When single rat hepatocytes were stimulated with the phospholipase C-activating hormone, vasopressin (from 300 pm to 1 pm), the [Ca\textsuperscript{2+}] signals were always "all-or-none" responses. At low concentrations of vasopressin, Ca\textsuperscript{2+} release was maximal because liberation of additional inositol 1,4,5-trisphosphate (IP\textsubscript{3}) by photolysis of its caged precursor at the top of the [Ca\textsuperscript{2+}], spike failed to increase [Ca\textsuperscript{2+}], further. However, if IP\textsubscript{3} was generated by photolysis of caged IP\textsubscript{3} in previously unstimulated cells, [Ca\textsuperscript{2+}], increased immediately, and the magnitude of the response was a graded function of the quantity of IP\textsubscript{3} released. We also analyzed the kinetics of activation of intracellular IP\textsubscript{3} receptor/Ca\textsuperscript{2+} channels by monitoring the quench of sequestered dye by the entry of cytoplasmic Mn\textsuperscript{2+} into fura-2-loaded intracellular IP\textsubscript{3}-sensitive organelles. This Mn\textsuperscript{2+}-induced quench was precipitous and always preceded by a delay inversely related to the vasopressin concentration. In hepatocytes stimulated with 10 nm vasopressin, IP\textsubscript{3} increased slowly, and the half-time of the IP\textsubscript{3} rise was comparable with the latency for the release of intracellular calcium. The slow rise in IP\textsubscript{3} would be predicted to produce accelerating Ca\textsuperscript{2+} release. This is consistent with the results of the Mn\textsuperscript{2+} quench experiments, which revealed accelerating activation of intracellular IP\textsubscript{3}-regulated calcium channels.

We conclude that this accelerating release of Ca\textsuperscript{2+}, which does not occur with instantaneous increases in IP\textsubscript{3} due to flash photolysis, is likely to be important for generating the all-or-none Ca\textsuperscript{2+} mobilization that initiates the processes of intracellular [Ca\textsuperscript{2+}], oscillations.

In hepatocytes, submaximal concentrations of phospholipase C-linked hormones induce regular and repetitive spikes, or base-line oscillations of [Ca\textsuperscript{2+}], first described by Woods et al. (1). Increasing the agonist concentration does not modify the amplitude of the spikes, but it enhances their frequency. Similarly, the latency before the first [Ca\textsuperscript{2+}], spike is inversely related to agonist concentration (2). This constancy of amplitude with varying latency is indicative of "all-or-none" behavior such as that seen in the regenerative process underlying action potentials in excitable cells (3). However, in some situations, for example muscarinic-cholinergic activation of lacrimal acinar cells, [Ca\textsuperscript{2+}], increases with little or no latency, and the average rise in [Ca\textsuperscript{2+}], is graded with concentration of agonist (4). This implies that the mechanisms underlying the initial delayed precipitous Ca\textsuperscript{2+} release may be important in initiating the cyclical pattern of base-line [Ca\textsuperscript{2+}], oscillations. In this study, we have examined the factors that may be responsible for this kinetic behavior by using two different techniques to examine intracellular calcium signaling in vasopressin-stimulated hepatocytes. We have also measured the time course of IP\textsubscript{3} accumulation under similar experimental conditions. We conclude that the slow kinetics of accumulation of IP\textsubscript{3} are important for initiating delayed all-or-none Ca\textsuperscript{2+} release.

**EXPERIMENTAL PROCEDURES**

Preparation of Hepatocytes, Fura-2/AM Loading and Fluorescence Measurements—Procedures for preparing hepatocytes and measurement of [Ca\textsuperscript{2+}], in single fura-2/AM loaded cells were similar to those described by Glennon et al. (5). Hepatocytes were prepared by collagenase perfusion of livers from male Sprague-Dawley rats (200-300 g). Cells were maintained in ice-cold Dulbecco’s modified Eagle’s medium (pH 7.4 with 2% bovine serum albumin) equilibrated with 5% CO\textsubscript{2} and 95% O\textsubscript{2}. Hepatocytes were plated on polylysine-coated glass coverslips (500 ng/ml) and incubated in the presence of 2 μm fura-2/AM at 37 °C under 5% CO\textsubscript{2} for 15-30 min for measurement of [Ca\textsuperscript{2+}]. These conditions resulted in substantial dye present in the cytoplasm, whereas longer incubations (45-60 min) resulted in more compartmentalized fura-2 (for example, Fig. 1) and were thus better suited for Mn\textsuperscript{2+} quench experiments (described below). After the incubation period, the coverslip with attached fura-2-loaded cells was mounted in a Teflon microscope chamber (Bionique Laboratories, Lake Saranac, NY) and washed three times with 1 ml of a Hepes-buffered Krebs-Ringer media (KRH) containing, in mm: NaCl (120), KCl (5.4), MgSO\textsubscript{4} (0.8), CaCl\textsubscript{2} (1.8), glucose (11), Hepes (20), and 0.2% bovine serum albumin at pH 7.4. All experiments in this study were then carried out in medium lacking added Ca\textsuperscript{2+}. Single cell fluorescence measurements were obtained by using a Nikon Diaphot inverted microscope equipped with a 40x Neofluor objective and a Delta-Scan 1 dual excitation system (Photon Technologies International, Princeton, NJ). The excitation wavelengths were 340 and 380 nm, and fluorescence emission was measured at 530 nm. All experiments were performed at room temperature, and experimental agents were applied by exchanging the solutions of the microscope chamber.

Because fura-2 is partly compartmentalized in hepatocytes after loading with the acetoxymethylester form of the dye, we did not calculate apparent values for [Ca\textsuperscript{2+}]. The data are presented in one of two ways. In experiments examining the latency to the rise in [Ca\textsuperscript{2+}], ratios of fluorescence with excitation at 340 and 380 nm (F340/F380), which increase as [Ca\textsuperscript{2+}], increases, are shown. In experiments determining the latency to the activation of intracellular IP\textsubscript{3} receptors in the absence of Ca\textsuperscript{2+} mobilization, intracellular Ca\textsuperscript{2+} pools were discharged by thapsigargin, and Mn\textsuperscript{2+} was allowed to enter the cytoplasm until all cytoplasmic fura-2 was quenched and free Mn\textsuperscript{2+} was present in excess. After a wash to remove all extracellular Mn\textsuperscript{2+}, activation of vasopressin of intracellular IP\textsubscript{3} receptors resulted in the penetration of cytoplasmic Mn\textsuperscript{2+} into the IP\textsubscript{3}-sensitive, Ca\textsuperscript{2+} storage organelle pressur-
Unambiguously through the IP$_3$-regulated channel, and quench of compartmentalized fura-2 occurred. Rather than monitor fluorescence directly with a second spike. Statistical analysis confirmed that the maximal rate of rise of the [Ca$^{2+}$], spike was not significantly affected by increasing the concentration of vasopressin from 300 pm (F$\text{Ca}_1$ spike: 1.77 ± 0.25 s; mean ± S.E., n = 11) to 1 pm (F$\text{Ca}_1$ = 1.31 ± 0.13 s; mean ± S.E., n = 5). Furthermore, as reported previously (9), the release of intracellular Ca$^{2+}$ was all-or-none in that, at the lowest concentrations tested, it appeared that only a percentage of cells showed a Ca$^{2+}$ spike. As shown in Fig. 4, in marked contrast to the situation with vasopressin (all-or-none [Ca$^{2+}$] spike), graded photolysis of the caged precursor produced "quantal" [Ca$^{2+}$] signals that occurred with no measurable delay. Similar results were obtained

![Image](https://example.com/image.jpg)

**FIG. 1.** Compartmentalization of fura-2 in fura-2/AM-loaded hepatocytes. A single hepatocyte loaded with fura-2/AM for 45 min as described under "Experimental Procedures" was treated with 2 mm Mn$^{2+}$ in the absence of external Ca$^{2+}$. This caused a quench of fura-2, which was measured by monitoring F$_{340}$/F$_{380}$ (see "Experimental Procedures"). The initial drop in F$_{340}$ was due to Mn$^{2+}$ quench of cytoplasmic dye because permeabilization of the plasma membrane with 50 μg/ml saponin produced no further quench. Addition of 10 μM of the divalent cationophore, ionomycin, resulted in quench of the remaining indicator, indicating its presence in intracellular organelles.

**FIG. 2.** Delays for Ca$^{2+}$ mobilization and for the quench of internalized dye are inversely related to the concentration of vasopressin. The top panel shows four representative traces of F$_{340}$/F$_{380}$ (related to [Ca$^{2+}$]) from hepatocytes loaded with fura-2/AM for 45–50 min. Traces are aligned to allow comparison of the latencies before Ca$^{2+}$ mobilization. Vasopressin (1 μM, 10, 3, and 1 nM) was added at time zero for each cell (dashed line). Due to the variable amount of compartmentalized dye within each cell, differences in the amplitude of the F$_{340}$/F$_{380}$ responses do not necessarily reflect differences in the magnitude of the responses. As in all of the experiments shown in this study, the extraacellular medium contained no Ca$^{2+}$. The bottom panel shows traces from four other hepatocytes stimulated by vasopressin after the addition of thapsigargin (2 μM) and Mn$^{2+}$ (2 μM) as described under "Experimental Procedures." Only the end of the trace, when vasopressin was added, is shown. Again, the magnitude of the quenches at different concentrations cannot be compared due to the variability of the compartmentalization (from 50 to 80% when individual hepatocytes were loaded for 45 min at 37°C with 2 μM fura-2/AM).

**FIG. 3.** Summarized latencies for [Ca$^{2+}$] spike, and Mn$^{2+}$ quench of sequestered fura-2. Data obtained from experiments performed as in Fig. 2 are summarized. The concentrations of vasopressin used were: 1 μM, 10, 3, and 1 nM. For the mobilization (F$_{340}$/F$_{380}$, closed circles), 5, 3, 12, and 20 cells were used, respectively; for the quench (F$_{340}$/F$_{380}$, open circles), 4, 4, 16, and 17 cells were used. For Ca$^{2+}$ mobilization, the latency is the time from the application of vasopressin to the start of the sudden spike. For F$_{340}$, the delay is the time from the hormonal stimulation to the beginning of the precipitous quench, which was determined from the intersection of the prestimulatory base-line and the tangent at the maximum speed of quench. Results are means ± S.E.

**RESULTS**

When fura-2/AM-loaded hepatocytes were stimulated with different concentrations of vasopressin, an initial spike of calcium (F$_{340}$/F$_{380}$ increase) was observed after a delay that was inversely related to the hormone concentration (Fig. 2, top; Fig. 3, closed circles). Note that all of the experiments shown in this study were carried out with freshly isolated hepatocytes and in nominally calcium-free medium; in our hands under these conditions we seldom saw continuous base-line oscillations or even a second spike. Statistical analysis confirmed that the maximal...
The cell, in a medium lacking external Ca\(^{2+}\), was exposed to increasing amounts of vasopressin (300 PM) activated a maximal [Ca\(^{2+}\)] spike with green and caged-IP, as described under "Experimental Procedures." On top of the delayed Ca\(^{2+}\) peak, a subsequent flash intervals when indicated, and then a near threshold concentration of vasopressin was added. The bottom panel shows the vasopressin [Ca\(^{2+}\)] response on an expanded time scale.

To attempt to understand this apparent paradox (graded versus all-or-none behavior), we next exploited the ability of Mn\(^{2+}\) to quench the entire agonist-sensitive pool, consistent with previously published observations (5, 9, 12, 13). The lag in the onset of the quench thus represents a delay in the activation of the IP\(_{3}\)-regulated channels, allowing Mn\(^{2+}\) entry, rather than a delay in the quenching of the dye by Mn\(^{2+}\) because (i) the latency depends on vasopressin concentration (see below) and (ii) Mn\(^{2+}\) has a very high affinity for fura-2 (Ref. 14), and thus it quenches the dye almost instantaneously and with high binding affinity when it enters the pool. Furthermore, with this experimental protocol, Ca\(^{2+}\), which might compete with Mn\(^{2+}\) for binding to fura-2, is depleted from the intracellular IP\(_{3}\)-sensitive store by prior treatment with thapsigargin.

As previously observed for the experiments measuring the delay to the rise in [Ca\(^{2+}\)], Fig. 2, top; Fig. 3, closed circles), and in confirmation of the findings of Hajnoczy et al. (13), the delay preceding the quench of sequestered dye by Mn\(^{2+}\) was also inversely related to the concentration of vasopressin (Fig. 2 bottom; Fig. 3, open circles). As for the [Ca\(^{2+}\)], spiking experiments, the Mn\(^{2+}\) quench was all-or-none. As shown in Fig. 6, even low concentrations of vasopressin caused a complete quench of the fura-2 trapped in the IP\(_{3}\)-sensitive pool. Note that for the cell in Fig. 6A, a very low concentration of vasopressin was capable of activating quench, and when this was complete, 1 μM vasopressin produced little or no additional quench. The cell in Fig. 6B was apparently less sensitive to vasopressin; the near threshold concentrations of 300 PM and 1 nM did not cause significant quench, but 10 nM vasopressin induced a precipitous quench. Again, a supramaximal concentration of vasopressin caused no additional quench. However, unlike the case for the [Ca\(^{2+}\)], spikes, the maximal rate of Mn\(^{2+}\) quench increased as a function of vasopressin (T\(_{0}\) at 300 PM = 46.5 ± 8.30 s, mean ± S.E., n = 5, and T\(_{0}\) at 1 μM = 19.36 ± 2.74 s, mean ± S.E., n = 5). The ability of Mn\(^{2+}\) to quench the entire agonist-sensitive calcium pool, at a rate that depends on the agonist concentration, has been reported previously (13). Finally, the data summarized in Fig. 3 show that the average latency for full activa-
confirmation of earlier studies (2), we found that the initial [Ca\(^{2+}\)], the phospholipase C-activating hormone, vasopressin. In contrast, when IP₃ was increased more rapidly and was at least transiently higher after a delay that was inversely related to the concentration of vasopressin applied. However, the maximal rate of rise of [Ca\(^{2+}\)], was independent of the vasopressin concentration, and the extent of release appeared to be maximal; i.e., in responsive cells, all IP₃-releasable calcium was mobilized, even by very low concentrations of vasopressin. In contrast, when IP₃ was increased either by photolysis of caged-IP₃ or caged-GPIP₃, the calcium signal always occurred without delay, and the extent of release was graded with the quantity of inositol trisphosphate agonist released.

To determine the reasons for this apparent paradox, we examined the kinetics of the IP₃-induced increase in permeability of intracellular calcium stores by using the intracellular Mn²⁺ quench method first described by Glennon et al. (5) as an assay. This technique relies on the fact that in some cell types, such as hepatocytes, loading with the acetoxymethyl ester derivative of fura-2 leads to dye accumulation in intracellular organelles, including the agonist and IP₃-sensitive stores. When Mn²⁺ is present in the cytoplasm, activation of the intracellular IP₃ receptor/channel allows retrograde flow of Mn²⁺ into the store, resulting in quench of the sequestered dye. By using this approach, we found that, following agonist stimulation, the permeability of the intracellular stores does not immediately increase, as would be predicted by the photolysis of the IP₃ receptor-channel measured as intracellular Mn²⁺ quench of sequestered fura-2. A, the isosbestic fluorescence of a single fura-2/AM loaded hepatocyte was monitored as described under "Experimental Procedures." The cell was treated with thapsigargin and Mn²⁺ and then washed as indicated in Fig. 5. 300 pm vasopressin, 1 μM vasopressin, and ionomycin (10 μM) were added when indicated. This experiment was carried out three times with similar results. B, another hepatocyte, with a different sensitivity to vasopressin was monitored in the same manner. In this case 300 pm, 1 μM, 10 nm, and 1 μM vasopressin were added. Note that for both cells, 1 μM vasopressin does not produce a further quench, but some dye is still quenched by ionomycin addition.

**DISCUSSION**

The purpose of this work was to gain insight into the mechanisms underlying the delayed, all-or-none intracellular Ca²⁺ mobilization observed when hepatocytes are stimulated with the phospholipase C-activating hormone, vasopressin. In confirmation of earlier studies (2), we found that the initial [Ca²⁺], spike due to low concentrations of vasopressin occurred after a delay that was inversely related to the concentration of vasopressin applied.

**Fig. 6. Activation of the IP₃ receptor-channel measured as intracellular Mn²⁺ quench of sequestered fura-2.** A, the isosbestic fluorescence of a single fura-2/AM loaded hepatocyte was monitored as described under “Experimental Procedures.” The cell was treated with thapsigargin and Mn²⁺ and then washed as indicated in Fig. 5. 300 pm vasopressin, 1 μM vasopressin, and ionomycin (10 μM) + Mn²⁺ (5 mM) were added when indicated. This experiment was carried out three times with similar results. B, another hepatocyte, with a different sensitivity to vasopressin was monitored in the same manner. In this case 300 pm, 1 μM, 10 nm, and 1 μM vasopressin were added. Note that for both cells, 1 μM vasopressin does not produce a further quench, but some dye is still quenched by ionomycin addition.

**Fig. 7. Ca²⁺ mobilization and subsequent Mn²⁺ quench of sequestered dye from the same cell.** A single hepatocyte was loaded with fura-2/AM (2 μM) for 45 min at 37°C, and 1 nm vasopressin was added. This caused [Ca²⁺], mobilization (F₃40/F₃80). The cell was washed, and allowed to recover for 30 min. It was then challenged with thapsigargin and Mn²⁺, and washed as in Fig. 5, and then a second stimulation with vasopressin (1 nm) induced a further quench (F₃40/F₃80). Both responses are aligned such that the additions of vasopressin are at time zero (dotted line). This experiment was carried out three times with similar results.

**Fig. 8. Effect of 10 nm vasopressin on (H)IP₃ accumulation.** (H)inositol-labeled cells were treated with 10 nm vasopressin (at t = 0) in calcium-free medium and were then analyzed by HPLC for content of [H]IP₃ at the times indicated. In the experiments indicated by open circles, the cells were pretreated with 2 μM thapsigargin as in the Mn²⁺ quench experiments (n = 4). In two experiments, 2 nm Mn²⁺ was added along with thapsigargin (filled triangles), with no significant effect. Control cells, indicated by filled circles, were treated with a similar quantity of vehicle (dimethyl sulfoxide) (n = 6). The data are expressed as cpm [H]IP₃/cpm [H]inositol (×1,000) (means ± S.E.).
experiments, but increases gradually and then precipitously after a considerable delay that again depends on the concentration of vasopressin.

The simplest explanation for this behavior is that the permeability of the intracellular stores begins to significantly increase only when the level of IP₃ reaches some critical level. If IP₃ rises rapidly, and almost instantaneously as in photolysis of caged precursors, the permeability will rapidly increase and remain constant for a period, and Ca²⁺ will exit at a constant rate. However, if IP₃ levels increase more slowly, as happens when low concentrations of vasopressin are used, then the permeability of the stores will also increase with a similar long time course, causing the apparent accelerating rate of Mn²⁺ quench. In addition, the binding of IP₃ to its receptor is known to be a cooperative process (15), and this could also contribute to the rapid acceleration of activation.

In the Ca²⁺ replete condition, it would also be expected that the rate of Ca²⁺ release would gradually increase. This acceleration could be important in the eventual initiation of a calcium-induced calcium release-triggered (16), all-or-none [Ca²⁺]ᵢ spike. Indeed, when we measured IP₃ accumulation in hepatocytes, we found that with 10 mM vasopressin, about 30 s were required for IP₃ levels to reach their peak. Since the cellular half-life for IP₃ is much shorter (for example, see Ref. 17), this most likely reflects the time course of activation of the phospholipase C-signalizing system by vasopressin. In support of the interpretation that this slow rate of IP₃ accumulation determines the latency to the [Ca²⁺]ᵢ spike, prior depletion of intracellular stores with thapsigargin increased the delay to full activation (measured as intracellular Mn²⁺ quench) and also prolonged the rise in IP₃ to about the same extent (Fig. 8). Note that this may indicate a role for [Ca²⁺]ᵢ, in the initial kinetics of activation of phospholipase C by vasopressin. A role of [Ca²⁺]ᵢ in activating phospholipase C is a component of the model proposed by Meyer and Stryer (18) for calcium spiking. However, our data indicate that only the rate of increase in IP₃ is facilitated by intracellular [Ca²⁺], release; the steady-state level of IP₃ was apparently unaffected. Additional experimental work will be required to ascertain whether or not [Ca²⁺]ᵢ, activation of phospholipase C and slowly oscillating levels of IP₃ play a role in the maintenance of sustained [Ca²⁺]ᵢ spiking in hepatocytes.

Prior reports have pointed out the necessity of calcium priming mechanisms to achieve a delayed, all-or-none calcium response (19). As a result of the current study, we suggest that an important determinant of this calcium signaling pattern is the kinetics of IP₃ accumulation. In fact, previous experimental results suggesting a role of calcium in priming the spiking behavior may reflect calcium effects on the rate of rise of IP₃. Since the kinetics of IP₃ accumulation will differ for different agonists and receptor types, the likelihood of obtaining all-or-none [Ca²⁺]ᵢ spikes and continuous base-line spike oscillations may also similarly vary. An interesting case is the activation of the muscarinic cholinergic receptor, which often does not give delayed all-or-none responses; rather, as was the case in this study with caged compounds, muscarinic stimuli induce immediate, graded rises in [Ca²⁺]ᵢ (20, 21). It has been considered possible that even in the same cell type, the pancreatic acinar cell, a peptide agonist, cholecystokinin, causes delayed all-or-none base-line spikes, while activation of the muscarinic pathway does not (21). We note that the agonists used to activate muscarinic receptors typically have receptor affinities orders of magnitude less than peptide hormones for their receptors. It is expected, therefore, that the rate of equilibration of muscarinic agonists with their binding sites would be much faster than for peptide agonists. Thus, the reason that muscarinic agonists do not generally give rise to delayed, base-line spikes could result from the rapid accumulation of IP₃ to steady-state levels; i.e. the IP₃ kinetics may be more similar to those artificially generated in the flash photolysis experiments.

What might be the physiological consequences of these kinetic distinctions? It has been argued that base-line spiking, generated by very low but prolonged stimulation by peptide hormones, allows for detectable signal-to-noise and thus may be important for long term responses such as those involved in cell growth and differentiation (22). With the neurotransmitter acetylcholine, perhaps it is more important to provide rapidly increasing, graded, and rapidly reversible changes in [Ca²⁺]ᵢ, solely to regulate acute responses, such as secretion or smooth muscle contraction.

In single corticotropin pituitary cells (23), it has been shown that corticotropin releasing factor, a CAMP-stimulating agent, stimulates corticotropin secretion in a concentration-dependent manner. With higher concentrations of corticotropin releasing factor, each single cell releases more corticotropin (i.e. a graded response). In contrast, with the IP₃ generating hormone vasopressin, the extent of corticotropin secretion (measured in single cells with the reverse hemolytic plaque assay method) is not concentration dependent (all-or-none response). Rather, as is the case in hepatocytes, the percentage of responding cells was increased with higher concentrations.

In summary, our data provide evidence that the kinetics of IP₃ accumulation is an important determinant of delayed all-or-none calcium spiking behavior. However, there are likely other significant factors that regulate the kinetics of [Ca²⁺]ᵢ, spiking in oscillating cells. The phenomenon of calcium-induced calcium release by the IP₃ receptor (24, 25) is probably responsible for the all-or-none nature of the spike. Consistent with this suggestion, we observed that the rate of rise of [Ca²⁺]ᵢ, spikes was independent of agonist concentration, but the rate of Mn²⁺ quench, when no Ca²⁺ release occurred, was dependent of the agonist concentration. Based on our data, we suggest that the delay preceding the first spike occurs primarily because initially the IP₃ level is low and the rate of Ca²⁺ release is slow due to the various brakes and negative feedbacks on cytoplasmic calcium (for example, intracellular calcium buffers, pumps, and the inhibitory effects of decreasing luminal calcium concentration). However, as the level of IP₃ increases, the rate of Ca²⁺ release will at some point exceed these negative influences, [Ca²⁺]ᵢ, will begin to rise, and the process of calcium induced calcium release will trigger an all-or-none spike.

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REFERENCES
1. Woods, N. M., Cuthbertson, K. S., and Cobbold, P. H. (1986) Nature 219, 650-652
2. Monch, J. R., Reynolds, E. E., Thomas, A. P., and Williamson, J. R. (1988) J. Biol. Chem. 263, 4569-4575
3. Meyer, T., and Stryer, L. (1991) Annu. Rev. Biochem. 60, 153-174
4. Bird, G. S. J., Fossier, M. F., Obie, J. F., and Putney, J. W., Jr. (1992) J. Biol. Chem. 267, 8425-8438
5. Glennon, R. C., Bird, G. S. J., Kwan, C.-Y., and Putney, J. W., Jr. (1993) J. Biol. Chem. 263, 7130-7133
6. Bird, G. S. J., Fossier, M. F., Obie, J. F., and Putney, J. W., Jr. (1994) J. Biol. Chem. 269, 15382-15390
7. Bird, G. S. J., Fossier, M. F., Obie, J. F., and Putney, J. W., Jr. (1992) J. Biol. Chem. 267, 17722-17725
8. Burgess, G. M., Bird, G. S. J., Obie, J. F., and Putney, J. W., Jr. (1991) J. Biol. Chem. 266, 14727-14731
9. Hajnoczky, G., Noma, T., Hoek, J. B., and Thomas, A. P. (1993) Biochem. J. 293, 413-422
10. Di Virgilio, F., Steinberg, T. H., and Silverstein, S. C. (1990) Cell Calcium 11, 97-112
11. Blatter, L. A., and Wier, W. G. (1990) Biochem. J. 288, 1491-1499
12. Bernard-Rooney, D., Hajnoczky, G., Seitz, M. B., Schneider, T. G., and Thomas, All-or-None Calcium Signaling
All-or-None Calcium Signaling

A. P. (1993) *J. Biol. Chem.* 268, 23601–23610

13. Hajnoczy, G., Lin, C., and Thomas, A. P. (1994) *J. Biol. Chem.* 269, 10280–10287

14. Kwan, C. Y., and Putney, J. W., Jr. (1990) *J. Biol. Chem.* 265, 678–684

15. Meyer, T., Holowka, D., and Stryer, L. (1988) *Science* 240, 653–656

16. Berridge, M. J. (1992) *Adv. Second Messenger Phosphoprotein Res.* 26, 1–223

17. Hughes, A. R., Takemura, H., and Putney, J. W., Jr. (1988) *J. Biol. Chem.* 263, 16314–16319

18. Meyer, T., and Stryer, L. (1988) *Proc. Natl. Acad. Sci. U. S. A.* 85, 5051–5055

19. Rooney, T. A., Sass, E. J., and Thomas, A. P. (1989) *J. Biol. Chem.* 264, 17131–17141

20. Gray, P. T. A. (1988) *J. Physiol. (Lond.*) 406, 35–53

21. Petersen, C. C. H., Toese, E. C., and Petersen, O. H. (1991) *EMBO J.* 10, 527–533

22. Putney, J. W., Jr., and Bird, G. S. J. (1990) *Endocrinol. Rev.* 14, 610–631

23. Canny, B. J., Jia, L. G., and Leong, D. A. (1992) *J. Biol. Chem.* 267, 8325–8329

24. Finch, E. A., Turner, T. J., and Goldin, S. M. (1991) *Science* 252, 443–446

25. Missiaen, L., Taylor, C. W., and Berridge, M. J. (1991) *Nature* 351, 241–244