Vol. 93, No. 3, 1980 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
April 14, 1980 Pages 746-754

ADRIAMYCIN AFFECTS PLASMA MEMBRANE REDOX FUNCTIONS

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Received February 20,1980

Summary

Adriamycin (Doxorubicin) stimulates NADH oxidase activity in liver plasma membrane, but does not cause NADH oxidase activity to appear where it is not initially present, as in erythrocyte membrane. NADH dehydrogenase from rat liver and erythrocyte plasma membranes shows similar adriamycin effects with other electron acceptors. Both NADH ferricyanide reductase and vanadate-stimulated NADH oxidation are inhibited by adriamycin, as is a cyanide insensitive ascorbate oxidase activity, whereas NADH cytochrome c reductase is not affected. The effects may contribute to the growth inhibitory (control) and/or deleterious effects of adriamycin. It is clear that adriamycin effects on the plasma membrane dehydrogenase involve more than a simple catalysis of superoxide formation.

Introduction

Plasma membranes from a variety of cells contain redox systems that oxidize NADH (1,2,3), xanthine (4,5) NADPH (6) or ascorbic acid. The NADH dehydrogenases of plasma membrane are unique in that they are stimulated by catabolic hormones, such as glucagon (7), but are inhibited by anabolic hormones, such as insulin (8,9). Since the hormone - controlled adenylate cyclase (10) and the cyclic AMP phosphodiesterase (11) from the plasma membrane are inhibited by NADH, a control mechanism has been proposed whereby,the response to hormones can be regulated by the redox state of the cell (12). NADH oxidation by the plasma membrane may also supply energy for metabolite transport (13), function in changing the fluidity of

0006-291X/80/070746-09$01.00/0

the plasma membrane by catalyzing fatty acid desaturation (14) or exert other roles in energizing membrane flux or stimulus-responses of the plasma membrane (15).

We now find that adriamycin affects the plasma membrane enzyme oxidizing both NADH and ascorbic acid. The stimulations and/or inhibitions by adriamycin should be considered in any interpretation of adriamycin effects on growth of tumors or in relation to undesirable side effects,which limit its therapeutic value.

Materials and Methods

Plasma membranes were prepared from rat (16) or mouse liver (2), and human or pig erythrocytes (17), as described. Contamination by mitochondria, endoplasmic reticulum or outer mitochondrial membrane was monitored with succinate cytochrome c reductase (12), NADPH cytochrome c reductase (12) and kynurenine hydroxylase (18). NADH oxidase activity was assayed spectrophotometrically (12) or with an oxygen electrode. Vanadate stimulation of NADH oxidase was measured as for NADH oxidase with 3×10^{-4} M vanadate (expressed as $VO_4^{\equiv}$) added. Cytochrome c reductase was determined by absorbance increases at 550 nm (12). NADH ferricyanide reductase was determined either by absorbance changes at 400 nm (19) or by a decrease in pH in 10^{-3} M phosphate buffer. The pH changes were calibrated with standard 0.01 M HCl. Ascorbate oxidase activity was measured by an oxygen electrode in 0.1 M sodium phosphate buffer, pH 7.0 with 10^{-3} M potassium cyanide.

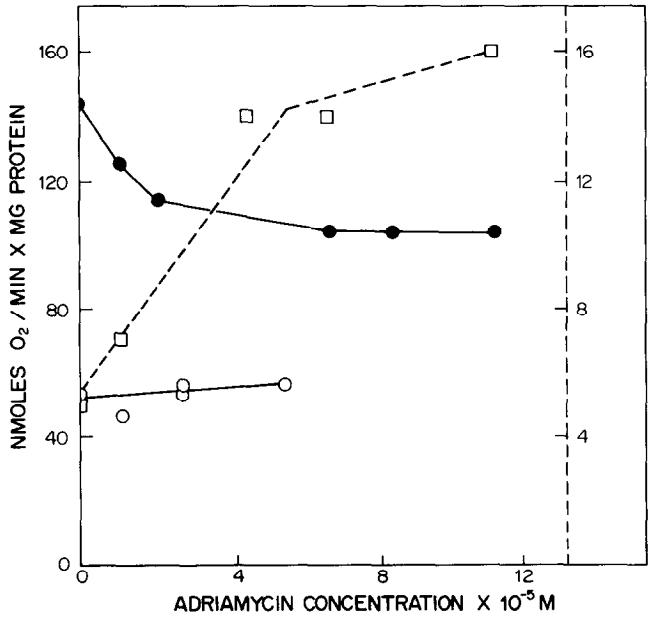


Fig. 1: Effect of Adriamycin on NADH Oxidase, NADH Cytochrome c Reductase and Ascorbate Oxidase on Mouse Liver Plasma Membrane. □ - NADH oxidase was assayed in 0.1 M potassium phosphate buffer, pH 7.0 by an oxygen electrode. O - NADH cytochrome c reductase was assayed in 0.1 M potassium phosphate buffer, pH 7.2 by absorbancy increases at 550 nm. ● - Ascorbate oxidase was assayed in 0.1 sodium phosphate pH 7.0 with 10^{-3} M potassium cyanide by an oxygen electrode.

TABLE 1. Effect of Vanadate on NADH Oxidase Activity of Mouse Liver and Pig Erythrocyte Plasma Membranes.

Sample		Activity (nmoles min^{-1} mg^{-1}) NADH Dissappearance	Oxygen Uptake
Mouse Liver P.M.	-vanadate	10	-
	+ vanadate	196	223
Pig Erythrocyte P.M.	- vanadate	0	0
	+ vanadate	197	151

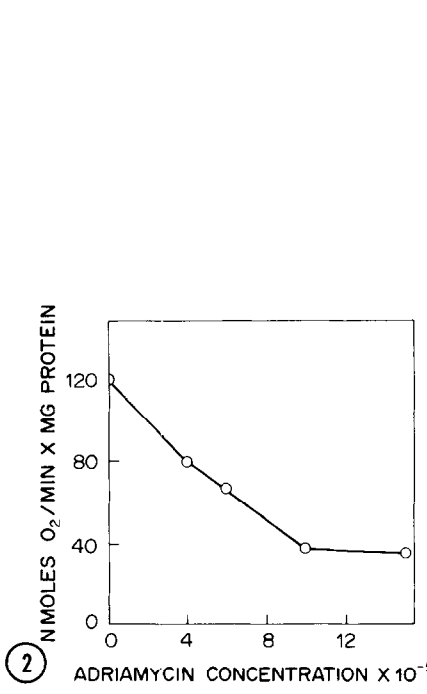
Results

NADH oxidase activity in mouse or rat liver plasma membrane was stimulated and ascorbate oxidase was inhibited by adriamycin (fig. 1). Human erythrocyte plasma membrane (open ghosts) showed no NADH oxidase activity, and oxidase activity was not induced by addition of adriamycin. NADH cytochrome c reductase in both mouse liver (fig. 1) and human erythrocyte membranes was not affected by adriamycin Ascorbate oxidase activity (cyanide-insensitive) was inhibited with 1/2 maximum inhibition as 1.5×10^{-5} M. The response of NADH ferricyanide reductase to adriamycin showed a stimulation at concentrations below 1×10^{-5} M and inhibition above 2×10^{-5} M (fig. 3).

When NADH and vanadate were added to plasma membrane, the rate of NADH oxidation, measured either as disappearance of NADH or of oxygen, was stimulated (Table 1). Since this activity was inhibited by superoxide dismutase and did not function in the absence of oxygen, it appeared that superoxide was involved in the NADH oxidation (Table 2). If adriamycin stimulated superoxide production in

TABLE 2. Effect of Superoxide Dismutase on Vanadate Stimulated NADH Oxidase of Pig Erythrocyte Plasma Membrane.

Superoxide Dismutase ug/ml	Activity (nmoles $min^{-1}mg^{-1}$)	% Control
–	197	100%
0.2	58	29%
0.4	41	21%
0.6	41	21%
0.8	36	18%



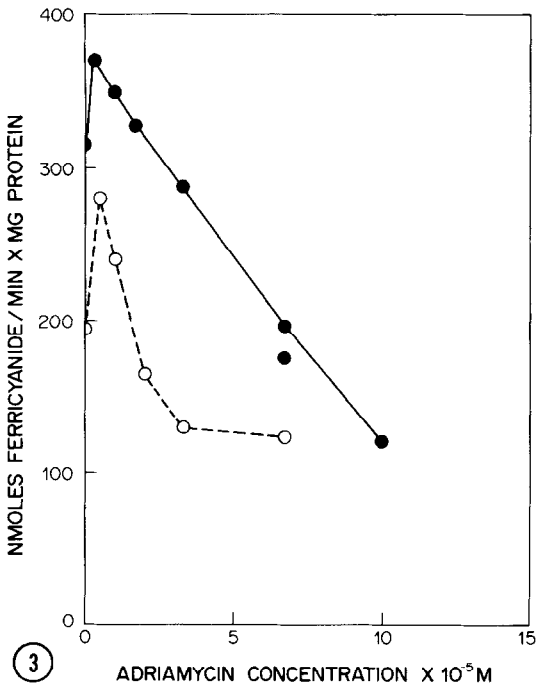


Fig. 2: Inhibition of Vanadate Stimulated NADH Oxidase in Pig Erythrocyte Plasma Membrane by Adriamycin. The assay system consisted of NADH (0.51 mM), vanadate (0.6 mM), EDTA (0.36 mM), $CaCl_2$ (0.36 mM), Na phosphate buffer (50 mM, pH 7.0) and pig erythrocyte plasma membrane (0.24 mg protein) in a total volume of 1 ml. The reaction was monitored by oxygen uptake at 22°C with an oxygen electrode.

Fig. 3: Effect of Adriamycin on NADH Ferricyanide Reductase Activity in Human Erythrocyte and Rat Liver Plasma Membranes. ● - rat liver plasma membranes assayed in 0.05 M potassium phosphate, pH 7.0 (0.025 mg protein per 3 ml). O - human erythrocyte ghosts assayed in 1 mM sodium phosphate, pH 7.0. Protein at 0.07 mg/ 3 ml assay. Assayed by decrease in absorbance of ferricyanide at 400 nm and expressed as a one electron reaction.

plasma membrane, then adriamycin would be expected to stimulate the NADH oxidase. On the contrary, the vanadate-stimulated NADH oxidase was inhibited by adriamycin (fig. 2). This inhibition was at concentrations of adriamycin from 2 to 9.9×10^{-5} M, which were similar to concentrations required for inhibition of NADH ferricyanide reductase activity. The adriamycin inhibition of vanadate stimulated NADH oxidase was also observed in mouse liver and human erythrocyte plasma membranes. Carminomycin, an anthracycline analogue, produced an inhibition of the vanadate stimulated oxidase similar to that with adriamycin, but maximum effects required a higher concentration.

Discussion

Adriamycin has been shown to stimulate superoxide production and induce lipid peroxidation in mouse heart (20). Goodman and Hochstein (21) have shown that much of the superoxide formation can derive through the NADPH-P450 reductase by increasing the oxidation of NADPH. A similar stimulation of NADPH oxidation was observed in rat liver microsomes with both adriamycin and daunomycin. Daunomycin, and to a lesser extent adriamycin, also increased NADPH induced lipid peroxidation in microsomes. NADH oxidation and associated lipid peroxidation were stimulated much less by daunomycin and even less by adriamycin. The lower rate of NADH oxidation through the NADH cytochrome b_5 reductase has been observed previously, whereas the NADPH dehydrogenase reacts more readily with oxygen (22) and causes quinone radical formation (40,41). Overall, the increased NADPH and NADH oxidation induced by anthracycline quinones may be attributed to increased autoxidation of the dehydrogenase or an anthracycline semiquinone radical to form superoxide (23).

The increased NADH oxidation rate induced in plasma membrane by adriamycin is at least as great as the activity increase in endoplasmic reticulum. This high oxidase rate is surprising, since the NADH dehydrogenase activity in plasma membrane is one tenth the activity found in endoplasmic reticulum (2,12). It should also be noted that,even though erythrocyte membranes have a rate of NADH dehydrogenase activity similar to that of liver plasma membrane, the addition of adriamycin does not induce NADH oxidase activity. Furthermore, the amount of NADH oxidase activity induced by adriamycin in plasma membrane and endoplasmic reticulum is not directly proportional to the NADH dehydrogenase levels. Therefore, it appears that the oxidase and dehydrogenase represent non-identical activities.

If the stimulation of NADH oxidase involved the formation of superoxide by autoxidation of the adriamycin, then an increased rate of NADH cytochrome c reductase activity should be observed, since superoxide reduces cytochrome c (24,25). Additionally, the increased rate of cytochrome c reduction should be inhibited by superoxide dismutase. Since there was neither an increase of NADH

TABLE 3. Effect of Superoxide Dismutase on NADH Cytochrome c Reductase Activity (with 4 x 10^{-5} M Adriamycin Added) of Mouse Liver Plasma Membrane

Superoxide Dismutase ug/ml	Activity (nmoles $min^{-1}mg^{-1}$)
-	28
0.4	28
0.6	27
0.8	28

cytochrome c reductase activity with adriamycin (fig. 1),nor an inhibition of NADH cytochrome c reductase activity with adriamycin and superoxide dismutase added (Table 3), it would appear that the increased NADH oxidation rate in plasma membrane was not accompanied by increased superoxide formation, as appears to be the situation with endoplasmic reticulum (21).

The lack of stimulation of NADH ferricyanide reductase by adriamycin and lack of superoxide dismutase inhibition of NADH ferricyanide reductase indicates,that this activity is not mediated by superoxide formation. The inhibition suggests an action of adriamycin close to the dehydrogenase.

Vanadate at 10^{-4} M increased the rate of NADH oxidase activity in mouse liver plasma membrane and induced oxidase activity in completely inactive erythrocyte membrane. This activity is clearly dependent on superoxide formation. If adriamycin caused increased superoxide formation in plasma membrane, as it does in endoplasmic reticulum, then adriamycin would be expected to increase the rate of vanadate stimulated NADH oxidase. The adriamycin inhibition of the vanadate stimulated oxidase (fig. 2) again indicates that adriamycin is not stimulating superoxide production by the plasma membrane dehydrogenase.

Since adriamycin also affects ascorbate oxidase activity, which is sensitive to chelating agents, and adriamycin is a weak chelator for copper (26), it is possible that adriamycin may act as a chelating agent. In addition to effects on plasma membrane, adriamycin has been reported to inhibit coenzyme Q function (27) and respiration localized at site 2 in mitochondria (unpublished data). The latter appears to involve one of the iron-sulfur proteins.

The effects of adriamycin on plasma membrane redox enzymes may be related to control of plasma membrane functions, such as transport, synthesis and breakdown of cyclic nucleotides, or mitosis. Effects of adriamycin on sodium transport (29) and the Na^+/K^+ stimulated ATPase (30), as well as adriamycin induced change in membrane potential (31), or rate of aggregation of lectin receptors (39), have been suggested. The NADH driven amino acid transport (13) could also be affected. Increased cardiotoxicity of adriamycin at high calcium concentration has suggested an effect on calcium exchange (28).

A balance of cAMP and cGMP has been implicated as a controlling factor in cell development. An alteration of this balance by changing redox control of a cyclase may be a part of the growth control by adriamycin (32,33),as well as explain inhibition of DNA or RNA synthesis (34,35,37). Thus the action of adriamycin derivatives, which show poor intercalation with DNA (36,38) may be mediated through a direct interaction with membranes in the control of redox functio

Acknowledgements

Supported by grants from the National Institutes of Health AM 25235 and CA 18801, the Indiana Elks and Swedish Medical Research Council. F.L. Crane is supported by a Career Award K06-GM 21839 from the National Institute for General Medical Research.

Adriamycin was kindly provided by Dr. M. Ghione, Farmitalia, Milano, Italy. Adriamycin analogs were supplied by Dr. W.T. Bradner, Bristol Laboratories, Syracuse, NY.

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