The 52nd Annual Meeting and Symposium of the Society of General Physiologists

Local Calcium Signaling in Cell Physiology

(organized by W. Jonathan Lederer and Richard S. Lewis)

Several features, taken together, make Ca^{2+} stand out among the physiologically important cations: first, the concentration of "free" Ca^{2+} ($[Ca^{2+}]$) in the cytoplasm is only ~100 nM; second, Ca^{2+} binds with high affinity (dissociation constants from ~100 nM to ~10 μM) to many intracellular proteins that activate/control a wide variety of biochemical/cell physiological events; and third, the extracellular $[Ca^{2+}]$ is four orders of magnitude larger than the cytoplasmic $[Ca^{2+}]$ with similar concentration ratios for organellar vs. cytoplasmic $[Ca^{2+}]$, which means that transient increases in the plasma or organelle membrane permeability to Ca^{2+} may produce large relative changes in cytoplasmic $[Ca^{2+}]$. The spatial and temporal evolution of these $[Ca^{2+}]$ transients is determined by the magnitude and time course of Ca^{2+} release, Ca^{2+} influx, and the kinetics of Ca^{2+} buffering by diffusible and fixed buffers in the cytoplasm, as well as by cellular organelles. Ca^{2+} signaling therefore reflects the amplitude of the change in $[Ca^{2+}]$, its spatial extent, and its kinetics. This spatio-temporal organization of the Ca^{2+} signal forms the foundation for signaling in local domains, which is a unique characteristic of Ca^{2+} signaling as it allows this single messenger to regulate a wide variety of different physiological functions. Fig. 1 illustrates some key events in Ca^{2+} signaling from the perspective of a T lymphocyte.

The intricacies of Ca^{2+} signaling in local domains were the focus of the 52nd Annual Meeting of the Society of General Physiologists, which took place in Woods Hole, MA, September 10–12, 1998. W. Jonathan Lederer (University of Maryland School of Medicine) and Richard S. Lewis (Stanford University School of Medicine) organized the symposium on Local Calcium Signaling in Cell Physiology, which provided new insights into the mechanisms by which cytoplasmic $[Ca^{2+}]$ is controlled by the plasma membrane and the intracellular organelles, as well as the Ca^{2+} dependence of many cell physiological events. Attendance at the meeting and symposium was high, with more than 250 participants. More than 130 abstracts covering a broad range of topics were presented. The discussions were lively and put things into perspective for the expert and nonexpert alike.

Ca^{2+} control of cell function arises from changes in steady state cytoplasmic $[Ca^{2+}]$, which is controlled exclusively by the plasma membrane, and from $[Ca^{2+}]$ transients, which may arise at either the plasma or organellar membranes. Many $[Ca^{2+}]$ transients arise from "elementary" events, the so-called Ca^{2+} sparks or puffs, that can be visualized using fluorescent Ca^{2+} indicators. Sparks and puffs differ in their kinetics and origin: Ca^{2+} sparks are relatively brief and result from Ca^{2+}-induced Ca^{2+} release (CICR) through ryanodine receptors (RYR); Ca^{2+} puffs results are more prolonged and result from Ca^{2+} release through IP_3 receptors (IP_3 receptors support CICR, but at higher $[Ca^{2+}]$ than is the case for RYR). In either case, the increase in $[Ca^{2+}]$ is localized at least initially and limited by the Ca^{2+} buffering kinetics.

Given the local, punctate nature of Ca^{2+} release, it becomes important to know the localization of the major Ca^{2+} release sites. A.O. Jorgensen (University of Toronto) presented the anatomy of the sarcoplasmatic reticulum (SR), which can be subdivided into three distinct parts: the junctional SR, in close apposition to transverse tubules, T-tubules, which couple voltage-dependent changes in the plasma membrane dihydropyridine receptors (DHPR) to RYR activation in the SR membrane; the corbular SR, close to the *z* line, which supports Ca^{2+} release through RYR-mediated CICR; and the network SR, connecting the junctional and corbular components that contain the Ca^{2+}-ATPase that pumps Ca^{2+} into the SR lumen. Junctional and corbular SR differ in their protein composition, but they both contain the Ca^{2+} binding protein calsequestrin, which acts as Ca^{2+} buffer/storage to support Ca^{2+} release. Given the limitations in optical microscopy, Ca^{2+} sparks close to the *z* line could arise in either the junctional or the corbular SR.

Ca^{2+} sparks were one of the major themes of the meeting. The interest in sparks arises because they constitute the elementary events in sarcoplasmic Ca^{2+} release, and allow for insight into the local properties of Ca^{2+} handling and CICR. An important remaining question, however, is whether a spark arises from the activation of a single RYR or from the coordinated acti-

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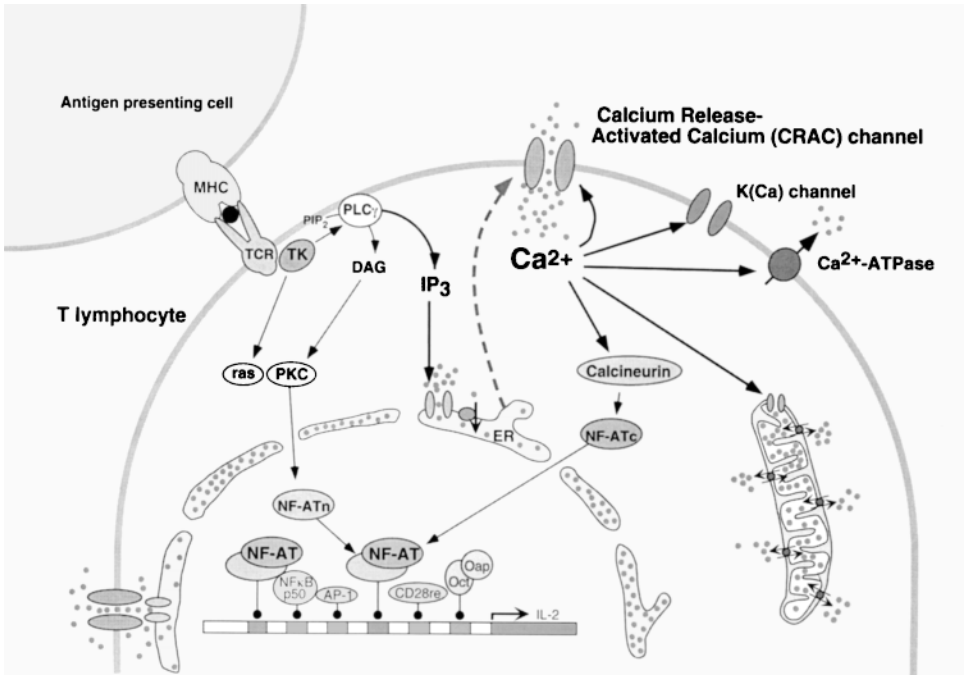

FIGURE 1. Cellular Ca^{2+} signaling using the T lymphocyte as an example. The cytoplasmic $[Ca^{2+}]$ is determined by organellar Ca^{2+} release and influx across the plasma membrane. The cytoplasmic $[Ca^{2+}]$ transients initially are localized to the release/influx channels; the time course of the $[Ca^{2+}]$ transients is determined by buffering Ca^{2+} buffers and organelles. Long-term changes in $[Ca^{2+}]$ activate gene transcription by causing a movement of transcription factors from the cytoplasm to the nucleus, which may be initiated by Ca^{2+}-dependent changes in the transcription factors phosphorylation state. We thank Richard S. Lewis and Markus Hoth for permission to use the figure.

vation of a cluster of RYRs; e.g., all the RYRs at a SR/transverse tubule junction. Three different models were discussed by various speakers (Fig. 2): several uncoupled (asynchronous) low-conductance channels (Fig. 2 a), several coupled (synchronous) low-conductance channels (Fig. 2 b), and a single high-conductance channel (Fig. 2 c). Evidence in support of all three models was presented at the meeting. (The origin of Ca^{2+} sparks will be discussed in a *Perspectives in General Physiology* in the March issue of *The Journal*.)

A technical problem that affects the field is that sparks are recorded by confocal microscopy in the line scan mode, in which the microscope rapidly and repetitively scans a line along the fiber axis. Temporal information is obtained by stacking the successive line scans side-by-side and is therefore limited by the scan and data acquisition rates (~500 lines/s). The spatial resolution is determined by the confocal volume, ~1 μm^3; but spatial definition is lacking as sparks may originate at a site on the scan line or at some site off the line. The distinction is important because the shape (amplitude and time course) of a spark is determined by (at least) four factors: the number and gating kinetics of the RYRs underlying the spark, the time course of Ca^{2+} release from the calsequestrin in the SR, the kinetics of Ca^{2+} binding to the fluorescent indicator and Ca^{2+} buffering and diffusion the cytoplasm, and the distance from the release site to the scan line. The deconvolution of these factors, which is necessary for translating the optical signal into Ca^{2+} release, becomes a tour-de-force involving sophisticated optical measurements in combination with detailed modeling. Important technical limitations are the sampling rate, the optical image blurring, and noise.

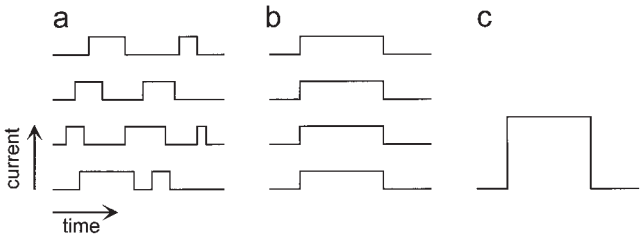

FIGURE 2. Three different schemes for the calcium release channel activity that underlies a spark. (a) A spark is generated by many low-conductance channels that gate asynchronously. (b) A spark is generated by many low-conductance channels that gate synchronously. (c) A spark is generated by a single high-conductance channel. For all three models, the measured Ca^{2+} signal (fluorescence change) reflects the kinetics of Ca^{2+} flux through the channel(s) in combination with the kinetics of buffering and diffusion in the cytoplasm.

To overcome some of these limitations, M.F. Schneider (University of Maryland School of Medicine) described a data acquisition method based on video array laser scanning, which allows for a 30-fold increased data acquisition rate (over video frame-based methods) with a corresponding increase in noise. The increased acquisition rate allows for better definition of the time course of the spark, especially of the rising phase and peak of the fluorescence transient (see the article by Lacampagne et al., in this issue of *The Journal* on pp. 187–198). The results show that the shape of the transient varies considerably among the sparks detected in a

given experiment, and that the fluorescence time course is described better as the sequence of two exponential functions (Fig. 3 b), as compared with the product of two exponentials (Fig. 3 a). Notwithstanding that the fluorescence transients have yet to be deconvoluted to yield the actual [Ca^{2+}] changes, the results tend to rule out "uncoupled small channel" models (Fig. 2 a).

Given that sparks reflect the elementary events in Ca^{2+} release, how are they regulated? The relationship between the plasma membrane depolarization and spark frequency was described by M.B. Cannell (University of Aukland, Auckland, New Zealand). Sparks begin to appear at ~40 mV, close to the threshold potential for L-type calcium channels. Based on the voltage dependence of the spark frequency, one L-type calcium channel could activate one spark consistent with the organization of DHPR and RYR at the SR/T-tubule junctions. The probability of spark activation varies with the square of the single-channel current amplitude, which could suggest that two Ca^{2+} are needed for spark activation. But the magnitude and speed of the [Ca^{2+}] transients in SR/T-tubule interspace complicates mechanistic interpretations. In any case, the results leave open the question of whether a spark originates from a single RYR, as opposed to models in which the activation of one RYR activates many (all) RYRs in a given SR/T-tubule contact. Support for the latter was obtained in experiments on mouse myocytes, which allow for repetitive measurements on a single (a few) Ca^{2+} release site. The resulting amplitude distribution shows discrete peaks, suggesting that sparks result from the activation of multiple channels. When the [Ca^{2+}] time course is extracted from the fluorescence time course, the calculated release rate corresponds to a Ca^{2+} current of ~50 pA (~20 RYRs based on the results of Meija-Alvarez et al. in this issue of *The Journal* pp. 177–186). The issue of whether sparks result from multiple/cooperative release channels was discussed further by A. Gonzalez and E. Rios (both from Rush Medical College), who gave New Faces/New Ideas presentations on spark modulation by Mg^{2+} and caffeine. Mg^{2+} decreases the spark amplitude, caffeine increase it. Neither maneuver affects the spark kinetics, but caffeine shifts the distribution of peaks in spark amplitude histograms toward higher multiples—suggesting that the release involves multiple channels.

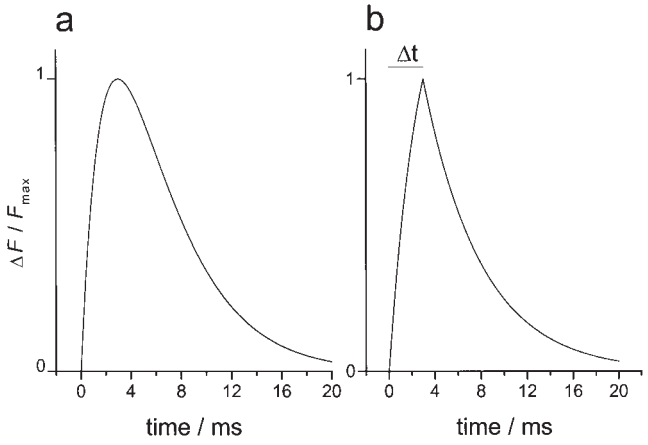

FIGURE 3. Two different time courses for a spark. (a) The time course to be expected for the model in Fig. 2 a, in which the time course can be approximated as (disregarding many of the complexities in the kinetics of Ca^{2+} buffering and diffusion): $\Delta F/\Delta F_{max} \approx [1 - \exp(-t/\tau_1]) \cdot \exp(-t/\tau_2)$. Note the "rounded" peak. (b) The time course to be expected for the models in Fig. 2, b and c, in which the time course can be approximated as (again disregarding many of the complexities in the kinetics of Ca^{2+} buffering and diffusion): $\Delta F/\Delta F_{max} \approx [1 - \exp(-t/\tau_1])$ for $0 \leq t \leq \Delta t$ and $\Delta F/\Delta F_{max} \approx [1 - \exp(-t/\tau_2])$ for $\Delta t \leq t$, where Δt denotes a variable time (the time the channel(s) are open).

Just as plasma membrane events trigger the sparks, the [Ca^{2+}] increase in the spark triggers plasma membrane events such as spontaneous transient outward currents (STOC) due to the activation of Ca^{2+}-dependent potassium channels, K_{Ca}, in smooth muscle cells. M.T. Nelson (University of Vermont) showed that every spark gave rise to a STOC, but that "sparkless" STOCs could be recorded most likely because all STOCs would be recorded in a whole-cell patch clamp measurement, whereas some sparks may be missed. (For a more complete description, see the article by Pérez et al. on pp. 229–237 in this issue of *The Journal*.) The K_{Ca} open probability increased by $>10^4$ during a spark, which could suggest that the plasma membrane K_{Ca} channels are clustered in apposition to RYRs in SR close to the plasma membrane, again emphasizing the importance of the cell microanatomy.

Channel clustering and colocalization is a common feature among ion channels. K_{Ca} channels are activated not only by organellar calcium channels, but also plasma membrane calcium channels. W.M. Roberts (University of Oregon) described the coupling between calcium channels and K_{Ca} in the frog sacculus, and showed that the K_{Ca} activation could be explained only by channel clustering. The functional coupling among the channels is strongly affected by Ca^{2+} buffering, where calretinin is the major native Ca^{2+} buffer. Detailed modeling of the effects of buffering and clustering shows that

the cell's frequency response is improved by Ca^{2+} buffering, and is optimized by having relatively small clusters. Nevertheless, the experimental evidence favors rather large clusters in which there may be buffer depletion. Not all events mediated by local Ca^{2+} accumulation are due to clustering, however. D. Yue (Johns Hopkins University School of Medicine) showed that the Ca^{2+}-dependent inactivation of L-type calcium channels results from an auto-inhibition in which the Ca^{2+} influx through a given channel causes the inactivation of that channel. The rate of inactivation varies as a function of the subunit type. This was shown in gene transfer experiments using replication-deficient adenoviruses, where the exogenous DNA was attached to the virus' exterior surface using poly-lysine, a method that seems to have unlimited possibilities.

Local Ca^{2+} regulation is important not only in excitable cells. Several speakers focused on the role of Ca^{2+} in mediating communication between the plasma membrane and intracellular organelles in nonexcitable cells. R.S. Lewis described the operation of the receptor-stimulated Ca^{2+} entry through the Ca^{2+} release-activated calcium channels (CRAC), which are activated by the depletion of organelle Ca^{2+} stores. Because the Ca^{2+} current through CRAC (I_{CRAC}) is activated when the Ca^{2+} stores are empty, the Ca^{2+} influx is termed capacitative Ca^{2+} entry. I_{CRAC} inactivates rapidly because Ca^{2+} entering through the pore binds to a site only a few nanometers from the pore where the local $[Ca^{2+}]$, due to the Ca^{2+} influx, will be much higher than the average cytoplasmic $[Ca^{2+}]$. The activation of CRAC is also regulated by the mitochondria, which are close to the plasma membrane and somehow "feel" the local $[Ca^{2+}]$ gradients established by CRAC. Mitochondria are essential for maintaining Ca^{2+} influx by depleting Ca^{2+} locally near a site that inactivates the CRAC channel, and then redistributing Ca^{2+} more diffusely to other regions of the cell. Mitochondrial Ca^{2+} uptake and release, through catalyzed diffusion of Ca^{2+} and mitochondrial Na^+/Ca^{2+} exchange, is a prerequisite for high I_{CRAC}, and the increased mitochondrial $[Ca^{2+}]$ activates key dehydrogenases, thereby increasing ATP production. Long-lasting CRAC activation, which causes a sustained increase in the cytoplasmic $[Ca^{2+}]$, is important for T cell activation because a rise in $[Ca^{2+}]$ will activate the protein phosphatase calcineurin, which in turn dephosphorylates the transcription factor NF-AT (activated T cell) and thereby enables its translocation into the nucleus (see also Fig. 1).

The interrelationships between Ca^{2+} gradients, mitochondrial Ca^{2+} handling, and (IP_3-stimulated) Ca^{2+} release from the endoplasmic reticulum, ER, were another major theme of the meeting.

To obtain insights into the physiological significance of the different IP_3 receptor subtypes, M.H. Nathanson (Yale University School of Medicine) compared the effects of IP_3 on Ca^{2+} dynamics in hepatocytes and rat insulinoma (RIN) cells. These cells were chosen because they differ in their complement of IP_3 receptors: RIN cells express only type I, whereas hepatocytes express types I and III. The two IP_3 receptor subtypes differ in their Ca^{2+} sensitivity (Fig. 3), which forms the basis for two cell types' different response to extracellular ATP, which causes a single $[Ca^{2+}]$ transient in RIN cells but $[Ca^{2+}]$ oscillations in hepatocytes. The underlying mechanism was probed using acetylcholine-induced Ca^{2+} release. In hepatocytes, acetylcholine induces apical $\rightarrow$ basal Ca^{2+} waves, and the type III IP_3 receptors are localized in the trigger zone at the apex, which could suggest that the "positive feedback" behavior of the type III receptor initiates (and maintains) the wave. This suggestion was supported by results in nonpigmented ciliary epithelial cells, which have type I receptors at the basal membrane and type III receptors at the apical membrane. Acetylcholine induced Ca^{2+} waves that progress from apex $\rightarrow$ base.

R. Rizzuto (University of Padua, Padua, Italy) described $[Ca^{2+}]$ measurements in ER and mitochondria using the Ca^{2+}-sensitive photoprotein aequorin, which was modified to have specific targeting sequences (e.g., to the ER or the mitochondria). The organelles could be visualized using similarly engineered green fluorescent protein (GFP) that were further modified so that the GFP in each organelle has a characteristic emission wavelength. High-resolution three-dimensional imaging of living cells using GFP targeted to the ER and the mitochondria show that both organelles have a tubular appearance, undergo incessant motion, and have numerous close contacts ($<$80 nm). Release of Ca^{2+} from the ER causes rapid mitochondrial Ca^{2+} uptake, and experiments with permeabilized cells shows that mitochondrial Ca^{2+} uptake is specific for Ca^{2+} released from the ER. It is not known, however, whether the IP_3 receptor density is higher at the ER–mitochondrial contacts. The mitochondrial $[Ca^{2+}]$ increase (and metabolic activation) is transient even in the presence of a maintained increase in the cytoplasmic $[Ca^{2+}]$. The Ca^{2+}-induced increase in ATP production varies, however, with the cells' energy metabolism. In HELA cells, which can maintain their ATP production by glycolysis (when fed glucose), the mitochondrial $[Ca^{2+}]$ transient may not increase ATP production, which suggests that the ATP production is secondary to the dihydrogenase activation.

The dynamics of IP_3 receptors were further developed by I. Parker (University of California, Irvine), who showed that IP_3-mediated Ca^{2+} release after flash photolysis of caged IP_3 was surprisingly variable (varying $>$100-fold), reflecting a wide variation in the number of IP_3 receptors involved in a given puff, as well as the

duration of the individual puffs. (For a more complete description, see the article by Callamaras and Parker, on pp. 199–213 in this issue of *The Journal.*) When the IP_3 concentration is increased, using photolysis flashes of increasing strength, one observes two phases of Ca^{2+} release. First, the amount of Ca^{2+} release increases with increasing flash intensity, meaning that IP_3 activates Ca^{2+} release without regenerative Ca^{2+} release. When a threshold is reached, $[Ca^{2+}]$ waves appear, which are initiated by IP_3 but maintained by regenerative Ca^{2+} release. The wave front progresses almost linearly, showing that the wave is saltatory from one cluster of IP_3 receptors to the next. The regulation of IP_3 receptor function was developed by D.-O.D. Mak (University of Pennsylvania School of Medicine) and I. Bezprozvanny (University of Texas, Southwestern Medical Center) in New Faces/New Ideas presentations. Mak examined the coupling between Ca^{2+} and IP_3 in channel activation using patch-clamp results on IP_3 receptors in the outer nuclear membrane—and concluded that the channels are activated by Ca^{2+} binding; the primary role of IP_3 is to alter the IP_3 receptor's Ca^{2+} affinity. Bezprozvanny showed that the ability of IP_3 to activate the channels is inhibited by phosphatidylinositoldiphosphate, PIP_2; binding of the IP_3 receptor to PIP_2 serves to ensure efficient coupling between phospholipase C activation and IP_3 receptor activation.

The relationship between Ca^{2+} stores and cell physiology was explored from another point of view by D. Clapham (Harvard Medical School), who showed how the state of Ca^{2+} loading of the nuclear envelope controls the transfer of material between the cytoplasm and the nucleoplasm. (For a more complete description, see the article by Strübing and Clapham, on pp. 239–248 in this issue of *The Journal.*) The nuclear envelope is a Ca^{2+} store, with a Ca^{2+}-ATPase of the smooth endoplasmic reticulum subtype (SERCA) and IP_3 receptors. When the nuclear envelope stores are depleted by activating the IP_3 receptors, the nuclear pore complex becomes "plugged" so that it will allow for passage only of compounds with a molecular weight $<$500 D. The active import of GFP-tagged glucocorticoid receptors is not, however, blocked by physiological stimuli that would be expected to empty Ca^{2+} stores. The membrane-permeable form of the Ca^{2+} chelator BAPTA (BAPTA-AM) appears to be more effective in emptying the nuclear envelope Ca^{2+} stores, store depletion using BAPTA-AM does block glucocorticoid receptor import. Surprisingly, nuclear export of MAP kinase-activated protein kinase 2, which is stimulated by cell "stress" is not affected by Ca^{2+} store depletion.

Fast synapses in the central nervous system exhibit the extreme example of a requirement for speed in triggering release of neurotransmitter with a synaptic delay of 1 ms, including a multiplicity of signaling events within the postsynaptic terminal. The problem is solved by a colocalization of calcium channels and Ca^{2+} sensors associated with synaptic vesicles, enabling local domains of Ca^{2+} to trigger a low affinity receptor. G. Borst (Max-Planck-Institut, Heidelberg, Germany) reported on current progress in dissecting the Ca^{2+} requirements for transmitter release using recording of pre- and postsynaptic signals simultaneously in brain slices from the medial nucleus of the trapezoid body. The use of an action potential waveform as a presynaptic command stimulus showed that Ca^{2+} influx is maximally activated under normal physiological conditions, with transmitter release being a supralinear function of $[Ca^{2+}]_i$. Using specific toxins to block selected calcium channels, in combination with the use of intracellular EGTA as a slow Ca^{2+} buffer, the relative contributions of P/Q and N-type calcium channels are being dissected.

Efforts to visualize local domains of Ca^{2+} using imaging techniques must contend with a multiplicity of technical difficulties. T. Fisher (Mayo Clinic) showed how one can visualize the spatial pattern of $[Ca^{2+}]$ shortly after plasma membrane calcium channels open by using a pulsed laser flash (lasting 30 ns) synchronized to the voltage stimulus to excite the Ca^{2+} indicator dyes. In adrenal chromaffin cells, the influx appeared patchy, perhaps indicating local aggregations of calcium channels. When secretion was monitored using amperometry, the pattern of "hotspots" correlated well with the amperometry signals, indicating release of vesicle contents in regions of high calcium channel density. Local calcium channel clustering may not be necessary for secretion, or the cluster size and distribution may vary among secretory cells, as Ca^{2+} influx was rather uniform in pituitary cells, resulting in a homogeneous ring of elevated Ca^{2+}. Immunolabeling indicated the presence of P/Q-, N-, and L-type calcium channels, but selective channel blockade did not reveal evidence for selective clustering of specific calcium channel subtypes.

M. Nowycky (Allegheny University of the Health Sciences) continued the theme of Ca^{2+} dependence of secretion in neuroendocrine cells, using membrane capacitance changes as a secretion assay. When voltage-gated calcium channels are opened to trigger secretion, the same supralinear relation between secretion and Ca^{2+} entry was found, but evidence was presented that a store-operated Ca^{2+} influx mechanism may also be present. Triggered by thapsigargin, which causes Ca^{2+} depletion from the intracellular stores (and thereby activates I_{CRAC}), a Ca^{2+}-selective inward current was revealed that, despite its small amplitude, can evoke very large increases in membrane capacitance. The involvement of store-operated channel presents the opportunity for multiple mechanisms to modulate secretion by triggering membrane receptors.

F. Helmchen (Bell Laboratories) presented an amazing look at cortical neurons in living rat brain, using two-photon imaging of Ca^{2+} indicator dyes. Two-photon microscopy permits excellent spatial resolution of fluorescence images hundreds of microns into living tissue, because near-infrared light penetrates tissue better than shorter wavelength light in the visible portion of the spectrum. Ca^{2+} transients evoked by action potentials were visualized during stimulation of the rat's whiskers, which provide for "triggered" physiological activity. This technique offers unique opportunities to examine the structure and signaling of neurons in the intact nervous system. The images served to emphasize, again, the spatio-temporal organization of the Ca^{2+} signal and the importance of being able to examine living tissue (or cells).

What does the Ca^{2+} signal do inside postsynaptic cells, aside from eliciting fast transmitter release? M. Kennedy (Caltech) presented a biochemical approach to determine proteins localized to the postsynaptic densities in the hippocampus. Her work, and that of others, has shown that a phosphorylation cascade is initiated by Ca^{2+} influx through NMDA receptors. Calmodulin-dependent kinase II (CaM kinase II) may provide the initial molecular switch, as its autophosphorylated form continues to be active after the Ca^{2+} signal initiated by activity. To get further insight into the functional significance of this putative switch, the levels of autophosphorylated and nonphosphorylated forms of CaM kinase II were quantified using specific monoclonal antibodies. Stimuli that induce long-term potentiation in the hippocampus greatly increase the level of the phosphorylated form in dendrites and cell bodies—and increase overall CaM kinase levels in the dendrites, which may reflect increased synthesis. Synaptic forms of GAP, a Ras GTPase-activating protein, also are localized to the postsynaptic density in a scaffold of proteins that includes the NMDA receptors. Activation of CaM kinase II causes phosphorylation of synaptic GAP, and may lead to an increase in Ras activity that provides another path for connecting plasma membrane events to transcription (and other cell functions).

R.W. Tsien (Stanford University School of Medicine) continued this theme with two additional downstream signaling pathways in hippocampal neurons. Calmodulin, located initially in the dendrites, is involved in communicating the Ca^{2+} signal to the cell body. The translocation of calmodulin correlates well with phosphorylation of the cAMP-responsive transcription factor CREB. Ca^{2+} entry through L-type calcium channels appears to be essential for initiating the calmodulin translocation. This translocation required cytoskeletal machinery, which was directly revealed by imaging using a form of GFP directly tagged to calmodulin. In addition, the transcription factor NF-AT was shown to migrate to the nucleus when cells were stimulated by phorbol ester and ionomycin or during depolarization by elevated K^+ (see Fig. 1). This pathway is critical for activation in the immune system, where it is the primary target for inhibition by cyclosporin A. Its presence in the nervous system serves to further accentuate the similarities that exist between the immune and nervous systems.

A traditional feature of the symposia organized by the Society of General Physiologists is the New Ideas/New Faces sessions, where the speakers are chosen by the organizers based on the free abstracts submitted to the meeting. This is, indeed, where the new ideas are presented—usually by young investigators. In addition to the presentations mentioned above, the final New Faces/New Ideas session extended the theme of localized Ca^{2+} signaling and signaling cascades to other systems. A. Tepikin (University of Liverpool, Liverpool, UK) visualized calmodulin translocation to the nucleus using fluorescein-labeled calmodulin delivered from a patch pipette in pancreatic acinar cells. The agonist, cholecystokinin triggered Ca^{2+} oscillations and translocation of calmodulin to both the secretory pole and to the nucleus. The calmodulin levels at the secretory pole were oscillating, the calmodulin increase in the nucleus was slower—and sustained. These results are consistent with the notion that a local (transient) rise in $[Ca^{2+}]_i$ facilitates secretory events, whereas global (sustained) $[Ca^{2+}]_i$ increases are more important for nuclear events such as gene transcription. E.A. Finch (Duke University Medical Center) demonstrated that Ca^{2+} signaling triggered by IP_3 can be highly localized, using cerebellar Purkinje cells and caged IP_3 with focal uncaging to generate a tiny source of IP_3 in dendrites. The signals did not spread very far, in seeming contradiction of the dogma that IP_3 can diffuse readily in cytoplasm. Perhaps IP_3 is phosphorylated or otherwise buffered rapidly. Other signaling pathways were found to be correlated with Ca^{2+} gradients visualized by aequorin in *Drosophila* dorsal embryo cells. R. Creton (Marine Biological Laboratory) showed marked differences in Ca^{2+} in the dorsal vs. ventral parts of the embryo during development. Expression of dorsal markers was inhibited by BAPTA and stimulated by Ca^{2+} ionophores, which suggests a role in embryo dorsalization. W.A. Yuhas (Johns Hopkins University School of Medicine) described a role for Ca^{2+} in the efferent inhibition mechanism of cochlear hair cells, a mechanism by which auditory sensitivity can be controlled by the central nervous system. Acetylcholine causes a biphasic current response consisting of an early inward current, which includes Ca^{2+} influx, followed by a later outward Ca^{2+}-activated potassium current—as would be expected for coclustering of calcium and K_{Ca} channels.

For the meeting's grand finale, the Nobel Laureate Erwin Neher (Max-Planck-Institut, Göttingen, Germany)

gave the keynote address, in which he developed the theory to depict the relationship between local and global Ca^{2+} signals by analyzing the diffusion of Ca^{2+} ions from the site of influx. The presence of both mobile buffers and Ca^{2+} indicator dyes results in Ca^{2+} not being at local equilibrium. He discussed cases in which local buffering is either sustained or partially depleted, resulting in gradients of $[Ca^{2+}]$ as Ca^{2+} diffuses away from a point source. Ca^{2+} is handed off to different buffers based on the association and dissociation rates, like a relay race in which buffers in effect transmit the Ca^{2+} signal through the cell. Finally, he pointed out that changes in global $[Ca^{2+}]$ can drastically affect the local $[Ca^{2+}]$ near a site of Ca^{2+} influx by depleting the local concentrations of the uncomplexed buffers, especially if the buffers have reasonably high affinity. Neher's talk reminded us of the interplay between theory and experiment, of the limitations of present techniques to visualize what is happening in living cells within a few nanometers of the membrane, and of the potential of the mind to create a picture of what really is happening within this important juxta-membranous domain where so much of the downstream signaling is initiated.

The meeting succeeded admirably in its purpose, emphasizing the importance of local domains in, and the spatio-temporal organization of, Ca^{2+} signaling. The experimental results presented throughout the meeting highlight the importance of being able to explain the spatio-temporal trajectory of a Ca^{2+} domain as it migrates from a channel either in the plasma membrane or from intracellular organellar membranes. The complexity of the topic could have become overwhelming, but the organizers and speakers provided a remarkably clear picture of where we need to go. Not that any problem could be regarded as solved (or even close to being solved), but the presentations defined the scope of the problem in a way that inspires further work. The importance of Ca^{2+} in cell physiology has been evident for a long time. Only recently, however, have the appropriate tools been developed to examine Ca^{2+} signaling as a four-dimensional problem in a complex inhomogeneous geometry. As evident from the articles in this issues of *The Journal*, whether from the symposium or not, there are reasons to be optimistic about the future.

Olaf S. Andersen
Editor
The Journal of General Physiology
and
Michael D. Cahalan
President
The Society of General Physiologists