PERSPECTIVES

A new stretch for muscle spindle research

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Molecular neurobiology over the last few decades has provided detailed insight into many neural signalling mechanisms. However, an obvious gap in our knowledge is the nature of the mechanosensitive (MS) molecules and mechanisms operating within vertebrate specialized mechanosensors (e.g. touch, auditory and proprioceptors). This gap does not reflect any long-standing research neglect, since over the last 100 years some of the most renowned physiologists, including Ruffini, Sherrington, Adrian, Katz, Kuffler and Hunt, have at one time or another focused their research efforts on one of these mechanosensors – the muscle spindle or stretch receptor. As a consequence, the spindle became the first sense organ from which electrical activity was recorded, and the receptor where the sensory code was discovered (see Chapleau, 2007). In more recent years, behavioural studies have demonstrated that it is mainly sensory data from our ∼25 000 spindle receptors that the brain uses to construct an internal representation of our body's position and movement and thereby also our global sense of self (Proske & Gandevia, 2009).

What has hampered molecular progress in the field has been the difficulty of applying patch-clamp and molecular biological techniques to the mechanosensory nerve endings that spiral around intrafusal muscle fibres encapsulated within tiny (∼5 mm long and 100–200 μm in diameter) fluid-filled 'spindles' interspersed amongst the larger and more numerous extrafusal muscle fibres. As an alternative approach, several groups have succeeded in patch-clamping and mechanically stimulating MS channels/currents in the cell body and neurites of cells isolated from dorsal root ganglia (DRG) (Drew *et al.* 2002; Hu & Lewin, 2006, and see references cited within). These studies have revealed cation-selective and Na^+-selective MS conductances that show different adaptation/desensitization kinetics, and are blocked by 10 μM Gd^{3+}, but not by 500 μM amiloride (Drew *et al.* 2002) or by 100 μM benzamil (Hu & Lewin, 2006). This pharmacology alone would seem to exclude a MS role for members of the amiloride-sensitive epithelial Na^+ channel (ENaC) family. However, there are several caveats associated with extrapolating the results to spindle mechanosensors. To begin with, it is difficult to know the sensory modality (i.e. touch, stretch, temperature or pain) of the isolated neurone once the afferent projection is lost. Furthermore, with this approach there will be a significant sampling problem because, out of the more than 5 million DRG neurones in man, only ∼50 000 innervate muscle spindles (i.e. <1 in 100 cells sampled). This problem may be overcome by retrogradely labelling the cell bodies of spindle afferents before DRG dissociation. However, one will still be left with the basic question of whether the isolated cell body/neurites express the same mechanisms seen in the mechanosensory nerve terminal, and if so, whether they are preserved following cell isolation and growth under culture conditions. Even given the best scenario, isolated cell studies cannot provide information on how the viscoelastic properties of intrafusal muscle shape the nerve response to passive muscle stretch or γ fibre-induced muscle contraction.

In a recent issue of *The Journal of Physiology*, Simon and colleagues (2010), presumably recognizing the Sisyphean challenges to interpreting isolated cell studies, have sort molecular clues from a more intact and physiologically relevant spindle preparation involving the 4th lumbrical muscle of the rat's hind paw and its attached saphenous nerve. This preparation has the advantage that the deep paw muscles lack Golgi tendon organs so that any muscle stretch-evoked discharge will reflect purely spindle output. Prompted by previous studies implicating ENaC members in invertebrate and vertebrate mechanotransduction, they first demonstrated that 1 μM amiloride (or benzamil) significantly reduced spindle discharge. In contrast, 1000-fold higher concentrations did not alter the electrically evoked compound action potential in the isolated saphenous nerve. Based on the order of blocking potency of several amiloride analogues, they concluded that a low-affinity amiloride-sensitive channel may be involved in spindle transduction. Moreover, by using immunofluorescence they were able to demonstrate that several ENaC members (α-, β-, γ-ENaC and ASIC-2 subunits) were highly colocalized in the mechanosensory nerve terminal with synaptophysin, a marker for the synaptic-like vesicles (SLVs). Interestingly, glutamate released from the SLVs regulate spindle excitability, but whether the ENaC subunits are conducted to the cell surface via the same SLVs remains to be determined. Another, subunit, the δ-ENaC, which has previously been shown to combine with β- and γ-subunits to form a low-affinity amiloride-sensitive ENaC could not be detected by either Western blots or immunofluoescence, even though two different δ subunit-selective agonists, icilin and capsazepine, were effective in increasing spindle discharge. One possibly therefore is that the δ-subunit is present in low abundance in the terminal but is below the detection limits of the antibody.

The results of Simon *et al.* (2010) clearly put new focus on amiloride-sensitive channels and ENaC subunits in muscle spindle signalling. Future studies should reveal the timing of ENaC subunit activation in the primary transduction event and also the role of other amiloride-sensitive channels, particularly the low voltage threshold Ca^{2+} channel implicated in spindle terminal Ca^{2+} spike generation and therefore also SLV release. The availability of specific genetic channel knockouts and the development of improved procedures for recording channel activity from the intact spindle would certainly help in these research endeavours.

References

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