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TRANSPLASMALEMMA NADH DEHYDROGENASE IS INHIBITED BY ACTINOMYCIN D.

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Summary

Actinomycin D and puromycin inhibit the NADH dehydrogenase activity of mouse liver and pig erythrocyte plasma membrane. Actinomycin is effective at 10^{-6} M and inhibits the transmembrane ferricyanide reduction by whole erythrocytes. The NADH dehydrogenase of endoplasmic reticulum is not inhibited. Puromycin inhibits the plasma membrane dehydrogenase but does not inhibit ferricyanide reduction by whole erythrocytes. Effects of these drugs on metabolism after short term exposure may be mediated through the plasma membrane dehydrogenase.

Introduction

Actinomycin D inhibition of cell growth and mitosis (1,2) has primarily been related to inhibition of RNA and protein synthesis by binding to DNA (3,4). There is, however, increasing consideration for an effect on membranes (5,6) and evidence has been presented that the drug can induce changes in protein conformation (7) and superoxide formation in membranes (8,9).

There is also increasing evidence for an NADH dehydrogenase in plasma membranes of both animal (10) and plant cells (11) which is transmembranous in orientation. NADH inside the cell can be oxidized by ferricyanide outside the cell. This enzyme may be involved in control of metabolic function since it is affected by physiological levels of anabolic or catabolic hormones (12, 13,14). The transmembrane enzyme can also be involved in generation of a transmembrane proton gradient (11) and in transport of selected amino acids (15,16). We now find that this plasma membrane enzyme is inhibited by actinomycin D in concentration ranges known to inhibit protein synthesis (1) and mitosis (2).

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Methods and Materials

Plasma membrane and endoplasmic reticulum were isolated from mouse liver (17). Pig erythrocyte ghost preparation was based on Steck and Kant (18). NADH ferricyanide reductase and NADH cytochrome c reductase were assayed as described (17) using the aminco-DW-2a spectrophotometer dual wavelength mode substracting absorbance change at 500 from 420 nm for ferricyanide reduction and 541 from 500 nm for cytochrome c reduction respectively. Inhibitors were added to membrane one minute before addition of ferricyanide (0.2 mg) to start the reaction. Buffer was 0.05 M sodium phosphate pH 7.0. For whole erythrocyte ferricyanide reduction the method of Avron and Shavit (19) was used. 1 ml cells were suspended in 9 ml of the "standard medium" which contained NaCl 100 mM, KCl 5 mM, Na_2HPO_4 20 mM, glycylglycine 10 mM and glucose 10 mM plus 0.001 M ferricyanide. After incubation for the designated time the cells were removed by centrifugation and a 0.1 ml aliquot of the supernatant assayed for ferrocyanide.

Results

Actinomycin D inhibits the NADH ferricyanide reductase activity in plasma membrane preparations from both mouse liver and pig erythrocytes. Inhibitory effects are found at concentrations starting at 1×10^{-6} M (fig. 1). Maximum inhibition is seen in the range from 5 to 7.5×10^{-6} M with both membranes. There is a decrease in the inhibition at concentrations above 1×10^{-5} M. In contrast to plasma membrane the NADH ferricyanide reductase activity of endoplastic reticulum is not strongly inhibited by actinomycin D up to 1.5×10^{-5} M (fig. 2).

The NADH cytochrome c reductase activity in the plasma membrane preparations is also inhibited by actinomycin D at concentrations similar to those which inhibit the NADH ferricyanide reductase. Table I. NADH cytochrome c reductase and NADPH cytochrome c reductase activities of mouse liver endoplasmic reticulum, however, are not inhibited by actinomycin D at 7.5×10^{-6} M.

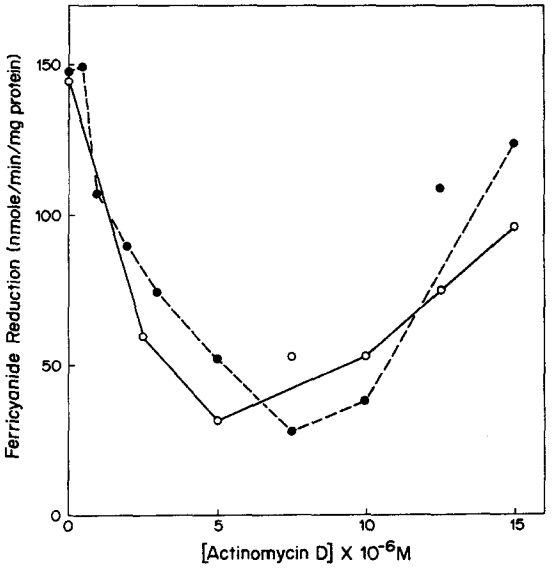

Fig. 1. Effect of actinomycin D on NADH ferricyanide reductase activity of mouse liver -o- and pig erythrocyte -●- plasma membranes. Assay as described in methods.

Intact erythrocytes reduct external ferricyanide without addition of substrate and this activity has been attributed to a transmembrane NADH dehydrogenase which transfers electrons from internal NADH to external ferricyanide. (20,21) Addition of actinomycin D to the incubation mixture inhibits the reductio of ferricyanide by the intact erythrocytes by 30 percent (fig. 3).

Puromcyin, another well known inhibitor of protein synthesis (22), also inhibits the NADH ferricyanide reductase activity of erythrocyte and mouse liver plasma membrane. The concentration required for maximum inhibition is about ten fold higher than required for actinomycin. Maximum inhibition is found at 5 to 10 x 10^{-5} M. (fig. 4). At the same concentration puromycin does not inhibit the NADH ferricyanide reductase of mouse liver endoplasmic reticulum. We have not found any inhibition of the reduction of ferricyanide by intact erythrocytes by puromycin at 7.5 x 10^{-5} M.

Table I

Effect of antinomycin D on cytochrome c reductase activity of membranes from mouse liver and pig erythrocytes.

Membrane	Substrate	Cytochrome c reductase nmole/min/mg protein control	7.5×10^{-6} M actinomycin D	Percent of Control
erythrocyte	NADH	5.5	0.8	15
liver plasma membrane	NADH	17	4	24
liver endoplasmic reticulum	NADH	485	575	119
liver endoplasmic reticulum	NADPH	40	43	108

Discussion

It is clear that actinomycin D inhibits the NADH ferricyanide reductase activity of plasma membrane, but does not inhibit similar type activity in endoplasmic reticulum. Selective inhibition of plasma membrane NADH dehydrogenase activity has been shown by atebrin (1×10^{-3} M) (23) and insulin (5×10^{-11} M) (12,14). Adriamycin has also been found to inhibit the plasma membrane dehydrogenase (24). Since this dehydrogenase has been related to selective amino

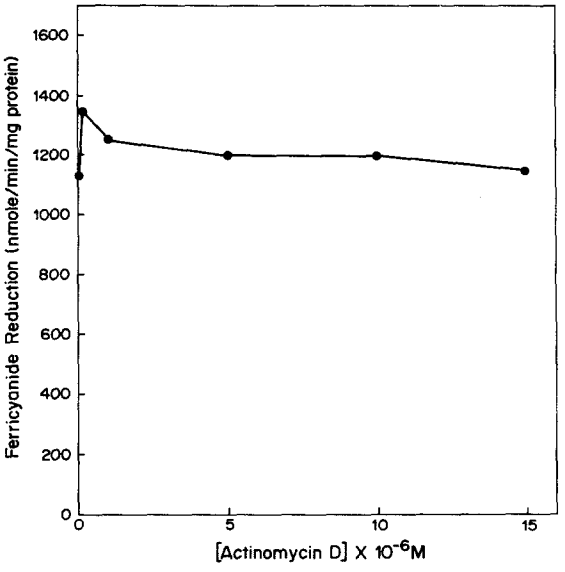

Fig. 2. Effect of actinomycin D on NADH ferricyanide reductase activity of mouse liver endoplasmic reticulum membranes.

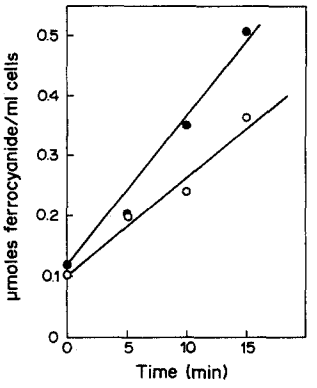

Fig. 3. Ferricyanide reduction by whole pig erythrocytes -o- control, -●- plus 7.5 x 10^{-6}M actinomycin (see methods).

acid transport it is reasonable to consider that the inhibition of the dehydrogenas by actinomycin could contribute to the effect of this drug on cell functions. The effects which we observe may also be related to actinomycin induced changes in membrane protein conformation (7). A relation to the stimulation of superoxide formation in liver microsomes (8,9) is less likely because the inhibition

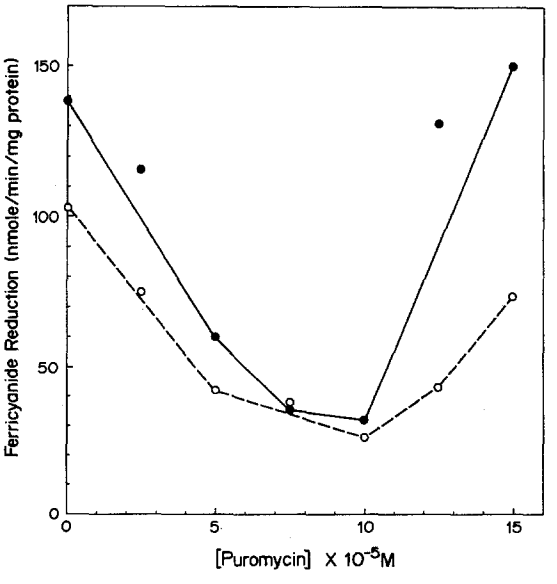

Fig. 4. Effect of puromycin on NADH ferricyanide reductase activity of mouse liver -●- and pig erythrocyte -o- plasma membranes.

effect is restricted to plasma membrane and if superoxide were formed it would increase the reduction of cytochrome c and ferricyanide (25).

The actinomycin effect on ferricyanide reduction by intact cells indicates that the transmembrane dehydrogenase is inhibited. In contrast, puromycin is not able to inhibit dehydrogenase activity when applied to the intact erythrocyte and may only act at an interior site.

It has been clear for some time that membrane functions can be affected by antineoplastic drugs (26). The NADH dehydrogenase(s) of the plasma membrane is now seen to be inhibited by anthracyclines, actinomycin and puromycin which emphasizes that the plasma membrane dehydrogenase may be involved in control of cell function.

Short term (30 min) treatment of fat cells with actinomycin D(8×10^{-6} M) decreases glucose metabolism 25% in the presence of insulin (27). Basal respiration in rat mammary gland is inhibited 20% by 0.8×10^{-6} M actinomycin D(28). Since the transmembrane dehydrogenase is sensitive to insulin it is possible that the actinomycin effects on ferricyanide reduction and glucose metabolism are both related to the dehydrogenase. Puromycin inhibits basal respiration 50% at 0.5 mM in rat fat pads but does not inhibit insulin stimulated respiration (29). The basis for this selective inhibition must be reconsidered in relation to inhibitor effects on plasma membrane function.

Acknowledgements

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