melatonin profile precedes the presence of malignant tissue it will be necessary to conduct longitudinal and familial studies.

A number of studies have suggested that ER concentrations in breast tumors indicate the degree of hormonal dependence of an individual's breast cancer (*14*). The present data suggest that the lower the peak concentration of plasma melatonin, the greater the probability that the primary tumor may be hormonally dependent. Thus, the absence of nocturnal peak melatonin may serve as a biochemical marker for increased risk of developing ER+ breast cancer.

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Insulin Stimulation of Nucleoside Triphosphatase Activity in Isolated Nuclear Envelopes

Abstract. *The activity of nucleoside triphosphatase, an enzyme that regulates nuclear messenger RNA transport, was measured in highly purified nuclear envelopes isolated from rat liver. Addition of picomolar concentrations of insulin to freshly prepared nuclear envelopes directly increased the enzyme activity. The major effect of insulin on this enzyme was to increase the maximum velocity of its activity; no significant effects were seen on the affinity constant. These studies raise the possibility, therefore, that the nuclear envelope is a site where insulin regulates nuclear functions.*

The nuclear envelope is a double-membrane structure that separates the nucleoplasm from the cytoplasm. Although the exact organization and function of the nuclear envelope are unknown, several studies (*1*) have demonstrated that the nuclear envelope contains a distinctive enzyme, nucleoside triphosphatase (E.C. 3.6.1.15). This Mg^{2+}-dependent enzyme has several features generally not shared by other triphosphatases, including a broad substrate specificity (that is, the triphosphates of adenosine, uridine, cytidine, and guanosine are all effective), stimulation by RNA (*2*), and regulation by adenosine 3′,5′-monophosphate (cyclic AMP) (*3*).

Several studies in liver and other tissues indicate that a major function of this nucleoside triphosphatase is to provide the energy necessary for the transport of messenger RNA (mRNA) out of the nucleus. Either adenosine triphosphate (ATP) or another nucleoside triphosphate is necessary for this transport process to occur, and mRNA transport increases linearly with increasing nucleoside triphosphate concentrations (*4*). Also, nucleoside triphosphatase activity and RNA transport have a similar activation energy and a similar Michaelis constant (K_m) for ATP (*3*). Furthermore, several compounds regulate both processes in a similar manner; for example, cyclic AMP increases mRNA transport and nuclear envelope nucleoside triphosphatase activity (*3*, *5*), whereas compounds such as sodium fluoride, colchicine, and proflavin inhibit both functions (*3*, *6*).

There are a number of reports that insulin increases mRNA levels in liver and other tissues (*7*). Schumm and Webb (*8*) observed that the direct addition of insulin to isolated rat liver nuclei

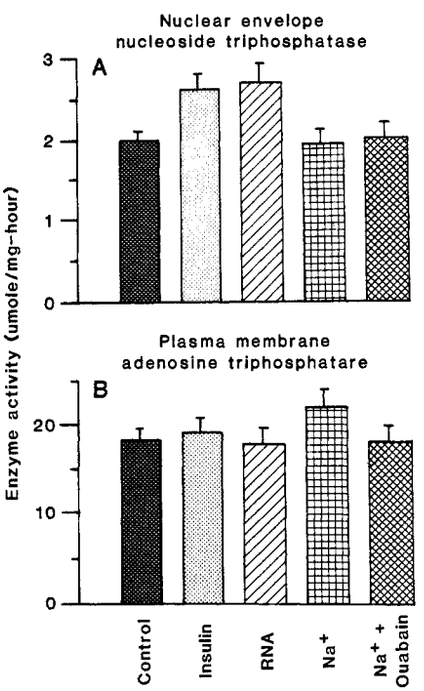

Fig. 1. Effects of insulin and other agents on the activity of (A) nuclear membrane nucleoside triphosphatase and (B) plasma membrane adenosine triphosphatase. Female Sprague-Dawley rats, 140 to 160 g, were deprived of food for 24 hours and injected through the tail vein with streptozotocin (Sigma) at 75 mg/kg. When the rats were studied 7 to 14 days later, the blood glucose concentrations were greater than 500 mg/dl. Purified liver nuclei were isolated (*20*), and nuclear envelopes were prepared from these nuclei as in (*21*), except that glycerol was omitted during the gradient centrifugation step. Purified plasma membranes were isolated (*22*), and nucleoside triphosphatase and adenosine triphosphatase activities were measured (*3*). In brief, either freshly prepared nuclear envelopes (0.25 to 0.5 mg of protein per milliliter) or plasma membranes (0.05 to 0.2 mg of protein per milliliter) were placed into buffer at 37°C containing 25 m*M* KCl, 5 m*M* $MgCl_2$, 50 m*M* tris-HCl (*p*H 7.5), and bovine serum albumin (0.1 g/dl). To this was added 10 p*M* insulin (Elanco), RNA (Millipore) at 200 μg/ml, 100 m*M* Na^+, or 100 μ*M* ouabain plus 100 m*M* Na^+. After a 10-minute incubation, the reaction was started with adenosine 5′-[γ-^{32}P]triphosphate (Amersham) added to a final concentration of 1 m*M*. The reaction was then stopped after 10 minutes with the addition of perchloric acid at 4°C; the intact ATP was adsorbed to charcoal; and the hydrolyzed ^{32}P was measured by liquid scintillation counting (*3*). Samples without membrane protein were included in each assay, and their values were subtracted from each result. Each value is the mean ± S.E. of three separate experiments.

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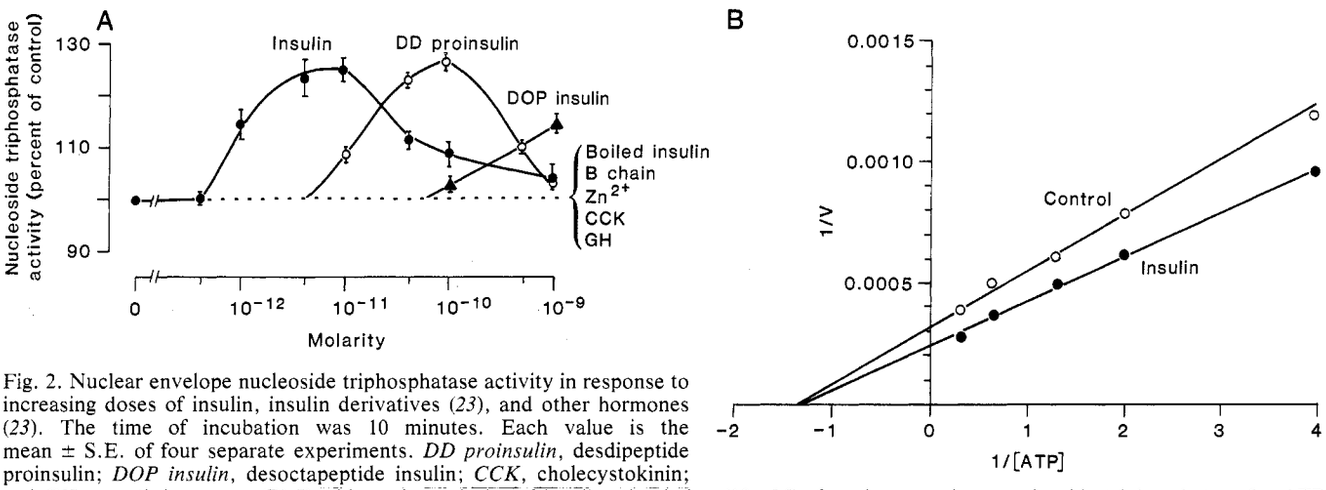

Fig. 2. Nuclear envelope nucleoside triphosphatase activity in response to increasing doses of insulin, insulin derivatives (*23*), and other hormones (*23*). The time of incubation was 10 minutes. Each value is the mean ± S.E. of four separate experiments. *DD proinsulin*, desdipeptide proinsulin; *DOP insulin*, desoctapeptide insulin; *CCK*, cholecystokinin; and *GH*, growth hormone. (B) Double reciprocal plot of insulin stimulation (10 p*M*) of nuclear envelope nucleoside triphosphatase for ATP concentrations of 0.25 to 3.0 m*M*. Time of incubation was 10 minutes. A representative of four experiments is shown. Each value is the mean of three determinations. *V* is the velocity of the reaction, measured as millimoles of ATP hydrolyzed per 10 minutes, and [*ATP*] is the concentration of ATP in millimoles per liter.

causes a significantly increased efflux of mRNA, but not of transfer RNA or ribosomal RNA. These data suggest that insulin might raise cellular mRNA levels by increasing mRNA transport from the nucleus. In view of the role of the nuclear envelope nucleoside triphosphatase in regulating mRNA transport, we studied the effect of the direct addition of insulin to purified rat liver nuclear envelopes.

Insulin at concentrations of 1 to 100 p*M* stimulated the nucleoside triphosphatase activity of nuclear envelopes from diabetic rats (Figs. 1 and 2A) (*9*). The magnitude of stimulation was similar to that seen with RNA, a known activator of this enzyme. In contrast, there was no stimulation of nucleoside triphosphatase activity when sodium was added, and thus no inhibition of this effect with ouabain (Fig. 1). For comparison, adenosine triphosphatase activity was also studied in purified rat liver plasma membranes. In this preparation, as expected, the addition of sodium enhanced adenosine triphosphatase activity, and this effect was inhibited by ouabain (Fig. 1). In contrast to their effects on nuclear envelopes, insulin and RNA did not stimulate plasma membrane adenosine triphosphatase activity (Fig. 1).

Nuclear membrane nucleoside triphosphatase activity in both the presence and absence of insulin was linear with time for up to 20 minutes (data not shown). A detectable effect of insulin was seen at 1 p*M*, with maximal stimulation occurring at 5 to 10 p*M* (Fig. 2A). At higher insulin concentrations, the increased enzyme activity decreased toward control levels. This biphasic effect is similar to that seen in other subcellular systems (*8, 10, 11*). In ten separate experiments with 10 p*M* insulin, enzyme activity was increased by 27.1 ± 1.1 percent (mean ± standard error). Two insulin analogs, desdipeptide proinsulin and desoctapeptide insulin, stimulated nucleoside triphosphatase activity with potencies approximately 10 percent and 1 percent of that of native insulin; these potencies are in agreement with the relative biological activities of these hormones (*12*). In contrast, boiled insulin, insulin B chain, Zn^{2+}, cholecystokinin, and growth hormone were without effect.

The effect of insulin on nucleoside triphosphatase was to influence the maximum velocity (V_{max}) of its activity without an appreciable effect on the apparent K_m (Fig. 2B). In four separate experiments, insulin raised the V_{max} by 29.5 ± 2.1 percent (mean ± S.E.).

Insulin increases mRNA concentrations in liver and other tissues (*7*), but how insulin produces this effect is unknown. One possibility is that insulin acts on nuclear and other functions by the generation of a second messenger. In support of this hypothesis, a low molecular weight substance has been isolated from insulin-stimulated perfused livers, which then increases the RNA polymerase activity of isolated nuclei (*13*). Furthermore, the direct addition of insulin to intact muscle and rat adipocytes and to hepatocyte plasma membranes can produce a similar type of low molecular weight substance, which then stimulates the activities of pyruvate dehydrogenase and glycogen synthase phosphoprotein phosphatase (*10*).

However, insulin may act directly on nuclear functions. In support of this hypothesis, insulin has been demonstrated to be internalized by various target cells (*14*). In electron microscopic autoradiographs of human cultured lymphocytes and of the liver of fasted rats, we have observed the concentrations of insulin grains over the nuclear envelope (*15*). Others, using immunofluorescence, have also observed the presence of insulin on the nuclear envelopes of fibroblasts and other cells (*16*). Furthermore, specific binding sites for insulin are present in highly purified nuclei (*17*), and these binding sites have been shown to be in the nuclear envelope (*18*). Presumably, after insulin is internalized, it is able to interact with intracellular organelles. Finally, the direct addition of insulin to isolated nuclei is reported to increase mRNA transport (*8*), and the present study demonstrates a direct effect of insulin on the nuclear envelope nucleoside triphosphatase, the enzyme that controls mRNA transport (*19*). The possibility exists, therefore, that insulin regulates the nuclear envelope to increase mRNA transport either directly or by the generation of a second messenger at the nuclear surface.

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Corticotropin-Releasing Factor Stimulates Accumulation of Adenosine 3′,5′-Monophosphate in Rat Pituitary Corticotrophs

Abstract. *The presence of synthetic ovine corticotropin-releasing factor leads to a rapid and marked stimulation of adenosine 3′,5′-monophosphate accumulation in an enriched population of rat pituitary corticotrophs in primary culture. The increase, observed as early as 60 seconds after the addition of corticotropin-releasing factor, suggests that changes in the intracellular concentration of the cyclic nucleotide coincide with or precede the secretion of adrenocorticotropic hormone in response to corticotropin-releasing factor.*

Vale *et al.* (*1*) recently elucidated the structure of an ovine peptide, corticotropin-releasing factor (CRF), with potent adrenocorticotropic hormone (ACTH)–releasing activity in vivo and in vitro in the rat. This knowledge has opened new avenues for studying the control of adrenocortical activity and for achieving a better understanding of the mechanisms controlling the pituitary response to stress. The first suggestion that adenosine 3′,5′-monophosphate (cyclic AMP) acts as an intracellular mediator of pituitary ACTH secretion was based on the observation that theophylline, an inhibitor of cyclic nucleotide phosphodiesterase, stimulates ACTH release in intact pituitary glands (*2*). In addition, cyclic AMP derivatives are powerful stimuli of ACTH secretion in intact pituitaries (*2*) and in cultured pituitary cells (*3, 4*). Although the observation of a stimulatory effect of theophylline and cyclic AMP derivatives on ACTH release indicated that the cyclic nucleotide has a role in the control of ACTH secretion, definitive proof of the role of the adenylate cyclase system had to be obtained by

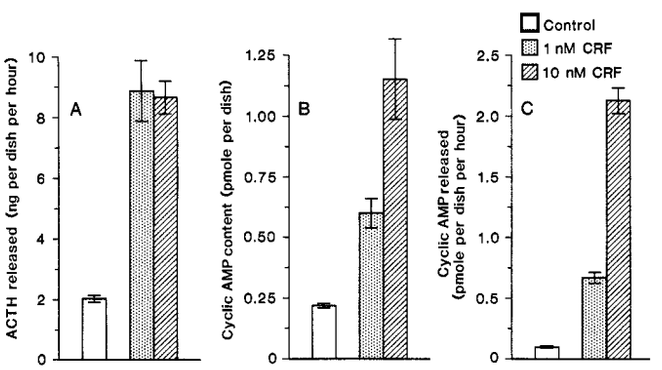

Fig. 1. Effect of synthetic ovine CRF on ACTH release and on cyclic AMP content and release in rat corticotrophs. ACTH and cyclic AMP were measured by specific radioimmunoassays (*4, 9*) after a 1-hour incubation period in the absence or presence of 1 or 10 n*M* CRF. Anterior pituitary cells were enzymatically dispered as described (*10*). Adult female Sprague-Dawley rats (Charles River CD strain) were obtained from Canadian Breeding Farms and ovariectomized. Two weeks later they were killed and their pituitary glands were removed. An enriched population of corticotrophs was prepared by centrifugation for 9 minutes at 65*g* through a continuous gradient of a 3 to 7.5 percent solution (weight to volume) of Ficoll (Pharmacia) in sterile Hepes buffer. The collected cells were plated in Eagle's minimum essential medium (Dulbecco's modification), containing 10 percent horse serum and 2.5 percent fetal calf serum, at a density of 2×10^5 cells per milliliter in Flow dishes with multiple wells. The cell fraction used contained 30 percent corticotrophs, 1 percent thyrotrophs, 50 percent mammotrophs, and 14 percent somatotrophs. This represents an approximately threefold enrichment of corticotrophs. Cell numbering was performed (*11*) after immunostaining for rat ACTH, luteinizing hormone, follicle-stimulating hormone, prolactin, thyrotropin, and growth hormone with antiserums provided by A. F. Parlow. After 4 days in culture, the cells were washed and incubated for the various time periods with the indicated concentrations of CRF prepared by solid-phase methods and purified by preparative reverse-phase high-performance liquid chromatography (HPLC). Homogeneity was determined by analytical HPLC on 300 Å C_{18} silica columns and by peptide mapping of enzymatic digests with HPLC (*12*). Radioimmunoassay data were analyzed with a program derived from that of Rodbard and Lewald (*13*). Statistical significance was measured using the multiple-range test of Duncan and Kramer (*14*). All assays were performed in duplicate on samples from triplicate dishes. Data are means ± standard errors.

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