Hormone Effects on NADH-Oxidizing Enzymes of Plasma Membranes of Rat Liver

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Abstract

Hormone binding influences both oxidase activity and adenylate cyclase activity in the plasma membrane of rat liver. The findings suggest a coupling between redox function and adenylate cyclase. Furthermore, since NADH inhibits adenylate cyclase of PM, whereas NAD has no effect, the NADH dehydrogenase may act as a sensor for the oxidation-reduction state of the cell.

Introduction

Polypeptide hormones, growth hormones, and catecholamines bind with receptors localized on the outer surface of the plasma membrane. This binding of hormones has been correlated with the activation in adenylate cyclase of the inner membrane surface. Recently, Crane and Low have shown that a distinct NADH dehydrogenase exists in plasma membranes (2) and that it, too, is stimulated by hormones (4). The NADH dehydrogenase activity in the plasma membranes is influenced by glucagon, ACTH, fluoride, atebrin, and azide at concentrations which stimulate or inhibit the activity of adenylate cyclase (4).

The catecholamines (epinephrine, norepinephrine, isoproterenol) and azide activate adenylate cyclase activity (9,10) whereas the flavin antagonist, atebrin, inhibits adenylate cyclase activity (5). In this report, these known stimulators or inhibitors of the adenylate cyclase activity were tested for their effect on the oxidation of NADH with oxygen or juglone as electron acceptors. Membrane sources were plasma membrane from rat liver (PM), milk fat globular membranes (MFGM), smooth endoplasmic reticulum (SER), rough endoplasmic reticulum (RER), Golgi apparatus (GA), and mitochondria (MITO). Only in PM, did the effects of the stimulators and the inhibitor on NADH dehydrogenase correlate with their effects on adenylate cyclase. The other membrane preparations showed differences from PM with respect to stimulation or inhibition.

Materials and Methods

Membranes were prepared from rat liver as described: PM (11), GA (7), SER(7), RER(7), and MITO (7). MFGM (6) were prepared from cows milk.

Both NADH oxidase and NADH juglone reductase assays were based on oxygen uptake at 37°C monitored with an oxygen electrode. The reaction mixture contained in a final volume of 1.5 ml, membrane preparation, sodium phosphate buffer (0.03 M; pH 7), and an appropriate amount of test substance. Incubations were for 3 min. NADH (260 μg), with or without juglone (60 μg), was added to measure NADH oxidase or NADH juglone reductase, respectively.

Isoproterenol, epinephrine, 3,5,3′-triidothyronine, propranolol, NADH and juglone were from Sigma Chemical Company. Norepinephrine and atebrin were from Aldrich Chemical Company.

Results

Atebrin (10^{-3}M) is a flavin antagonist that inhibits adenylate cyclase activity (3). When NADH oxidase activity in various membrane preparations was measured as a function of atebrin concentration (Fig. 1), NADH oxidase activity was inhibited in PM and MFGM. No inhibition of NADH oxidase was observed with GA, SER, or MITO. With GA, NADH oxidase was stimulated. Similar results were obtained with atebrin when NADH juglone reductase was measured (Fig. 2). Azide stimulated both NADH oxidase and NADH juglone reductase activity in PM (Fig. 3 and Fig. 4). Again the effect of azide on NADH oxidase and NADH juglone activity in the other membrane preparations was insignificant, except for MFGM. MFGM was inhibited by azide which may be due to higher amounts of xanthine oxidase contained in this type of plasma membrane (1).

A selected hormone, 3,5,3′-triiodothyronine (T_3) was tested for stimulation of NADH dehydrogenase with MFGM, PM, GA, RER, and MITO.

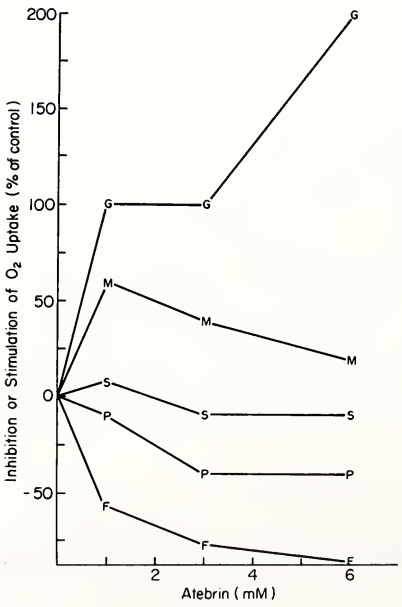

FIGURE 1. *The inhibition or stimulation of NADH oxidase activity with various membrane preparations when treated with atebrin. Control activity of membranes (nmoles/min/mg protein): P-plasma membrane, 14; F-milk fat globular membrane, 24; M-mitochondria, 13; G-Golgi apparatus, 10; S-smooth endoplasmic reticulum, 59.*

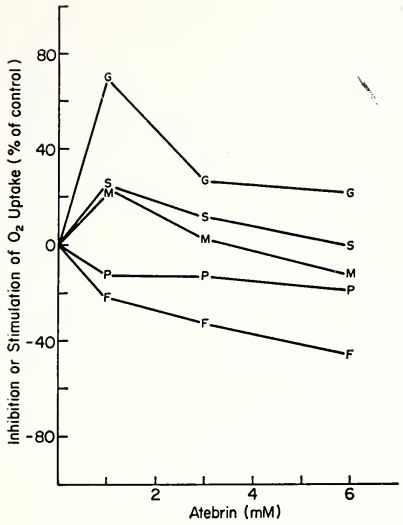

FIGURE 2. *The inhibition or stimulation of NADH juglone reductase activity with various membrane preparations when treated with atebrin. Control activity of membranes, (nmoles/min/mg protein): P-plasma membrane, 37; F-milk fat globular membrane, 290; M-mitochondria, 290; G-Golgi apparatus, 132; S-smooth endoplasmic reticulum, 720.*

NADH oxidase of T_3 (10^{-6}M)-treated PM was stimulated 250%, while that of T_3 (10^{-6}M)-treated MFGM was stimulated 80%. In contrast, no stimulation of NADH oxidase was measured in T_3-treated GA, MITO, or RER.

The β-adrenergic catecholamines stimulate the enzyme adenylate cyclase in virtually all tissue (10). The catecholamine, isoproterenol, (10^{-5}M) also stimulated NADH oxidase in PM and MFGM (Fig. 5). Isoproterenol gave successively less stimulation of oxidase with other membranes in the order GA, SER, RER and MITO. Furthermore, the

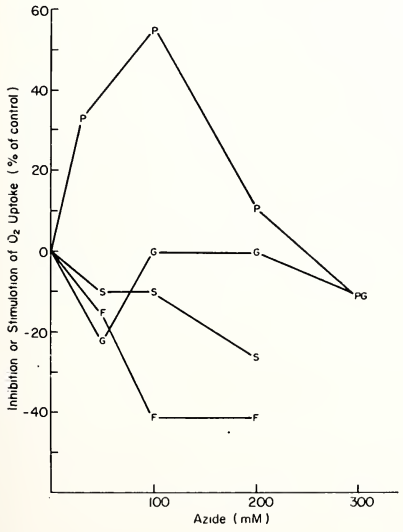

FIGURE 3. *The inhibition or stimulation of NADH oxidase activity with various membrane preparations when treated with azide. Control activity of membranes (nmoles/min/mg protein): P-plasma membrane, 25; F-milk fat globular membrane, 32; G-Golgi apparatus, 18; S-smooth endoplasmic reticulum, 38.*

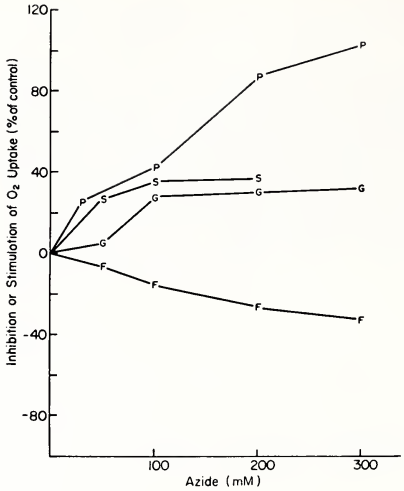

FIGURE 4. *The inhibition or stimulation of NADH judglone reductase activity with various membrane preparations when treated with azide. Control activity of membranes (nmoles/min/mg protein): P-plasma membrane, 390; F-milk fat globular membrane, 275; G-Golgi apparatus, 175; S-smooth endoplasmic reticulum, 1075.*

isoproterenol stimulating effect on PM and MFGM was inhibited by propranolol (10^{-3}M), a β-adrenergic antagonist.

The effect of two other β-adrenergic catecholamine agonists on NADH oxidase activity was measured with MFGM (Table 1). Epinephrine stimulated NADH oxidase 130%, whereas, norepinephrine stimulated NADH oxidase only 50%. Murad et al. (8) found stimulation of adenylate cyclase by the catecholamines in broken cell preparations.

The effect of an α-adrenergic agonist, phenylephrine on NADH oxidase was also tested with MFGM (Table 1). No inhibition or stimulation on NADH oxidase was found upon treatment with phenylephrine (10^{-6}M) or ephedrine, (10^{-6}M).

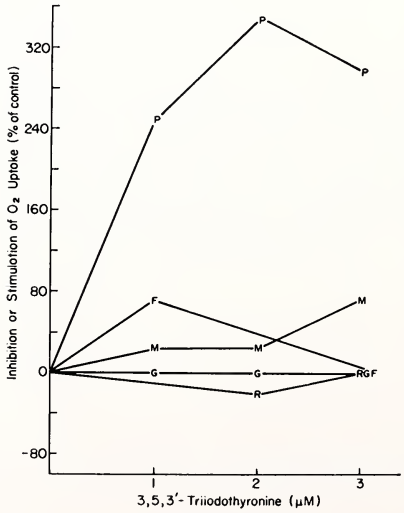

FIGURE 5. *The inhibition or stimulation of NADH oxidase activity with various membrane preparations when treated with 3,5, 3'-triiodothyronine. Control activity of membranes (nmoles/min/mg protein): P-plasma membrane, 16; F-milk fat globular membrane, 24; M-mitochondria, 19; G-Golgi apparatus, 18; R-rough endoplasmic reticulum, 33.*

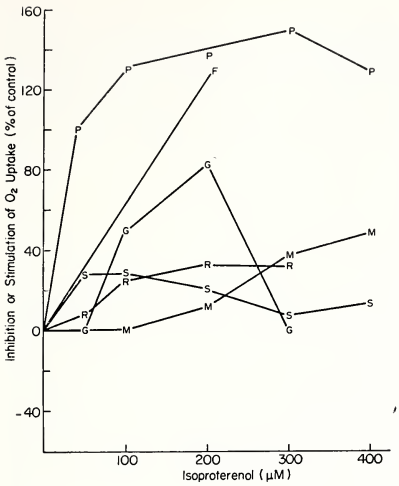

FIGURE 6. *The inhibition or stimulation of NADH oxidase activity with various membrane preparation when treated with isoproterenol. Control activity of membranes (nmolcs/min/mg protein): P-plasma membrane, 22; F-milk fat globular membrane, 23; M-mitochondria, 19; G-Golgi apparatus, 18; S-smooth endoplasmic reticulum, 96; R-rough endoplasmic reticulum, 40.*

Studies on NADH dehydrogenase by Crane and Low showed effects of atebrin, azide, and T_3 on cytochrome C reductase activity and indophenol reductase activity (2). Atebrin inhibits cytochrome C reductase activity and indophenol reductase activity in PM, so it inhibits all NADH dehydrogenase activity. Azide and T_3, however, differ in their effects depending on the redox acceptor used. NADH cytochrome C reductase activity and NADH indophenol reductase activity are both inhibited whereas the NADH oxidase is stimulated by both T_3 and azide and NADH juglone reductase activity is stimulated by azide and not affected by T_3. Thus, PM has been shown to contain a distinct NADH dehydrogenase (2). It appears now that a connection between NADH dehydrogenase of PM and adenylate cyclase activity exists as well. A redox function in the coupling of hormone binding with the activation of adenylate cyclase is suggested. Since NADH inhibits adenylate cyclase of PM, whereas NAD has no effect (5), the NADH dehydrogenase may act as a sensor for the oxidation-reduction state of the cell and thereby function as part of a more complicated homeostatic control mechanism.

TABLE 1. *Stimulation or inhibition of NADH oxidase activity with adrenergic agonists on MFGM*

Adrenergic agonist	Stimulation (% of control)
isoproterenol	+150
epinephrine	+130
norepinephrine	+ 50
phenylephrine	0
ephedrin	0

Control activity of MFGM is 23 nmoles/min/mg.

Acknowledgments

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