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Characterization of the inositol 1,4,5-trisphosphate-induced calcium release from permeabilized endocrine cells and its inhibition by decavanadate and *p*-hydroxymercuribenzoate

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The inositol 1,4,5-trisphosphate (IP_3)-sensitive Ca^{2+} compartment of endocrine cells was studied with α-toxin- and digitonin-permeabilized rat insulinoma (RINA2) and rat pheochromocytoma (PC12) cells. The Ca^{2+} uptake was ATP-dependent, and submicromolar concentrations of IP_3 specifically released the stored Ca^{2+}. Half-maximal Ca^{2+} release was observed with 0.25–0.5 μmol of IP_3/l, and the amount of Ca^{2+} released due to IP_3 could be enhanced by additional loading of the Ca^{2+} compartment. Consecutive additions of the same concentration of IP_3 for 1–2 h always released the same amount of Ca^{2+} without desensitization, providing an ideal basis to further characterize the IP_3-induced Ca^{2+} release. Here we describe for the first time a reversible inhibitory effect of decavanadate on the IP_3-induced Ca^{2+} release. Among the vanadium species tested (decavanadate, oligovanadate and monovanadate), only decavanadate was inhibitory, with a half-maximal effect at 5 μmol/l in both cell types. The effect of decavanadate could be overcome by increasing the amount of sequestered Ca^{2+} or added IP_3. Decavanadate did not affect the ATP-driven Ca^{2+} uptake but oligovanadate was inhibitory on Ca^{2+} uptake. *p*-Hydroxymercuribenzoate (pHMB) at concentrations between 10 and 30 μmol/l also inhibited the Ca^{2+} release due to IP_3. Thiol compounds such as dithiothreitol (DTT; 1 mmol/l) added before pHMB removed all its inhibitory effect on the IP_3-induced Ca^{2+} release, whereas the inhibition caused by decavanadate was unaffected by DTT. Thus, the decavanadate-dependent inhibition functions by a distinctly different mechanism than pHMB and could serve as a specific tool to analyse various aspects of the IP_3-induced Ca^{2+} release within endocrine cells.

INTRODUCTION

The receptor-stimulated hydrolysis of inositol phospholipids and the mobilization of intracellular Ca^{2+} stores is a common mechanism for transmembrane signalling when cells respond to external stimuli as diverse as hormones, neurotransmitters and antigens (cf. Berridge, 1987). Rises in the intracellular free Ca^{2+} concentration are key events in a variety of cellular processes including the control of exocytosis. The intracellular free Ca^{2+} concentration is regulated by Ca^{2+} transport systems present in the plasma membrane as well as intracellular sequestering systems such as mitochondria, secretory vesicles and the endoplasmic reticulum. The latter is characterized by the ability to sequester Ca^{2+} in an ATP-dependent manner and to release it upon stimulation with micromolar concentrations of inositol 1,4,5-trisphosphate (IP_3). A common problem encountered when working with this IP_3-sensitive Ca^{2+} compartment is the desensitization of the Ca^{2+} release upon repeated application of a given concentration of IP_3 (Rossier *et al.*, 1987). In the present work it is demonstrated that appropriately permeabilized cells respond to IP_3 for periods longer than 1 h without desensitization. With such cellular preparations, a careful analysis of the IP_3-induced Ca^{2+} release and its characterization with specific reagents is possible.

EXPERIMENTAL

Materials

Defined species of vanadate were prepared as described below using ortho- and metavanadate from Sigma (St. Louis, MO, U.S.A.). The pore-forming protein α-toxin from *Staphylococcus aureus* was purified as described by Lind *et al.* (1987). Inositol 1,4-bisphosphate (IP_2) and IP_3 were from Boehringer (Mannheim, Germany). Inositol 1,3,4,5-tetrakisphosphate was kindly given by Dr. R. F. Irvine (Cambridge, U.K.). Ca^{2+}-selective membranes containing the neutral carrier ETH 129 were kindly provided by W. Simon (ETH, Zürich, Switzerland). The firefly assay for determination of ATP was obtained from Boehringer. KCl (suprapure) was from Merck (Darmstadt, Germany) and Azur A was from Fluka (Neu-Ulm, Germany). All other chemicals were analytical grade.

Cell culture and preparation for Ca^{2+} measurement

Rat insulinoma (RINA2) and pheochromocytoma (PC12) cells were cultured in 60 mm wells containing 3 ml of RPMI 1640 medium supplemented with 10% (v/v) fetal calf serum in an atmosphere of 10% CO_2 as described previously (Ahnert-Hilger *et al.*, 1985; Lind *et al.*, 1987). Before permeabilization, the cells were washed 3 times with medium A containing KCl

Abbreviations used: IP_3, inositol 1,4,5-trisphosphate; IP_2 inositol 1,4-bisphosphate; IP_4, inositol 1,3,4,5-tetrakisphosphate; pHMB, *p*-hydroxymercuribenzoate; DTT, dithiothreitol; h.u., haemolytic units.

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(150 mmol/l), NaN_3 (5 mmol/l), Mops (20 mmol/l), pH 7.2, and EGTA (1 mmol/l). For permeabilization with α-toxin, the cells were suspended in medium A and incubated with 300 haemolytic units (h.u.) of α-toxin/10^7 cells for 10 min on ice followed by 30 min at 30 °C. Digitonin permeabilization was carried out in the cold directly in the culture wells (25 μmol of digitonin/l in medium A containing 0.1% dimethyl sulphoxide; 10 min) (Ahnert-Hilger *et al.*, 1989*b*). Permeabilization was controlled by counting the Azur A-stained cells [0.25% Azur A in NaCl (150 mmol/l)/NaN_3 (5 mmol/l)/Mops (20 mmol/l), pH 7.2] in a Neubauer chamber. Following permeabilization, the cells were washed three times with medium B (medium A without EGTA) before the Ca^{2+} measurements.

Measurement of the ambient free Ca^{2+} concentration

Ca^{2+}-specific mini-electrodes were prepared as described by Simon *et al.* (1978) and were calibrated before and after each experiment. The poly(vinyl chloride) membrane of the electrode contained the recently developed neutral carrier ETH 129, which allows reliable measurements of Ca^{2+} down to the nanomolar range (Ammann *et al.*, 1987). All chemicals used in the experiments were tested for Ca^{2+} contamination and Ca^{2+}-binding activity in medium B. At experimental concentrations no electrode interference was detected. Experiments were carried out at room temperature and pH 7.2 in 300–500 μl of medium B containing $(2.5–10) \times 10^6$ cells.

Other methods

Protein was measured according to Smith *et al.* (1985) using bovine serum albumin as standard. For determination of cell volume, the mean cell diameter was estimated under a microscope and the volume calculated under the assumption of a spherical cell form. ATP was determined using the firefly assay as described elsewhere (Lind *et al.*, 1987).

Characterization and preparation of vanadate solutions

The speciation of vanadate in aqueous solution is strongly dependent on both the pH and the absolute vanadate concentration as analysed with ^{51}V n.m.r. spectroscopy. At pH 7.2, vanadate (total 1.25 mmol/l) exists as 50% tetramer, 30% monomer, 10% dimer and 10% other species. At pH 3.75, vanadate exists solely as decavanadate (Pettersson *et al.*, 1983).

Concentrated stock solutions of vanadate were prepared by dissolving crystals of either ortho- or metavanadate as purchased from the supplier in 1 mol of KOH/l (monovanadate). All other vanadate solutions used were prepared freshly from this stock solution.

Decavanadate was prepared by dilution of the stock solution with KCl (150 mmol/l)/Mops (20 mmol/l) pH 7.2, and titration to pH 3.75 with concentrated HCl. Solutions were subsequently diluted to 5 mmol/l and stored at 4 °C until use. Solutions prepared in this manner were effective even after several months (results not shown).

Decavanadate is the thermodynamically stable species at pH 3.75. Its yellow colour is due to a broad shoulder around 350–400 nm. Upon adjustment of the decavanadate solution from pH 3.75 to pH 7.2, decavanadate decomposes slowly to oligomeric forms. We found at experimental conditions (room temperature and pH 7.2) a half-life of decavanadate (200 μmol/l) in medium B of approx. 2 h, thus allowing ample time for completion of all experiments without significant influence of oligomeric vandate species.

Oligovanadate solutions were prepared by titration of stock solutions to pH 7.2 with conc. HCl. The yellow colour formed during titration is due to decavanadate as identified by spectroscopy and disappeared upon boiling. After cooling to room temperature, the pH was checked again. This procedure was repeated until a colourless solution of pH 7.2 was obtained containing oligovanadate and monovandate. The solution was then diluted to a final concentration of 50 mmol/l and stored at room temperature. In this manner, stable oligovanadate solutions free of decavanadate can be prepared.

RESULTS

Characterization of the ATP and IP_3-sensitive Ca^{2+} pool in permeabilized PC12 and RINA2 cells

Cell poration by α-toxin is restricted to the plasma membrane and allows the passage of low-molecular-mass substances (Ahnert-Hilger *et al.*, 1985, 1989*b*; Bhakdi & Traunum-Jensen, 1987). By contrast, permeabilization with digitonin in addition allows the diffusion of higher molecular mass proteins. Since no difference in the ATP-dependent Ca^{2+} uptake or IP_3-induced Ca^{2+} release was found using either method of

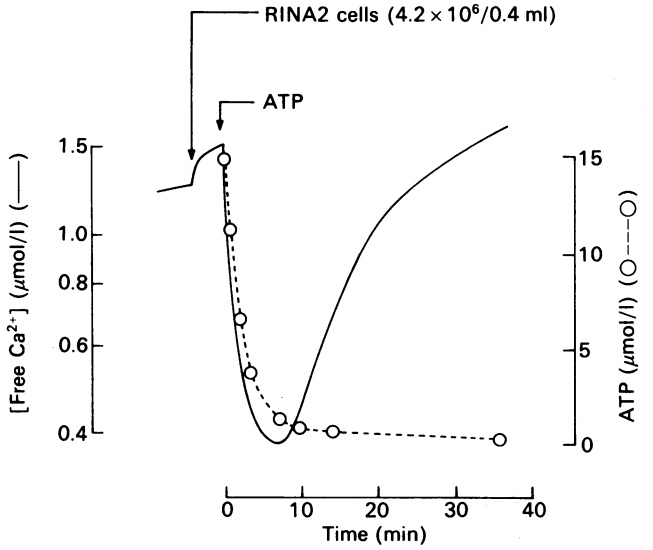

Fig. 1. Ca^{2+} uptake and ATP hydrolysis by digitonin-permeabilized RINA2 cells incubated with low concentrations of ATP

Cells (4.2×10^6) were incubated at room temperature in medium B (150 mmol of KCl/l/20 mmol of Mops/l, pH 7.2/5 mmol of NaN_3/l) containing 1 mmol of Mg^{2+}/l. Arrows indicate the addition of cells, permeabilized with 25 μmol of digitonin/l, and ATP. For determination of ATP, 20 μl aliquots were removed and diluted in 10 vol. of preheated buffer (assay buffer for ATP determination) and boiled for 5 min. After cooling, the denatured cells were centrifuged for 2 min at 10000 *g*. The supernatant was analysed by means of the firefly assay for ATP. Concentrations of ATP stock solutions were controlled spectrophotometrically at 259 nm with the molar absorption coefficient given by Mahoney *et al.* (1986).

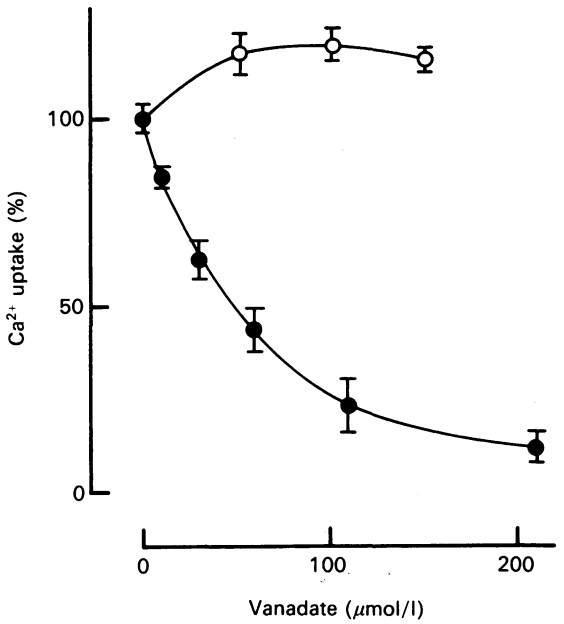

Fig. 2. Effect of decavanadate and oligovanadate on ATP-dependent Ca^{2+} uptake

Ca^{2+} uptake at different oligovanadate (●) or decavanadate (○) concentrations was determined with 5 μmol of ATP/l as shown in Fig. 1. 100% Ca^{2+} uptake corresponds to the sequestration of 1.25 nmol of Ca^{2+} by 10^7 cells. Vanadate concentrations are referred to the monomeric form.

Table 1. ATP-dependent Ca^{2+} uptake and IP_3 (5 μmol/l)-induced Ca^{2+} release from RINA2 and PC12 cells

Results are expressed as means ± S.D. of three independent experiments.

Cells	Ca^{2+} uptake (nmol/mg of protein)	Ca^{2+} release (nmol/mg of protein)	Release (% of uptake)
RINA2	10.6 ± 2.7	7.1 ± 1.7	66.9 ± 7.7
PC12	5.8 ± 1.3	4.0 ± 0.9	68.9 ± 22.2

permeabilization, an involvement of soluble cytoplasmic proteins in both processes is unlikely.

ATP-dependent Ca^{2+} uptake and its inhibition by oligovanadate

Intact PC12 and RINA2 cells suspended in a buffered KCl medium (medium B) containing 5 mmol of NaN_3/l and 1–2 μmol of free Ca^{2+}/l exhibited no Ca^{2+} uptake. Permeabilized cells sequestered Ca^{2+} provided that ATP was present. Small amounts of ATP (10–20 μmol/l) resulted in a transient Ca^{2+} storage in the permeabilized cells (Fig. 1). The stored Ca^{2+} was released again upon the ATP concentration dropping below 1 μmol/l (Fig. 1). Under these conditions, the cells required 100–200 μmol of ATP/l to maintain steady-state Ca^{2+} levels of approx. 0.2–0.4 μmol/l. Most experiments were routinely carried out in the presence of 2 mmol of Mg–ATP/l to ensure an excess of ATP for longer incubations (up to 4 h). Ca^{2+} uptake determined as shown in Fig. 1 was not influenced by decavandate, but oligovanadate was strongly inhibitory (Fig. 2). This finding allowed us to analyse the effect of different vanadate species on the IP_3-induced Ca^{2+} release.

Table 2. Correlation of cell number to cell volume and cell protein

Values represent the means ± S.D. of numbers of measurements given in parentheses.

Cells	Cell volume (μl/10^7 cells)	Protein (mg/10^7 cells)
RINA2	16.1 ± 2.4 (n = 100)	1.51 ± 0.09 (n = 9)
PC12	14.9 ± 2.6 (n = 100)	1.1 ± 0.3 (n = 4)

Release of stored Ca^{2+} by IP_3

Submicromolar concentrations of IP_3 caused the release of Ca^{2+} (Figs. 3*a* and 3*b*). This release was highly specific for IP_3, since IP_4, IP_2 and inositol were ineffective (results not shown). For both RINA2 and PC12 cells, the minimal releasing concentration was at 0.01 μmol of IP_3/l. Half-maximal release was obtained between 0.25 and 0.5 μmol of IP_3/l (Figs. 3*a* and 3*b*). An immediate reuptake of Ca^{2+} followed each release. The level of Ca^{2+} reached in the presence of 2 mmol of Mg^{2+}–ATP/l with permeabilized rat insulinoma or pheochromocytoma cells was around 0.1 μmol of free Ca^{2+}/l. IP_3 was commonly added when the cells reached an ambient free Ca^{2+} concentration of approx. 0.2–0.4 μmol of Ca^{2+}/l (Figs. 3*a* and 3*b*). Maximal IP_3 concentrations (1–5 μmol/l) resulted in a rise of ambient free Ca^{2+} to around 0.6–0.8 μmol/l (Figs. 3*a* and 3*b*), corresponding to a release of 60–80% of the sequestered Ca^{2+} (Table 1). From these data, it can be calculated that RINA2 and PC12 cells in the presence of 2 mmol of Mg–ATP/l sequester approx. 10.6 ± 2.7 and 5.8 ± 1.3 nmol of Ca^{2+}/mg of protein respectively (Table 1).

Since 1 mg of cell protein corresponds to a cell volume of approx. 10–13 μl (Table 2), the IP_3-sensitive Ca^{2+} storage system could give rise to intracellular Ca^{2+} concentrations up to or higher than 100 μmol/l. Prolonged incubation did not change the ability to store Ca^{2+} as well as to release it upon a given IP_3 concentration, indicating that no desensitization occurred (Fig. 4*a*). Furthermore, the IP_3-induced Ca^{2+} release directly depended upon the amount of sequestered Ca^{2+} (Figs. 4*a* and 4*b*). Sequential additions of small amounts of Ca^{2+} after the initial Ca^{2+} uptake increased the Ca^{2+} release when stimulated with an equal IP_3 concentration (Fig. 4*b*), indicating that the amplification of the IP_3 signal in secretory cells depends on the 'charge' of the Ca^{2+} battery. From Fig. 4(*a*), it was calculated that one molecule of IP_3 could release up to 30 Ca^{2+} ions.

Inhibition of the IP_3-induced Ca^{2+} release

Decavanadate and pHMB inhibited the IP_3-induced Ca^{2+} release in both RINA2 and PC12 cells. Decavanadate (5 μmol/l) resulted in an almost half-maximal inhibition of Ca^{2+} release induced by 0.25 μmol of

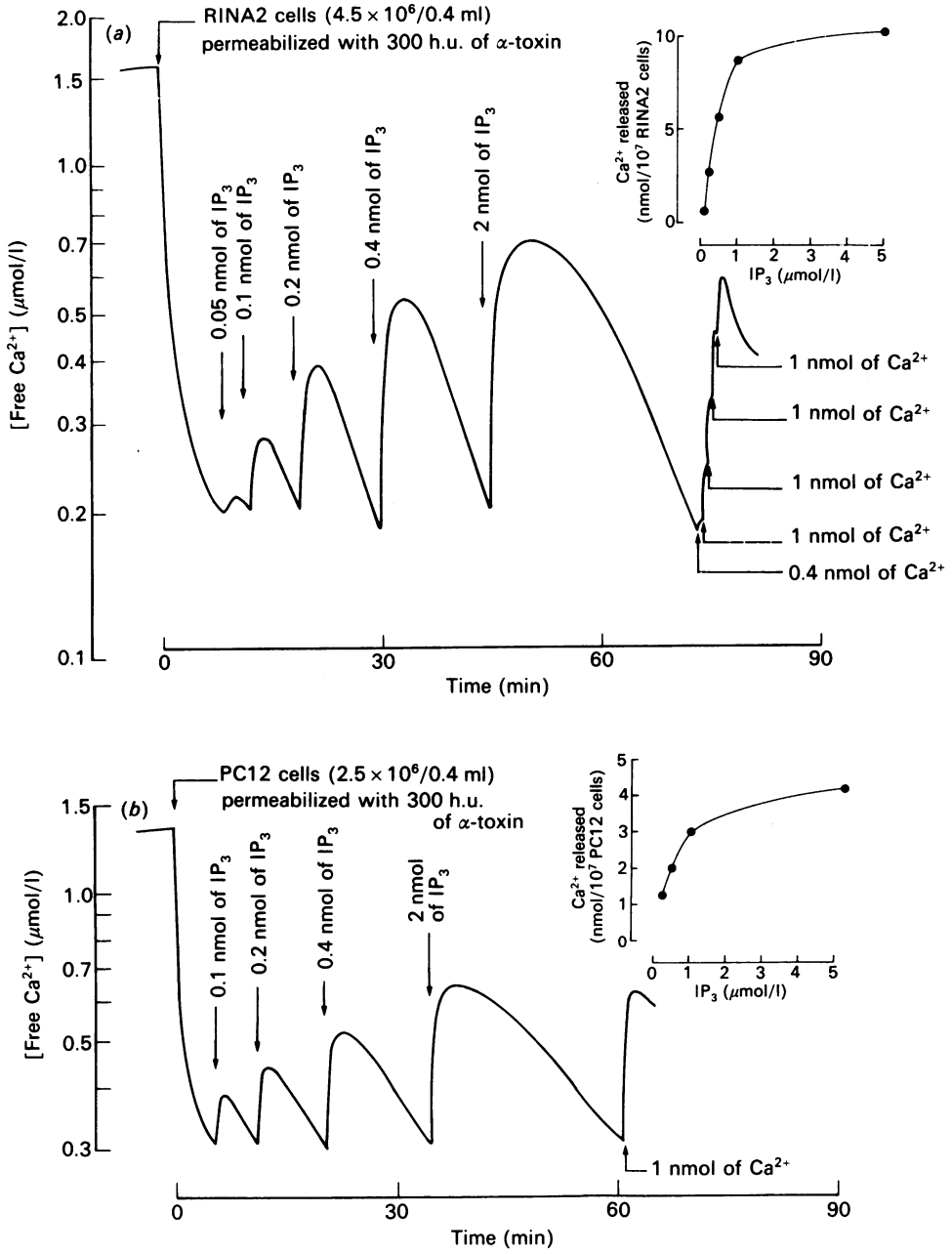

Fig. 3. Ca^{2+} release induced by different concentrations of IP_3 from α-toxin-permeabilized RINA2 (*a*) and PC12 (*b*) cells

Cells were incubated in medium B containing 2 mmol of Mg^{2+}/l and 2 mmol of ATP/l. The arrows indicate the addition of increasing amounts of IP_3 (between 0.05 and 2.0 nmol) corresponding to final concentrations of between 0.125 and 5 μmol/l. The calibration of the Ca^{2+} release was carried out by sequential addition of a Ca^{2+} standard. The absolute Ca^{2+} release/10^7 cells as calculated from these data is shown in the inserts of this Figure.

IP_3/l (Fig. 5*a*). Further addition of decavanadate (10–15 μmol/l) inhibited the release by up to 90% (Fig. 5*a*). At higher concentrations, decavanadate released Ca^{2+} (results not shown). The effect of decavanadate was found to be reversible (Fig. 5*b*) and could be overcome by increasing the amount either of sequestered Ca^{2+} or of added IP_3 (Fig. 5*c*). Neither oligovanadate (Fig. 5*d*) nor the closely related arsenate (results not shown) were inhibitory on the IP_3-induced Ca^{2+} release. Low concentrations of the thiol-reactive reagent pHMB exhibited similar effects. The effective concentrations for inhibition of IP_3-induced Ca^{2+} release by pHMB were observed at about 10–30 μmol/l (Fig. 6*a*). Again, higher concentrations resulted in a total release of sequestered Ca^{2+} (result not shown). The pHMB effect could be abolished by an excess of thiol compounds such as DTT (1 mmol/l) (Fig. 6*b*). By contrast, the addition of DTT had no effect on the decavanadate-induced inhibition (results not shown). Nitro-group-containing compounds such as nitroprusside or nitroglycerin, which were reported to interfere with the mobilization of intracellular Ca^{2+} in smooth muscle (cf. Shibata *et al.*, 1987), were not

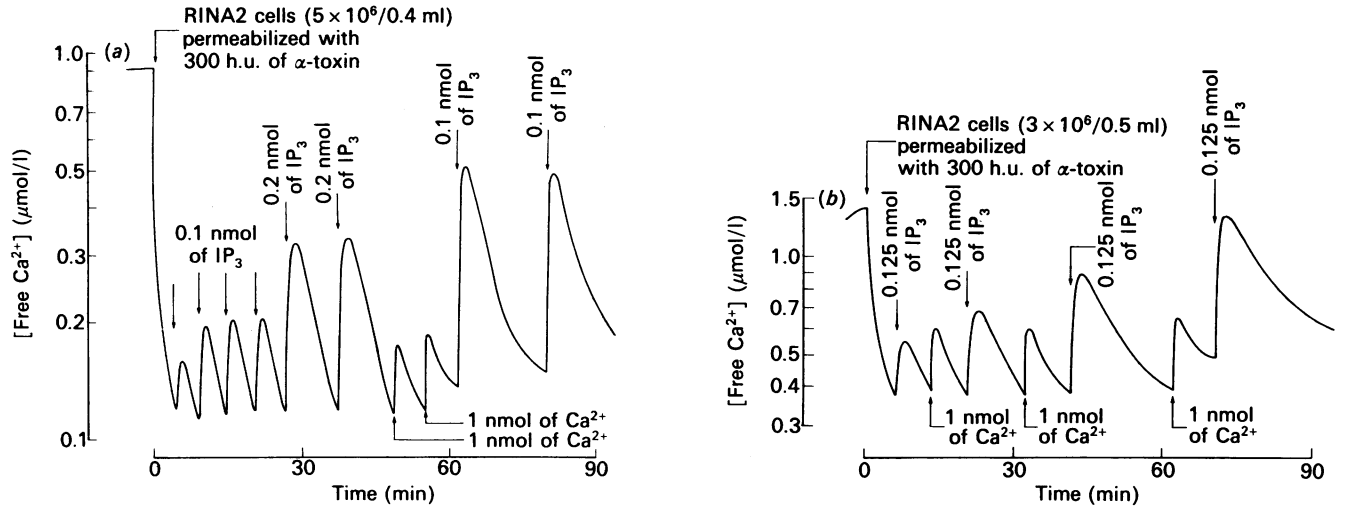

Fig. 4. Ca^{2+} release by the same amount of IP_3 from α-toxin-permeabilized RINA2 cells is constant (*a*) but can be increased by additional loading with Ca^{2+} (*b*)

Arrows indicate the addition of IP_3 and Ca^{2+}. Note the increased IP_3-induced Ca^{2+} release after sequential additions of 1 nmol of Ca^{2+}.

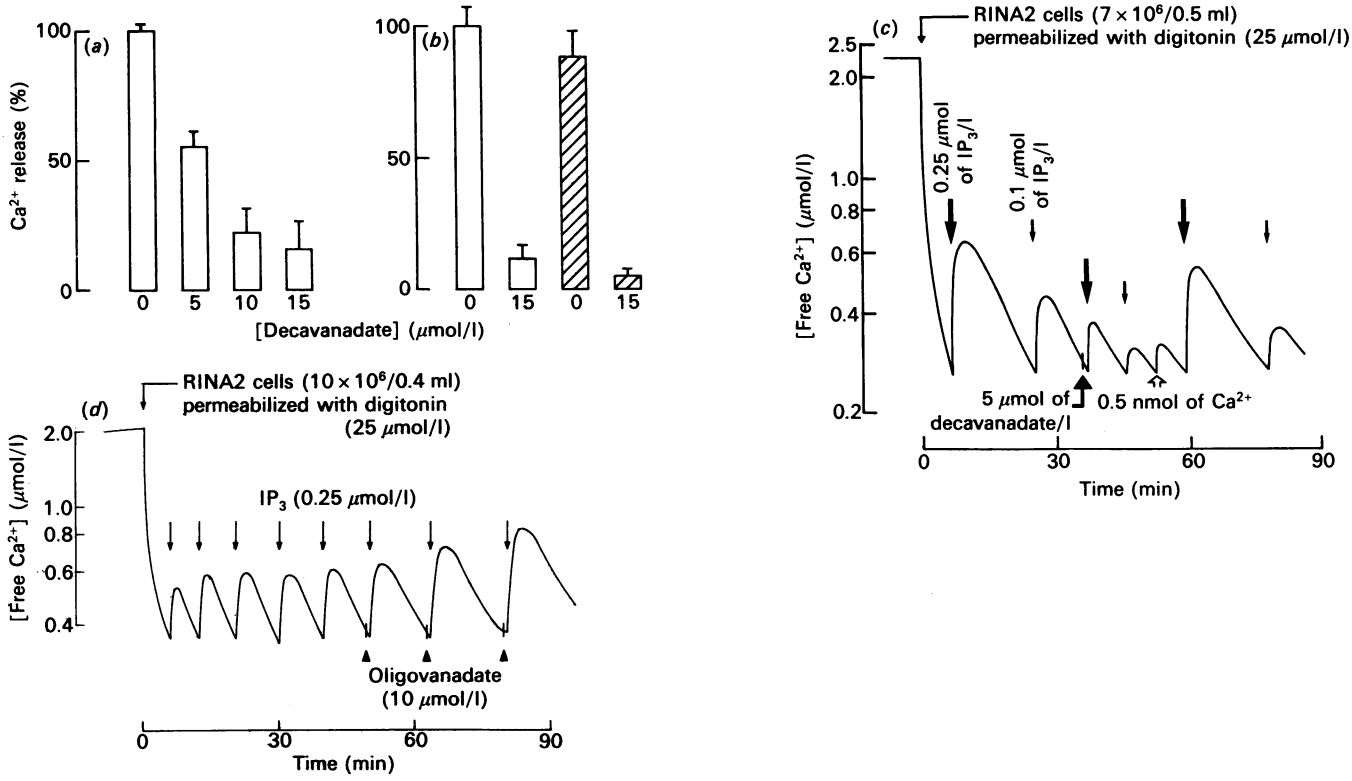

Fig. 5. Effect of decavanadate (*a*, *b*, *c*) and oligovanadate (*d*) on IP_3-induced Ca^{2+} release in digitonin-permeabilized RINA2 cells

(*a*) Inhibition of the IP_3-induced Ca^{2+} release by increasing concentrations of decavanadate. The values are expressed as the percentage of Ca^{2+} released from permeabilized RINA2 cells as compared with the release elicited by 0.25 μmol of IP_3/l (100 %; $n = 3$; means ± S.D.). (*b*) The inhibitory effect of decavanadate on the IP_3-induced Ca^{2+} release is reversible. Permeabilized cells were treated with 15 μmol of decavanadate/l (hatched bars) or an equivalent amount of medium B (open bars). Following centrifugation (400 *g*, 2 min) the amount of Ca^{2+} released by 0.25 μmol of IP_3/l (100 %; $n = 4$) in the presence or absence of 15 μmol of decavanadate/l was determined in the resuspended cells. (*c*) The inhibition of the IP_3-induced Ca^{2+} release by decavanadate can be overcome by increasing the amount of stored Ca^{2+}. Experimental conditions were the same as in (*a*). Arrows indicate the addition of IP_3 (final concentrations 0.1 and 0.25 μmol/l). The arrowhead indicates the addition of decavanadate (final concentration 5 μmol/l). The inhibition by decavanadate is overcome by additional loading of Ca^{2+} into the compartment. (*d*) The influence of oligovanadate on the IP_3-induced Ca^{2+} release. Experimental conditions were as in (*c*). Each arrow indicates the addition of 0.1 nmol of IP_3 (corresponding to 0.25 μmol/l). Each arrowhead indicates the addition of 4 nmol of oligovanadate (corresponding to 10 μmol/l).

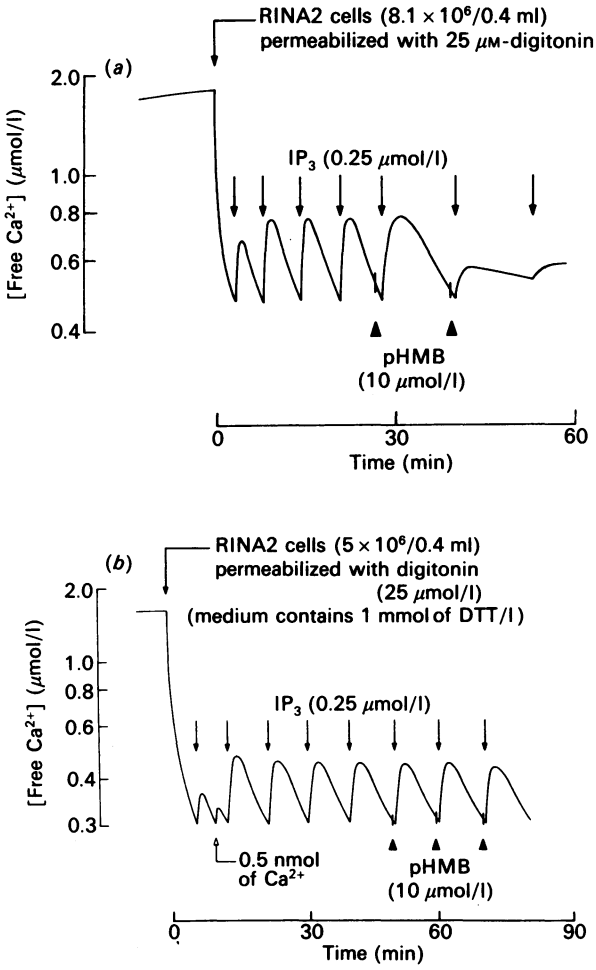

Fig. 6. The inhibition of the IP_3-induced Ca^{2+} release in digitonin-permeabilized RINA2 cells by pHMB (*a*) is abolished by DTT (*b*)

Experimental conditions were the same as in Fig. 5*c*. Each arrow indicates the addition of 0.1 nmol of IP_3 (corresponding to 0.25 μmol/l); each arrowhead indicates the addition of 4 nmol of pHMB (corresponding to 10 μmol/l).

effective in inhibiting the IP_3-induced Ca^{2+} release (results not shown).

DISCUSSION

The regulation of cytosolic free Ca^{2+} is accomplished by several cellular Ca^{2+} transport systems, comprising the plasma membrane, mitochondria and endoplasmic reticulum. Mobilization of Ca^{2+} by IP_3 from the latter is part of an intracellular signal-transducing system common to many different cell types. Experimental conditions excluded the plasma membrane by permeabilization and the mitochondria by addition of NaN_3. Thus the free Ca^{2+} concentration was mainly regulated by the endoplasmic reticulum or the so-called IP_3-sensitive Ca^{2+} compartment (Streb *et al.*, 1983; Berridge, 1987).

As shown here, pHMB inhibits the IP_3-induced Ca^{2+} release in rat insulinoma and rat pheochromocytoma cells. This finding correlates well with the situation seen in platelets (Adunyah & Dean, 1986) and the inhibition of the binding of IP_3 to its receptor in brain cell preparations by an analogue of pHMB, *p*-chloromercurisulphonate (Supattapone *et al.*, 1988). Thus the inhibition of the IP_3-induced Ca^{2+} release by pHMB is probably the result of decreased binding of IP_3 to its receptor protein. Supattapone *et al.* (1988) also found, as reported here for the IP_3-induced Ca^{2+} release, that this inhibition could be prevented by the addition of DTT.

Decavanadate similarly inhibits the IP_3-induced Ca^{2+} release. However, although almost equally potent, the mechanisms of inhibition by decavanadate and pHMB appear to be different. The inhibition by decavanadate is unaffected by the addition of DTT, indicating that decavanadate does not act by oxidation of essential thiol groups of the IP_3 receptor protein. However, the observed inhibition of the IP_3-induced Ca^{2+} release by low concentrations of decavanadate suggests a specific interaction of decavanadate with the IP_3 receptor protein.

As stated above, vanadate exists in several forms, each of which influences a different component of the intracellular Ca^{2+} uptake and release systems. As observed in this study, decavanadate specifically inhibits the IP_3-induced Ca^{2+} release. On the other hand oligo- and monovanadate inhibit the ATP-dependent Ca^{2+} uptake by permeabilized endocrine cells (Prentki *et al.*, 1984). This blockade is most probably due to the binding of the various oligomeric vanadate species to the Ca^{2+} transport ATPase (cf. Csermely *et al.*, 1983; Varga *et al.*, 1985). Indeed, we observed here that oligovanadate but not decavanadate inhibits ATP-driven Ca^{2+} uptake.

Permeabilized cell preparations have been instrumental in the investigation of intracellular Ca^{2+} transport (Prentki *et al.*, 1984), the effects of IP_3 on intracellular Ca^{2+} compartments (Streb *et al.*, 1983; Berridge, 1987), IP_3 binding to its intracellular receptor (Spät *et al.*, 1986) and in the analysis of the intracellular requirements for exocytosis (Knight & Baker, 1982; Ahnert-Hilger *et al.*, 1985, 1987; Bader *et al.*, 1986). The present investigation enlarges data on Ca^{2+} metabolism in endocrine tumour cell lines and shows for the first time the inhibition of the IP_3-induced Ca^{2+} release by decavanadate and pHMB in these cell types. Inhibition of the IP_3-induced Ca^{2+} release by decavanadate was reversible and could be overcome by increasing either the amount of sequestered Ca^{2+} or the IP_3 concentration used.

The IP_3-induced Ca^{2+} release from permeabilized RINA2 cells was, when analysed in the presence of millimolar concentrations of ATP, similar in quality and quantity to data described earlier (Biden *et al.*, 1984). The properties of PC12 cells may be compared with those of chromaffin cells in primary culture (Stöhr *et al.*, 1986; Kao, 1988). Chromaffin cells exhibited an IP_3-induced Ca^{2+} release of approx. 20% of that of PC12 cells (Stöhr *et al.*, 1986) or even less (Kao, 1988), which led to the conclusion that Ca^{2+} release induced by IP_3 may play only a small role in the physiological stimulation by acetylcholine. Furthermore, upon repeated addition of IP_3, a 'complete desensitization' occurred in these cells, i.e. a second addition of IP_3 failed to elicit further Ca^{2+} release (Stöhr *et al.*, 1986). Therefore, long-term investigations as described here for PC12 cells cannot be carried out with such chromaffin cell preparations.

The endocrine cells used in this study have been permeabilized in two different ways. The stable pores provided by α-toxin (Füssle *et al.*, 1981; Bhakdi & Traunum-Jensen, 1987) are an almost ideal tool for the investigation of the role of small molecules (up to 1 kDa) on intracellular Ca^{2+} metabolism. On the other hand, the

use of digitonin and other pore-forming proteins like streptolysin O allows the extraction of large molecules from permeabilized cells (Dunn & Holz, 1983; Wilson & Kirshner, 1983; Ahnert-Hilger *et al.*, 1985) and the introduction of others (Ahnert-Hilger *et al.*, 1989*a*,*b*). Since, as reported here, Ca^{2+} uptake and release can be measured for long periods of time (even longer than 2 h) using both protocols for permeabilization, the membrane structure which accumulates Ca^{2+} with the aid of ATP and releases it upon addition of IP_3 can now be investigated with a variety of different substances. A closer definition of the endoplasmic reticulum or other related structures (Volpe *et al.*, 1988) engaged in intracellular Ca^{2+} regulation is now feasible. Specific inhibitors of IP_3 function, such as decavanadate described here, could then be used as valuable tools to further analyse the mechanism of the IP_3-induced Ca^{2+} release in cellular signal transduction.

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