Mechanisms of Subcellular Cytosolic Ca\textsuperscript{2+} Signaling Evoked by Stimulation of the Vasopressin V\textsubscript{1a} Receptor*

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Michael H. Nathanson, M. Susan Moyer, Angela D. Burgstahler, Anne-Marie O'Carroll, Michael J. Brownstein, and Stephen J. Loll

From the Liver Center, Yale University School of Medicine, New Haven, Connecticut 06510 and the Laboratory for Cell Biology, National Institute of Mental Health, Bethesda, Maryland 20892

Receptor activation may result in distinct subcellular patterns of Ca\textsuperscript{2+} release. To define the subcellular distribution of Ca\textsuperscript{2+}, signals induced by stimulation of the vasopressin V\textsubscript{1a} receptor, we expressed the cloned receptor in Xenopus oocytes. Oocytes were then loaded with fluo-3 and observed using confocal microscopy. Vasopressin induced a single concentric wave of increased Ca\textsuperscript{2+} that radiated inward from the plasma membrane. With submaximal stimulation, however, regions of the Ca\textsuperscript{2+} wave spontaneously reorganized into repetitive (oscillatory) waves. Focal stimulation of a small part of the plasma membrane resulted in a Ca\textsuperscript{2+} wave which began at the point of stimulation, radiated toward the center of the cell, then reorganized into multiple foci of repetitive, colliding waves and spirals of increased Ca\textsuperscript{2+}. The pattern of Ca\textsuperscript{2+} signaling induced by focal or global stimulation was not altered in Ca\textsuperscript{2+}-free medium, although signals did not propagate as fast. Finally, subcellular Ca\textsuperscript{2+} signaling patterns induced by vasopressin were inhibited by caffeine, while neither vasopressin nor microinjection of inositol trisphosphate blocked caffeine-induced increases in cytosolic Ca\textsuperscript{2+}. Thus, stimulation of the V\textsubscript{1a} receptor in this cell system induces a complex pattern of Ca\textsuperscript{2+} signaling which is influenced by (1) the magnitude of the stimulus, (2) the distribution of the surface receptors that are stimulated, and (3) mobilization of Ca\textsuperscript{2+} from the extracellular space as well as from two distinct endogenous Ca\textsuperscript{2+} pools. The manner in which a single type of receptor is activated may represent an important potential mechanism for subcellular Ca\textsuperscript{2+} signaling.

The temporal pattern of a cytosolic Ca\textsuperscript{2+} (Ca\textsuperscript{2+}); signal is thought to be important for its effect as a second messenger (1–5), and this pattern is often influenced by the magnitude of agonist stimulation (1, 2, 5). The spatial pattern of a Ca\textsuperscript{2+}; signal may also be important for cell regulation (6–8), and stimulation of different types of surface receptors on the same cell induces different subcellular patterns of Ca\textsuperscript{2+} signaling (9). Stimulation of the vasopressin V\textsubscript{1} receptor leads to an inositol trisphosphate (IP\textsubscript{3})-mediated Ca\textsuperscript{2+}, increase (3, 4, 10), but the subcellular pattern of V\textsubscript{1}-induced increases in Ca\textsuperscript{2+}; has only recently been explored (4, 11). The V\textsubscript{1} receptor is found in a number of tissues, including liver, brain, kidney, vascular smooth muscle, and platelets (10), and the V\textsubscript{1} sub-type of the receptor has been cloned from rat liver (12). The subcellular distribution of vasopressin-induced Ca\textsuperscript{2+}; signals has been difficult to investigate in individual hepatocytes because of the small size of these cells (20–25 μm in diameter) relative to the speed at which Ca\textsuperscript{2+}; waves may traverse them (up to 100 μm/s) (11, 13). To better define Ca\textsuperscript{2+}; signals generated by stimulation of the V\textsubscript{1} receptor, and to insure that the Ca\textsuperscript{2+}; signals were specifically induced by that receptor, we used the cloned cDNA for the receptor (12) to express it in much larger cells, Xenopus oocytes (~1000 μm in diameter). Such cells are too thick for detailed subcellular resolution of fluorescence from Ca\textsuperscript{2+} dyes if epifluorescence imaging is used, so the oocytes were observed using confocal microscopy (9, 14). The specific aims of this work were (1) to characterize subcellular Ca\textsuperscript{2+} signaling patterns induced by stimulation of the V\textsubscript{1} receptor and (2) to determine the mechanisms responsible for these signaling patterns.

MATERIALS AND METHODS

Ang\textsuperscript{2-vasopressin, caffeine, HEPES, and EGTA were obtained from Sigma, fluo-3 and IP\textsubscript{3} were obtained from Molecular Probes, and ionomycin was obtained from Calbiochem. Xenopus oocytes were harvested and incubated at 18 °C in a modified Barth's medium. After 24 h, the oocytes were injected with mRNA (15–20 ng) synthesized from cDNA for the V\textsubscript{1} receptor previously cloned from rat liver (12). After an additional 48–72 h, oocytes were microinjected with the Ca\textsuperscript{2+}-sensitive fluorescent dye fluo-3 (15) (48 ng). Within 30–120 min, the cells were transfected to a chamber on the stage of a Zeiss Axiovert microscope, perifused at room temperature with a HEPES-buffered solution (1.2 ml/min), and observed using a Bio-Rad MRC-600 confocal imaging system. For studies in Ca\textsuperscript{2+}-free medium, EGTA (1 mM) was added to the HEPES buffer and Ca\textsuperscript{2+} was withheld. To fokally stimulate oocytes, a WPI Pneumatic PicoPump was used to deliver 125 nl of vasopressin (1 μM) onto the surface of the oocyte over 1–2 s. To minimize the surface area of membrane exposed to vasopressin, the microinjection apparatus delivered the vasopressin in the direction opposite to the direction of flow in the chamber. Microinjections of IP\textsubscript{3} or CaCl\textsubscript{2} were performed using this same apparatus, although injection volumes were 30–80 nl (approximate oocyte volume, 1.5 μl). For microinjections, IP\textsubscript{3} (10 or 100 μM) or CaCl\textsubscript{2} (1 mM) was in a solution containing KCl (150 mM) and HEPES (1 mM) at pH 7.25. For all studies, an argon laser was used to excite the fluo-3 at 488 nm and emission signals above 515 nm were collected. Optical sections less than 30 μm in thickness and with a

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† To whom correspondence should be addressed: Liver Study Unit, 1080 LMP, Yale University School of Medicine, New Haven, CT 06510.

\textsuperscript{1}The abbreviations used are: IP\textsubscript{3}, inositol 1,4,5-trisphosphate; CICR, Ca\textsuperscript{2+}-induced Ca\textsuperscript{2+} release.
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**Fig. 1.** Subcellular changes in cytosolic \( \text{Ca}^{2+} \) in response to maximal (1 mM) vasopressin stimulation in a fluo-3-loaded Xenopus oocyte expressing the V₁₆ receptor. A–H, serial confocal microscopic images of the oocyte before and 20, 40, 60, 80, 100, 120, and 140 s after stimulation, respectively. Increasing fluorescence intensity corresponds to increasing cytosolic \( \text{Ca}^{2+} \), but \( \text{Ca}^{2+} \) concentrations are not quantified further, because fluo-3 cannot be ratio-imaged (15). Oocyte diameter in this focal plane is 1175 \( \mu \text{m} \), and depth of focus is 20 \( \mu \text{m} \). I, change in fluorescence over time at locations 1, 2, and 3 indicated in A. There is an abrupt, sustained increase in \( \text{Ca}^{2+} \) at each location and the lag time between stimulation and response increases as a function of distance from the plasma membrane.

Resolution of 1.76 \( \mu \text{m/pixel} \) were obtained; these cells typically have a diameter of 1000–1500 \( \mu \text{m} \). Images were recorded at a rate of 1 s⁻¹ using a Panasonic TQ3031F optical memory disc recorder and were subsequently analyzed using an Itex Series 151 image processor. In selected experiments, confocal line scanning microscopy (11, 13) was performed instead of time-lapse confocal microscopy. In the line scanning mode of confocal microscopy, fluorescence measurements are restricted to a single line across the plane of focus. This approach induces negligible photobleaching of fluo-3 (13) and allows the subcellular distribution of \( \text{Ca}^{2+} \); signals over time to be displayed in a single image, provided that \( \text{Ca}^{2+} \) wave propagation occurs along the line which is scanned (11, 13). Changes in fluorescence intensity were used to reflect changes in cytosolic \( \text{Ca}^{2+} \), but \( \text{Ca}^{2+} \) concentrations were not quantified further, because fluo-3 cannot be ratio-imaged (15). Although the distribution of fluo-3 within the cytosol appeared slightly inhomogenous in some oocytes, oocytes were studied at least 30 min after fluo-3 microinjection and redistribution of the dye was never seen during experiments. Results for all studies are expressed as mean \pm S.E., and comparisons between groups were made using the two-sample \( t \) test.
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RESULTS AND DISCUSSION

Stimulation with vasopressin (1 nM–1 µM) induced a single concentric wave of increased Ca²⁺ that radiated inward from the plasma membrane (Figs. 1 and 2). Each wave traveled at a constant rate and in a nondiminished fashion, suggestive of an autocatalytic mechanism of wave propagation such as Ca²⁺-induced Ca²⁺ release (CICR) (16–18). Of 26 oocytes examined, the wave speed was the same in oocytes stimulated with 1, 10, or 100 nM vasopressin (2.9 ± 0.4, 2.7 ± 0.4, and 3.0 ± 0.3 µm/s, respectively; mean ± S.E.), but was over twice as great in cells stimulated with 1 µM vasopressin (6.8 ± 0.6 µm/s; p < 0.0001). In half of the 10 oocytes stimulated with 1 or 10 nM VP, regions of the Ca²⁺ wave spontaneously reorganized into rapid repetitive (oscillatory) waves (Fig. 2). Stimulation with 0.1 nM vasopressin (n = 4) induced similar oscillatory waves, but which began circumferentially at or near the plasma membrane (Fig. 2). No response to vasopressin (1 µM) was observed in oocytes injected with fluo-3 but not mRNA (n = 5). These observations indicate that the magnitude of a stimulus may affect the subcellular pattern of Ca²⁺ release. A similar phenomenon was previously reported in acetylcholine-stimulated Xenopus oocytes expressing the cloned muscarinic M₂ receptor (9). However, the current work extends those observations by demonstrating that the different Ca²⁺ signaling patterns seen with increasing concentrations of agonist represent a progression. Specifically, minimal concentrations of vasopressin (0.1 nM) induce repetitive subplasmalemmal Ca²⁺ waves, while higher concentrations (1–10 nM) induce a single concentric wave that reorganizes into these repetitive waves, and maximal stimulation (1 µM) leads to a single, faster wave that exhibits no repetitive behavior (Figs. 1 and 2). These Ca²⁺ signaling patterns appear to be the spatial correlate of the progression of temporal patterns that often occurs with increasing concentrations of agonist; low concentrations induce sustained Ca²⁺ oscillations, higher concentrations induce transient Ca²⁺ elevations that may become (dampened) oscillations as the original signal decays, and maximal stimulation leads to a single sustained Ca²⁺ elevation, often of greater amplitude than is seen in oscillatory signals (2, 3, 16). Thus, these findings demonstrate a parallel between the spatial and temporal aspects of cytosolic Ca²⁺ signals, as has previously been suggested (16).

Perfusion of a small part of the plasma membrane with vasopressin (1 µM, n = 8) resulted in an intracellular Ca²⁺ wave which began nearest the point of stimulation, radiated across that region of the cell at 8.7 ± 2.1 µm/s (no different than the Ca²⁺ wave speed induced by global stimulation with 1 µM vasopressin), then reorganized along the wavefront into multiple foci of smaller, repetitive, colliding waves and spirals of increased Ca²⁺, which persisted for over 10 min. The average speed of these repetitive waves was over 40% greater than that of the original wave (12.4 ± 0.6 µm/s, p < 0.0005), and the frequency of these Ca²⁺ oscillations ranged from 6 to 10 min⁻¹. This periodic behavior within the cytosol demonstrates that the intracellular milieu behaves as an excitable medium (14, 19). In such systems, the radius of curvature of these waves is linearly related to wavefront velocity, and this relationship has been described in detail (14, 19). The change in cytosolic Ca²⁺ over time, as measured at a single point within the oocyte, was also very different depending on which point was chosen. In fact, sustained oscillations, dampened oscillations, and sustained elevations of Ca²⁺ could all be observed simultaneously at different locations within a single oocyte. These observations demonstrate that the distribution of surface receptors which are stimulated also may affect the subcellular pattern of Ca²⁺ release within a cell.

No new or additional Ca²⁺ signal was elicited by re-exposure of a small region of plasma membrane to vasopressin (oocytes were restimulated within 1–2 min; n = 3). In addition, oocytes in which a small region of membrane was stimulated, followed by stimulation of the entire membrane, responded to the latter by a single, concentric inward-directed Ca²⁺ wave that excluded the intracellular region responding to the initial stimulus (n = 2). These findings demonstrate that a refractory period follows local release of Ca²⁺ stores, which also supports the previous observation that the interior of Xenopus oocytes behaves like a regenerative excitable medium (14). However, refractory periods of over 1 min, as observed here, are over an order of magnitude longer than predicted (14). Longer refractory periods appear to result from more intense stimulation of receptors and are associated with faster Ca²⁺ wave propagation, which may reflect greater depletion of local Ca²⁺ stores (20).

It has been postulated that complex subcellular patterns of Ca²⁺ signaling are mediated by linkage of receptors to G proteins (9, 14). To determine whether activation of plasma membrane Ca²⁺ channels (which are thought to be receptor-operated for V₄ receptors (21, 22)) is also involved, we investigated signaling patterns in Ca²⁺-free medium (Fig. 3). The
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FIG. 3. Subcellular changes in cytosolic Ca\textsuperscript{2+} in response to focal perfusion in Ca\textsuperscript{2+}-free medium with vasopressin (1 \( \mu \)M) in a fluo-3-loaded Xenopus oocyte expressing the \( V_{1a} \) receptor. A–H, serial confocal microscopic images of the oocyte before and 10, 30, 150, 151, 153, 155, and 160 s after stimulation, respectively. B and C illustrate a Ca\textsuperscript{2+} wave that begins at the cell membrane near the location of the micropipette tip (not seen) and radiates into the cell. D illustrates an intracellular focus (arrow), 175 \( \mu \)m from the cell membrane, from which an expanding elliptical wavefront emanates (E–G). Note that the time interval between each of frames D–G ranges from only 1–2 s, indicating a more rapid wave speed than that of the initial Ca\textsuperscript{2+} wave seen in frames B and C. H illustrates regeneration of the intracellular focus seen in D. Basal fluorescence in this oocyte is markedly less than in Fig. 1, and the black half of the cell (left side) is the highly pigmented animal pole. Oocyte diameter in this plane of focus is 875 \( \mu \)m and depth of focus is 25 \( \mu \)m. I, change in fluorescence over time at locations 1, 2, and 3 indicated in A. The Ca\textsuperscript{2+} signaling patterns include 1) a single large but transient increase in Ca\textsuperscript{2+}, 2) a similar transient increase followed by dampened oscillations, and 3) sustained oscillations. This figure illustrates the extent to which the temporal pattern of Ca\textsuperscript{2+} signaling can vary depending on the intracellular location at which the signal is observed.

basic pattern of Ca\textsuperscript{2+}, signaling induced by local (n = 7) or global (n = 5) stimulation with 1 \( \mu \)M vasopressin was not altered, although initial signals did not propagate as fast (4.7 ± 0.7 \( \mu \)m/s; \( p < 0.005 \)) or as far. Thus, influx of extracellular Ca\textsuperscript{2+} contributes to the \( V_{1a} \)-induced Ca\textsuperscript{2+} response by accelerating the speed of the initial Ca\textsuperscript{2+} wave formed by release of endogenous stores. Neither the speed nor the frequency of oscillatory Ca\textsuperscript{2+} waves was decreased in Ca\textsuperscript{2+}-free medium,
**FIG. 4. Effect of caffeine (20 mM) on cytosolic Ca\(^{2+}\) in a Xenopus oocyte loaded with fluo-3.** A, confocal image of the oocyte prior to stimulation with caffeine. A line scan was subsequently performed along the white line (see text). Oocyte diameter in this plane of focus is 1140 \(\mu\)m and depth of focus is 20 \(\mu\)m. B, confocal line scan along the line indicated in A. Each measurement of fluorescence along that line is stacked horizontally on top of subsequent measurements. Fluorescence along the line was measured every 0.5 s, for a total of 4.3 min (top to bottom). Caffeine induces a gradual increase in fluorescence which begins peripherally and moves symmetrically toward the center of the oocyte. This is typical of the pattern seen in all caffeine-stimulated oocytes. C, caffeine-induced change in fluorescence over time at a single point within the oocyte. Caffeine (20 mM) is added to the perifusate at \(t = 0\) s and induces a gradual, sustained increase in \(\text{Ca}^{2+}\), which begins after a brief latency period. Fluorescence was monitored 30 \(\mu\)m from the cell membrane, but as indicated in the line scan in B, the same pattern of increased fluorescence occurs throughout the cell interior (although the latency period increases toward the center of the oocyte).

**FIG. 5. Effect of caffeine (20 mM) on cytosolic Ca\(^{2+}\) in a fluo-3-loaded Xenopus oocyte in Ca\(^{2+}\)-free medium.** Fluorescence over time was determined by time-lapse confocal microscopy, and the fluorescence at a single intracellular location is shown. The oocyte was perfused with Ca\(^{2+}\)-free medium beginning at \(t = 0\) s and though. These observations indicate that the basic patterns of subcellular \(\text{Ca}^{2+}\) signaling (i.e. \(\text{Ca}^{2+}\) waves, oscillations, and spirals) occur by mobilization of endogenous \(\text{Ca}^{2+}\) stores, although influx of extracellular \(\text{Ca}^{2+}\) across specific \(\text{Ca}^{2+}\) channels can contribute to certain features of these patterns.

Stimulation of the \(V_{1a}\) receptor induces an increase in cytosolic \(\text{Ca}^{2+}\) which is initiated largely by \(\text{Ca}^{2+}\) release from IP\(_3\)-sensitive stores (4, 12, 15, 16). This results in propagation of single or repetitive \(\text{Ca}^{2+}\) waves across the cytosol, in both isolated rat hepatocytes expressing the native \(V_{1a}\) receptor (4, 11, 13) and Xenopus oocytes expressing the cloned receptor. It is hypothesized that such \(\text{Ca}^{2+}\) waves propagate by an autocatalytic reaction-diffusion network (i.e. CICR) rather caffeine was added subsequently (arrow). Caffeine induces a gradual, sustained increase in \(\text{Ca}^{2+}\), no different from the pattern seen in \(\text{Ca}^{2+}\)-containing medium (see Fig. 4c).
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FIG. 6. Effect of caffeine (20 mM) on cytosolic Ca\textsuperscript{2+} in a fluo-3-loaded Xenopus oocyte first stimulated with vasopressin (1 \muM). Fluorescence over time was determined by time-lapse confocal microscopy, and the fluorescence at a single intracellular location is shown. The oocyte was stimulated with vasopressin beginning at \( t = 0 \) s, and caffeine was added subsequently (arrow). Caffeine induces a gradual, sustained increase in Ca\textsuperscript{2+}, no different from the pattern seen in the absence of vasopressin (see Fig. 4c).

FIG. 7. Effect of caffeine (20 mM) on cytosolic Ca\textsuperscript{2+} in a fluo-3-loaded Xenopus oocyte first microinjected with IP\textsubscript{3} (100 \muM). Fluorescence over time was determined by time-lapse confocal microscopy, and the fluorescence at a single intracellular location was shown. The oocyte was injected with IP\textsubscript{3} at \( t = 0 \) s and caffeine was added subsequently (arrow). IP\textsubscript{3} induces an immediate, transient increase in Ca\textsuperscript{2+}, while caffeine induces a gradual, sustained increase in Ca\textsuperscript{2+}, no different from the pattern induced by caffeine in the absence of IP\textsubscript{3} microinjection (see Fig. 4c). Tracing is representative of findings from three experiments in which caffeine was introduced 2-5 min after IP\textsubscript{3}. In contrast, re-injection of IP\textsubscript{3} within 2-6 min of the initial microinjection failed to elicit a detectable increase in fluorescence (\( n = 3 \); data not shown).

than by simple diffusion of the Ca\textsuperscript{2+} released from IP\textsubscript{3}-sensitive stores (3, 16, 18). The CICR pool is thought to be distinct from IP\textsubscript{3}-sensitive Ca\textsuperscript{2+} stores (3, 16), in part because Ca\textsuperscript{2+} oscillations can be induced independent of changes in IP\textsubscript{3} (23, 24) but are dependent upon mobilization of CICR stores (25).

We used caffeine to investigate the role of CICR in the generation of subcellular Ca\textsuperscript{2+} signals, since the open probability of CICR Ca\textsuperscript{2+} channels is increased in a concentration-dependent manner by caffeine (26, 27), while caffeine has little affinity for the IP\textsubscript{3} receptor (28). Oocytes perfused with 20 mM caffeine displayed a gradual but sustained increase in cytosolic Ca\textsuperscript{2+} (\( n = 7 \)). The Ca\textsuperscript{2+} increase began at the plasma membrane and spread slowly inward (Fig. 4). Caffeine also induced this subcellular Ca\textsuperscript{2+} pattern in oocytes in Ca\textsuperscript{2+}-free medium (\( n = 6 \); Fig. 5), in oocytes pretreated with 1 \muM vasopressin (\( n = 4 \); Fig. 6), and in oocytes in which a supra-maximal concentration of IP\textsubscript{3} (100 \muM) had been microinjected (\( n = 3 \); Fig. 7). In contrast, in oocytes in which IP\textsubscript{3} had been microinjected, re-injection of IP\textsubscript{3} failed to elicit a detectable increase in fluorescence (\( n = 3 \); data not shown). Together, these findings suggest that oocytes contain endogenous caffeine-sensitive Ca\textsuperscript{2+} stores that are not depleted by mobilization of the IP\textsubscript{3}-sensitive Ca\textsuperscript{2+} pool. An alternative explanation is that caffeine instead mobilizes the IP\textsubscript{3}-sensitive Ca\textsuperscript{2+} pool itself. Although this hypothesis cannot be excluded by the current work, it may be less likely because caffeine is thought not to interact with the IP\textsubscript{3} receptor (27-30). In addition, both caffeine and ryanodine slow the speed of agonist-induced Ca\textsuperscript{2+} waves but neither agent abolishes the waves or alters their direction (31), which further supports the hypothesis that caffeine acts on the ryanodine-sensitive rather than the IP\textsubscript{3}-sensitive Ca\textsuperscript{2+} pool. Oocytes pretreated with caffeine displayed no subsequent Ca\textsuperscript{2+} increase in response to 1 nM vasopressin (\( n = 5 \)), and those oocytes stimulated instead with 1 \muM vasopressin (\( n = 8 \)) displayed either no global Ca\textsuperscript{2+} increase (\( n = 1 \) of 8) or a faint circumferential Ca\textsuperscript{2+} wave which propagated to the center of the cell (\( n = 7 \) of 8). The fluorescence increase was quantified in those seven oocytes showing a global response to vasopressin by dividing maximal fluorescence by basal fluorescence at a specific point within each cell (32). This increase in fluorescence was one-fifth the increase seen in paired-matched oocytes stimulated with vasopressin but not caffeine (\( p < 0.01 \), Wilcoxon signed rank test). These findings suggest that vasopressin-induced Ca\textsuperscript{2+} signals depend on mobilization of Ca\textsuperscript{2+} from caffeine-sensitive stores. This is consistent with the previous observation that caffeine inhibits the IP\textsubscript{3}-mediated increase in current across Ca\textsuperscript{2+}-activated Cl\textsuperscript{−} channels (33). It was also previously observed that caffeine alone fails to increase current across Ca\textsuperscript{2+}-activated Cl\textsuperscript{−} channels (33), so it was concluded that caffeine does not elevate Ca\textsuperscript{2+}. In the current work, however, Ca\textsuperscript{2+} signals in the oocyte were observed directly using confocal fluorescence microscopy. Since caffeine induces an increase in fluo-3 fluorescence even in Ca\textsuperscript{2+}-free medium, these observations suggest that caffeine indeed releases Ca\textsuperscript{2+} from endogenous stores, despite the fact that caffeine has been reported not to increase current across Ca\textsuperscript{2+}-activated Cl\textsuperscript{−} channels (33). Finally, microinjection of IP\textsubscript{3} (10 or 100 \muM) resulted in a single Ca\textsuperscript{2+} wave that radiated across the cell from the point of injection, even when IP\textsubscript{3} was not injected near the plasma membrane (Fig. 8). In contrast, and in agreement with previous observations (19, 33), microinjection of CaCl\textsubscript{2} (1 mM) resulted in a nonpropagating Ca\textsuperscript{2+} wave that extended only 126 ± 8 \muM from the injection site and dissipated within 14 ± 3 s (\( n = 10 \) oocytes, two to four injections/oocyte). Similarly, perfusion with the Ca\textsuperscript{2+} ioni-
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Fig. 9. Effect of ionomycin (1 μM) on cytosolic Ca\textsuperscript{2+} in a Xenopus oocyte loaded with fluo-3. A, confocal image of the oocyte prior to stimulation with ionomycin. A line scan was subsequently performed along the white line (see text). Oocyte diameter in this plane of focus is 838 μm, and depth of focus is 25 μm. B, confocal line scan along the line indicated in A. Each measurement of fluorescence along that line is stacked horizontally on top of subsequent measurements. Fluorescence along the line was measured every 0.3 s, for a total of 2.6 min (top to bottom). Ionomycin induces a gradual increase in fluorescence which is limited to an annular region beneath the cell membrane. This is typical of the pattern seen in all ionomycin-stimulated oocytes. C, confocal image of the oocyte after injection of CaCl\textsubscript{2}. The subcellular distribution of Ca\textsuperscript{2+} signals is typical of the pattern seen in all ionomycin-stimulated oocytes. D, ionomycin-induced change in fluorescence over time at two distinct points within the oocyte. A gradual, sustained increase in Ca\textsuperscript{2+}, which begins after a brief latency period is seen just beneath the cell membrane (solid line), but no increase in Ca\textsuperscript{2+} is evident 113 μm further into the oocyte (dotted line). Ionomycin (1 μM) is added to the perifusate at t = 0 s and is present continuously thereafter.

Ionomycin (1 μM) induced an increase in cytosolic Ca\textsuperscript{2+} that was limited to the subplasmalemma (n = 7; Fig. 9). Thus, a focal increase in cytosolic Ca\textsuperscript{2+}, induced either by microinjection of CaCl\textsubscript{2} or by perfusion with ionomycin, failed to elicit a nondiminished Ca\textsuperscript{2+} wave which propagates across the oocyte. Together, these findings suggest that the subcellular pattern of vasopressin-induced Ca\textsuperscript{2+} signals depends on the sequential release of Ca\textsuperscript{2+}, first from IP\textsubscript{3}-sensitive and then from caffeine-sensitive stores. Although caffeine-sensitive Ca\textsuperscript{2+} stores are thought to be responsible for CICR, the finding by us and others (19, 33) that focal increases in cytosolic Ca\textsuperscript{2+} per se do not lead to CICR suggests that different or additional messengers are needed to trigger Ca\textsuperscript{2+} release from CICR stores (19, 33). Since cytosolic Ca\textsuperscript{2+} modulates Ca\textsuperscript{2+} release from IP\textsubscript{3}-sensitive stores (34, 35), it has been hypothesized that the source of CICR in Xenopus oocytes is the IP\textsubscript{3}-sensitive rather than an IP\textsubscript{3}-insensitive Ca\textsuperscript{2+} pool (19). However, the observation that caffeine-sensitive Ca\textsuperscript{2+} stores can be mobilized even after maximal stimulation with vasopressin (1 μM) or IP\textsubscript{3} (10 or 100 μM) suggests that caffeine-sensitive and IP\textsubscript{3}-sensitive Ca\textsuperscript{2+} stores are distinct.

Vasopressin-induced Ca\textsuperscript{2+} signals regulate a number of functions in the hepatocyte, ranging from glycogenolysis (36) to canalicular contraction (37, 38) to bile secretion (39). Intercellular coordination of Ca\textsuperscript{2+} signals occurs in hepatocytes (11, 40) and other epithelia (41), and such coordination may be dependent in part upon the concentration of the stimulus (42). The subcellular distribution of Ca\textsuperscript{2+} signals within individual cells may also be important for the regulation of epithelial cell function (6), and this work suggests that stimulation of the hepatocyte V\textsubscript{1a} receptor may result in patterns of subcellular Ca\textsuperscript{2+} signaling which are more complex than previously realized. The current work also illustrates that the subcellular pattern of Ca\textsuperscript{2+} release induced by stimulation of the V\textsubscript{1a} receptor differs from the pattern induced by stimulation of either the muscarinic M\textsubscript{2} or M\textsubscript{3} receptor, which are the only other receptors for which such patterns have been reported (9). Specifically, the V\textsubscript{1a}-induced response always begins circumferentially along the cell membrane and radiates inward. In contrast, the M\textsubscript{2}-induced response begins in the cell interior and is manifested by multiple foci of repetitive Ca\textsuperscript{2+} pulsations (9), while the M\textsubscript{3}-induced response at lower acetylcholine concentrations is similar to the M\textsubscript{2} response, but at higher concentrations is a single Ca\textsuperscript{2+} wave that envelops the oocyte (9). The precise relationship between such complex Ca\textsuperscript{2+} signals and Ca\textsuperscript{2+}-mediated cell functions remains to be established, however.

In summary, stimulation of the vasopressin V\textsubscript{1a} receptor in this cell system induces a complex subcellular pattern of Ca\textsuperscript{2+}, signaling which is influenced by the magnitude of the stimulus, the distribution of the surface receptors that are stimulated, and mobilization of endogenous as well as exogenous Ca\textsuperscript{2+}. Furthermore, this Ca\textsuperscript{2+} signaling pattern appears to require sequential mobilization of endogenous Ca\textsuperscript{2+}, first from IP\textsubscript{3}-sensitive and then from caffeine-sensitive Ca\textsuperscript{2+} stores. It has been suggested that the spatial organization of a Ca\textsuperscript{2+} signal is intimately related to its temporal organization (16); these findings support that hypothesis, since we observed that...
the spatial and temporal components of Ca\textsuperscript{2+} signals. Modulation of the subcellular distribution of Ca\textsuperscript{2+} may represent an important potential mechanism by which activation of a single type of receptor can regulate cell function.

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