Imaging and modelling calcium microdomains around individual and clustered channels

I. Parker, A. Demuro, J.W. Shuai and I. Smith, Departments of Neurobiology & Behavior and Physiology & Biophysics, University of California, Irvine, CA 92697, USA. (Introduced by Dirk van Helden)

The Ca^{2+} microdomains generated around the mouth of open ion channels represent the basic building blocks from which cytosolic Ca^{2+} signals are constructed. Improvements in optical imaging techniques now allow these microdomains to be visualized as single channel calcium fluorescence transients (SCCaFTs). A key requirement is that fluorescence signals reported by a cytosolic Ca^{2+} indicator dye should be sampled from a very small imaging volume so as to maximize kinetic resolution, and modeling studies indicate that a volume of a few tens of attoliters represents an optimal compromise between temporal resolution and signal-to-noise ratio (Shuai & Parker, 2005). In this regard, total internal reflection fluorescence (TIRF) microscopy provides a near-ideal imaging methodology for visualizing Ca^{2+} transients in close proximity to the plasma membrane, because fluorescence excitation is restricted by the extremely thin (ca. 100 nm) evanescent wave formed by total internal reflection between the microscope cover glass and cells bathed in aqueous medium. In conjunction with fast (500 fps), highly sensitive imaging using an electron-multiplied ccd camera, TIRF imaging enables an "optical patch-clamp" technique that can provide information about channel properties previously accessible only by electrophysiological recording, but with further advantages of being massively parallel and mapping channel locations and motility (Demuro & Parker, 2006).

We have used the expression of Ca^{2+}-permeable nicotinic acetylcholine receptor channels (nAChR) in *Xenopus* oocytes as a model system with which to develop optical patch-clamping of individual plasmalemmal channels (Demuro & Parker, 2005). Oocytes (obtained after euthanasia of donor frogs) are loaded with the Ca^{2+} indicator fluo-4 dextran, bathed in Ringers solution with elevated (6 mM) [Ca^{2+}] containing low concentrations of ACh and are voltage clamped at hyperpolarized potentials to increase the driving force for Ca^{2+} influx. TIRF imaging then reveals SCCaFTs arising stochastically at as many as several hundred discrete sites within the imaging field. Fluorescence signals from individual sites are pulsatile and closely resemble electrophysiological single channel records. Consistent with their arising through openings of individual nicotinic channels, SCCaFTs are seen only when a nicotinic agonist is present in the bathing solution, are blocked by curare, increase in frequency as roughly the second power of [ACh], and have mean durations in good agreement with expected channel open lifetimes. The ability to record simultaneously from hundreds of channels reveals a surprisingly large variability in gating properties (e.g. P_{open}, τ_{open}) among nominally identical nAChR expressed from the same genes, and our preliminary data suggest that gating kinetics may be spatially modulated, as correlations between P_{open} are higher among closely neighbouring channels than among distant channels.

More recently, we have extended single channel image techniques to study the local calcium puffs generated by the concerted openings of several clustered IP_3R to liberate Ca^{2+} from ER stores. Questions remain regarding the numbers of IP_3R involved in a puff, and the mechanisms by which their activity is coordinated to initiate and terminate local calcium liberation. To address these issues, we utilize cultured SH-SY5Y neuroblastoma cells, in which a majority of puff sites are located adjacent to the plasma membrane, thereby permitting use of TIRF microscopy to monitor near-membrane (ca. 100 nm) calcium signals with high spatial (ca. 300 nm) and temporal (2 ms) resolution. In addition, intracellular loading with membrane–permeant EGTA-AM is used to buffer cytosolic Ca^{2+}, so that fluo-4 fluorescence signals more closely reflect instantaneous release flux rather than accumulation of Ca^{2+} in the cytosol. Flash photolysis of membrane-permeant caged IP_3 evokes persistent (minutes) activity at several sites per cell. Individual puff sites generally display a mix of 'square' quantal events that likely represent openings of a single IP_3R, together with larger puffs that often show abrupt step-wise transitions on their rising and falling phases. Measurements of event and step amplitudes follow a multi-modal distribution, suggesting a quantal composition of puffs as multiples (8-fold or greater) of single-IP_3R calcium flux.

In summary, we believe that functional imaging of calcium microdomains opens a new approach to study of Ca^{2+}-permeable ion channels, having particular advantages over electrophysiological patch-clamp recording in that it is massively parallel, provides high-resolution spatial as well as kinetic information, and is applicable to plasmalemmal and intracellular channels in intact cells with minimal perturbation.

Demuro A & Parker I. (2005) *Journal of General Physiology*, **126:** 179-192.
Demuro A & Parker I. (2006) *Cell Calcium*, **40:** 413-422.
Shuai JW & Parker I. (2005) *Cell Calcium*, **37:** 283-299.

Supported by grant GM48071 by the US National Institutes of Health.