Intracellular Ca^{2+} pools in Jurkat T-lymphocytes

Andreas H. GUSE,* Edith ROTH and Frank EMMRICH

Max-Planck-Society, Clinical Research Unit for Rheumatology/Immunology at the Institute for Clinical Immunology of the University of Erlangen-Nürnberg, Schwabachanlage 10, D-8520 Erlangen, Germany

Jurkat T-lymphocytes comprise at least four intracellular Ca^{2+} pools. Pool I was agonist-sensitive and contained $23 \pm 8\%$ ($n = 18$) of the total Ca^{2+} -storage capacity, as shown in intact cells in the presence of EGTA. The time courses of the agonist-induced formation of $\text{Ins}(1,4,5)\text{P}_3$ and of the Ca^{2+} release from pool I were nearly superimposable, indicating that the agonist-sensitive pool I is emptied by $\text{Ins}(1,4,5)\text{P}_3$. Likewise, in permeabilized cells, the size of the $\text{Ins}(1,4,5)\text{P}_3$ -sensitive Ca^{2+} pool I was $27 \pm 11\%$ ($n = 14$). Pool II contained $26 \pm 5\%$ ($n = 9$) of intracellularly stored Ca^{2+} and was liberated by thapsigargin, an inhibitor of the endoplasmic-reticulum (ER) Ca^{2+} -ATPase. Addition of thapsigargin before addition of agonist abolished the agonist-induced Ca^{2+} release in both intact and permeabilized cells, indicating that pool I is a subcompartment of the ER Ca^{2+} pool. The content of this ER Ca^{2+} pool (pools I and II) amounted to $51 \pm 15\%$ ($n = 9$) in intact cells and $49 \pm 16\%$ ($n = 16$) in permeabilized cells. Caffeine released Ca^{2+} even when the ER

pool (pools I and II) was emptied by previous addition of thapsigargin, indicating the presence of a third pool independent of pools I and II. Pool III contained $23 \pm 6\%$ ($n = 8$) in intact cells, but $41 \pm 8\%$ ($n = 5$) in permeabilized cells. The remaining intracellularly stored Ca^{2+} was released by addition of the Ca^{2+} ionophore ionomycin. This fourth pool contained $27 \pm 8\%$ ($n = 9$) in intact cells, but less than 10% in permeabilized cells. The size of pool III was increased when pools I and II were emptied before addition of caffeine, whereas the size of pool IV was decreased under such conditions. In conclusion, this first comprehensive description of intracellular Ca^{2+} pools in Jurkat T-lymphocytes demonstrates the presence of four different Ca^{2+} pools, provides estimates of their sizes and describes relationships between each other. Release of Ca^{2+} from pool I [$\text{Ins}(1,4,5)\text{P}_3$ -sensitive] has previously been shown to play a major role in T-cell activation, whereas the physiological role of pools II–IV remains to be established.

INTRODUCTION

Stimulation of a rapidly growing number of receptors results in the formation of inositol polyphosphates and subsequently in an increase of the free cytosolic Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$; for reviews see [1–3]). In many cell types the increase in $[\text{Ca}^{2+}]_i$ consists of two different processes, namely (i) release of Ca^{2+} from intracellular non-mitochondrial stores and (ii) Ca^{2+} entry via the plasma membrane. Although a current opinion favours Ca^{2+} entry as a process that works via refilling of intracellular stores emptied by $\text{Ins}(1,4,5)\text{P}_3$, recently published data support the idea of an $\text{Ins}(1,3,4,5)\text{P}_4$ -induced Ca^{2+} influx via the plasma membrane directly into the cytosol [4–6].

Agonist- and caffeine (Cf)-sensitive intracellular Ca^{2+} pools have been described in a number of cell types, such as smooth-muscle cells, neurons, endocrine cells and pancreatic acinar cells [7–11]. Main candidates for the $\text{Ins}(1,4,5)\text{P}_3$ -sensitive Ca^{2+} store in non-muscle cells are the endoplasmic reticulum (ER) [12] and the ‘calciosomes’ [13]. Only recently $\text{Ins}(1,4,5)\text{P}_3$ -binding intracellular compartments were purified from rat liver [14] and from HL-60 cells [15]. Agonist-insensitive Ca^{2+} pools have been characterized only in a minority of cells, e.g. PC12 cells [16].

In the work presented here, we have identified the $\text{Ins}(1,4,5)\text{P}_3$ -sensitive Ca^{2+} store of T-lymphocytes as a subcompartment of the ER. Furthermore we report on three further distinct Ca^{2+} pools not yet described in T-cells. One of these pools is emptied by Cf, an agonist of the ryanodine receptor. From this result, Ca^{2+} -induced Ca^{2+} release (CICR) might be involved in the propagation of the Ca^{2+} signal in T-cells.

EXPERIMENTAL

Materials

Thapsigargin (Tg), ionomycin, Fura2 free acid and Fura2/AM were from Calbiochem, Bad Soden, Germany. Cf, dithiothreitol, saponin, oligomycin, phosphocreatine and ATP were bought from Sigma Chemicals, Deisenhofen, Germany. $\text{Ins}(1,4,5)\text{P}_3$ was purchased from Bio-Trend, Köln, Germany. Anti-CD3 monoclonal antibody OKT3 was purified from hybridoma supernatant on Q-Sepharose, Phenyl-Sepharose and Superdex 200 (Pharmacia, Freiburg, Germany). All other chemicals were of the highest purity available.

Cell culture

Jurkat T-lymphocytes were cultured as described recently [17].

Measurement of $[\text{Ca}^{2+}]_i$ in intact cells

$[\text{Ca}^{2+}]_i$ was measured by using the fluorescent indicator Fura2/AM as described by Treves et al. [18]. In brief, 10^7 cells were loaded with Fura2/AM, and fluorescence was determined in batches of 1.5×10^6 cells with an LS-3B fluorimeter (Perkin–Elmer) at excitation and emission wavelengths of 340 ± 3 nm and 505 ± 10 nm respectively. Measurements were carried out at 20 °C. In all experiments, shortly before addition of stimulants (1–2 min), EGTA/Tris (3 mM/30 mM, pH 7.4) was added to chelate extracellular Ca^{2+} . At the end of each single measurement the maximal fluorescence (F_{max}) was determined by

Abbreviations used: $[\text{Ca}^{2+}]_i$, free cytosolic Ca^{2+} concentration; Cf, caffeine; CICR, Ca^{2+} -induced Ca^{2+} release; ER, endoplasmic reticulum; Tg, thapsigargin.

* To whom correspondence and reprint requests should be addressed.

addition of ionomycin ($2 \mu\text{M}$) and Ca^{2+} (5 mM), and the minimal fluorescence ($F_{\min.}$) was determined by addition of EGTA/Tris ($4 \text{ mM}/40 \text{ mM}$, pH 7.4). $[\text{Ca}^{2+}]_i$ was calculated from the formula $[\text{Ca}^{2+}]_i = K_D[(F - F_{\min.})/(F_{\max.} - F)]$ where K_D is 224 nM [19] and F is fluorescence. Finally, data were corrected graphically and/or numerically for artefacts due to addition of large volumes (e.g. Cf was added in $200 \mu\text{l}$ volumes to the cuvette, with a concomitant decrease in the fluorescence) or to autofluorescence of compounds or of cells.

Preparation of permeabilized cells

Permeabilized Jurkat T-cells were prepared and Ca^{2+} measurements were carried out exactly as described previously [6].

H.p.l.c. analysis of $\text{Ins}(1,4,5)\text{P}_3$

Intracellular masses of $\text{Ins}(1,4,5)\text{P}_3$ were determined by anion-exchange h.p.l.c. and a post-column complexometric dye system as described recently [17].

RESULTS

Addition of EGTA to chelate extracellular Ca^{2+} allows measurement of agonist-induced Ca^{2+} release from intracellular pools in intact Fura2-loaded cells. We have recently demonstrated that $\text{Ins}(1,4,5)\text{P}_3$ released Ca^{2+} from permeabilized Jurkat and HPB.ALL T-lymphocytes [6]. In Figure 1 the time course of the agonist-induced Ca^{2+} release in intact cells in the presence of EGTA and the time course of agonist-induced $\text{Ins}(1,4,5)\text{P}_3$ formation as measured by anion-exchange h.p.l.c. mass analysis are shown. As the curves are nearly superimposable, further evidence is provided that agonist-induced $\text{Ins}(1,4,5)\text{P}_3$ -mediated Ca^{2+} release from an intracellular store is a transient short-lived phase in early T-cell activation.

However, $\text{Ins}(1,4,5)\text{P}_3$ released only a minor part of the total Ca^{2+} -storage capacity of Jurkat T-lymphocytes. In a series of experiments using different pharmacological tools such as the ER Ca^{2+} -ATPase inhibitor Tg, the ryanodine-receptor agonist Cf and the Ca^{2+} ionophore ionomycin, the presence and sizes of at least four different Ca^{2+} pools were investigated. Ca^{2+} -pool sizes were calculated as follows. Ionomycin released the total Ca^{2+} -storage capacity of intact Fura2-loaded cells as well as of permeabilized cells. The value obtained by this procedure was regarded as 100 % of the intracellular Ca^{2+} -storage capacity. Portions released by individual agonists and/or drugs are expressed as percentages in relation to the value obtained with ionomycin. Since individual stimuli can probably cause loading of other pools, e.g. by activated Ca^{2+} -ATPase or by direct Ca^{2+} transfer from pool to pool, the order of addition of the drugs was reversed or changed when possible, i.e. if an individual pool could be emptied independently.

Agonists such as the monoclonal anti-CD3 antibody OKT3 released Ca^{2+} from an $\text{Ins}(1,4,5)\text{P}_3$ -sensitive pool (pool I). In intact cells as well as in permeabilized cells, this pool appears to be a subcompartment of the ER, since Tg released Ca^{2+} when given after OKT3 in intact cells or after $\text{Ins}(1,4,5)\text{P}_3$ in permeabilized cells (Figures 2a and 3a), but OKT3 in intact cells or $\text{Ins}(1,4,5)\text{P}_3$ in permeabilized cells both failed to release Ca^{2+} when given after Tg (Figures 2b and 3b). Moreover, the proportion of Ca^{2+} released by Tg when given after OKT3 or after $\text{Ins}(1,4,5)\text{P}_3$ was significantly decreased (Figures 2 and 3).

The size of the ER Ca^{2+} store as emptied by Tg was calculated to be $51 \pm 15 \%$ ($n = 9$) of the total Ca^{2+} -storage capacity of intact cells and $49 \pm 16 \%$ ($n = 13$) in permeabilized cells. One section of this store, pool I, was $\text{Ins}(1,4,5)\text{P}_3$ -sensitive and comprised approximately one-half, as demonstrated in intact and permeabilized cells, whereas the second part, pool II, was not $\text{Ins}(1,4,5)\text{P}_3$ -sensitive and also contained half of the Tg-sensitive Ca^{2+} pool.

To test whether CICR plays a role in Jurkat T-lymphocytes, the effects of Cf on Ca^{2+} release from intracellular stores were investigated. Cf released Ca^{2+} in a dose-dependent manner in intact Fura2-loaded cells in the absence of extracellular Ca^{2+} (Figure 4). Optimal concentrations of Cf were about 15 mM . Higher concentrations did not improve the yield, probably due to the low solubility of Cf in water. A surprising result, however, was that Cf induced Ca^{2+} release when added after OKT3 and also when given after Tg in intact cells (Figures 5a and 5b).

Figure 1 Time courses of $\text{Ins}(1,4,5)\text{P}_3$ and $[\text{Ca}^{2+}]_i$.

Jurkat T-cells were stimulated by OKT3 ($10 \mu\text{g}/\text{ml}$; time point 0 min) briefly after addition of EGTA (3 mM). $\text{Ins}(1,4,5)\text{P}_3$ (a) was measured as detailed elsewhere [17], and $[\text{Ca}^{2+}]_i$ (b) was measured in Fura2-loaded cells as described in the Experimental section. For $[\text{Ca}^{2+}]_i$ a representative tracing out of five is shown; values for $\text{Ins}(1,4,5)\text{P}_3$ are presented as means $\pm$ S.D. ($n = 3$).

Figure 2 Effects of OKT3 and Tg on $[\text{Ca}^{2+}]_i$.

(a) After addition of EGTA to the medium, Fura2-loaded Jurkat T-cells were stimulated by OKT3 ($10 \mu\text{g}/\text{ml}$) and subsequently by Tg ($1 \mu\text{M}$). After emptying of the ER Ca^{2+} store, ionomycin (IM; $2 \mu\text{M}$) was added three times to ensure emptying of the total Ca^{2+} -storage capacity. (b) After addition of EGTA to the medium, the ER Ca^{2+} pool of Fura2-loaded Jurkat T cells was emptied by Tg ($1 \mu\text{M}$). Thereafter OKT3 ($10 \mu\text{g}/\text{ml}$) did not liberate further Ca^{2+} , whereas ionomycin (IM; $2 \mu\text{M}$) did. Representative tracings of at least four experiments are displayed.

Figure 3 Effects of $\text{Ins}(1,4,5)\text{P}_3$ and Tg on Ca^{2+} release in permeabilized cells

Saponin-permeabilized cells were prepared as described in [6] and a portion of $(6-7) \times 10^7$ cells was challenged by $\text{Ins}(1,4,5)\text{P}_3$ ($2 \mu\text{M}$), Tg ($1 \mu\text{M}$) and ionomycin (IM; $2 \mu\text{M}$) in the presence of Fura2 free acid ($1 \mu\text{M}$).

Likewise, in permeabilized cells Cf released Ca^{2+} when pool I or the Tg-sensitive pool had been liberated by $\text{Ins}(1,4,5)\text{P}_3$ or Tg previously (Figures 6a and 6b). An explanation for the different Ca^{2+} tracings in Figures 6(a) and 6(b) is that in Figure 6(a) Ca^{2+} release from pool I by $\text{Ins}(1,4,5)\text{P}_3$ was transient, due to Ca^{2+} -ATPase activity. Likewise, Ca^{2+} released by Cf was pumped back quickly. In Figure 6(b) Tg induced a sustained elevated level of $[\text{Ca}^{2+}]$ in the cuvette, due to its inhibitory effect on the Ca^{2+} -ATPase. Therefore, further Ca^{2+} release by Cf also resulted in a sustained signal due to the ongoing inhibitory action of Tg.

These results from both intact and permeabilized cells indicate a third, Cf-sensitive, Ca^{2+} pool (pool III), which is not a part of the ER Ca^{2+} store. The size of pool III differed, depending on the experimental conditions (Table 1). When Cf was added as the first drug or added directly after OKT3, $10 \pm 3\%$ ($n = 8$) and $14 \pm 6\%$ ($n = 8$) respectively of the Ca^{2+} -storage capacity was

Figure 4 Effect of caffeine on $[\text{Ca}^{2+}]_i$

(a) After addition of EGTA to the medium, Fura2-loaded Jurkat T-cells were challenged with Cf (15 mM), OKT3 ($10 \mu\text{g/ml}$) and IM ($2 \mu\text{M}$). (b) Dose-response curve for Cf in Jurkat T-cells. Data are presented as means $\pm$ S.D. ($n = 5-9$).

Figure 5 The caffeine-sensitive Ca^{2+} pool is independent of the ER Ca^{2+} pool

(a) After addition of EGTA to the medium, the agonist-sensitive Ca^{2+} pool of Fura2-loaded Jurkat T cells was emptied by OKT3 ($10 \mu\text{g/ml}$). A second addition of OKT3 did not release further Ca^{2+} . Subsequent addition of Cf (15 mM) released Ca^{2+} from an agonist-independent pool. (b) After addition of EGTA to the medium, the ER Ca^{2+} pool was emptied by Tg ($1 \mu\text{M}$). Subsequent addition of Cf (15 mM) released Ca^{2+} from a pool different from the ER. Tracings representative of at least four experiments are shown. Abbreviation: IM, ionomycin.

Figure 6 Caffeine-induced Ca^{2+} release in permeabilized cells

Saponin-permeabilized cells were prepared as described in [6] and a portion of $(6-7) \times 10^7$ cells was challenged by $\text{Ins}(1,4,5)\text{P}_3$ ($2 \mu\text{M}$), Tg ($1 \mu\text{M}$), Cf (15 mM) and ionomycin (IM; $2 \mu\text{M}$) in the presence of Fura2/free acid ($1 \mu\text{M}$). Representative tracings of at least four experiments are displayed.

Table 1 Ca^{2+} pools in intact and permeabilized Jurkat T-cells

Ca^{2+} release from individual pools was measured as described in the Experimental section. Ca^{2+} pool sizes were calculated as detailed in the Results section. Data are presented as means $\pm$ S.D. Abbreviations: sens., sensitive; IM, ionomycin.

Ca^{2+} pool	Pool size (% of total capacity)	
	Intact cells	Permeabilized cells
I (agonist-sens.)	23 ± 8 ($n = 18$)	—
I (InsP_3 -sens.)	—	27 ± 11 ($n = 14$)
II (Tg-sens., InsP_3 -insens.)	26 ± 5 ($n = 9$)	28 ± 9 ($n = 5$)
I + II (Tg-sens.)	51 ± 15 ($n = 9$)	49 ± 16 ($n = 13$)
III (Cf-sens.)	10 ± 3 ($n = 8$)	14 ± 7 ($n = 4$)
Cf given as first drug		
IV (IM-sens.)	46 ± 9 ($n = 6$)	48 ± 14 ($n = 7$)
III (Cf-sens.)	14 ± 6 ($n = 8$)	29 ± 10 ($n = 10$)
Cf given after OKT3		
IV (IM-sens.)	40 ± 14 ($n = 8$)	32 ± 13 ($n = 5$)
III (Cf-sens.)	23 ± 6 ($n = 8$)	42 ± 14 ($n = 13$)
Cf given after Tg		
IV (IM-sens.)	27 ± 8 ($n = 9$)	< 10 ($n = 6$)

released in intact cells. Moreover, when Tg was added before stimulation with Cf, a significantly larger portion of Ca^{2+} ($23 \pm 6\%$, $n = 8$) was released. These increases of pool III correlated well with decreases in the size of pool IV under identical conditions in intact cells (Table 1). Similarly, even more pronounced effects were observed in permeabilized cells (Table 1). Although the size of the Cf-sensitive pool III increased from $14 \pm 7\%$ ($n = 4$; Cf added as the first drug) to $42 \pm 10\%$ ($n = 13$; Cf added after Tg), a concomitant decrease in pool IV from $48 \pm 11\%$ ($n = 7$; Cf added as the first drug) to $< 10\%$ ($n = 6$; Cf added after Tg) was observed (Table 1). These data indicate a Ca^{2+} flux from pool IV into pool III under conditions where pool I and/or pool II are empty.

The rest of the intracellularly pooled Ca^{2+} could only be released by the Ca^{2+} -ionophore ionomycin. This fourth pool contained $27 \pm 8\%$ of the Ca^{2+} -storage capacity of intact cells and $< 10\%$ in permeabilized cells under conditions where Cf-induced Ca^{2+} release reached a maximum.

DISCUSSION

Only recently, agonist-sensitive and -insensitive Ca^{2+} pools have been examined extensively in intact PC12 cells, a cell line commonly used as model for neurosecretory cells [16,20]. In this study on Jurkat T-lymphocytes we have used a similar methodology to that of Zachetti et al. [16] and Fasolato et al. [20], but in extension a verification of the data was achieved by using saponin-permeabilized cells as a second model system. Regarding pool types and sizes, similarities and differences between the PC12 cells and the Jurkat T cell line were observed. The $\text{Ins}(1,4,5)\text{P}_3$ -sensitive pool was calculated to contain about 25% of the total PC12-cell exchangeable Ca^{2+} [20]. We found that 23–27% of the total Ca^{2+} -storage capacity was agonist- and $\text{Ins}(1,4,5)\text{P}_3$ -sensitive in Jurkat T cells, which is in good agreement with results for PC12 cells. Since the size of the agonist-sensitive pool as determined in intact Jurkat cells and the size of the $\text{Ins}(1,4,5)\text{P}_3$ -sensitive pool as determined in permeabilized cells were not significantly different, it can be assumed that the agonist OKT3 ($10 \mu\text{g/ml}$), via formation of $\text{Ins}(1,4,5)\text{P}_3$, saturated intracellular $\text{Ins}(1,4,5)\text{P}_3$ receptors and thus emptied pool I completely. Furthermore, in PC12 cells as well as in Jurkat T cells, the $\text{Ins}(1,4,5)\text{P}_3$ -sensitive store is emptied by the Ca^{2+} -ATPase inhibitor Tg, whereas agonists do not empty the whole ER pool, indicating that the $\text{Ins}(1,4,5)\text{P}_3$ -sensitive pool is not located in the entire ER, but appears to be an ER subcompartment ([20]; and the Results section). This finding is not due to sub-maximal $\text{Ins}(1,4,5)\text{P}_3$ production in agonist-stimulated cells, as pointed out above. Moreover, our finding that pool I is part of the ER is also supported by immuno-gold labelling of the $\text{Ins}(1,4,5)\text{P}_3$ receptor in an ER subcompartment in cerebellar Purkinje cells [21]. However, in a recent report, $\text{Ins}(1,4,5)\text{P}_3$ -binding vesicles from rat liver did not co-purify with ER marker enzymes [14]. Instead, $\text{Ins}(1,4,5)\text{P}_3$ -binding vesicles showed enriched plasma-membrane marker activity, which was altered by disruption of microfilaments by cytochalasin B [14]. It was concluded that $\text{Ins}(1,4,5)\text{P}_3$ -sensitive Ca^{2+} stores are linked to the plasma membrane by actin microfilaments. In contrast, for HL-60 cells it was reported that an $\text{Ins}(1,4,5)\text{P}_3$ -binding compartment moderately co-purified with ER markers, but not with plasma-membrane markers [15]. This report also favoured the concept of the $\text{Ins}(1,4,5)\text{P}_3$ -sensitive pool to be a subcompartment of the ER, perhaps the 'calciosomes' [16].

As novel observations, we report here (i) that a Cf-sensitive Ca^{2+} -pool exists in Jurkat T-lymphocytes, as demonstrated in both intact and permeabilized cells, and (ii) that the Cf-sensitive

pool increases in size, probably by a direct transfer of Ca^{2+} from pool IV, when pool I and/or pool II had been emptied previously. In contrast with PC12 cells, the Cf-sensitive pool in Jurkat T cells is not identical to the $\text{Ins}(1,4,5)\text{P}_3$ -sensitive store [16]. Also, the Cf-sensitive pool of Jurkat T cells is not a part of the ER, since prior addition of Tg enhanced the effect on Cf-induced Ca^{2+} release, rather than diminished or inhibited it. This observation is consistent with two recent reports demonstrating (i) independence of the $\text{Ins}(1,4,5)\text{P}_3$ - and Cf-sensitive stores [11], and (ii) the Tg-insensitivity of a Cf-sensitive Ca^{2+} store in permeabilized adrenal chromaffin cells [22]. Similar results were obtained by Stauderman et al. [23] in the same cell type, although they postulated a third pool which, beside the $\text{Ins}(1,4,5)\text{P}_3$ -sensitive and the Cf-sensitive pools, was sensitive to both drugs. Furthermore, by using Ca^{2+} monitoring at the single-cell level, it was shown that CICR plays a major role in agonist-induced Ca^{2+} signalling in the chromaffin cells [23]. Whether this also applies to T-cells remains to be demonstrated. Since the time courses of agonist-induced formation of $\text{Ins}(1,4,5)\text{P}_3$ and $[\text{Ca}^{2+}]_i$ are nearly superimposable under Ca^{2+} -free conditions (Figure 1), CICR seems not to be required. On the other hand, emptying of pools I [$\text{Ins}(1,4,5)\text{P}_3$ -sensitive!] and II caused an increase in the size of pool III, indicating its potential role as source for CICR and thereby for propagation of Ca^{2+} waves as hypothesized by Berridge and Irvine [2]. However, further studies on the function of different Ca^{2+} stores during T-cell stimulation will have to consider the influence of Ca^{2+} entry from the extracellular space. This might be of particular importance, since we observed that depletion of some of the pools resulted in rapid refilling from the extracellular space (A. H. Guse, unpublished work).

The Max-Planck Research Units for Rheumatology are funded by the Federal Ministry for Research and Technology (BMFT grant no. 01 VM 8702).

REFERENCES

- Berridge, M. J. (1987) *Annu. Rev. Biochem.* **56**, 159–193
- Berridge, M. J. and Irvine, R. F. (1989) *Nature (London)* **341**, 197–205
- Catt, K. J., Hunyady, L. and Balla, T. (1991) *J. Bioenerg. Biomembr.* **23**, 7–27
- Lückhoff, A. and Clapham, D. E. (1992) *Nature (London)* **355**, 356–358
- Khan, A. A., Steiner, J. P. and Snyder, S. H. (1992) *Proc. Natl. Acad. Sci. U.S.A.* **89**, 2849–2853
- Guse, A. H., Roth, E. and Emmrich, F. (1992) *Biochem. J.* **288**, 489–495
- Iino, M., Kobayashi, T. and Endo, M. (1988) *Biochem. Biophys. Res. Commun.* **152**, 417–422
- Lipscombe, D., Madison, D. V., Poenie, M., Reuter, H. and Tsien, R. Y. (1988) *Proc. Natl. Acad. Sci. U.S.A.* **85**, 2398–2402
- Malgaroli, A., Fesce, R. and Meldolesi, J. (1990) *J. Biol. Chem.* **265**, 3005–3008
- Wakui, M., Osipchuk, Y. V. and Petersen, O. H. (1990) *Cell* **63**, 1025–1032
- Cheek, T. R., Barry, V. A., Berridge, M. J. and Missiaen, L. (1991) *Biochem. J.* **275**, 697–701
- Streb, H., Irvine, R. F., Berridge, M. J. and Schulz, I. (1983) *Nature (London)* **306**, 67–68
- Volpe, P., Krause, K.-H., Hashimoto, S., Zorzato, F., Pozzan, T., Meldolesi, J. and Lew, D. P. (1988) *Proc. Natl. Acad. Sci. U.S.A.* **85**, 1091–1095
- Rossier, M. F., Bird, G. St. J. and Putney, J. W., Jr. (1991) *Biochem. J.* **274**, 643–650
- VanDelden, C., Favre, C., Spät, A., Cerny, E., Krause, K.-H. and Lew, D. P. (1992) *Biochem. J.* **281**, 651–656
- Zachetti, D., Clementi, E., Fasolato, C., Lorenzon, P., Zottini, M., Grohovaz, F., Fumagalli, G., Pozzan, T. and Meldolesi, J. (1991) *J. Biol. Chem.* **266**, 20152–20158
- Guse, A. H. and Emmrich, F. (1991) *J. Biol. Chem.* **266**, 24498–24502
- Treves, S., DiVirgilio, F., Cerundolo, V., Zanollo, P., Collavo, D. and Pozzan, T. (1987) *J. Exp. Med.* **166**, 33–42
- Gryniewicz, G., Poenie, M. and Tsien, R. Y. (1985) *J. Biol. Chem.* **260**, 3440–3450
- Fasolato, C., Zittini, M., Clementi, E., Zachetti, D., Meldolesi, J. and Pozzan, T. (1991) *J. Biol. Chem.* **266**, 20159–20167
- Satoh, T., Ross, C. A., Villa, A., Supattapone, S., Pozzan, T., Snyder, S. H. and Meldolesi, J. (1990) *J. Cell Biol.* **111**, 615–624
- Robinson, I. M. and Burgoyne, R. D. (1991) *J. Neurochem.* **56**, 1587–1593
- Stauderman, K., McKinney, R. A. and Murawsky, M. M. (1991) *Biochem. J.* **278**, 643–650