PERSPECTIVES IN PHYSIOLOGY

To quark or to spark, that is the question

P. Lipp and M. D. Bootman

The Babraham Institute, Babraham Hall, Babraham, Cambridge CB2 4AT, UK

A rise in the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) is the first and last signal that most animal cells generate during the span of their life. The question of how these whole-cell Ca^{2+} signals (e.g. for contraction, secretion, information processing) are generated, is still a matter of great debate. Advancements in optical techniques are constantly revealing new layers of complexity in the mechanisms responsible for cellular Ca^{2+} signals. For excitable cells, the question was at least partially answered a few years ago by the description of spatially confined Ca^{2+} transients in resting rat cardiac muscle cells, the so-called 'Ca^{2+} sparks' (Cheng *et al.* 1993). This initial observation in cardiac muscle cells was thereafter confirmed throughout the entire diversity of excitable cells, from cardiac and smooth to skeletal muscle and recently spatially restricted Ca^{2+} release was also observed in neuronal cells (for a review see Lipp & Niggli, 1996*b*). The idea of these Ca^{2+} sparks being the elementary building blocks of Ca^{2+} signalling has been established during recent years. The original concept was that the gating of a single sarcoplasmic reticulum (SR) Ca^{2+} release channel (ryanodine receptor or RyR) gives rise to the occurrence of Ca^{2+} sparks. Spatio-temporal recruitment of these Ca^{2+} sparks then leads to a global or whole-cell Ca^{2+} transient.

An accumulating body of evidence, including recent papers on cardiac muscle (Lipp & Niggli, 1996*a*; Parker *et al.* 1996) and the report in this issue on skeletal muscle fibres (Shirokova & Ríos, 1997), suggests that Ca^{2+} sparks can be devolved into even smaller entities. Monitoring Ca^{2+} sparks by scanning along the transverse axis of cardiac myocytes revealed 'sub-spark' Ca^{2+} release with small, but still discrete amplitudes (Parker *et al.* 1996). Based on the lack of spatial inhomogeneities (i.e. occurrence of Ca^{2+} sparks) in Ca^{2+} signals triggered following photolytic liberation of caged Ca^{2+} in cardiac muscle cells we suggested the existence of fundamental Ca^{2+} signalling events ('Ca^{2+} quarks', Lipp & Niggli, 1996*a*). These serve as the fundamental building blocks of Ca^{2+} signalling in excitable cells. The unresolved nanoscopic Ca^{2+} quark is proposed to reflect the gating of a single RyR, whereby recruitment of several quarks gives rise to the microscopic Ca^{2+} sparks.

In skeletal muscle, Ca^{2+} sparks have also been described as elementary Ca^{2+} release events (Tsugorka *et al.* 1995) despite the fact of different excitation–contraction coupling (E–C coupling) mechanisms present in cardiac and skeletal muscle. In cardiac muscle, Ca^{2+}-induced Ca^{2+} release (CICR) is responsible for both the initial and further Ca^{2+} release from the SR. Conversely, in skeletal muscle the primary step in E–C coupling is a purely voltage-dependent release mechanism originally suggested to recruit Ca^{2+} spark-like Ca^{2+} release from the SR. Further SR Ca^{2+} release, also manifested as Ca^{2+} sparks, is then triggered by CICR (Klein *et al.* 1996). This initial scheme is now challenged by Shirokova & Ríos (1997) in a paper in this issue on subcellular aspects of SR Ca^{2+} release in skeletal muscle fibres.

Using confocal line scanning of voltage clamped muscle fibres from the frog, they were able to resolve two different types of SR Ca^{2+} release. Threshold stimulation triggers the sparse occurrence of Ca^{2+} sparks (1 spark per 6 s per triad) on top of a pedestal of Ca^{2+} increase which was of a very low amplitude (less than 10% of a typical spark) suggesting a second type, i.e. non-spark type, of SR Ca^{2+} release in skeletal muscle. The latter release was characterized by low amplitude fluctuations of $[Ca^{2+}]_i$ at membrane voltages insufficient to trigger spark-type Ca^{2+} release. Increased depolarizations were indeed able to induce spark-type Ca^{2+} release as indicated by the occurrence of identifiable Ca^{2+} sparks and the associated increased fluctuations in $[Ca^{2+}]_i$ over time. Besides voltage discrimination, the authors isolated non-spark-type Ca^{2+} release by applying tetracaine and D600. Tetracaine is an inhibitor of the fast Ca^{2+} release component in skeletal muscle which is attributed to CICR, but it has little effect on the steady-state Ca^{2+} release, which is under the control of the membrane voltage. A similar effect is described by Shirokova & Ríos. Application of tetracaine abolished Ca^{2+} sparks but did not suppress the non-spark release component. This inhibition could not be overcome by stronger depolarizations. In contrast, application of D600, a well-known inhibitor of the membrane voltage sensor, suppressed spark-type Ca^{2+} release (without inhibiting non-spark release) at low voltages, but this inhibition could be overcome by stronger membrane depolarizations.

In summary, the work described above supports the concept of a twofold mechanism for skeletal muscle E–C coupling, but challenges the idea of the Ca^{2+} spark being the sole elementary event. The primary SR Ca^{2+} release, i.e. the voltage-dependent step, is characterized by a lack of measurable discrete events, while the subsequent Ca^{2+}-induced release occurs as distinct Ca^{2+} sparks. This points to a striking similarity between cardiac and skeletal muscle Ca^{2+} signalling, in that both use single-channel events (Ca^{2+} quarks) as the primary event. Subsequent regeneration occurs via clusters of RyRs (Ca^{2+} sparks). However, due to the nature of E–C coupling in skeletal muscle, Ca^{2+} signals will be solely triggered by voltage-operated Ca^{2+} quarks, whereas in cardiac muscle a CICR mechanism predominates.

This hierarchy of events (i.e. quarks → sparks → global signals) is not unique to excitable cells. It has also been shown for Ca^{2+} signalling in non-excitable cells, which primarily relies on the gating of inositol 1,4,5-trisphosphate-operated Ca^{2+} release channels (Bootman *et al.* 1997; Berridge, 1997). It is tempting to speculate that future studies will confirm this hierarchical concept among all cell types, regardless of the precise coupling mechanism. This unification of ideas across excitable and non-excitable cells is striking given the different physiological consequences of $[Ca^{2+}]_i$ signals in various cell types, but will certainly enhance our understanding of cellular Ca^{2+} signalling.

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