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INFLUENCE OF HORMONES ON NADH-DEHYDROGENASE IN MOUSE LIVER PLASMA MEMBRANE

H. Goldenberg[+], F.L. Crane, D.J. Morré*

Department of Biological Sciences

and

Department of Medicinal Chemistry and Pharmacognosy*,

Purdue University

West Lafayette, Indiana 47907

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Summary

Highly purified mouse liver plasma membranes have been used to define the properties of an NADH dehydrogenase activity associated with plasma membrane. The NADH indophenol reductase activity is two-fold stimulated at 5×10^{-8} M glucagon and the stimulation is inhibited by atebrin. Corresponding activity in endoplasmic reticulum is not stimulated by glucagon. The NADH indophenol reductase is 90% inhibited by insulin at 7×10^{-11}M and shows a return to the original activity at higher insulin concentrations. NADH dehydrogenase activity in endoplasmic reticulum is inhibited up to 50% by insulin at a similar concentration. Triiodothyronine at 10^{-7}M also inhibits the plasma membrane dehydrogenase whereas thyroxine has little effect. The response of this dehydrogenase to hormones suggests a role in regulation of cellular function.

Introduction

The inhibition of basal and hormone-stimulated adenylate cyclase of plasma membranes of fat cells by NADH and the flavin antagonist atebrin [1] suggested the presence of an NADH-dependent redox system of the cell membrane which is hormone sensitive [2]. This redox system might influence the action of the hormone-stimulated cyclase, or alternatively, may in itself respond to hormones. A possible connection between redox function and hormone action might be confined to the plasma membrane, or demonstrable in other endomembranes possessing a similar redox system such as endoplasmic reticulum or Golgi apparatus. The redox enzymes of endoplasmic reticulum are well described for liver [3] but

+ Department of Medical Chemistry, University of Vienna, Austria.

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have been studied less in plasma membrane and Golgi apparatus.

Using four hormones with different metabolic effects, we have obtained evidence that a hormone-responsive redox function of cellular membranes may be of general functional significance. The hormones examined were glucagon, insulin, and two thyronines, 3,3'5 - triiodo-thyronine (T_3) and thyroxin (T_4). Each binds to liver plasma membrane [4,5,6], but has different effects on adenylate cyclase: glucagon stimulates [6,7], insulin inhibits [8,9], and triiodothyronine is without effect [10]. Triiodothyronine does stimulate Na^+ - K^+ - ATPase [11].

Livers of Swiss male mice were used. This organ yields a highly purified (97%) plasma membrane fraction, so the NADH-dehydrogenase activity measured is not complicated by contributions from contaminating mitochondria or endoplasmic reticulum. The composition and characteristics of the plasma membrane fraction as well as correlative biochemical and cytochemical evidence for localization of redox activities in mouse liver plasma membrane will be published separately (H. Goldenberg, F. L. Crane and D. J. Morré, in preparation).

Methods

The NAD(P)H-dehydrogenase activity was assayed as 2,6-dichlorophenol indophenol-reductase (Fig. 1) in the presence of 0.1 M KCl. The KCl enhances coupling between insulin and its receptor [12], and results in more reproducible hormone responses. Glucagon and insulin were dissolved in aqueous 0.3% bovine serum albumin; the latter has no effect on the reductase activity. T_3 and T_4 were dissolved in 0.1 M NaOH in 70% ethanol. The fractions were preincubated with hormone for 2 min before assay.

Results and Discussion

Glucagon stimulated the NADH-dichloroindophenol reductase in plasma membranes but not that of endoplasmic reticulum (Fig. 1). The stimulation of plasma membrane activity was inhibited by 3 mM atebrin. Maximum stimulation was observed at 5×10^{-8} M glucagon, and at higher concentrations the stimulation was reversed. In endoplasmic reticulum the NADH-dehydrogenase showed slight inhibition (25%) by glucagon.

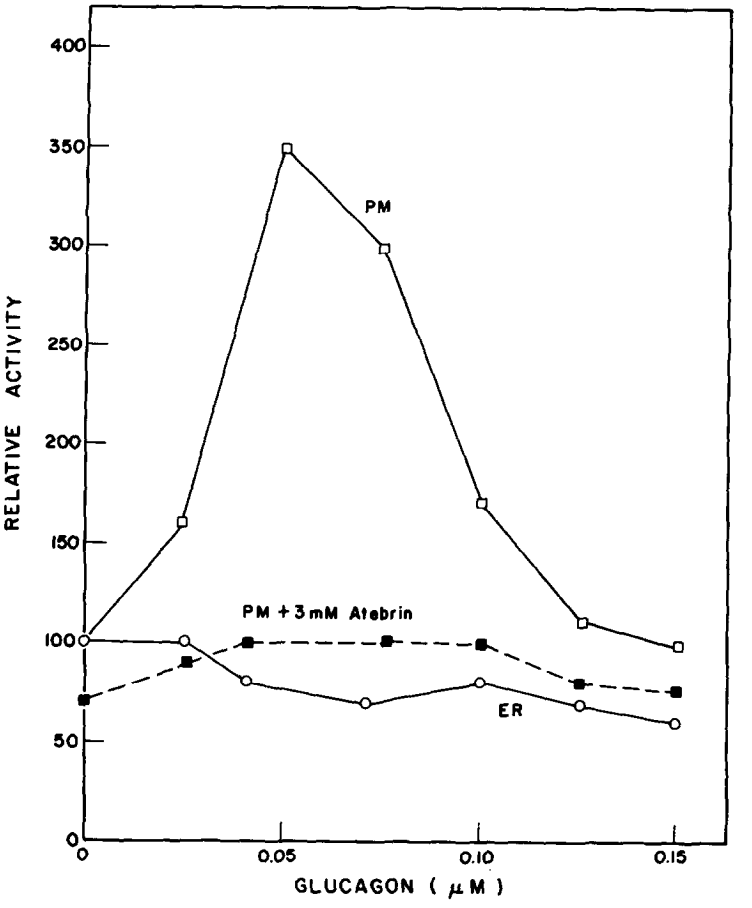

Figure 1: Effect of glucagon on NADH-dichlorophenolindophenol-reductase in plasma membranes and endoplasmic reticulum of mouse liver prepared as described (30). Assays were in a total volume of 3 ml at 37° C in the presence of 0.05 M potassium phosphate, pH 6.0, 0.1 M KCl, 20 μg 2, 6-DCIP, 20 μm NADH, 30-50 μg of fraction protein, and hormones as indicated. Change in absorbance at 600 nm was monitored using a Beckman Acta III spectrophotometer. - □ - □ - plasma membrane, -● - ● - plasma membrane in presence of 3mM atebrin, - O - O - endoplasmic reticulum. Basic (100%) activities were as follows in nanomoles DCIP reduced per minute per mg protein: Plasma membrane NADH-dehydrogenase 40, NADPH dehydrogenase 3, endoplasmic reticulum NADH-dehydrogenase 400, NADPH-dehydrogenase 80.

The NADH reductase of plasma membrane was strongly inhibited by insulin at physiological concentrations (90% at 8 μU/ml) and slightly stimulated at concentrations below 6 μU/ml (Fig. 2). Insulin deactivated by incubation in 0.1 N NaOH at 37° for 30 min (13) was ineffective, and a 600 times higher concentration of proinsulin is needed for 60% inhibition. Both the NADH-and NADPH-reductases

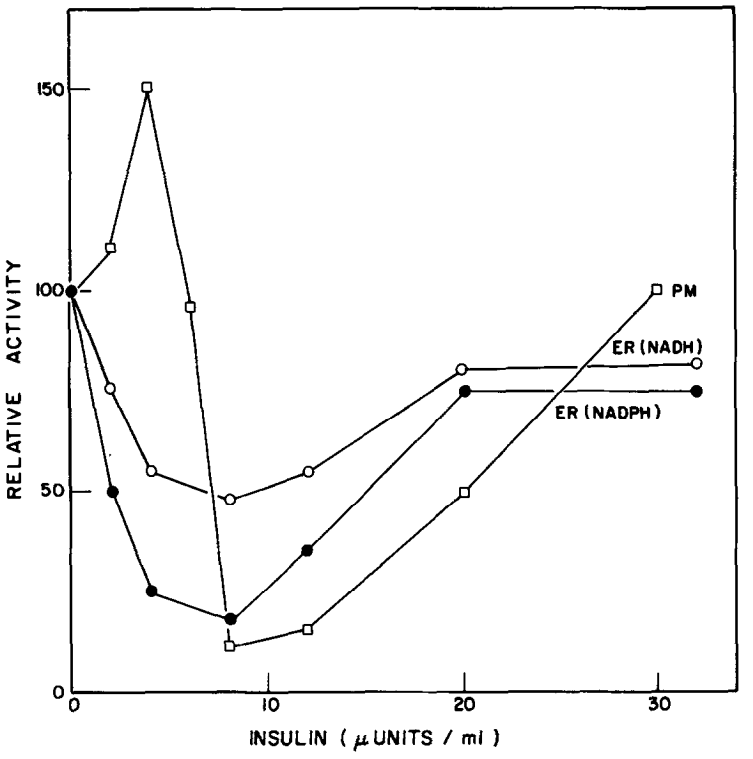

Figure 2: Effect of insulin on NADH-DCIP reductase in plasma membranes, and NADH- and NADPH-DCIP reductase in endoplasmic reticulum. Assays were as described for Figure 1. - □ - □ - plasma membrane, -0 - 0 - endoplasmic reticulum NADH-dehydrogenase, - ● - ● - endoplasmic reticulum NADPH-dehydrogenase.

of endoplasmic reticulum were inhibited by insulin but not to the same extent as those of plasma membrane (Fig. 2).

Insulin at concentrations similar to those used here has been found by some workers to be an adenylate cyclase inhibitor with plasma membranes [8,9]. Mukherjee and Lynn [15], have proposed a mechanism for this inhibition, which involves NADPH-oxidase stimulation and subsequent hydrogen peroxide production in fat cell membranes. We have found NADH-oxidase in liver plasma membrane, but this activity was not stimulated by insulin. That insulin and glucagon affect both the NADH dehydrogenase and adenylate cyclase equally and in the same direction (inhibition by insulin; stimulation by glucagon) suggests that the two actions are related. Glucagon stimulation and insulin inhibition of the NADH-dehydrogenase

are additive in our system. Reversals of hormone effects at higher concentrations have not been observed with adenylate cyclase [7]. Thus the redox and cyclase systems may not be obligatorily coupled.

The significance of the inhibition of the reductase of endoplasmic reticulum by insulin is problematic. Insulin binding to endoplasmic reticulum of rat liver has been described [14], but there is little, if any, binding of insulin to endoplasmic reticulum of mouse liver (E. Schilling, H. Goldenberg, D. J. Morré and F. L. Crane, unpublished). NADPH-dehydrogenase activity in plasma membranes was too low to be evaluated for hormonal effects.

Effects of thyronines on redox enzymes are well known, notably stimulation of α-glycerolphosphate-dehydrogenase[16] and inhibition of DT-diaphorase[17]. For these effects, there are no differences between T_3 (3,3',5-triiodothyronine) and T_4 (thyroxin). Yet T_3 is much more effective than T_4 in the stimulation of amino acid transport [18] and also binds more effectively to plasma membranes [6]. Figure 3 shows that T_3 is also more effective in inhibiting plasma membrane NADH-dehydrogenase than is T_4. The concentrations required suggest involvement of the low affinity receptor [6]. T_3 is without effect on endoplasmic reticulum (data not shown).

An influence of the redox state of the plasma membrane on membrane transport has been evidenced from previous studies [19-22]. Influences of redox state of the cell on cyclic AMP levels have been described for liver [23-25] and transformed cells [26-28]. Thus alterations of membrane redox components by hormones like insulin or T_3 might affect transport, as well as exert an effect on adenylate cyclase. The latter effect, however, seems not to be obligatorily linked to the redox system for either plasma membrane or endoplasmic reticulum. An adenylate cyclase activity of endoplasmic reticulum is stimulated by glucagon[29] whereas the redox system is slightly inhibited. The findings may provide the basis for a new mechanism whereby effects of hormones on the cell membrane are modulated to exert transmembrane control over cytoplasmic activities.

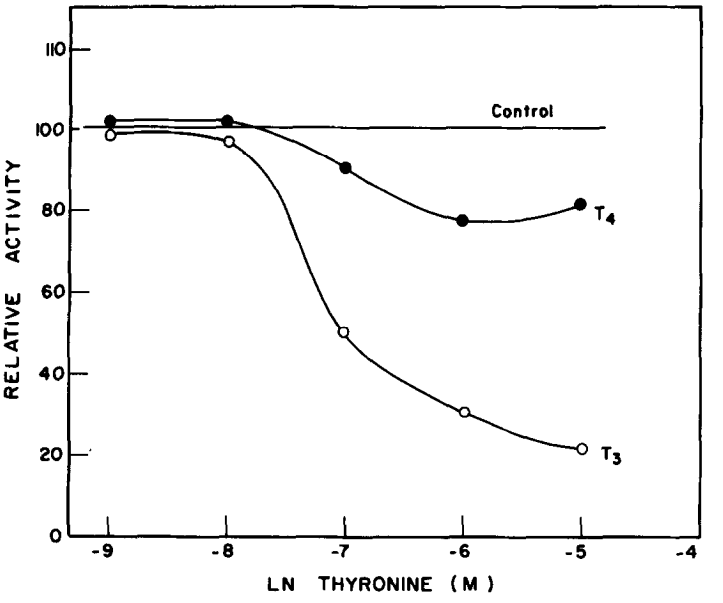

Figure 3: Effect of thyronines on NADH-DCIP- reductase in plasma membranes. Assays were as described for Figure 1. - O - O - T_3, - ● - ● - T_4.

Acknowledgements

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