NADH OXIDATION IN LIVER AND FAT CELL PLASMA MEMBRANES

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1. Introduction

Evidence that transformed cells have an increased NADH/NAD ratio [1] and decreased adenylate cyclase [2] and evidence that NADH will inhibit the AMP cyclase of plasma membrane [3] has brought up the question of a sensor of NADH in the plasma membrane.

The fact that the flavin antagonist atebrin inhibits cyclase suggested that an NADH dehydrogenase might be the site of the NADH inhibition [3].

There have been reports of NADH dehydrogenase activity in plasma membrane preparations. In most work the NADH cytochrome *c* reductase activity has been considered to be derived from mitochondrial or microsomal contamination [4]. On the other hand there has been consistant evidence for NADH ferricyanide reductase activity [5,6] and [7] have shown histochemical evidence for NADH ferricyanide reductase in plasma membrane in electron micrographs of liver cells.

We find considerable NADH dehydrogenase activity in liver and fat cell plasma membranes with the use of different redox acceptors. Selective inhibition of this activity as compared to mitochondrial or microsomal activity by atebrin, azide and triiodothyronine provides evidence of a distinct NADH dehydrogenase in the plasma membrane. Since the NADH dehydrogenase in the plasma membrane is inhibited by atebrin this dehydrogenase may be related to the atebrin inhibition site for adenyl cyclase and the control of cyclase by NADH [3].

2. Methods

Preparation of the membranes were carried out as follows: Plasma membrane from fat cell as described earlier [8]. Liver plasma membranes were prepared according to Morré et al. [9] with the following modifications. The finely cut liver was homogenized with a 'Polytron PT10' at the lowest speed for 15 sec. The final isolation of the membranes was done in a discontinous sucrose gradient with the following concentration of the sugar in the layers: 81%, 54.3%, 48.0% and 42.9%. The plasma membranes were recovered from the interface between the 48.0% and the 42.9% layers. The mitochondrial and microsomal fractions from liver were isolated by fractionated centrifugation of a 10% homogenate in 0.25 M sucrose. The washed final pellets were taken up in 1 mM potassium bicarbonate.

Dehydrogenase assays were carried out spectrophotometrically in 0.9–1.0 ml. Buffer, enzyme (2–20 μg) and compounds to be tested were preincubated for 2 min in the cuvette before starting the reaction. For cytochrome *c* reductase activity 100 μg NADH, 0.3 mg cytochrome *c* and 1×10^{-3} M KCN were added and absorbancy recorded at 550 nm. 160 μg succinate or 100 μg NADPH were substituted for NADH in other cytochrome *c* reductase determinations. For NADH indophenol reductase 30 μg of NADH and 15 μg of indophenol were added and absorbancy recorded at 600 nm. Below pH 7.0 the lower level of NADH reduces non-enzymatic reaction between NADH and indophenol. For NADH glyoxylate reductase 80 μg of NADH and 200 μg of glyoxylate were added and absorbancy recorded at 340 nm. mM extinction coefficients used were 19.0 for cytochrome *c*, 20.0 for indophenol and 6.22 for NADH. The addition of KCN to prevent reoxidation of reduced cytochrome *c* is not always necessary in plasma membrane preparations.

Table 1
NADH Dehydrogenase activity in liver plasma membranes

	Plasma membranes	Microsomes	Mitochondria
NADH → cytochrome *c*	145	1640	470
NADH → indophenol	50	1100	210
NADH → glyoxylate	1100	320	50
NADH → ferricyanide	314	2302	3764
NADH → juglone	80	1056	386
NADH → oxygen	0–20	10	301[a]
Succinate → cytochrome	42	0	250
NADPH → cytochrome *c*	5	169	28
5′-nucleotidase	0.50	0.010	0.008
Glucose-6-phosphatase	0.067	0.490	0.163
F^- stimulated adenylate cyclase	0.10		

[a]+Cytochrome *c*
Cytochrome *c* reductase at pH 7.2, all other redox assays at pH 7.0 in 0.1 M potassium phosphate, nmol/min/mg protein

Assay for adenylate cyclase [10], glucose-6-phosphatase [11] and 5′-nucleotidase [11] was carried out as described to evaluate plasma membrane and microsomal membrane levels. The purity of the plasma membrane preparations was evaluated by measuring succinate cytochrome *c* reductase for comparison with mitochondria and NADPH cytochrome reductase and glucose-6-phosphatase for comparison with microsomes (table 1). From this data 2–10% mitochondrial and 5–15% microsomal contamination is indicated in the liver plasma membranes.

3. Results

The NADH cytochrome *c* reductase activity in liver cell plasma membranes is low compared to the activity in microsomes and mitochondria. In all fractions the activity is not inhibited by antimycin A or rotenone so it would not be attributed to mitochondrial inner membranes. In plasma membranes the activity is inhibited by azide and atebrin in contrast to no inhibition in microsomes and less inhibition in mitochondria (table 2). Triiodothyronine (T_3) at low levels also

Table 2
Relative inhibition of liver plasma membranal microsomal and mitochondrial NADH dehydrogenase activity

	Inhibition, per cent of basal		
	T_3, 1 μM	Azide, 0.1 M	Atebrin, 3 mM
L PM DCIP	37	–	54
Mitochondria DCIP	58	–	100
Microsomes DCIP	76	–	100
L PM cyt *c*	74	49	80
Mitochondria cyt *c*	78	89	65
Microsomes cyt *c*	100	110	127

Uninhibited activity (equals 100%) T_3 inhibition with DCIP as acceptor at pH 7.0, with cytochrome c as acceptor at pH 8.0. Azide inhibition measured at pH 7.2. Atebrin inhibition with DCIP as acceptor at pH 6.0 and with cytochrome *c* as acceptor at pH 7.0

Table 3
NADH Dehydrogenase activity in fat cell plasma membranes

	Specific activity nmol/min/mg/prot	NADH Dehydrogenase Inhibition (per cent of basal)		
		Atebrin, 3 mM	T_3, 1 μM	Azide, 0.1 M
NADH → cytochrome *c*	1620	47	55	51
NADH → indophenol	750	50	40	–
NADH → glyoxylate	125	–	64	53
NADH → juglone	860	–	–	–
NADH → oxygen	20	–	–	–
F^- stimulated adenylate cyclase (pH 7.5)	2.8			

*Inhibition of cytochrome *c* reductase by T_3 was at pH 8.0, all other inhibitions are at pH 7.0

inhibits the plasma membrane activity but not the microsomal activity.

The NADH indophenol reductase activity in the liver plasma membranes is also low in comparison to that of mitochondria and microsomes. As with the cytochrome *c* reductase it also shows a difference in inhibitor sensitivity in that it is inhibited by atebrin and low levels of T_3 whereas mitochondria and microsomes are not inhibited. Azide effects cannot be tested with indophenol because of non-enzymatic reactions.

The NADH glyoxylate reductase activity is very apparent in liver cell plasma membranes in contrast to lower activity in microsomes and mitochondria. A relationship between this activity and the NADH indophenol or cytochrome *c* reductase is indicated by the fact that the activity is also inhibited by azide and T_3. The effect of atebrin has not been tested because of absorbancy by atebrin at 340 nm.

The NADH oxidase activity in the plasma membrane is very low at pH 7.0 but is more active at pH 6.0. This activity is not inhibited by cyanide, antimycin or rotenone and is stimulated by azide so that it does not appear to come from mitochondria and since azide does not stimulate microsomal NADH oxidase it does not appear to be a microsomal activity. The juglone reductase in liver cell plasma membranes is also stimulated by azide. Fat cell plasma membranes have much higher NADH cytochrome *c* reductase and indophenol reductase and lower NADH glyoxylate reductase than liver plasma membranes. These activities show a similar pattern of inhibition by atebrin, azide and T_3 to that of the liver cell membranes.

4. Discussion

Our objective in this study was to determine if there was an NADH dehydrogenase in plasma membranes which could be related to NADH and atebrin inhibition of the adenyl cyclase. Since the activity with the usual acceptors for NADH dehydrogenase such as cytochrome *c* and indophenol was very low in liver cells as compared to microsomes and mitochondria it was necessary to determine if the plasma membrane enzyme differed in its response to inhibitors or stimulators. We have found three reagents which show selective partial inhibition of the dehydrogenase activity in plasma membrane. They are atebrin, azide and triiodothyronine. NADH cytochrome *c* reductase has often been found to be low in liver plasma membranes [4] but azide has often been used in these assays to inhibit cytochrome *c* oxidase. From the fact that Fleischer et al. [4] find no cytochrome *c* reductase in liver plasma membranes assayed with azide it would appear that the most purified plasma membranes will contain no azide insensitive NADH cytochrome *c* reductase. Studies of NADH indophenol reductase activity in plasma membranes have not been extensive but significant NADH ferricyanide reductase activity has often been reported [5–7].

The presence of a high level of NADH glyoxylate reductase in liver cell plasma membranes is unexpected

although this activity has been reported in erythrocyte membranes [12]. Since it is also found in fat cell membranes and is low in microsomes and mitochondria it may be a useful marker for plasma membranes. In contrast to indophenol or cytochrome *c* the glyoxylate may represent a natural substrate for the plasma membrane dehydrogenase.

The NADH cytochrome *c* reductase and NADH indophenol reductase in fat cell membranes is higher than in the liver cell membranes but shows the same sensitivity to atebrin, azide and T_3 as in the liver membranes. Since the atebrin inhibition in both liver and fat cell membranes occurs at the same concentration previously reported for inhibition of the cyclase [3] it is possible to suggest that this plasma membrane dehydrogenase is related to control of the adenylate cyclase.

Xanthine oxidase has been reported in milk fat globule membranes [13]. We find no xanthine oxidase activity but do find xanthine cytochrome *c* reductase and xanthine methylene blue reductase in the liver cell plasma membranes in the range of 10–30 nmol/mg protein/min. This activity indicates that the NADH dehydrogenase activity of xanthine oxidase may only account for a small part of the NADH dehydrogenase activity which we find.

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References

[1] Schwartz, J. P., Passoneau, J. V., Johnson, G. S. and Pastan, I. (1974) J. Biol. Chem. 249, 4138.
[2] Anderson, W. B., Gallo, M. and Pastan, I. (1974) J. Biol. Chem. 249, 7041.
[3] Löw, H. and Werner, S. (1976) FEBS Lett. 65, 96.
[4] Fleischer, B. and Fleischer, S. (1969) Biochim. Biophys. Acta 183, 265.
[5] Ferber, E., Resch, K., Wallach, D. F. H. and Imm, W. (1972) Biochim. Biophys. Acta 266, 494.
[6] Warley, A. and Cook, G. M. W. (1973) Biochim. Biophys. Acta 323, 55.
[7] Vigil, E. L., Morré, D. J., Frantz, C. and Huang, C. M. (1973) J. Cell. Biol. 59, 353a.
[8] Harwood, J. P., Löw, H. and Rodbell, M. (1973) J. Biol. Chem. 248, 6239.
[9] Morré, D. J., Yunghans, W. N., Vigil, E. L. and Keenan, T. W. (1974) in: Methodological Developments in Biochemistry, (Reid, E., ed.) Vol. 4, Longman, London.
[10] Solomon, Y., Londos, C. and Rodbell, M. (1974) Anal. Biochem. 58, 541.
[11] Aronson, N. A. and Touster, O. (1975) in: Methods in Enzymology, (Colowick, S. P. and Kaplan, N. O., eds.) Vol. 31, p. 90, Academic Press, New York.
[12] Wins, P. and Schoffeniels, E. (1969) Biochim. Biophys. Acta 185, 287.
[13] Briley, M. S. and Eisenthal, R. (1975) Biochem. J. 147, 147, 417.