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Bovine adrenal chromaffin cells contain an inositol 1,4,5-trisphosphate-insensitive but caffeine-sensitive Ca^{2+} store that can be regulated by intraluminal free Ca^{2+}

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We have characterized some properties of the caffeine-sensitive Ca^{2+} store in bovine chromaffin cells. Addition of 10 mM-caffeine to permeabilized cells that were allowed to sequester Ca^{2+} in the presence of the precipitating anion pyrophosphate induced a transient rise in free Ca^{2+} concentration that was blocked by 10 μM-Ruthenium Red. Caffeine was able to release Ca^{2+} after the InsP_3-sensitive Ca^{2+} pool had been completely emptied, and 10 μM-InsP_3 still released Ca^{2+} in the presence of a high dose (50 mM) of caffeine, indicating that there are selectively sensitive Ca^{2+} pools in these cells. The progressive hydrolysis of pyrophosphate by a cytosolic pyrophosphatase induced a spontaneous Ca^{2+} release after a latency. Caffeine prevented this spontaneous Ca^{2+} release, indicating that the pyrophosphate-sensitive Ca^{2+} pool was caffeine-sensitive. On varying the free Ca^{2+} concentration within the caffeine-sensitive pool (by using methylenediphosphonic acid, pyrophosphate or no precipitating anion), we observed that the Ca^{2+}-releasing effect of caffeine was dependent on an elevated intraluminal free Ca^{2+} concentration. In conclusion, the caffeine-sensitive Ca^{2+} store in bovine chromaffin cells is largely distinct from the InsP_3-sensitive Ca^{2+} store, and its release mechanism shares characteristics with the ryanodine receptor of muscle cells.

INTRODUCTION

Many hormones, neurotransmitters and other extracellular stimuli activate the phosphoinositide cascade by binding to cell-surface receptors that are linked to phospholipase C. The subsequent increase in inositol 1,4,5-trisphosphate (InsP_3) leads to release of Ca^{2+} from an InsP_3-sensitive Ca^{2+} store (Berridge, 1987; Putney, 1987) that is probably some part of the endoplasmic reticulum (Meldolesi *et al.*, 1990).

In bovine adrenal chromaffin cells, both the InsP_3-sensitive store (Burgoyne *et al.*, 1989) and the intracellular Ca^{2+} signal (Cheek *et al.*, 1989) appear to be spatially restricted to a small region near the nucleus. By contrast, a caffeine-sensitive Ca^{2+} store is diffusely distributed throughout the cell (Burgoyne *et al.*, 1989; Cheek *et al.*, 1990). Caffeine-sensitive Ca^{2+} stores have also been identified in some smooth-muscle cells (Saida & van Breemen, 1983; Kanaide *et al.*, 1987), sympathetic (Lipscombe *et al.*, 1988*a*,*b*) and sensory neurons (Thayer *et al.*, 1988), pancreatic acinar cells (Osipchuk *et al.*, 1990), and rat chromaffin cells (Malgaroli *et al.*, 1990). The role that these stores play in Ca^{2+} signalling in non-muscle cells is unknown, but possibilities include the generation of Ca^{2+} oscillations and the propagation of Ca^{2+} waves by a process of Ca^{2+}-induced Ca^{2+} release (Berridge & Irvine, 1989; Berridge, 1990; Rooney *et al.*, 1990) similar to that first described in skeletal muscle (for review see Endo, 1977). We have used permeabilized cells incubated with fluorescent Ca^{2+} indicator fluo-3 to investigate some characteristics of the caffeine-sensitive store in bovine chromaffin cells. The results show that chromaffin cells contain a caffeine-sensitive store that is InsP_3-insensitive, and furthermore that release of Ca^{2+} from the caffeine-sensitive store can be regulated by the concentration of free Ca^{2+} ($[Ca^{2+}]$) in the lumen of the store. This characteristic of the caffeine-sensitive store would be consistent with it playing a role in the propagation of sub-plasmalemmal Ca^{2+} signals in these cells.

MATERIALS AND METHODS

Chromaffin cells were dissociated from bovine adrenal medullas as described previously (Cheek *et al.*, 1990) and used within 4 h. Cells were washed twice and finally resuspended at a density of 5×10^7 cells/ml in the following medium: 120 mM-KCl, 20 mM-Hepes (pH 7.2), 1 mM-$MgCl_2$, 1 mM-ATP, 10 mM-NaN_3, 25 mM-phosphocreatine, 25 units of creatine kinase/ml and 5 μM fluo-3 free acid. Under these conditions, the cells occupied about 33% of the incubation volume. Cell suspensions (300 μl) were transferred to a stirred thermostatically controlled cuvette at 37 °C, and Ca^{2+} uptake was started by adding 50 μM-digitonin, 25 mM-phosphocreatine and (unless otherwise indicated) 5.8 mM-pyrophosphate. Pyrophosphate was used to increase the Ca^{2+}-accumulating capacity of the non-mitochondrial Ca^{2+} pools (Palade, 1987). Fluorescence was continuously monitored with a Perkin–Elmer LS-5 luminescence spectrometer (excitation 503 nm, emission 530 nm). The traces were calibrated from the equation:

$$[Ca^{2+}] = K_d \frac{F - F_{min.}}{F_{max.} - F}$$

$F_{max.}$ was obtained by adding 1 mM-$CaCl_2$ to the cuvette at the end of the experiment, and $F_{min.}$ by subsequently adding 10 mM-K-EGTA (pH 7.2). A K_d of 864 nM was used (Merritt *et al.*, 1990). We used caffeine instead of ryanodine to activate the putative Ca^{2+}-induced Ca^{2+}-release channel in the cells because ryanodine binds very slowly to the channel ($t_{\frac{1}{2}} = 24.8$ min), and because the $[Ca^{2+}]$ required for one-half of optimal binding is 20 μM, which is 1–2 orders of magnitude above our working range (Pessah *et al.*, 1986). Where caffeine was added during an experiment, a dose of 10 mM was used. This dose was not maximal, but the poor solubility of caffeine in water limits the final concentration that can be used. We used this same dose in our previous experiments on intact chromaffin cells (Burgoyne *et al.*, 1989; Cheek *et al.*, 1990). Such an addition of 10 mM-

Abbreviations used: $[Ca^{2+}]$, free Ca^{2+} concentration; InsP_3, inositol 1,4,5-trisphosphate.

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caffeine induced a slight quench of the fluo-3 fluorescence. The calculated levels of free Ca^{2+} are therefore only correct for that part of the trace after the caffeine addition; the free $[Ca^{2+}]$ before the caffeine addition is slightly overestimated.

ATP, creatine kinase and phosphocreatine were from Boehringer. Methylenediphosphonic acid, Ruthenium Red, caffeine and digitonin were from Sigma. Fluo-3 was from Molecular Probes (Eugene, OR, U.S.A.). $InsP_3$ was a gift from Dr. Robin Irvine (AFRC, Babraham, Cambridge, U.K.). All other reagents were of the highest chemical purity commercially available.

RESULTS

Chromaffin cells are $InsP_3$- and caffeine-sensitive

We routinely supplemented our assay medium with 5.8 mM-pyrophosphate to increase the Ca^{2+}-accumulating capacity of those pools that possess the specific anion transporter required for accumulation of this anion (Martonosi, 1982). Under these conditions, cytosolic $[Ca^{2+}]$ was lowered to 53.8 ± 4.4 nM (mean $\pm$ S.E.M.; $n = 6$) as a consequence of the efficient uptake of Ca^{2+} into the stores. From a resting $[Ca^{2+}]$ of 58 nM, 10 μM-$InsP_3$ induced a rise in $[Ca^{2+}]$ to a peak of 392 nM that was transient over 8 min (Fig. 1*a*). From a resting $[Ca^{2+}]$ of 52 nM, 10 mM-caffeine induced a rise in $[Ca^{2+}]$ to a peak of 255 nM that was transient over 2 min (Fig. 1*b*). The amplitudes of the $InsP_3$- and caffeine-induced $[Ca^{2+}]$ rises varied between different batches of cells, but were very reproducible within any given batch. These results indicate that chromaffin cells incubated in the presence of digitonin remain sensitive to both caffeine and $InsP_3$. The caffeine response was inhibited by 62.8 ± 10.4% ($n = 4$) after preincubation with 10 μM-Ruthenium Red (results not shown), indicating that caffeine was probably acting on the chromaffin-cell ryanodine receptor (Robinson & Burgoyne, 1991).

Chromaffin cells are still sensitive to caffeine after depletion of the $InsP_3$-sensitive store

Addition of a supra-maximal (40 μM) dose of $InsP_3$ to permeabilized cells resulted in a large rise in $[Ca^{2+}]$ that was transient over about 8 min (Fig. 2*a*). A subsequent addition of 10 μM-$InsP_3$ resulted in only a very small rise in $[Ca^{2+}]$, indicating that either the $InsP_3$-sensitive store had not refilled or that the $InsP_3$ receptor had desensitized. A subsequent addition of 10 mM-caffeine produced a much larger rise in $[Ca^{2+}]$ that was transient over 8 min. This Ca^{2+} presumably originated from a store that was insensitive to $InsP_3$. That this was the case was confirmed by a second addition of 10 μM-$InsP_3$, which this time resulted in a large rise in $[Ca^{2+}]$, presumably because the $InsP_3$ added before the caffeine had been metabolized, allowing refilling of the $InsP_3$-sensitive store. Note that the caffeine-induced transient was longer-lasting than that recorded in the absence of $InsP_3$ (Fig. 1*b*). Since caffeine is presumably not metabolized under these conditions, the recovery of the Ca^{2+} transient largely depends upon the uptake of Ca^{2+} into the $InsP_3$-sensitive store, and this was delayed, owing to the time required to metabolize the 10 μM pulse of $InsP_3$ (Fig. 2*a*).

Pretreatment of the cells with 50 mM-caffeine modulated both the Ca^{2+}-sequestering ability of the cells and the response to $InsP_3$ (Fig. 2*b*, cf. Fig. 2*c*). The Ca^{2+} was sequestered more slowly at the beginning of the experiment, and the resting level of $[Ca^{2+}]$ achieved (198 nM; Fig. 2*c*) was higher than that in the control (57 nM; Fig. 2*b*). These results are consistent with caffeine having prevented its store from efficiently sequestering Ca^{2+}, thereby decreasing the ability of the preparation to accumulate Ca^{2+}. Under these conditions the cells were still able to respond with a rise in $[Ca^{2+}]$ to 10 μM-$InsP_3$ (Fig. 2*c*), but the rise in $[Ca^{2+}]$ was

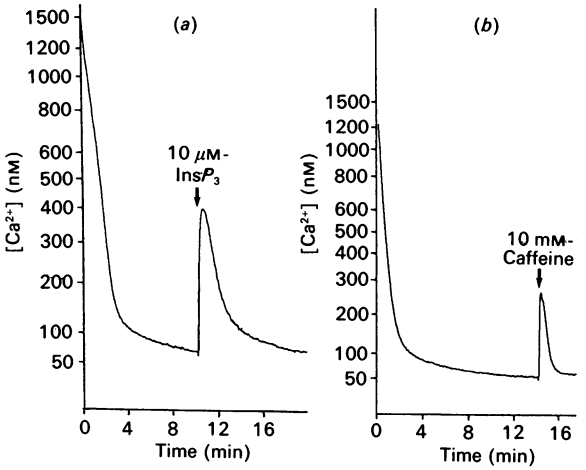

Fig. 1. $InsP_3$ and caffeine release Ca^{2+} from intracellular stores in permeabilized chromaffin cells

Ca^{2+} uptake by non-mitochondrial Ca^{2+} pools in the presence of 5.8 mM-pyrophosphate was measured as described in the Materials and methods section; 10 μM-$InsP_3$ (*a*) or 10 mM-caffeine (*b*) was added as indicated. Representative traces from three experiments on three different batches of cells are shown.

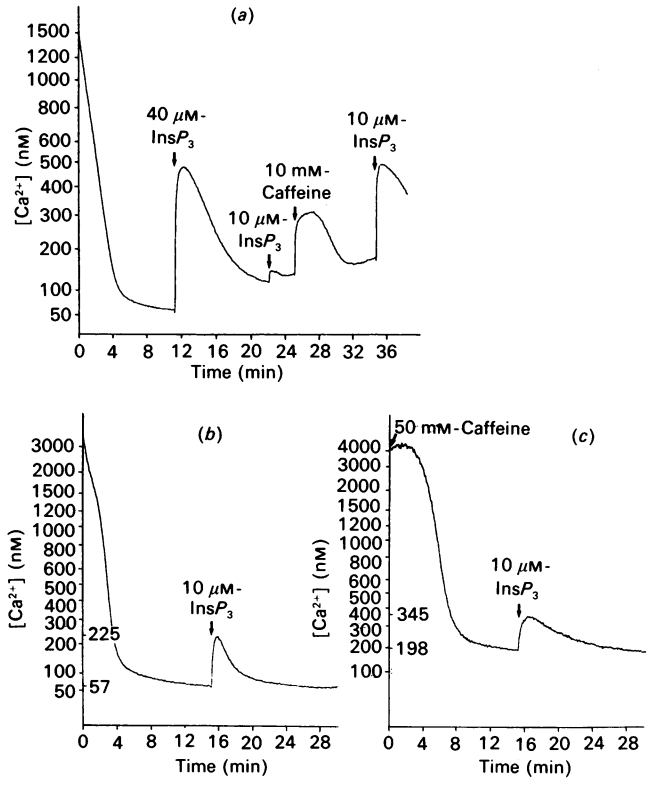

Fig. 2. $InsP_3$- and caffeine-sensitive Ca^{2+} pools are distinct

Trace (*a*) illustrates that 40 μM-$InsP_3$ was able to keep the $InsP_3$-sensitive Ca^{2+} pool empty for at least 10 min, as judged by the absence of a significant Ca^{2+} release by a subsequent addition of 10 μM-$InsP_3$. These additions of $InsP_3$ did not deplete the caffeine-sensitive Ca^{2+} pool, as indicated by the Ca^{2+} release by 10 mM-caffeine. The Ca^{2+} release by a later addition of 10 μM-$InsP_3$ indicates that the $InsP_3$-sensitive pool was able to refill subsequently. The experiments shown in traces (*b*) and (*c*) were performed on a different batch of cells from that shown in trace (*a*) and illustrate the $[Ca^{2+}]$ rise induced by 10 μM-$InsP_3$ in the absence (*b*) and presence (*c*) of 50 mM-caffeine. $InsP_3$ was able to induce a near-normal Ca^{2+} response even when the caffeine-sensitive store was largely empty.

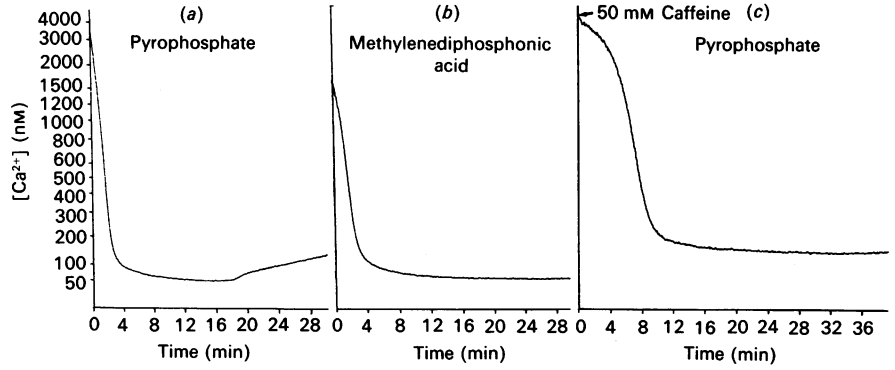

Fig. 3. Pyrophosphatase-induced Ca^{2+} release from the caffeine-sensitive Ca^{2+} store

Trace (*a*) illustrates that, in the presence of 5.8 mM-pyrophosphate, permeabilized chromaffin cells spontaneously released Ca^{2+} after a latency. This release did not occur in the presence of 5.8 mM-methylenediphosphonic acid, which is not a substrate for the cytosolic pyrophosphatase (*b*). Trace (*c*) illustrates that 50 mM-caffeine prevented loading of the pool that spontaneously released Ca^{2+} in the presence of 5.8 mM-pyrophosphate. The spontaneous-release store is therefore caffeine-sensitive. The traces shown are representative of three experiments carried out on different batches of cells.

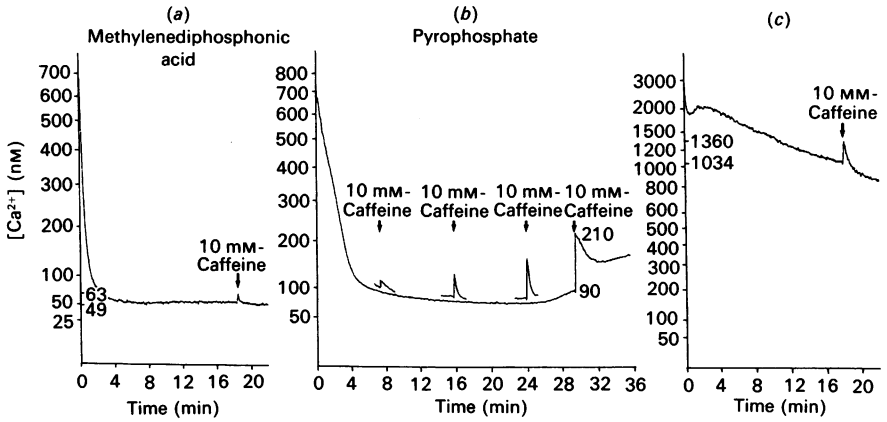

Fig. 4. Intraluminal free Ca^{2+} stimulates caffeine-induced Ca^{2+} release

Traces show the Ca^{2+} released by 10 mM-caffeine in the presence of 5.8 mM-methylenediphosphonic acid (*a*), 5.8 mM-pyrophosphate (*b*) or no precipitating anion (*c*). The time course in the presence of pyrophosphate (trace *b*) shows superimposed traces from four different experiments in which 10 mM-caffeine was added to the cells at different times as indicated. (To avoid confusion, only one complete trace is shown; the relevant regions of the other three traces are indicated during the response to caffeine added at 7.5, 16 and 24 min). All traces indicate that sensitivity to caffeine increases under conditions in which intraluminal free Ca^{2+} is expected to rise (see the text). Representative traces are shown that were typical of three experiments performed on different batches of cells. In trace (*c*), cells were washed three times, instead of twice, before permeabilization, to decrease the amount of contaminating Ca^{2+} (the extra wash was necessary to measure Ca^{2+} uptake in the absence of precipitating anions).

longer-lasting than the Ca^{2+} response to InsP_3 in control cells (Fig. 2*b*). This is also consistent with the decreased ability of the cells to re-sequester released Ca^{2+}. The amplitude of the InsP_3-induced rise in $[Ca^{2+}]$ was similar in control and caffeine-treated cells (Figs. 2*b* and 2*c*).

The caffeine-sensitive store is able to release Ca^{2+} spontaneously

When the stores were loaded in the presence of 5.8 mM-pyrophosphate, we always observed a spontaneous progressive rise in $[Ca^{2+}]$ to a new steady state after a latency (~ 18 min; Fig. 3*a*). This spontaneous rise in $[Ca^{2+}]$ was probably due to progressive metabolism of pyrophosphate by a cytosolic pyrophosphatase, because it did not occur in the presence of 5.8 mM-methylenediphosphonic acid, which is not a substrate for the pyrophosphatase (Halestrap & Davidson, 1989) (Fig. 3*b*), and did not occur after the cells were washed twice after permeabilization to remove the cytosol (results not shown). This suggests that the spontaneous release may only occur under conditions in which the level of uncomplexed (i.e. free) Ca^{2+} in the lumen of the store might be expected to rise. This is because phosphate anions, produced by the action of the endogenous pyrophosphatase, are less effective in complexing Ca^{2+}, so free $[Ca^{2+}]$ in the lumen rises, and this may trigger release.

Caffeine (50 mM) prevented the spontaneous rise in $[Ca^{2+}]$ in the presence of pyrophosphate (Fig. 3*c*), suggesting that the specific anion transporter for accumulating pyrophosphate is present in the membrane of the caffeine-sensitive store. A possible inhibitory effect of caffeine on the pyrophosphatase can be excluded, because caffeine did not affect pyrophosphatase-induced Ca^{2+} release in permeabilized hepatocytes (L. Missiaen & M. J. Berridge, unpublished work).

Is the sensitivity of the caffeine-sensitive store governed by the level of free Ca^{2+} in the lumen?

Because the spontaneous release is only apparent under conditions in which free Ca^{2+} in the lumen may be expected to

rise (see above), and the spontaneous-release store is related to the caffeine-sensitive store, we investigated the possibility that the sensitivity of this store to caffeine may be governed by the amount of uncomplexed Ca^{2+} in the lumen.

In the presence of methylenediphosphonic acid, where the level of uncomplexed Ca^{2+} in the lumen is expected to be low, there was virtually no rise in $[Ca^{2+}]$ in response to 10 mM-caffeine (Fig. 4*a*). In the presence of pyrophosphate, where the level of uncomplexed Ca^{2+} in the lumen would be expected to rise progressively, the cells become more sensitive to caffeine with time, as shown by the time course of caffeine addition in Fig. 4(*b*). In the absence of precipitating anions the rate of Ca^{2+} sequestration was greatly decreased (Fig. 4*c*), indicating that the ability of the cells to sequester Ca^{2+} was severely impaired. This was probably because the stores were full of free Ca^{2+} very soon after permeabilization. In this case, cells were extremely sensitive to caffeine, a rise in $[Ca^{2+}]$ of 326 nM being achieved in response to 10 mM-caffeine (Fig. 4*c*).

These results suggest that the sensitivity of the caffeine-sensitive store to caffeine can be influenced by the amount of free uncomplexed Ca^{2+} in the lumen of the store.

DISCUSSION

In this study we have used the low-affinity fluorescent Ca^{2+} indicator fluo-3 to investigate some characteristics of the intracellular Ca^{2+} stores in permeabilized chromaffin cells. The results demonstrate that chromaffin cells contain distinct Ca^{2+} stores that can be released by exposure to either $InsP_3$ or caffeine, and suggest that the sensitivity to caffeine can be regulated by the level of intraluminal free Ca^{2+}.

A necessary prerequisite when using fluo-3 to monitor Ca^{2+} fluxes is that the free Ca^{2+} in the medium must be low enough to detect the $[Ca^{2+}]$ rises in response to various stimuli. Some cell types, such as hepatocytes, sequester enough Ca^{2+} to lower medium $[Ca^{2+}]$ sufficiently (L. Missiaen & M. J. Berridge, unpublished work). Other cell types require an experimental modification in order for Ca^{2+} sequestration to be detected. In rat basophilic-leukaemia cells, the medium was filtered through a Ca^{2+}-chelating resin (Meyer *et al.*, 1990). In a recent study on permeabilized chromaffin cells, 10 μM-EGTA was added to the medium (Robinson & Burgoyne, 1991). In this study we used the precipitating anion pyrophosphate to increase the Ca^{2+}-buffering capability of the stores (Palade, 1987).

Chromaffin cells are $InsP_3$- and caffeine-sensitive

The results clearly demonstrate that pyrophosphate-loaded cells retain caffeine-sensitivity even after the $InsP_3$-sensitive store has been emptied and prevented from refilling by a supramaximal dose of $InsP_3$. In addition, a normal dose of $InsP_3$ was still able to mobilize Ca^{2+} in the continual presence of a high dose (50 mM) of caffeine, although the shape of the Ca^{2+} transient induced by $InsP_3$ was modified. These results directly demonstrate that intracellular stores exhibit selective sensitivity in chromaffin cells, as was proposed from earlier studies on intact (Burgoyne *et al.*, 1989) and permeabilized cells (Robinson & Burgoyne, 1991). Although the amplitude of the $InsP_3$-induced rise in $[Ca^{2+}]$ was similar in control and caffeine-treated cells, the amplitude in the presence of caffeine is exaggerated because the ability of the cells to re-sequester released Ca^{2+} is decreased under these conditions. This implies that the actual amount of Ca^{2+} released by $InsP_3$ is lower in the presence of caffeine than in its absence, and therefore that some of the $InsP_3$-sensitive stores are also caffeine-sensitive, as has been described in some smooth-muscle cells (Iino, 1987).

Caffeine-sensitivity is influenced by intraluminal free Ca^{2+}

When the intracellular stores were loaded in the presence of the Ca^{2+}-complexing anion pyrophosphate, we always observed a spontaneous rise in $[Ca^{2+}]$ to a new steady state after a latency. The same phenomenon was also observed in rat hepatocytes (L. Missiaen, C. W. Taylor & M. J. Berridge, unpublished work), where several lines of evidence indicated that it was due to the progressive metabolism of the pyrophosphate by a cytosolic pyrophosphatase. As the pyrophosphate is hydrolysed, the Ca^{2+}-accumulating capacity of the store decreases and as a result free Ca^{2+} in the lumen rises and is released. A similar phenomenon seems to occur in chromaffin cells, because the spontaneous rise in $[Ca^{2+}]$ was not observed when methylenediphosphonic acid, which is not a substrate for the pyrophosphatase (Halestrap & Davidson, 1989), was used instead of pyrophosphate, or when the cells were washed and the cytosol was removed. Interestingly, the spontaneous $[Ca^{2+}]$ rise also failed to occur in the continual presence of 50 mM-caffeine, even if pyrophosphate was present. This indicated that the 'spontaneous release' store may have been caffeine-sensitive.

The level of uncomplexed (i.e. free) Ca^{2+} in the lumen of this store seems to determine its sensitivity to caffeine. In the presence of pyrophosphate, where the level of free Ca^{2+} in the lumen progressively rises, as outlined above, caffeine was able to elicit a rise in $[Ca^{2+}]$ whose amplitude progressively increased with time (Fig. 4*b*). In the presence of methylenediphosphonic acid, where the level of uncomplexed free Ca^{2+} in the stores remains low because this compound is not hydrolysed by the pyrophosphatase, caffeine resulted in virtually no release of Ca^{2+}. In the absence of a complexing anion, the cells' ability to sequester Ca^{2+} from the medium is severely impaired, presumably because the stores become full of free Ca^{2+} very soon after permeabilization. Under these conditions a large rise in $[Ca^{2+}]$ was elicited by caffeine. Although the caffeine-sensitive stores in this case contained less Ca^{2+} than when loaded with precipitating anions, a greater proportion was in the form of free Ca^{2+}, hence the large release. These results indicate that the caffeine-sensitivity of bovine chromaffin cells increases under conditions in which the level of free Ca^{2+} in the lumen would be expected to rise. This phenomenon might account for an earlier observation on intact chromaffin cells, where caffeine-induced catecholamine secretion was potentiated if the cells were preloaded with Ca^{2+} (Poisner, 1973).

What is the physiological role of the caffeine-sensitive store?

In skeletal and cardiac muscle the caffeine-sensitive Ca^{2+} store functions in excitation–contraction coupling and is responsible for the process of Ca^{2+}-induced Ca^{2+} release (Endo, 1977). Caffeine-sensitive stores that are $InsP_3$-insensitive have also been proposed to occur in some smooth-muscle cells (Saida & van Breemen, 1983; Kanaide *et al.*, 1987) and non-muscle cells such as sensory neurons (Thayer *et al.*, 1988) and chromaffin cells from rat (Malgaroli *et al.*, 1990) and ox (Robinson & Burgoyne, 1991; the present study). The physiological role of the caffeine-sensitive store in non-muscle cells is unknown, but the existence of two internal Ca^{2+} stores has been invoked in a Ca^{2+}-induced Ca^{2+}-release model for the mechanism of Ca^{2+} oscillations (Berridge & Galione, 1988; Berridge, 1990) and the propagation of Ca^{2+} waves (Berridge & Irvine, 1989; Rooney *et al.*, 1990) seen in response to physiological agonists in non-muscle cells (Woods *et al.*, 1986; Rooney *et al.*, 1989). The ryanodine receptor in skeletal muscle, which is responsible for Ca^{2+}-induced Ca^{2+}

release, is regulated by the level of intraluminal free Ca^{2+} (Endo, 1977; Ohnishi, 1979; Nelson & Nelson, 1990). The fact that in the present study the caffeine-sensitivity of the store was modulated by the level of uncomplexed free Ca^{2+} in the lumen, and was blocked by Ruthenium Red, suggests that caffeine was acting on the ryanodine receptor of chromaffin cells (Robinson & Burgoyne, 1991) and that this channel could be involved in Ca^{2+}-induced Ca^{2+} release in these cells. One possibility is that the initial Ca^{2+} signal derived from membrane depolarization (O'Sullivan *et al.*, 1989; Augustine & Neher, 1990) is amplified and propagated into the centre of the cell in a manner similar to that proposed for sympathetic neurons (Lipscombe *et al.*, 1988*b*). A rapid release of Ca^{2+} from the caffeine-sensitive store, followed by an equally rapid re-uptake, may help to explain the observation that this type of spatially organized intracellular Ca^{2+} gradient can form and collapse very quickly (half-time $\sim$ 200 ms) in chromaffin cells (Augustine & Neher, 1990). Consistent with such a proposed role for the caffeine-sensitive store in these cells are the findings that these stores are evenly distributed throughout the cytoplasm of intact chromaffin cells (Burgoyne *et al.*, 1989) and that they play an important role in intracellular Ca^{2+} homoeostasis (Cheek *et al.*, 1990).

Further evidence consistent with a role for the caffeine-sensitive store in Ca^{2+} signalling in chromaffin cells has come from other studies on intact rat chromaffin cells, where spontaneous fluctuations in intracellular [Ca^{2+}] were found to be generated by a caffeine-sensitive but InsP_3-insensitive store (Malgaroli *et al.*, 1990). In bovine cells, over 90% of the internal Ca^{2+} released by the InsP_3-mobilizing stimuli histamine and angiotensin II originated from a store that was sensitive to caffeine (Stauderman & Murawsky, 1990). A similar phenomenon occurs in smooth muscle, where the caffeine-sensitive pool may contribute to agonist-evoked contractions (Kanmura *et al.*, 1988). It is therefore evident that the caffeine-sensitive store can contribute large amounts of signal Ca^{2+} during cell stimulation. However, the extent of overlap between the caffeine-sensitive and InsP_3-sensitive stores may vary, depending on cell types and experimental conditions, especially since the amount of Ca^{2+} released seems to depend upon the level of free Ca^{2+} in the lumen. The use of permeabilized cell systems in which the loading of Ca^{2+} into stores can be controlled should prove useful for further investigating the mechanisms underlying the regulation of internal Ca^{2+} stores.

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