# MATTERS ARISING

## Importance of cardiac cell membranes in vanadate-induced NADH oxidation

WE have reported previously[1] that purified cardiac cell membranes with high $(Na^+ + K^+)$ATPase activity have significant NADH–vanadate reductase activity. In our experiments, we used calf cardiac cell membranes prepared as described elsewhere[2], but similar results were obtained with cell membranes from cat and human ventricles and from human erythrocytes. Increasing concentrations of $Na_3VO_4$ (0.01–1 mM) in the presence of 10 mM imidazole/HCl buffer *p*H 7.25, 1.25 mM NADH and 0.1–0.2 mg cell membrane protein caused a concentration-dependent oxidation of NADH (followed photometrically by continuous recording at $A_{366}$), which was virtually absent when heat-denatured cell membranes were used. A more detailed analysis of these experiments has been published elsewhere[3].

Recently, Vyskočil and co-workers[4] reported that vanadate-induced oxidation of NADH does not require a specific enzyme. Moreover, they did not observe a vanadate-dependent oxidation of NADH in the presence of membrane fractions from rat heart and other tissues (skeletal muscle, brain and liver). They found that NADH oxidation in the presence of vanadate proceeded without cardiac cell membranes in the presence of several buffers, except imidazole/HCl, in which the reaction was completely inhibited. Although so far we agree with these results of Vyskočil *et al.*[4], we tested the vanadate–NADH reaction using rat heart cell membranes and again found vanadate-dependent oxidation of NADH in the presence of membrane fractions. Thus we maintain that cardiac cell membranes (also from rat) contain a heat-sensitive NADH-oxidoreductase that is stimulated by vanadate in the presence of 10 mM imidazole/HCl. Several authors[5–7] have found a NADH-oxidizing enzyme in plasma membranes of rat liver, rat skeletal muscle and other organs of various species.

A possible reason for the failure of Vyskočil *et al.* to detect the vanadate-dependent oxidation of NADH in the presence of membrane fractions from rat heart might be the procedure used to isolate the membranes or the preparation of the vanadate solution. We used a solution of $Na_3VO_4$ that was freshly prepared on each day of the experiment in twice distilled water and adjusted to *p*H 7.25 with HCl.

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VYSKOČIL *ET AL.* REPLY—The following remarks are pertinent to the discrepancies between our experimental data[1] and those of Erdmann *et al.*[2].

First, the preparation of the vanadate solutions may well have caused some of the differences. In previous papers[2], Erdmann *et al.* did not describe the preparation of the vanadate stock solutions; other papers[3–6] have shown that the degree of polymerization of vanadate species depends on the *p*H and concentration of vanadate in the solution, and on temperature and ionic strength. Particularly at vanadate concentrations of 0.1–10 mM) and within the *p*H range 7.0–8.0, it is uncertain which species predominates, as dimeric, trimeric and decameric clusters may be formed. The possibility cannot be excluded that Erdmann *et al.* worked with vanadate solutions in which decameric anions prevailed, even when freshly prepared. However, we observed no effect in imidazole solution when a stock solution of vanadate (V) was made with NaOH[7]. On the other hand, in the decavanadate solution prepared according to Choate and Mansour[4], we also observed vanadate-dependent oxidation of NADH in the presence of rat skeletal muscle membrane fractions in an imidazole buffer. It is possible (see ref. 6) that membrane fractions promote the NADH–vanadate (V) reactions by preferential binding of reduced vanadyl (IV), which is consistent with Rubinson's statement[6] that if free ligand(s)—probably some thermolabile components of membranes—are present in a *p*H-buffered solution, then vanadate (V) ions will tend to be reduced to vanadyl (IV) forms (which bind to the ligand more strongly than the V form) provided there is some electron-donating reagent such as NADH.

Moreover, in an imidazole-buffered medium, most vanadate (V) can be chelated preferentially with imidazole rather than with NADH. In this case, the NADH–vanadate redox reaction (which proceeds in other buffers within the NADH–vanadate complex[1,7]) is stopped and needs to be triggered by other ligands (for example, by flavines, F.V., H.D. and J.T., unpublished results).

Another possible source of discrepancy between our results and those of Erdmann *et al.* may have resulted from different treatment of membrane fractions with sodium iodide[8]. Other facts must be taken into account, such as the stability of the preparation[2] and the possibility of contamination of the cytoplasmic dehydrogenases, which can be attached to the membrane[8].

For these reasons we believe that within the cell, the oxidation of NADH by vanadate may proceed by forming a complex without any additional support from a specific enzyme, but we do not doubt the validity of the data obtained by Erdmann *et al.* in their specific experimental conditions.

*Note added in proof*: The rapid non-enzymatic oxidation of NADH ($10^{-3}$ M) by vanadate ($10^{-3}$ M) in phosphate buffer (*p*H 7.0) is accompanied by the appearance of equivalent amounts of vanadyl(IV) as revealed by the ESR method (Pilař, Vyskočil, Dlouhá and Teisinger, in preparation).

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