Commentary

# Studies on sarcolemma components may be misleading due to inadequate recovery

Otto Hansen, Torben Clausen

*Department of Physiology, University of Aarhus, DK-8000 Aarhus C, Denmark*

Received 21 February 1996

The efficiency of procedures for isolation of sarcolemma from cardiac tissue and skeletal muscle is remarkably low, of the order of at most a few per cent based upon the recovery of Na,K-ATPase [1,2]. 8 years ago we reviewed the specific problems related to studies on isolated components from sarcolemma. 16 papers aiming at elucidation of physiological mechanisms were documented to have very low recovery of Na,K-ATPase (Table 1 of [1]). The reason for the low yield of plasma membrane isolated from muscle tissue is not known and, to our knowledge, no methodological studies have focused on the problem. In fact, the recovery of plasma membrane markers is so low that the final yield may easily originate from contaminating nervous and vascular tissue, adipocytes, fibrocytes, etc. and not from the sarcolemma proper.

In a recent publication in FEBS Letters on recruitment of Na,K-ATPase and GLUT4 glucose transporters from intracellular membrane compartments data were given that would allow for calculation of the recovery on Na,K-ATPase and thus a comparison with studies on intact muscle tissue [3]. According to Table 1 in the paper the yield of plasma membrane from 1 g of rat skeletal muscle was 30 μg protein and the $K^+$-activated pNPPase activity 50 nmol $min^{-1}$ $mg^{-1}$. Using a generally accepted molar activity for $K^+$-pNPPase of 1430 $min^{-1}$ [4] it is seen that the yield and the specific activity are compatible with $0.03 \times 50/1450 \sim 1.05$ pmol Na,K-ATPase per g tissue. Data on the membrane pellet in Table 1 – erroneously called 'the starting muscle homogenate' in the text – is compatible with no more than 4.83 pmol ATPase per g tissue. Many studies on intact rat muscles as well as biopsies have shown that the number of ouabain receptors, i.e. Na,K-ATPase α-peptides, available from the extracellular aspect of the plasma membrane is about 700 pmol $g^{-1}$ in 28 day old rats [5] and 250–300 pmol $g^{-1}$ in 85 day old rats [6].

Thus, the recovery of plasma membrane in the above-mentioned study [3] is seen to be less than 1% and therefore not very likely to provide a representative sample of the total population of Na,K-ATPase. Similar methodological problems are of course associated with studies on other sarcolemma components when carried out after similar isolation procedures. In such studies the isolation procedure is usually justified by calculation of a purification factor along the last steps of the procedure whereas comparison with intact tissue or homogenate is omitted. The final product is assumed to be the sarcolemma and moreover to represent a significant fraction of this structure.

Over the years, a very large number of papers on sarcolemma receptors, channel proteins, etc. have been published in spite of the inherited problem of insignificant recovery of the plasma membrane. It is our impression that this could be due to the fact that editors of journals and reviewers of papers are unaware of this serious problem.

## References

[1] Hansen, O. and Clausen, T. (1988) Am. J. Physiol. 254 (Cell Physiol. 23), C1–C7.
[2] Lytton, J.L., Lin, J., DiAntonio, L., Brodsky, J., McGeoch, J., McGill, D. and Guidotti, G. (1994) in: The Sodium Pump (Bamberg, E. and Schoner, W. eds.) pp. 670–681, Steinkopff/Springer.
[3] Aledo, J.C. and Hundal, H.S. (1995) FEBS Lett. 376, 211–215.
[4] Ottolenghi, P. and Jensen, J. (1983) Biochim. Biophys. Acta 727, 89–100.
[5] Clausen, T. and Hansen, O. (1974) Biochim. Biophys. Acta 345, 387–404.
[6] Clausen, T. (1986) Physiol. Rev. 66, 542–580.


*SSDI* S0014-5793(96)00305-5