 min at 4°C in the presence or absence of insulin and other hormones. The reaction was initiated by addition of 20 μM [γ-^{32}P]ATP and carried out at 30°C for the indicated times. The reaction was terminated by 4 ml of 5% trichloroacetic acid at 4°C containing 1.5% sodium pyrophosphate and 1% monobasic sodium phosphate. The samples were then processed by the method of Lam and Kasper (36) on Millipore type HA (0.45 μm-pore-diameter) filters.

Gel Electrophoresis and Autoradiographic Analysis. For these studies, the phosphorylation reactions were terminated by adding 50 μl of a solution containing 30 mM Tris·HCl at pH 7.4, 10% sodium dodecyl sulfate, 15% (vol/vol) glycerol, and 0.05% bromophenol blue, and heating to 95°C for 3 min; 50 μl of 8% (vol/vol) 2-mercaptoethanol was then added, and the samples were subjected to sodium dodecyl sulfate/7.5% polyacrylamide gel electrophoresis (36). Autoradiography (24–48 hr) was performed with Kodak X-Omat AR film.

RESULTS

Insulin Regulation of Nuclear Envelope Phosphorylation and a Comparison with Plasma Membranes. Addition of [γ-^{32}P]ATP to both nuclear envelopes and plasma membranes led to a time-dependent increase in protein phosphorylation. The direct addition of 10 pM insulin produced a decrease in the incorporation of ^{32}P into the proteins of the nuclear envelope (Fig. 1). In contrast, insulin had no effect on plasma membranes (Fig. 1). In five experiments, 10 pM insulin decreased ^{32}P incorporation by 43% ± 2.9% (mean ± SEM). One factor that could influence the phosphorylation reaction is the rate of ATP hydrolysis. Because we previously demonstrated that insulin increased NTPase activity in nuclear envelopes, we tested whether the insulin-induced decrease in phosphorylation was subsequent to enhanced substrate depletion. However, in these studies no difference in [γ-^{32}P]ATP hydrolysis between control and insulin-treated nuclear envelopes (Fig. 2) was observed for up to 150 sec. At later times, insulin increased ATP hydrolysis (Fig. 2), suggesting that decreased ^{32}P-phosphorylation preceded increased NTPase activity.

[f] For two reasons we did not use liver cytosol in our efflux studies. First, it has been shown that efflux studies, under the conditions employed herein, yield efflux data similar to those from studies in which cytosol is added (44). Second, although we find that insulin stimulates mRNA efflux in the presence of cytosol, cytosol for unknown reasons increases the concentration of insulin necessary to elicit this effect by 1,000-fold or greater. Also, others have found that the addition of liver cytosol to nuclei blunts the effect of insulin (P. A. Agutter, personal communication).

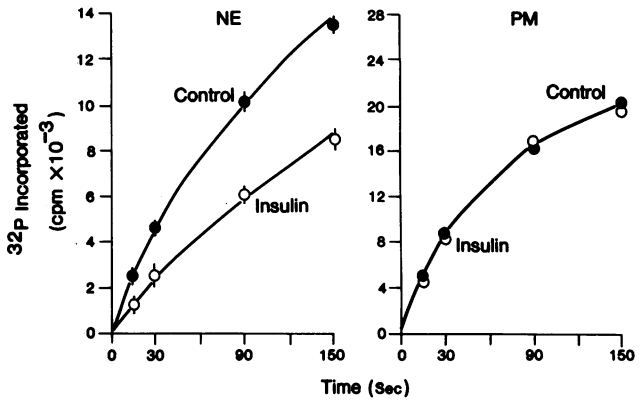

FIG. 1. Time course of the incorporation of [^{32}P]phosphate from [γ-^{32}P]ATP into nuclear envelope (NE) and plasma membrane (PM) proteins. Nuclear envelopes (100 μg) and plasma membranes (50 μg) were incubated in the presence (○) or absence (●) of 10 pM insulin for 10 min at 4°C and warmed to 30°C, and the phosphorylation reaction was started with 20 μM [γ-^{32}P]ATP. Each value is the mean ± SEM of triplicate determinations.

To study whether the insulin effect was due to inhibition of nuclear envelope protein kinase activity or stimulation of phosphatase activity, nuclear envelopes were first incubated with [γ-^{32}P]ATP to phosphorylate endogenous proteins and then a 50-fold excess of unlabeled ATP was added to prevent further ^{32}P incorporation (45). When insulin was added at this time, protein dephosphorylation was accelerated, suggesting that insulin was activating a phosphoprotein phosphatase (Fig. 3).[g] In other experiments, the effect of insulin on the labeling of nuclear envelope proteins was evaluated by using ATP[γ-^{35}S]; thiophosphorylated proteins have been shown to be phosphatase resistant (46). In these experiments, no effect of insulin on the incorporation of ^{35}S into trichloroacetic acid-precipitable proteins was observed (Fig. 4), further supporting the concept that insulin was acting through the activation of a phosphoprotein phosphatase.

[g] The inability of unlabeled ATP to completely reverse labeled ATP-mediated phosphorylation has been seen in other systems; it may be due to the inhibitory effects of ATP on phosphatase activity (45). Alternatively, the phosphatase activity in isolated nuclear envelopes may not be sufficient to dephosphorylate all proteins.

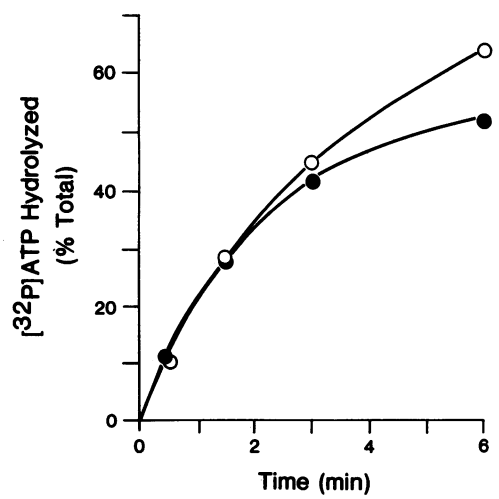

FIG. 2. Time course of ATP hydrolysis in the presence (●) or absence (○) of insulin. Nuclear envelopes were incubated as described in the legend for Fig. 1. The release of ^{32}P from [γ-^{32}P]ATP was determined after absorption of the nucleotide to charcoal. Each value is the mean of triplicate determinations.

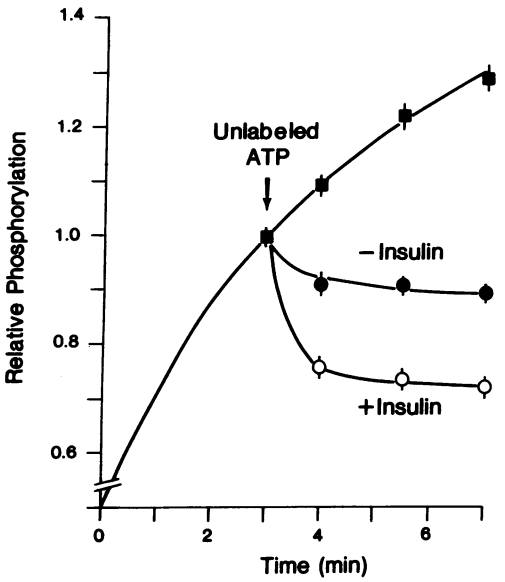

FIG. 3. Time course of nuclear envelope dephosphorylation in the presence and absence of insulin. Tubes of nuclear envelopes were incubated for 3 min at 30°C with 20 μM [γ-^{32}P]ATP. After this preincubation, one third (■) of the tubes received buffer, one third (●) received a 50-fold excess of unlabeled ATP, and one third (○) received excess unlabeled ATP plus 10 pM insulin, and the reaction then continued for the indicated times. A representative of four experiments is shown. Each value is the mean ± SD of triplicate determinations.

To determine which proteins insulin was influencing, the ^{32}P-labeled nuclear envelopes were solubilized in sodium dodecyl sulfate and subjected to electrophoresis in a 7.5% polyacrylamide gel, and autoradiograms were obtained. Several phosphorylated proteins were observed; the major bands had apparent molecular weights of 190,000, 72,000, 68,000, and 55,000. The two bands with apparent molecular weights of 68,000 and 72,000 represent the major constituents of the nuclear pore complex (47, 48). These studies indicated that insulin decreased ^{32}P incorporation into all major nuclear envelope protein bands by 40–50% (Fig. 5).

Effects of Insulin and Analogs. A detectable effect of insulin on ^{32}P incorporation was seen between 0.1 and 1 pM (Fig. 6).

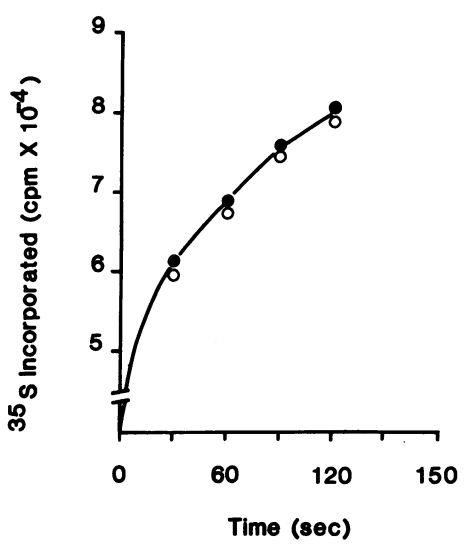

FIG. 4. Time course of incorporation of ^{35}S from ATP[γ-^{35}S] into purified nuclear envelopes. Nuclear envelopes were treated as described in the legend for Fig. 1, but ATP[γ-^{35}S] was used. ●, No insulin; ○, with insulin. Each value is the mean of triplicate determinations.

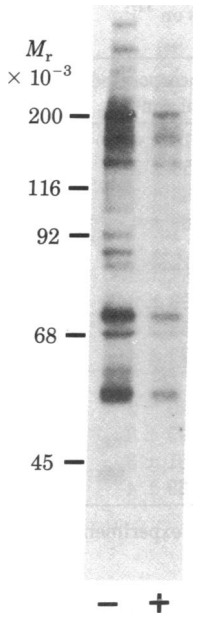

FIG. 5. Electrophoresis and autoradiography of purified nuclear envelopes after incubation with 20 μM [γ-^{32}P]ATP in the presence and absence of 10 pM insulin. Nuclear envelopes were incubated in the presence (+) or absence (−) of insulin for 10 min at 0°C and then warmed to 30°C. Next [γ-^{32}P]ATP was added and the incubation was continued for 30 sec. The reaction was then stopped and the mixtures were analyzed by sodium dodecyl sulfate gel electrophoresis, Coomassie blue staining, and autoradiography.

The maximal effect of the hormone was seen at 10 pM, whereas at higher concentrations the effects of insulin progressively diminished. Two insulin analogs were also tested (Table 1). Desdipeptide proinsulin had approximately 10% the activity and desoctapeptide insulin had approximately 1% the activity of native insulin; these potencies are in agreement with their known biological potencies in whole cells (49, 50) and nuclear envelopes (35). Other studies (not shown) indicated that Zn^{2+}, boiled insulin, insulin B chain, and growth hormone were all ineffective.

Potential Relationship to mRNA Metabolism. The direct addition of insulin to isolated nuclei stimulated the release of prelabeled [^{14}C]mRNA (Fig. 6). A detectable effect was seen between 0.1 and 1 pM, a maximal effect was seen at 10 pM, and the effect of insulin was diminished at higher concentrations. In addition, the stimulation of nuclear membrane NTPase by insulin produced a dose–response curve that was very similar to that for the stimulation of mRNA efflux. Moreover, both of these insulin dose–response curves were mirror images of the dose–response curve for insulin's inhibition of ^{32}P incorporation into nuclear envelope proteins.

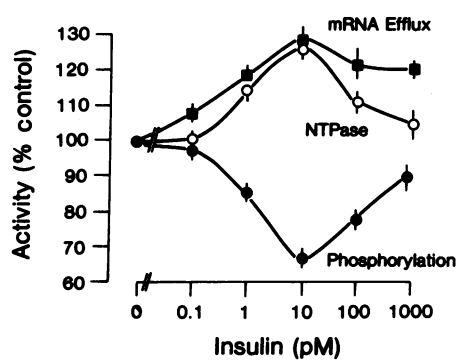

FIG. 6. Dose–response relationships between insulin effects on the efflux of mRNA-like material from isolated nuclei, NTPase activity, and incorporation of [^{32}P]phosphate from [γ-^{32}P]ATP in isolated nuclear envelopes. Basal mRNA efflux was 3.6% of total nuclear radioactivity released per 10 min. Basal NTPase activity was 2 μmol/mg of protein per hr. Basal ^{32}P incorporation was 3 pmol/mg of protein per min. Values are the mean of 5 mRNA efflux, 10 NTPase, and 3 phosphorylation studies.

Table 1. Effects of insulin and insulin analogs on ^{32}P incorporation into isolated nuclear envelopes

Addition	Conc., pM	^{32}P incorporated, % of control
Insulin	1	78 ± 4
	10	58 ± 3
	100	85 ± 3
	1,000	89 ± 4
Desdipeptide proinsulin	1	97 ± 3
	10	99 ± 2
	100	58 ± 5
	1,000	76 ± 4
Desoctapeptide insulin	1	99 ± 2
	10	95 ± 3
	100	81 ± 3
	1,000	69 ± 4

Results are the mean ± SEM of three separate experiments.

DISCUSSION

In the present investigation we have demonstrated that insulin, when added directly to purified nuclear envelopes prepared from liver of diabetic rats, decreases the incorporation of [^{32}P]phosphate from [γ-^{32}P]ATP into trichloroacetic acid-precipitable proteins.[h] Effects of insulin on phosphorylation were seen at levels as low as 1 pM, a concentration well within the physiological range. Further two insulin analogs also decreased phosphorylation with activities that were in concert with their known biological potencies. Moreover, inactive insulin fragments and unrelated hormones were without effect. These data indicated therefore that this effect of insulin was hormone specific.

Although the mechanism whereby insulin inhibits ^{32}P incorporation into nuclear envelope protein is unknown, three lines of evidence suggest that insulin may have been acting through an increase in phosphatase activity. First, insulin is known to activate phosphatase activity in other systems (2). Second, in nuclear envelopes insulin appeared to enhance protein dephosphorylation. Third, insulin had no effect on the phosphorylation of nuclear envelopes by ATP[γ-^{35}S], a phosphatase-resistant analog. These data suggested an effect of insulin on phosphatase activity, but an effect of insulin on protein kinase activity cannot be excluded.

The importance of this effect of insulin on ^{32}P incorporation relates to recent studies of the direct effects of insulin on both mRNA efflux from isolated nuclei and NTPase activity in isolated nuclear envelopes. In order to understand the mechanism of mRNA transport (51) from the nucleus, rats have been injected with either radiolabeled orotic acid or uridine and the efflux of labeled RNA from isolated liver nuclei into a surrogate cytoplasm has been studied (32, 52–54). A number of criteria suggest that the RNA transported under the conditions studied is mRNA [the criteria include size, base and poly(A) content, activity in directing protein synthesis, incorporation into polysomes, and inclusion into specific ribonucleoprotein particles] (32, 43, 44, 52, 53). RNA transport *in vitro* involves both intranuclear RNA processing and subsequent efflux, and a source of high-energy phosphate such as ATP is necessary for transport but not processing (32, 52, 53).

[h] We have also been able to reproducibly detect an effect of insulin on the phosphorylation of nuclear envelope proteins in nuclear envelopes prepared from nondiabetic fasted rats, but not from envelopes prepared from nondiabetic fed rats. Presumably the high endogenous levels of portal vein insulin in the latter group of animals activates these processes *in vivo* and thus obscures effects seen *in vitro*.

Schumm and Webb (30) recently reported that the direct addition of insulin to isolated nuclei from normal rats specifically stimulated the release of mRNA. In their studies, the concentration of insulin necessary to produce this effect was approximately 100 times higher than physiological concentrations. In the present studies with diabetic rats, we have extended their observations by demonstrating that picomolar concentrations of insulin regulate this process.[i]

There is considerable evidence that a nuclear membrane NTPase provides the energy for the transport of mRNA (32, 33, 43, 52–54). For instance, the activation energy for RNA transport is 13 kcal/mol and for NTPase activity it is 13.3–13.8 kcal/mol (1 kcal = 4.18 kJ) (33). The effective concentrations of ATP for both NTPase activity and facilitated RNA transport are similar (33), and cyclic AMP stimulates both functions whereas NaF inhibits them (32, 33, 52–54). Treatment with trypsin and sulfhydryl blockers inactivates both functions (32, 43). Moreover, histochemical studies have suggested that the NTPase resides in the nuclear pore complex (32) and is associated with ribonucleoproteins that are being translocated through the nuclear pore complex (51).

We have recently reported that insulin, when directly added to nuclear envelopes, stimulates the activity of NTPase (35), the enzyme that is believed to mediate mRNA nucleocytoplasmic

[i] It is possible, therefore, that the enhanced sensitivity to insulin of efflux from isolated nuclei in the present study was due in part to the use of diabetic animals and, as mentioned previously, was due in part to the omission of cytosol from the efflux system.

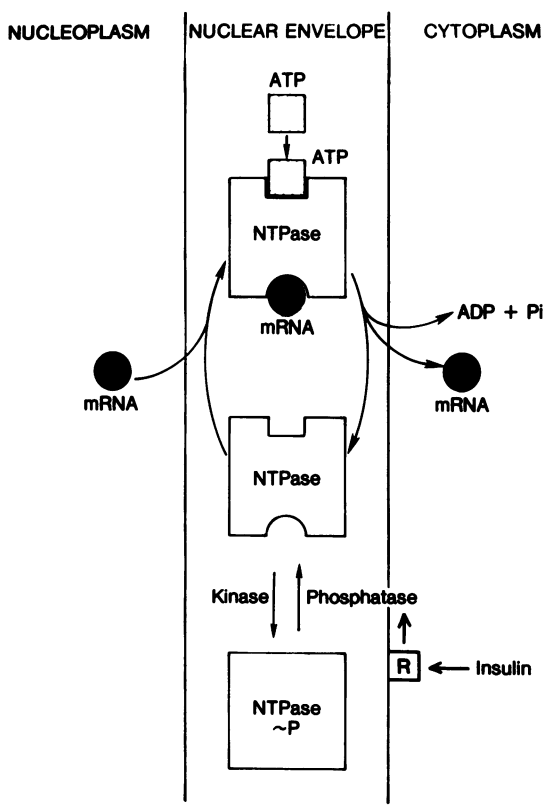

FIG. 7. Speculative proposed model of insulin regulation of mRNA efflux from the nucleus. The enzyme NTPase may be either phosphorylated (inactive) or dephosphorylated (active). Insulin, after binding to its receptor (R), increases a phosphatase activity, which in turn then activates NTPase, leading to increased ATP hydrolysis and mRNA transport. This model is an extension of the earlier models proposed by Agutter (43) and Ishikawa *et al.* (54). NTPase in our model most likely corresponds to "S" in their models.

transport (32). We suggested, therefore, that insulin may stimulate mRNA transport through the regulation of this enzyme. In the present study, we present evidence indicating that the effects of insulin on the transport of mRNA-like material, NTPase activity, and nuclear envelope phosphorylation may be related because the dose–response curves for these three functions are similarly shaped and occur over the same concentration range. Because inhibition of nuclear envelope phosphorylation precedes stimulation of nuclear envelope NTPase, it is possible, therefore, that decreased nuclear envelope phosphorylation is the first step in the regulation of nuclear functions by insulin.

These data therefore suggest a speculative model of how insulin may act directly on nuclei (Fig. 7). First, insulin binds to its receptor on the nuclear envelope. Because the insulin receptor on the nuclear envelope has a K_d of approximately 5 nM (15), whereas a detectable effect of insulin on nuclear functions occurs at 1 pM, it appears that only a very small fraction of these receptors need be occupied to regulate this function. Next, the phosphorylation of nuclear envelope proteins, including the NTPase, is inhibited. The NTPase of the nuclear envelope, like certain other enzymes (2), may be in either a phosphorylated (inactive) or dephosphorylated (active) state, and, when dephosphorylated by insulin, the enzyme has an increased ability to bind both mRNA and ATP. Thus, as a result of this activation, the hydrolysis of ATP is increased and the nucleocytoplasmic transport of mRNA is facilitated. This model is in agreement with the observation that ATPases are regulated by phosphorylation–dephosphorylation processes (55). Moreover, this model is also in agreement with the findings of Agutter, who has proposed that the dephosphorylation of the nuclear envelope may increase NTPase activity, and that poly(A)-containing RNA (a known activator of NTPase and mRNA efflux) may increase enzyme activity through a dephosphorylation reaction (43). Further, this model is in agreement with the cytochemical studies of Vorbrodt and Maul, who have observed that the NTPase of the nuclear envelope is associated with the nuclear pore complex and mRNA particles translocating through the nuclear pores (51).

The authors thank Dr. John A. Williams for helpful discussions during this study. This work was supported in part by National Institutes of Health Grant AM 26667 and in part by the Elise Stern Haas Research Fund, Mount Zion Hospital and Medical Center.

1. Czech, M. P. (1977) *Annu. Rev. Biochem.* **46**, 359–384.
2. Goldfine, I. D. (1981) in *Biochemical Actions of Hormones*, ed. Litwack, G. (Academic, New York), Vol. 8, pp. 273–305.
3. Denton, R. M., Browney, R. W. & Belsham, G. J. (1981) *Diabetologia* **21**, 347–362.
4. Greengard, P. (1978) *Science* **199**, 146–152.
5. Walsh, D. A. (1978) *Biochem. Pharmacol.* **27**, 1801–1804.
6. Krebs, E. G. (1979) *Annu. Rev. Biochem.* **48**, 923–959.
7. Seals, J. R. & Jarett, L. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 77–81.
8. Kiechle, F. L., Jarett, L., Katagae, N. & Popp, D. A. (1981) *J. Biol. Chem.* **256**, 2945–2951.
9. Larner, J., Galasko, G., Cheng, K., De Paoli Roach, A. A., Huang, L., Daggy, P. & Kellog, J. (1979) *Science* **205**, 1408–1410.
10. Rosen, O. M., Rubin, C., Cobb, M. H. & Smith, C. J. (1981) *J. Biol. Chem.* **256**, 3630–3633.
11. Walaas, O., Walaas, E., Lystad, E., Alertsen, A. R., Horn, R. S. & Fossum, S. (1977) *FEBS Lett.* **80**, 417–422.
12. Goldfine, I. D. & Smith, G. J. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 1427–1431.
13. Horvat, A., Li, E. & Katsoyannis, P. G. (1975) *Biochim. Biophys. Acta* **382**, 609–620.
14. Goidl, J. A. (1978) *Biochemistry* **18**, 3674–3679.
15. Vigneri, R., Goldfine, I. D., Wong, K. Y., Smith, G. J. & Pezzino, V. (1978) *J. Biol. Chem.* **253**, 2098–2103.
16. Goldfine, I. D., Vigneri, R., Cohen, D., Pliam, N. B. & Kahn, C. R. (1977) *Nature (London)* **269**, 698–700.
17. Brisson-Lougarre, A. & Blum, C. J. (1980) *C. R. Hedb. Seances Acad. Sci. Ser. D* **290**, 889–892.
18. Goldfine, I. D., Jones, A., Hradek, G., Wong, K. Y. & Mooney, J. S. (1978) *Science* **202**, 760–762.
19. Goldfine, I. D., Jones, A., Hradek, G. & Wong, K. Y. (1981) *Endocrinology* **108**, 1821–1828.
20. Lolait, S. J. & Toh, B. H. (1980) *Cell Tissue Res.* **210**, 145–153.
21. Steiner, D. F. & King, J. (1966) *Biochim. Biophys. Acta* **119**, 510–516.
22. Pilkis, S. J. & Salaman, D. F. (1972) *Biochim. Biophys. Acta* **272**, 327–339.
23. Younkin, B. & Martin, H. (1978) *J. Theor. Biol.* **74**, 491–498.
24. Peavy, D. E., Taylor, J. M. & Jefferson, L. S. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 5879–5883.
25. Roy, A. K., Chatterjee, B., Prasad, M. S. K. & Unakar, N. J. (1980) *J. Biol. Chem.* **255**, 11614–11618.
26. Bolander, F. P., Nicholas, K. R., Van Wyk, J. J. & Topper, Y. J. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 5682–5684.
27. Hill, R. E., Lee, K. L. & Kenney, F. T. (1981) *J. Biol. Chem.* **256**, 1510–1513.
28. Korc, M., Owerbach, D., Quinto, C. & Rutter, W. J. (1981) *Science* **213**, 351–353.
29. Pry, T. A. & Porter, J. W. (1981) *Biochem. Biophys. Res. Commun.* **100**, 1002–1009.
30. Schumm, D. E. & Webb, T. E. (1981) *Arch. Biochem. Biophys.* **210**, 275–279.
31. Agutter, P. S., McArdle, H. J. & McCaldin, B. (1976) *Nature (London)* **263**, 165–167.
32. Agutter, P. S., McCaldin, B. & McArdle, H. J. (1979) *Biochem. J.* **182**, 811–819.
33. Clawson, G. A., James, J., Woo, C. H., Friend, D. S., Moody, D. & Smuckler, E. A. (1980) *Biochemistry* **19**, 2748–2756.
34. Franke, W. W. (1974) *Int. Rev. Cytol. Suppl.* **4**, 71–236.
35. Purrello, F., Vigneri, R., Clawson, G. A. & Goldfine, I. D. (1982) *Science* **216**, 1005–1007.
36. Lam, K. S. & Kasper, C. B. (1979) *Biochemistry* **18**, 307–311.
37. Steer, R. C., Wilson, M. J. & Ahmed, K. (1979) *Exp. Cell Res.* **119**, 403–406.
38. Steer, R. C., Wilson, M. J. & Ahmed, K. (1979) *Biochem. Biophys. Res. Commun.* **89**, 1082–1087.
39. Agutter, P. S., Cockrill, J. B., Lavine, J. E., McCaldin, B. & Sim, R. B. (1979) *Biochem. J.* **181**, 647–658.
40. Blobel, G. & Potter, V. R. (1966) *Science* **154**, 1662–1665.
41. Monneron, A., Blobel, G. & Palade, G. E. (1972) *J. Cell Biol.* **55**, 104–125.
42. Ray, T. K. (1970) *Biochim. Biophys. Acta* **196**, 1–9.
43. Agutter, P. S. (1980) *Biochem. J.* **188**, 91–97.
44. Agutter, P. S. (1982) in *The Nuclear Envelope and the Nuclear Matrix*, ed. Maul, G. (Liss, New York), pp. 91–109.
45. Carpenter, G., King, L. & Cohen, S. (1979) *J. Biol. Chem.* **254**, 4884–4891.
46. Cassel, D. & Glaser, L. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 2231–2235.
47. Aaronson, R. P. & Blobel, G. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 1007–1011.
48. Krohne, G., Franke, W. W. & Scheer, V. (1978) *Exp. Cell Res.* **116**, 85–102.
49. Korc, M., Iwamoto, Y., Sankaran, H., Williams, J. A. & Goldfine, I. D. (1981) *Am. J. Physiol.: Gastrointest. Liver Physiol.* **240**, G56–G62.
50. Sankaran, H., Iwamoto, Y., Korc, M., Williams, J. A. & Goldfine, I. D. (1981) *Am. J. Physiol.: Gastrointest. Liver Physiol.* **240**, G63–G68.
51. Vorbrodt, A. & Maul, G. G. (1980) *J. Histochem. Cytochem.* **28**, 27–35.
52. Schumm, D. E. & Webb, T. E. (1978) *J. Biol. Chem.* **253**, 8513–8519.
53. Clawson, G. A., James, J., Woo, C. H., Friend, D. S., Moody, D. & Smuckler, E. A. (1980) *Biochemistry* **19**, 2756–2762.
54. Ishikawa, K., Sato-Odani, S. & Ogata, K. (1978) *Biochim. Biophys. Acta* **521**, 650–654.
55. Caroni, P. & Carafoli, E. (1981) *J. Biol. Chem.* **256**, 9371–9373.