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Regulatory role of translocation of Na^+-K^+ pumps in skeletal muscle: hypothesis or reality?

Torben Clausen

Institute of Physiology and Biophysics, University of Aarhus, Århus C, Denmark

TO THE EDITOR: The Perspectives article by Benziane and Chibalin (1) in this issue comments on part of the literature regarding the possible role of translocation in the regulation of Na^+-K^+ pumps in skeletal muscle. I have been invited to submit a letter to the editor about this review article.

The first evidence that Na^+-K^+ pumps might be translocated to the sarcolemma of skeletal muscle was that, at 20°C, insulin increased the binding of [^{3}H]ouabain by ~70% in frog sartorius muscle (6). The following year, it was shown that, at 30°C, insulin augmented the rate of [^{3}H]ouabain binding in rat soleus muscle in the first 15 and 30 min of incubation by 30 and 40%, respectively, but caused no change in the [^{3}H]ouabain binding measured after 4 h of incubation (4). We (4) proposed that inadequate saturation could account for the above-mentioned increase, and we could not obtain any evidence for translocation or "unmasking" of Na^+-K^+ pumps. However, Ref. 4 is not mentioned in the Perspectives article (1). Moreover, that article gives the impression that our technique for the detection of translocation is described in Ref. 5, which has no information about [^{3}H]ouabain binding or translocation. The Perspectives paper (1) mentions that "ouabain binding itself leads to a rather rapid internalization of the pump unit: ouabain complexes into cells." This is based on two studies on HeLa cells and rat atria. Another of the references mentioned (8) contains no data on the uptake of [^{3}H]ouabain into skeletal muscle. It is not mentioned that in the intact rat soleus no evidence of transmembrane penetration of [^{3}H]ouabain could be detected (3). In soleus, which had reached almost maximum binding of [^{3}H]ouabain at 30°C, the addition of unlabeled ouabain caused a prompt and four- to fivefold increase in the rate of release of [^{3}H]ouabain from the muscle, most likely reflecting displacement of [^{3}H]ouabain from binding sites at the outer surface of the cells. In contrast, making the muscle cells leaky by transverse cuts caused no loss of ^{3}H activity, showing that there was no intracellular accumulation (3).

Figure 1 in Ref. 1 shows that, at 30°C, rat soleus binds 48% more [^{3}H]ouabain than at 16–18°C. At 16–18°C, insulin or electrical stimulation increase [^{3}H]ouabain binding by 28 and 36%, respectively. It cannot be excluded that these increases reflect a faster rate of [^{3}H]ouabain binding due to a higher rate of active Na^+-K^+ transport induced by the higher temperature (30 vs. 16–18°C), insulin, or electrical stimulation. It is well documented that stimulation of the rate of active Na^+-K^+ transport increases the rate of [^{3}H]ouabain binding (2, 4). It is interesting that the experiments performed by Benziane and Chibalin at 30°C failed to detect any effect of insulin or electrical stimulation on [^{3}H]ouabain binding measured after 210 min of incubation, exactly in keeping with our observations (2, 4). As mentioned above, the values reached at 30°C are unlikely to reflect intracellular accumulation of [^{3}H]ouabain. Moreover, the major part of the [^{3}H]ouabain binding sites measured in intact rat soleus at saturation represents functional Na^+-K^+ pumps capable of operating close to their expected maximum theoretical transport rate (2).

Benziane and Chibalin point out that we (7) are emphasizing the many incertainties related to inadequate recovery of Na^+-K^+-ATPase in subcellular fractionation experiments. However, they do not quote that invited review (7), in which we analyzed this problem in detail and showed that, in 12 papers using such fractionation techniques on skeletal muscles, the recovery of Na^+-K^+-ATPase varied from 0.2 to 8.9%. Neither is it mentioned that the use of sarcolemmal giant vesicles gives rise to even lower recovery (0.3%). Such small samples of the Na^+-K^+ pumps may not be representative of the total pool of Na^+-K^+ pumps or of their regulatory changes (2). The intracellular pool of membranes, which should be the source of translocation of Na^+-K^+ pumps to the plasma membrane, is poorly defined and in a recent study on rat skeletal muscle could almost not be detected (10).

Benziane and Chibalin's article describes that, following exercise or electrical stimulation, cell surface biotinylation, outer membrane-enriched fractions, and sarcolemma giant vesicle isolation methods show similar increases (36–41%) in skeletal muscle. It is stated that "these findings provide evidence that the contraction-induced increase in the sodium pump activity in skeletal muscle is mostly due to an increase in cell surface pump content rather than changes in the intrinsic activity of the pump units." It should be recalled that in isolated rat skeletal muscles electrical stimulation induces up to a 1,000–2,000% increase in net Na^+ efflux (2), effects that cannot possibly be accounted for by the reported modest translocation of Na^+-K^+ pumps to the plasma membrane. Finally, recent studies failed to detect any significant effects of 60 min of running exercise on the content of [^{3}H]ouabain binding sites in rat soleus muscle, as measured in vitro or in vivo (9). The increase in active Na^+-K^+ transport induced by excitation or hormones in skeletal muscle seems to be the result of augmented affinity of the Na^+-K^+ pumps for $[Na^+]_i$ rather than translocation (2).

In conclusion, it is still not adequately documented that electrical or hormonal stimulation of skeletal muscle induces a regulatory translocation of Na^+-K^+ pumps to the plasma membrane. The Perspectives article raises more questions than it solves. It illustrates the need for better and quantitative analysis of the recovery of the Na^+-K^+ pumps situated in the plasma membrane and definition of cell membrane fractions from homogenates.

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Address for reprint requests and other correspondence: T. Clausen, Institute of Physiology and Biophysics, Univ. of Aarhus, DK-8000, Århus C, Denmark (e-mail: tc@fi.au.dk).

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