HORMONE CONTROL OF PLASMA MEMBRANE OXIDATION–REDUCTION REACTIONS

Effects of insulin

H. LÖW, F. L. CRANE, C. GREBING, M. TALLY and K. HALL
Department of Endocrinology and Metabolism, Karolinska Hospital, Stockholm, Sweden

Received 11 May 1978

1. Introduction

We have presented evidence for an NADH-oxidizing system in liver and fat cell plasma membranes [1] which can be stimulated by adenylcyclase-activating catabolic hormones [2]. It has further been proposed that this redox system may act as a regulator of adenylate cyclase stimulation by hormones, thus making the response to hormones sensitive to redox levels in the cell [2,3]. An NADH oxidase system with similar properties has also been proposed to function as an energy source for transport of neutral amino acids [4].

The relation between anabolic hormones such as insulin and adenylate cyclase is unclear. There is considerable evidence that insulin will suppress the glucagon- or epinephrine-induced increase of cyclic AMP in whole cells [5–8] and there are two reports that insulin can inhibit catabolic hormone stimulation of adenylate cyclase in liver or fat cell plasma membranes [9,10], but these latter studies have not received extensive support.

2. Methods

Rat liver and adipocyte plasma membranes were prepared as in [1] except for minor modifications to increase the purity of the rat liver preparation. NADH indophenol reductase and cytochrome *c* reductase were determined as in [1] except that 2,3′,6-trichloroindophenol was used instead of 2,6-dichloroindophenol.

3. Results

NADH indophenol reductase is inhibited by low concentrations of insulin in both rat liver and fat cell membranes. In rat liver membranes, at pH 6.0, the inhibition increases with increasing insulin concentration up to 35–50 μU/ml. There is less inhibition at higher insulin concentration and at about 150 μU/ml the inhibition may no longer be observed (fig.1). At pH 6.0, with very low insulin concentrations (about 5 μU/ml) there may be a slight stimulation of the activity. The inhibition which we consistantly observe in the rat liver membranes is in the range of 25–35%. Inhibition is observed in the range, from pH 6.0–8.0. The inhibition can be observed if enzyme is added to the reaction mixture last, but the best inhibition can be obtained by preincubation of membrane and insulin for 1 or 2 min before adding the NADH and indophenol to start the reaction.

Similar low concentrations of insulin also inhibit the NADH indophenol reductase in fat cell plasma membranes. Maximum inhibition is achieved at concentrations of 30–50 μU/ml similar to liver plasma membranes. The extent of inhibition is also similar (fig.2). In fat cell membranes we have not observed a return of activity with higher insulin concentration as is seen with liver membranes at pH 6.0. A return may be seen at pH 7.5.

Proinsulin at concentrations equal to inhibitory concentration insulin does not inhibit NADH indophenol reductase in liver cell membranes, but shows a 15% inhibition in fat cell membranes. Somatomedin A, which induces responses similar to insulin

Elsevier/North-Holland Biomedical Press

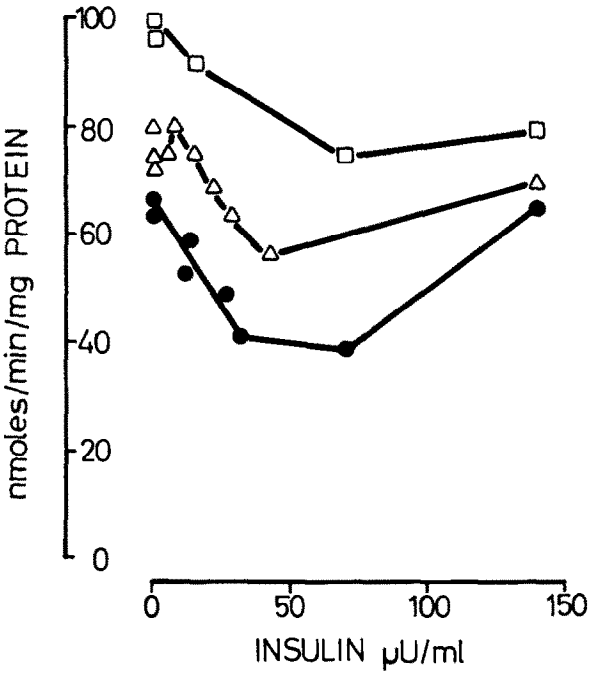

Fig.1. Insulin inhibition of NADH-indophenol reductase in rat liver membranes. At pH 6.0 and 7.0, the incubation medium contained 0.05 M potassium phosphate and 0.1 M KCl. At pH 7.5, the incubation medium contained 0.05 M Tris–HCl and 0.1 M KCl. The cuvette contained 15 μg trichloroindophenol and 30 μg NADH in final vol. 1 ml. Incubation temp, 30°C; absorbance, recorded at 600 nm.

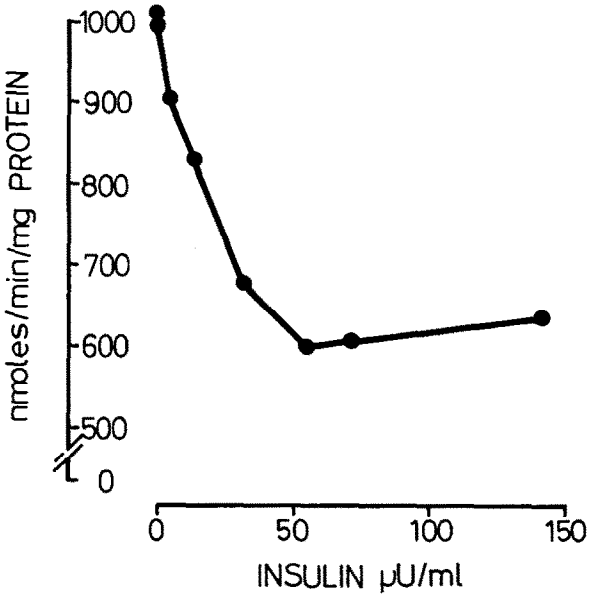

Fig.2. Insulin inhibition of NADH-TCPIP reductase in fat cell plasma membranes. Incubation was at pH 6.0. The incubation medium contained 0.05 M potassium phosphate and 0.1 M KCl. Otherwise the conditions were as in fig.1.

in other systems [8,11], shows an inhibition of indophenol reductase to similar degree as insulin at slightly higher concentration in fat cell membranes (table 1).

Mitochondrial and endoplasmic reticulum fractions from rat liver have NADH indophenol reductase activity which is inhibited only 7% and 10%, respectively, by insulin at concentrations which give maximum inhibition of plasma membrane at pH 7.5. Since these fractions contain only 8% and 4% plasma membrane on the basis of relative adenyl cyclase activity it remains to be established whether insulin can also cause a partial inhibition of the NADH dehydrogenase in these other membranes. Atebrin has been shown to inhibit the plasma membrane NADH indophenol reductase 50% at 3×10^{-3} M. The residual activity in presence of atebrin is not inhibited by insulin.

Liver plasma membranes have a very slow NADH oxidase activity which is cyanide insensitive and is stimulated by triiodothyronine, azide and fluoride. This activity is stimulated by insulin in the same concentration range which inhibits the NADH indophenol reductase activity.

Table 1
Comparison of insulin and related peptides effect on plasma membrane NADH indophenol reductase

	Maximum inhibition	
	Conc. (M)	Inhib. (%)
Fat cell membranes		
Insulin	3×10^{-10}	40
Proinsulin	5×10^{-10}	15
Somatomedin A	5×10^{-10}	37
Liver cell membranes		
Insulin	3×10^{-10}	25
Proinsulin	5×10^{-10}	0

Experimental conditions as in fig.2

4. Discussion

In our proposal [2,3] it was suggested that the plasma membrane NADH dehydrogenase acted as a sensor for the internal redox state of the cell and controlled the ability of hormones through their receptors to stimulate adenylate cyclase activity. In support of this it was shown that NADH inhibits adenylate cyclase activity in isolated membranes and cAMP is inversly correlated to NADH levels in whole cells [12]. Furthermore atebrin which inhibits the plasma membrane dehydrogenase inhibits cyclase at the same concentrations [13] and glucagon and ACTH stimulate both the dehydrogenase and cyclase in liver and fat cell membranes, respectively. We now find that insulin inhibits the dehydrogenase in isolated plasma membranes. This observation correlates very well with studies [9] showing insulin inhibition of hormone stimulated cyclase and the concentration response is strikingly similar.

References

[1] Crane, F. L. and Löw, H. (1976) FEBS Lett. 68, 153.
[2] Löw, H. and Crane, F. L. (1976) FEBS Lett. 68, 157.
[3] Löw, H. and Crane, F. L. (1978) Biochim. Biophys. Acta, in press.
[4] Garcia-Sancho, J., Sanchez, A., Handlogsen, M. E. and Christensen, H. N. (1977) Proc. Natl. Acad. Sci. USA 74, 1488.
[5] Jeffersson, L. S., Exton, J. H., Butcher, R. W., Sutherland, E. W. and Park, C. R. J. (1968) J. Biol. Chem. 243, 1031.
[6] Butcher, R. W., Baird, C. E. and Sutherland, E. W. (1968) J. Biol. Chem. 243, 1705.
[7] Kuo, J. F. and DeRenzo, E. C. (1969) J. Biol. Chem. 244, 2252.
[8] Zedermann, R., Grebing, C., Hall, K. and Löw, H. (1978) in preparation.
[9] Hepp, K. D. and Reuner, R. (1972) FEBS Lett. 20, 191.
[10] Illiano, G. and Cuatrecasas, P. (1972) Science 72, 906.
[11] Werner, S., Hall, K. and Löw, H. (1974) Hormon. Metab. Res. 6, 319.
[12] Zedermann, R., Löw, H. and Hall, K. (1977) FEBS Lett. 75, 291.
[13] Löw, H. and Werner, S. (1976) FEBS Lett. 65, 96.