The ins and outs of acid–base transport in skeletal muscle

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The tubular system (t-system) of skeletal muscle fibers arises from invaginations of the sarcolemma and forms bands of narrow, branching tubules that are transversely oriented across the fiber. The system is of paramount importance for excitation-contraction coupling in muscle fibers as it conveys the spread of excitation from the sarcolemma to the central parts of the cell where it causes activation of voltage-gated channels. However, because the surface area of the t-system by far exceeds the surface area of the sarcolemma, and the lumen of the t-system is continuous with the interstitial space of the muscles, the presence of the t-system also represents the largest interface between the extracellular fluid and the myoplasm. Combined with the observation that the t-system also contains large amounts of transport proteins, for example Na^+,K^+ pumps and glucose and monocarboxylate transporters (Jaimovich et al., 1986; Dohm et al., 1993; Hallerdei et al., 2010), an additional role for the t-system as an important site for exchange of solutes between the cytosol and the extracellular space has often been considered. In this issue of JGP, Launikonis et al. investigate proton flux across tubular membranes and characterize the amiloride-sensitive Na^+/H^+ exchange mechanism in fast-twitch muscles that removes protons from the myoplasm in exchange for an inward flux of Na^+.

In part related to their minute size and deep position within muscle fibers, the detailed physiological function of t-tubules has been difficult to establish. However, one approach that has provided substantial insight into their role, at least in excitation-contraction coupling within muscles, has been the use of mechanically skinned muscle fibers. In this method, the sarcolemma is physically peeled off from the muscle fiber by microdissection under paraffin oil, providing direct access to the myoplasm, which can then be controlled by placing the preparation in experimental solutions. During the skinning process, the tubular network seals off at the points of separation from the surface membrane and remains fully functional when the fiber preparation is later placed in an ionic environment that mimics the myoplasm (Stephenson, 2006). The functionality of the t-system in mechanically skinned fibers is underlined by the fact that the tubular membranes remain excitable and support activation of normal muscle contractions via electrical stimulation (Donaldson, 1985; Posterino et al., 2000).

In the study by Launikonis et al. (2018), the mechanically skinned preparation was not used for studies of excitation-contraction coupling, but was instead cleverly used as an inside-out preparation for studies of proton flux measurements across the tubular membranes. To accomplish this, the authors incubated bundles of intact muscle fibers in solutions containing a strong buffer and a pH-sensitive fluorescent probe for a sufficient amount of time to allow the chemicals to load into the t-system. The fibers were then skinned mechanically, causing the t-system to seal off and thereby trap both the buffer and the fluorescent probe within them. Net proton fluxes across the tubular membranes were subsequently estimated from changes in t-system pH recorded by fluorescent confocal microscopy. Using this approach, Launikonis et al. (2018) show that the t-system of at least fast-twitch muscles contains an Na^+/H^+ exchanger (NHE) that can remove protons from the myoplasm by exploiting an inward electrochemical gradient for Na^+. They also demonstrate that the capacity of the transport system is sufficient to theoretically maintain normal intracellular pH in resting muscles despite the inward electrochemical gradient for protons.

Although Launikonis et al. (2018) provide convincing evidence for functional expression of the Na^+/H^+ exchanger in the t-system, it will be important to confirm this conclusion by immunostaining of the Na^+/H^+ exchanger. It will also be of interest to understand whether there is additional Na^+/H^+ exchange activity at the sarcolemma, particularly because there is equally convincing evidence that cardiomyocytes lack functional expression of Na^+/H^+ exchangers in their t-system (Garciarena et al., 2013). In cardiomyocytes, this conclusion is supported by immunostaining, which shows expression of the Na^+/H^+ exchanger predominantly in intercalated discs and the sarcolemma, where Na^+/H^+ exchange activity was also demonstrated.

It is important to consider the physiological implication of the difference between these two muscle types.

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Launikonis et al. (2018) speculate that the difference might reflect the substantial glycolysis that can occur in skeletal muscle, which is not seen to the same extent in cardiomyocytes. This might require a large proton efflux capacity in the t-system, which is indeed a possibility. In this context, it is perhaps relevant that Garciarena et al. (2013) found intracellular pH gradients along both the long and radial axes in cardiomyocytes when the Na^+/H^+ exchanger was transiently activated by acute intracellular acidosis. If the acid–base transporters in skeletal muscles are organized in the same way as in cardiomyocytes, substantial gradients for pH would likely occur in the much larger skeletal muscle cells. This would mean that pH-sensitive intracellular proteins that are essential for glycolysis, such as phosphofructokinase (Chasiotis et al., 1982), would potentially behave in different ways at different locations in the cells. Because this would perturb muscle function, it is unlikely to be the case; thus, it is more likely that the organization of acid–base transport is different in skeletal muscle and cardiomyocytes.

Effective proton extrusion in the t-system could be accomplished by additional means, including monocarboxylate transporters and sodium-coupled bicarbonate influx pathways. Because lactate has metabolic effects such as the inhibition of phosphofructokinase, which are more pronounced at low pH (Costa Leite et al., 2007), there would be a need for an effective extrusion system for lactate as well as protons from the center of the cells. Indeed, evidence for lactate transport, both in the t-system and at the surface membranes of skeletal muscle cells, has convincingly been provided by measurements of lactate transport and the distribution of various carbonic anhydrase isoforms in wild-type mice and mice where the different carbonic anhydrase isoforms are knocked out (Hallerdei et al., 2010). There is also evidence that skeletal muscle—similarly to most other cell types—has one or more transporters that use the sodium gradient to transport HCO_3^- into cells against its electrochemical gradient.

The importance of HCO_3^- transport arose from the observation that simultaneous inhibition of Na^+/H^+ exchange, DIDS-sensitive HCO_3^- transport, and lactic acid transport is necessary to inhibit acid extrusion from rat spinotrapezius muscle in vivo (Tanaka et al., 2016). In others words, selective inhibition of Na^+/H^+ exchange activity is not sufficient to inhibit proton efflux. Therefore, although the study by Launikonis et al. (2018) demonstrates an important role for Na^+/H^+ exchange activity in the regulation of myoplasmic pH, it is very likely that HCO_3^- transport also contributes significantly. In skeletal muscle, the presence of the *slc4a4* gene product NBCe1 (which mediates Na,HCO_3^- cotransport with a stoichiometry of 1:2 or in some cases 1:3) has been demonstrated by Western blotting and immunohistochemistry (Kristensen et al., 2004; Thomas et al., 2007). Kristensen et al. (2004) reported expression of NBCe1 by Western blotting in several types of human and mice skeletal muscles. In addition, the *slc4a5* gene product NBCe2 (which also mediates Na,HCO_3^- with a stoichiometry of 1:2) was found in type I + IIa fibers (oxidative fibers; Kristensen et al., 2004). The transcript of *slc4a5* has also been found in skeletal muscle (Sassani et al., 2002), consistent with the expression of NBCe2 in this tissue. Interestingly, in the context of the paper by Launikonis et al. (2018), substantial staining was seen for both NBCe1 and NBCe2 on the surface membrane, but only faint staining was seen in the interior of the muscle cell, which most likely reflects tubular expression (Kristensen et al., 2004).

It has been suggested that lactate transport via the monocarboxylate transporter-1 (MCT1) may be facilitated by NBCe1 (Thomas et al., 2007), but this concept needs further assessment. The full-length *slc4a7* gene, which codes for NBCn1, was cloned from a skeletal muscle library (Pushkin et al., 1999). One year earlier, a partial cDNA was cloned that would later appear to be part of the *slc4a7* gene, and this product was also found in the skeletal muscle (Ishibashi et al., 1998). No evidence has been found for NBCn1 in human and rat muscle (Kristensen et al., 2004). However, immunostaining indicated that NBCn1 is expressed in the neuromuscular junction of skeletal muscle, but it was not possible to decide whether it is present in the neurons or muscle (Damkier et al., 2006). Functional evidence for Na^+-coupled Cl^-,HCO_3^- exchange in extensor digitorum longus has been provided by Grossie et al. (1988). However, an antibody against the *slc4a8* gene product NDCBE (which mediates Na^+-coupled Cl^-/HCO_3^- exchange) did not reveal a band of a relevant size in human vastus lateralis (Kristensen et al., 2004). There is thus a clear need to provide a better understanding of what HCO_3^- transporters are present in skeletal muscle, where they are localized, how they interact with Na^+/H^+ exchangers and monocarboxylate transporters, and how important they are for net proton extrusion under various conditions. The novel approach to assess net proton transport presented by Launikonis et al. (2018) would be one way to address some of these questions.

The evidence for significant capacity for solute membrane transport in the t-system, provided by both Launikonis et al. (2018) and several previous studies, is overwhelming and suggests that the large surface area of the tubules is important for solute exchange between the myoplasm and the interstitium. However, the lumen of the t-system is extremely small compared with its large membrane area (less than 2% of total fiber volume; Launikonis and Stephenson, 2002). Transport of solutes across the tubular membranes will therefore cause large changes in the concentration of t-system solutes that would impede further transport. In the

study of proton transport by Launikonis et al. (2018), this problem was overcome by placing a large buffer capacity for protons in the t-system before it was sealed from the interstitium. In intact muscle in the body, however, any solute transported from the t-system into the muscle fibers must be quickly replaced if the influx is to be maintained over time and vice versa for efflux of solutes. Thus, a prominent role for the t-system in the exchange of solutes between the muscle fibers and the extracellular space surrounding them requires that solutes be exchanged between the t-system and the interstitium. In this context, modeling studies of diffusion in the t-system have argued that, because of diffusion limitations caused by small tubular diameter and large tortuosity factor, the maintenance of ion homeostasis in the inner tubular compartments depends mainly on ion fluxes across the tubular membrane with little contribution from diffusion between the t-tubules and the interstitium (Mathias et al., 1977; Wallinga et al., 1999). In accordance with this, the t-system contains substantial amounts of Na^+,K^+ pumps (Jaimovich et al., 1986), permitting the maintenance of Na^+ and K^+ gradients across the tubular membranes without substantial exchange of ions between the t-system lumen and the interstitium.

With respect to proton transport via the NHE, Na^+,K^+ pumps would be able to maintain the necessary gradient for Na^+ to drive a net efflux of protons. However, the transport process would require that the protons be removed from the t-system lumen at the same rate that they are extruded from the myoplasm. Hallerdei et al. (2010) addressed the mechanisms for proton flux from the deep portion of the t-system to the surface and suggested that proton diffusion could be enhanced by a CO_2-HCO_3^- shuttle. This hypothesis incorporates diffusion of HCO_3^- from the surface into the deep portion of the tubules, where carbonic anhydrase supports buffering of protons followed by diffusion of CO_2 to the surface of the muscle fiber. Although this proposition would explain the removal of protons from the t-system, it would still require diffusion of HCO_3^- from the interstitium to the t-system lumen. To this end, it has been suggested that contractile activity in muscle fibers could cause a pumping action that would favor more rapid exchange of fluid between the t-system lumen and the interstitium. Such pumping action would also explain the relevance of lactate and glucose transporters in the t-system (Dohm et al., 1993; Hallerdei et al., 2010), despite the fact that diffusion of these molecules along the t-system is probably slow.

As indicated here, we are still far from understanding how acid–base homeostasis is maintained in skeletal muscle, especially during work. However, the approach taken by Launikonis et al. (2018), including the use of a mechanically skinned muscle fiber, gives us a new experimental tool to address the ins and outs of acid–base transport in skeletal muscle.

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