LETTERS

Calcium and energy transfer

A recent article in *The Journal of Physiology* by Gueguen *et al*. (2005) shows that an efficient metabolic coupling between mitochondrial creatine kinase (mtCK) or adenylate kinase (AK2), and ADP rephosphorylation via mitochondrial respiration is tissue specific: it is functioning in skinned muscle of oxidative type I and type IIa but not in IIx type muscles, and this coupling can be modified by calcium. We would like to add some critical considerations which may contribute to solving some of discrepancies in this paper, and point out some methodological difficulties in studies of the functional coupling phenomenon.

Calcium, ADP, AMP and creatine are known to act as important regulators of energy flux in muscle cells. High energy phosphates may be channelled out of mitochondria via direct ATP flux or by mtCK or AK2 via a phosphoryl-creatine (PCr) shuttle (Wallimann *et al.* 1992; Dzeja & Terzic, 2003; Saks *et al.* 2004). While all types of isolated mitochondria studied before show apparent K_m (ADP) of 5–17 μM, this parameter is tissue specific in permeabilized cells *in situ*: Gueguen *et al*. (2005) showed that it is about the same in fast twitch muscle fibres of IIx type, but very high (212 μM) in slow twitch oxidative fibres of the type I and intermediate in the mixed types of fibres IIax and IIb where two apparent K_m are observed. These results are in good agreement with earlier data (Veksler *et al*. 1995; Kuznetsov *et al.* 1996; Ventura-Clapier *et al.* 1998; Saks *et al.* 2004). Since the fibre diameter is usually bigger in fast glycolytic muscles, these data show very clearly the differences in the intracellular organization between different muscle types, and the very high apparent K_m values are caused by specific local restrictions of the diffusion of ADP inside the type I oxidative fibres (Abraham *et al.* 2002; Vendelin *et al*. 2004).

In their studies Gueguen *et al*. (2005) have made another remarkable observation: when the calcium concentration in the medium was elevated from 0.1 to 0.4 μM, the affinity of respiration for exogenous ADP was increased due to activation of actomyosin MgATPase. The increased affinity for exogenous ADP increased activation of respiration at submaximal ADP concentrations to the extent usually seen in the presence of creatine. Furthermore, calcium-induced activation of actomyosin ATPase also prevented the stimulation of respiration by creatine and AMP. The authors concluded from these results that mtCK and AK2 are no longer functionally coupled with mitochondrial respiration.

This is a very surprising conclusion since under high work load, that is under elevated energy demand, the flux through the mtCK reaction was shown *in vivo* to increase significantly, e.g. during sea urchin sperm activation (Dorsten *et al*. 1997) and in muscle during increase of contraction frequency when directly assessed by the *in vivo* ^{18}O labelling technique (Pucar *et al.* 2001; Janssen *et al.* 2003), or alternatively, to decrease when demand was lowered (Joubert *et al.* 2002). In addition, calcium has been shown to strengthen the interaction between mtCK and voltage-dependent anion channels (VDAC) (Schlattner *et al.* 2001), which also would favour the formation of a VDAC–mtCK–adenine nucleotide translocase (ANT) multienzyme channelling complex, and thus facilitate the export of PCr from mitochondria.

The experimental conditions described in the paper by Gueguen *et al*. (2005) are far from physiological conditions in the sense that the activating steady state level of calcium was continuously maintained at a high level and thus no relaxation was possible. Under these conditions, production of ATP by the activated mitochondrial respiration evidently results in hypercontraction of fibres, complete disturbance of myofibrillar architecture. This will also change the diffusion restrictions for ADP and results in a strong decrease of apparent K_m for exogenous ADP (Andrienko *et al.* 2003). The rather artifactual system may explain the observations made under calcium addition. The fact that in the presence of vanadate, where the fibres are prevented from hypercontraction, calcium no longer ablates creatine-stimulated respiration (see Fig. 7) is direct proof that the observed phenomenon is related to supercontraction of the fibres, much more than to calcium itself. Under these conditions, exogenous ADP added already maximally activates respiration, and there will be no way for creatine to increase respiration further even if functional coupling is intact. For exactly the same reason, the very high apparent affinity of type II fibres for ADP makes it impossible to see the functional coupling between mtCK or AK2 and respiration from measurements at the fixed and relatively high ADP concentration of 0.1 mM (Fig. 5). To study functional coupling under these conditions, more sophisticated approach should be used (Moreadith & Jacobus, 1982).

Clearly, metabolic microcompartments depend on the structural organization and integrity of the cells, and these considerations can no longer be ignored in studies of metabolic regulation of the muscle cell respiration (Schlattner & Wallimann, 2004).

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