HORMONE REGULATED REDOX FUNCTION IN PLASMA MEMBRANES

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1. Introduction

It has been shown that NADH can inhibit adenylate cyclase activity in fat cell plasma membranes [1] and NADH dehydrogenase has been shown to be present in plasma membranes [2]. We now find that the NADH dehydrogenase activity in the plasma membranes is influenced by hormones which stimulate the cyclase activity and that the hormone effects on the NADH dehydrogenase and the cyclase are found at similar levels of hormone concentration. This observation supports the idea that a redox center in the plasma membrane controls the response of the adenylate cyclase to hormones and that NADH dehydrogenase can act as a sensor for the redox state of the cell.

2. Methods

Plasma membranes were isolated from rat liver and epididymal fat pads by procedures which have been described [2]. Dehydrogenase activity was determined spectrophotometrically in 0.9–1.0 ml of 0.1 M buffer at pH 6,7 and 8. Buffers included potassium phosphate or Hepes. Buffer, enzyme (2–20 μg) and hormones were preincubated for 2 min in the cuvette before starting the reaction. For cytochrome c reductase activity 100 μg NADH, 0.5 mg cytochrome *c* and 1×10^{-3} M KCN (if necessary) were added and absorbancy recorded at 550 nm. NADH indophenol reductase was determined with 30 μg of NADH and 15 μg of 2.6 dichloro-indophenol added to the incubated mixture to start the reaction. Absorbancy was recorded at 600 nm. Below pH 7.0 a control with no enzyme is required to correct for non-enzymatic reduction of indophenol. For NADH glyoxylate reductase 80 μg of NADH and 200 μg of glyoxylate were added and absorbancy was recorded at 340 nm. Millimolar extinction coefficients used were 19.0 for cytochrome *c*, 20.0 for indophenol and 6.22 for NADH. The cytochrome *c* reductase activity is insensitive to antimycin A, rotenone and amytal. With many liver plasma membrane preparations it is not necessary to add cyanide to inhibit cytochrome oxidase. Cyanide is usually required for the fat cell membrane assay.

3. Results

The effect of glucagon has been studied using three assays for NADH dehydrogenase in liver cell plasma membrane. These assays are NADH cytochrome *c* reductase (atebrin sensitive), NADH indophenol reductase and NADH glyoxylate reductase. In all three reactions we find glucagon stimulation in the range of 0.2–2.0 μg glucagon per ml. Maximum stimulation of the dehydrogenase activity occurs at pH 6.0 but the effect is still seen at pH 8.0. The response on a per cent basis is largest in the cytochrome *c* reductase reaction and lowest in the glyoxylate reductase reaction. The absolute increase is similar for each activity. ACTH which has no effect on the adenylate cyclase activity in liver plasma membranes also shows no effect on the NADH dehydrogenase activity (fig.1). On the other hand when tested on fat cell membranes, ACTH stimulated the corresponding dehydrogenase activity almost 100% with a 70% stimulation at 3×10^{-8} M ACTH (fig.2).

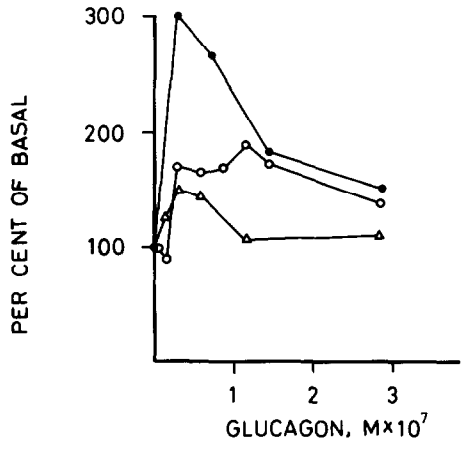

Fig.1. Effect of glucagon on the NADH dehydrogenase activity in liver plasma membranes under various conditions. Filled circles represent NADH-cytochrome *c* reductase activity in 0.1 M potassium phosphate pH 6.0. 100% corresponds to 65 nmol/min/mg protein. Open circles represent NADH-indophenol reductase in sodium Hepes buffer, pH 7.0. Specific activity for 100% 50 nmol/min/mg protein. Open triangles represent NADH-glyoxylate reductase in sodium HEPES buffer at pH 7.0 specific activity at 100% was 350 nmol/min/mg protein.

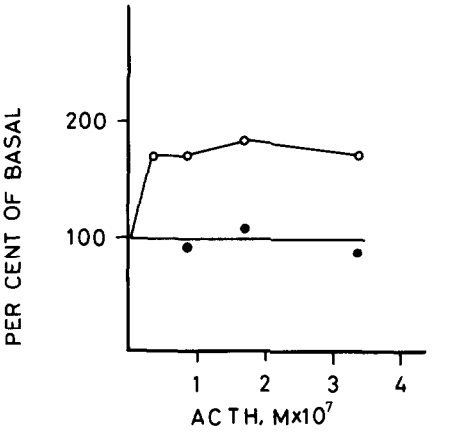

Fig.2. Effect of ACTH on NADH-indophenol reductase in plasma membranes from liver and fat. Filled circles represent liver plasma membranes. 100% corresponds to a specific activity of 80 nmol/min/mg protein. Open circles represent the fat plasma membrane. The specific activity was 400 nmol/min/mg protein. pH of the incubation media were 6.0 in 0.1 M potassium phosphate buffer.

As we have reported elsewhere [2] azide 0.1 M has a strong inhibitory effect on the NADH cytochrome *c* reductase and NADH glyoxylate reductase in both fat and liver cell plasma membranes. Fluoride 0.01 M also inhibits the indophenol and glyoxylate reductase activity of liver cell membranes at pH 7.0 up to 20%.

The NADH cytochrome *c* reductase in the plasma membrane preparations used was less than 10% inhibited by antimycin A (2.5 μg), rotenone (5 μg) or amytal (200 μg) when assayed in 0.1 M potassium phosphate buffer at pH 7.2 so it is evident that there is very little contribution to the activity from inner mitochondrial membrane. The NADH cytochrome *c* reductase in the plasma membrane fractions differs from microsomal activity in that the plasma membrane activity is inhibited by atebrin and azide which do not inhibit microsomal activity. Atebrin, 3×10^{-3} M, gives 20% inhibition and 0.1 M azide gives 50% inhibition at pH 7.0. The increase of dehydrogenase activity with glucagon in liver plasma membranes is 100% inhibited by 3×10^{-3} M atebrin. It should also be noted that glucagon did not stimulate the NADH cytochrome *c* reductase in rat liver microsomes under conditions for stimulation in plasma membrane.

4. Discussion

Glucagon affects on adenylate cyclase of isolated liver cell plasma membranes have been reported starting at 10^{-9} M with ½ maximum effects at 10^{-8} M [3]. The concentration of glucagon required for stimulation of the NADH dehydrogenase activity at pH 7.0 is in the same range (3×10^{-8} M or less).

ACTH is a hormone which has consistant stimulatory effects on the adenylate cyclase of fat cell plasma membranes. This hormone also induces up to 70% stimulation of the NADH indophenol reductase of the fat cell membranes at a concentration 3×10^{-8} M which will also cause a stimulation of adenylate cyclase activity. The very slight effects of ACTH on liver cell plasma membrane dehydrogenase activity is consistant with the small effect of ACTH on the cyclase activity of these membranes.

Fluoride (0.01 M) and azide 0.1 M are also effective stimulators of adenylate cyclase in liver and fat cell plasma membranes. Since they give either a weak or

strong inhibition of the dehydrogenase respectively it would appear that any connection between the cyclase and the dehydrogenase contains more than one component or that the inorganic ions have a different site of action than the hormones [4,5].

The basis for the similar effects of hormone on cyclase and dehydrogenase is of course not clear but the coincidence of the effects is so great that a connection is suggested. This connection would also be consistant with the fact that NADH can inhibit the cyclase [1]. The mechanism could be through a conformational change induced by the redox change in the proteins or in a region of the membrane as for example in the conformational changes found in mitochondrial membrane with redox change [6]. It is also possible to consider that the oxidation of the NADH through a membrane bound flavin and non-heme iron or cytochrome could produce a proton gradient which could influence cyclase activity and the hormones could stimulate by releasing this gradient through the cyclase.

The nature of natural redox acceptors for the NADH dehydrogenase system is unknown and it is unlikely that cytochrome *c* is involved. The fact that glyoxylate shows excellent activity with liver cell membranes and since the glyoxylate reductase is also present in the fat cell membranes and erythrocyte membranes [7] glyoxylate is one possible natural acceptor. The plasma membrane NADH dehydrogenase also can use oxygen as an acceptor at a slow rate (20 nmol/min/mg) in both liver and fat cells which provides a second possible acceptor. Oxidized ascorbate, thiols and fatty acid derivatives are other possibilities.

Acknowledgements

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