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Release of Ca^{2+} from a non-mitochondrial store site in peritoneal macrophages treated with saponin by inositol 1,4,5-trisphosphate

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The effects of inositol 1,4,5-trisphosphate, prepared from human erythrocyte ghosts, on Ca^{2+} release from intracellular store sites were studied in saponin-treated guinea pig peritoneal macrophages. Micromolar concentrations of inositol 1,4,5-trisphosphate released Ca^{2+} within 1 min from store sites which had accumulated Ca^{2+} in the presence of 10mM-NaN_3. In the presence of 10mM-NaN_3, the Ca^{2+} accumulated in the presence of oxalate was seen in the endoplasmic reticulum of saponin-treated macrophages by electron microscopy, indicating that the site of Ca^{2+} released by inositol 1,4,5-trisphosphate may be endoplasmic reticulum-like membranes. When the concentrations of free Ca^{2+} were over 3.5×10^{-6}M, the release of Ca^{2+} by this agent was inhibited. This inhibition may be due to either the higher concentration of extra-vesicular free Ca^{2+} or the larger accumulation of Ca^{2+} into the store site or perhaps both effects. $MgCl_2$ also had an inhibitory effect on the Ca^{2+} release. Inositol 1,4,5-trisphosphate also released Ca^{2+} from cardiac sarcoplasmic reticulum, but not from erythrocyte inside-out vesicles.

Activation of receptors for a wide variety of hormones and neurotransmitters leads to an increase in the intracellular concentration of free Ca^{2+}. Much of this Ca^{2+} is released from intracellular stores, but the link between surface receptors and the intracellular Ca^{2+} stores is as yet unknown. Hydrolysis of the phosphoinositides, which is another characteristic feature of the receptor activation (Michell, 1975; Berridge, 1981), has been implicated in Ca^{2+} mobilization (Michell, 1975). It was recently reported that InsP_3, which is a water-soluble product of phosphatidylinositol 4,5-bisphosphate hydrolysis, releases Ca^{2+} from a non-mitochondrial intracellular Ca^{2+} store in rat pancreatic acinar cells (Streb *et al.*, 1983). Thus, InsP_3 seems to be one of the candidates that may provide a link between surface receptors and the intracellular Ca^{2+} stores.

In the present study, we examined the effect of InsP_3 on Ca^{2+} release from the non-mitochondrial store sites of guinea pig peritoneal macrophages, and present some characteristics of this InsP_3-induced Ca^{2+} release.

Abbreviations used: InsP_3, inositol 1,4,5-trisphosphate; InsP_2, inositol 1,4-bisphosphate.

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Materials and methods

Preparation of guinea pig peritoneal macrophages and saponin treatment

Guinea pig peritoneal macrophages were prepared by the method of Yagawa *et al.* (1979). Saponin treatment was carried out as described previously (Hirata & Koga, 1982; Hirata *et al.*, 1983*a*).

Preparation of InsP_3 and ^{32}P-labelled InsP_3

InsP_3 was prepared by incubating human erythrocyte ghosts with $CaCl_2$ followed by a Dowex formate column separation, and desalted by elution from a Dowex chloride column with 1M-LiCl, followed by removal of the LiCl with ethanol (Downes *et al.*, 1982). ^{32}P-labelled InsP_3 was also prepared from ^{32}P-labelled erythrocyte ghosts, which were obtained by the method of Downes & Michell (1981). In a typical preparation starting

from 30ml of packed erythrocytes that had been labelled for 2h at 37°C with 1mCi of [^{32}P]P_i (sp. radioactivity 33mCi/mmol; Japan Atomic Energy Research Institute), we obtained 0.3μmol of InsP_3 (sp. radioactivity 2×10^6 c.p.m./μmol), which was comparable with the data of Downes *et al.* (1982).

Preparation of cardiac sacroplasmic reticulum and erythrocyte inside-out vesicles

Cardiac sarcoplasmic reticulum was prepared from canine ventricular muscle as described previously (Hirata & Inamitsu, 1983). Human inside-out vesicles were prepared by the method of Waisman *et al.* (1981). Since Ca^{2+} uptake by these preparations is enhanced by the presence of calmodulin (LePeuch *et al.*, 1980; Waisman *et al.*, 1981), calmodulin contents in these preparations were also measured as described previously (Hirata *et al.*, 1983*a*). Calmodulin contents in both preparations were less than 20ng/mg of vesicle protein. The protein concentration was determined by the method of Lowry *et al.* (1951).

Assays of Ca^{2+} uptake and Ca^{2+} release by InsP_3 in saponin-treated macrophages, cardiac sarcoplasmic reticulum and inside-out vesicles

Tris/maleate buffer, which was made by adding approx. 0.4M-maleic acid to 0.2M-Tris to bring it to pH6.8 at 37°C and diluting to 20mM, was used throughout this work. Ca^{2+} uptake was assayed as follows. The preparations (4×10^6 cells/ml for saponin-treated cells; 50μg/ml for sarcoplasmic reticulum and inside-out vesicles) were preincubated for 2min at 37°C in 5ml of a solution containing 0.1M-KCl, 20mM-Tris/maleate buffer (pH 6.8), 5mM-$MgCl_2$, 10mM-NaN_3, 0.12mM-$CaCl_2$ (containing 2μCi of ^{45}Ca/ml; radioactivity 39.9mCi/mg; New England Nuclear) and the concentration of EGTA required to obtain the desired free Ca^{2+} concentration. The Ca^{2+} uptake was started by the addition of 2mM-ATP. At 10min (for saponin-treated cells and sarcoplasmic reticulum) or 30min (for inside-out vesicles), 1ml of the above mixture was passed through a glass-fibre filter (for saponin-treated cells; Whatman GF/C, pore size 1.2μm) or a Millipore filter (for sarcoplasmic reticulum and inside-out vesicles; pore size 0.45μm). The filter was washed twice with 2ml of the above solution without ^{45}Ca, dried and then counted for radioactivity. At 11min (for saponin-treated cells and sarcoplasmic reticulum) or 31min (for inside-out vesicles), InsP_3 in 1/100 of the volume was added to the reaction mixture. At an appropriate time, 1ml of the mixture was passed through a filter. The filter was treated as described above. Desired free Ca^{2+} concentrations were obtained by the following equation:

$$[\mathrm{EGTA}]_{\mathrm{total}} = ([\mathrm{Ca}]_{\mathrm{total}} - [\mathrm{Ca}]_{\mathrm{free}}) \frac{1 + K_a[\mathrm{Ca}]_{\mathrm{free}}}{K_a[\mathrm{Ca}]_{\mathrm{free}}}$$

in which K_a is the apparent affinity constant of 1×10^6 M^{-1} at pH6.8 (Harafuji & Ogawa, 1980).

Assays of passive Ca^{2+} release in saponin-treated macrophages

Passive Ca^{2+} release was assayed as described previously (Hirata *et al.*, 1983*b*). The saponin-treated cells (4×10^6 cells) were incubated in 0.5ml of a solution containing 0.1M-KCl, 20mM-Tris/maleate buffer (pH6.8), 10mM-NaN_3, 1mM-$MgCl_2$, 0.12mM-$CaCl_2$ (containing 5μCi of ^{45}Ca), the required concentration of EGTA and 0.2mM-ATP at 37°C. At 10min, 100μl of the reaction mixture was passed through a glass-fibre filter. Thus the amount of Ca^{2+} uptake was determined. At 11min, 4ml of a solution containing 0.1M-KCl, 20mM-Tris/maleate buffer (pH6.8), 10mM-NaN_3, 2mM-EGTA and 5μM-InsP_3 was added to 400μl of the above mixture. Then, at a specified time, 1ml of the mixture was passed through a filter and the Ca^{2+} remaining in saponin-treated cells was determined.

Ca^{2+} uptake of saponin-treated macrophages for electron microscopy

Ca^{2+} uptake for electron microscopy was assayed as follows. Saponin-treated macrophages (2×10^6 cells/ml) were incubated in 10ml of a solution containing 0.1M-KCl, 20mM-Tris/maleate (pH6.8), 5mM-$MgCl_2$, 0.12mM-$CaCl_2$, 5mM-potassium oxalate, 2mM-ATP, 10mM-creatine phosphate and 2 units of creatine kinase (Sigma, type I)/ml, either in the presence or absence of 10mM-NaN_3 for 30min at 37°C. After centrifugation at 250*g* for 10min, the cell pellet was fixed with 2.4% (w/v) glutaraldehyde, and treated as described previously (McGraw *et al.*, 1980).

Results

Time course and dose-dependence of InsP_3-induced Ca^{2+} release from saponin-treated macrophages

Fig. 1 shows the time-course of Ca^{2+} release from the store sites of saponin-treated macrophages by application of 5μM-InsP_3. InsP_3 released Ca^{2+} within 20s and this released Ca^{2+} was about 25% of the accumulated Ca^{2+}. The Ca^{2+} ionophore A23187 (1μM) released all of the accumulated Ca^{2+}, whereas InsP_2 had no effect on the Ca^{2+} release. Fig. 2 shows the dose–response relationship of InsP_3-induced Ca^{2+} release. Near-maximal and half-maximal release of Ca^{2+} from the store sites were obtained at 3μM and 0.8μM,

respectively, such concentrations being comparable with the data of Streb *et al.* (1983). The Ca^{2+} released by InsP_3 was not taken up again within 20min, whereas Ca^{2+} released was taken up again within 20min in rat pancreatic acinar cells (Streb *et al.*, 1983). The lack of re-uptake of the released Ca^{2+} may not be due to the depletion of ATP, because we added 2mM-ATP in 5ml (10μmol of ATP) to the reaction mixture and ATP hydrolysis was about 0.07μmol/10min per 4×10^6 saponin-treated cells. Therefore, we next examined the hydrolysis of InsP_3 by saponin-treated macrophages by using ^{32}P-labelled InsP_3 (Table 1). ^{32}P radioactivity eluted from a Dowex formate column by 0.1M-formic acid and 1M-ammonium formate was decreased to about 59% and 49% by incubating InsP_3 with saponin-treated cells and sonicated macrophages, respectively. The result indicates that about 41% of the InsP_3 added was hydrolysed, and thus 2.95μM-InsP_3 remained 20min after the addition of 5μM-InsP_3. From the data of Fig. 2,

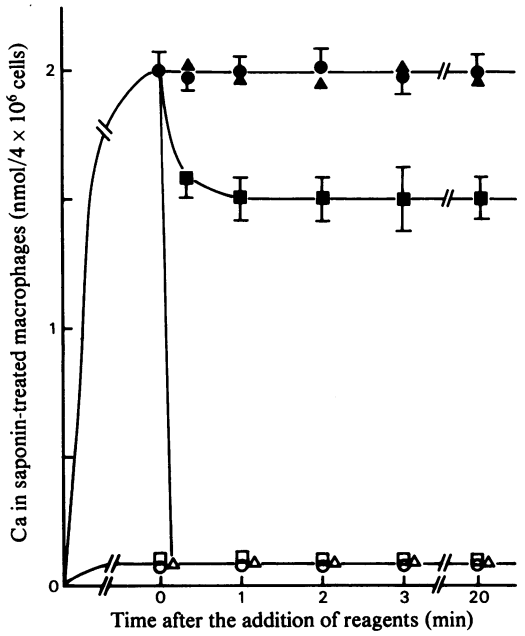

Fig. 1. *Time course of Ca^{2+} release induced by InsP_3 from the intracellular store site of saponin-treated macrophages*

Ca^{2+} was accumulated in the cells as described in the Materials and methods section for 10min. At 11min, reagents in 1/100 volume were added, and at the indicated times, the Ca^{2+} in saponin-treated cells was determined. ●, Control (the same volume of water was added); ■, 5μM-InsP_3; ▲, 1μM-InsP_2; △, 1μM-A23187; ○, ATP-free control; □, ATP-free + InsP_3. The vertical bars represent the S.E.M. for five independent experiments.

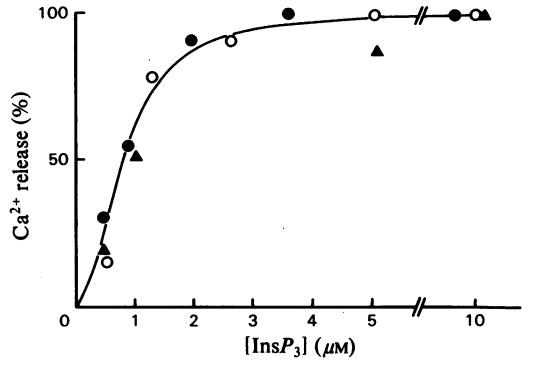

Fig. 2. *Dose–response relationship for InsP_3-induced Ca^{2+} release*

Ca^{2+} release was measured as described in the Materials and methods section and in Fig. 1 legend. Ca^{2+} release was relative to that 1min after the addition of 10μM-InsP_3. The different symbols represent experiments carried out using different preparation of InsP_3 and saponin-treated macrophages.

Table 1. *Hydrolysis of ^{32}P-labelled InsP_3 by saponin-treated macrophages and sonicated macrophages*

Saponin-treated macrophages (2×10^7 cells) or sonicated macrophages (2×10^7 cells) were incubated in 1ml of the same solution as that for Ca^{2+} uptake, except that 5μM-^{32}P-labelled InsP_3 (radioactivity approx. 10000 c.p.m.) was included, for 20min at 37°C. The reaction was stopped by the addition of 50% (w/v) trichloroacetic acid to give a final concentration of 5%. After centrifugation, trichloroacetic acid was removed by diethyl ether treatment (4 × 5ml). Then, fractionation on a Dowex-1 (formate form) column was employed as described previously (Downes *et al.*, 1982). As control, 5μM-^{32}P-labelled InsP_3 without cells was treated as described above. The results are means for two experiments.

	^{32}P-radioactivity (c.p.m.) eluted by:		
	0.2M-Ammonium formate/ 0.1M formic acid (P_i)	0.4M-Ammonium formate/ 0.1M-formic acid (InsP_2)	1.0M-Ammonium formate/ 0.1M-formic acid (InsP_3)
Control	502	822	8258
Saponin-treated macrophages	2735	2051	4896
Sonicated macrophages	3158	2384	4012

2.95 μM-InsP_3 had still near-maximal effect. Thus, the lack of re-uptake of the Ca^{2+} released may be due to the remaining InsP_3 which will induce Ca^{2+} release. When we added 1 μM-InsP_3 to induce Ca^{2+} release, re-uptake was clearly observed within 20 min (results not shown).

Site of Ca^{2+} released by InsP_3

Ca^{2+} uptake by saponin-treated macrophages was enhanced by oxalate as described previously (Hirata & Koga, 1982). McGraw *et al.* (1980) showed calcium oxalate precipitates in Ca^{2+}-accumulating vesicles in rat brain synaptosomes by electron microscopy. Therefore, we examined calcium oxalate precipitates in saponin-treated macrophages (Fig. 3). In the presence of 10 mM-NaN_3, the calcium oxalate precipitates were observed only in the endoplasmic reticulum, but not in the mitochondria (Fig. 3*c*). On the other hand, the precipitates were observed in the mitochondria in the absence of NaN_3 (Fig. 3*b*). Since staining with uranyl acetate and lead was carried out to

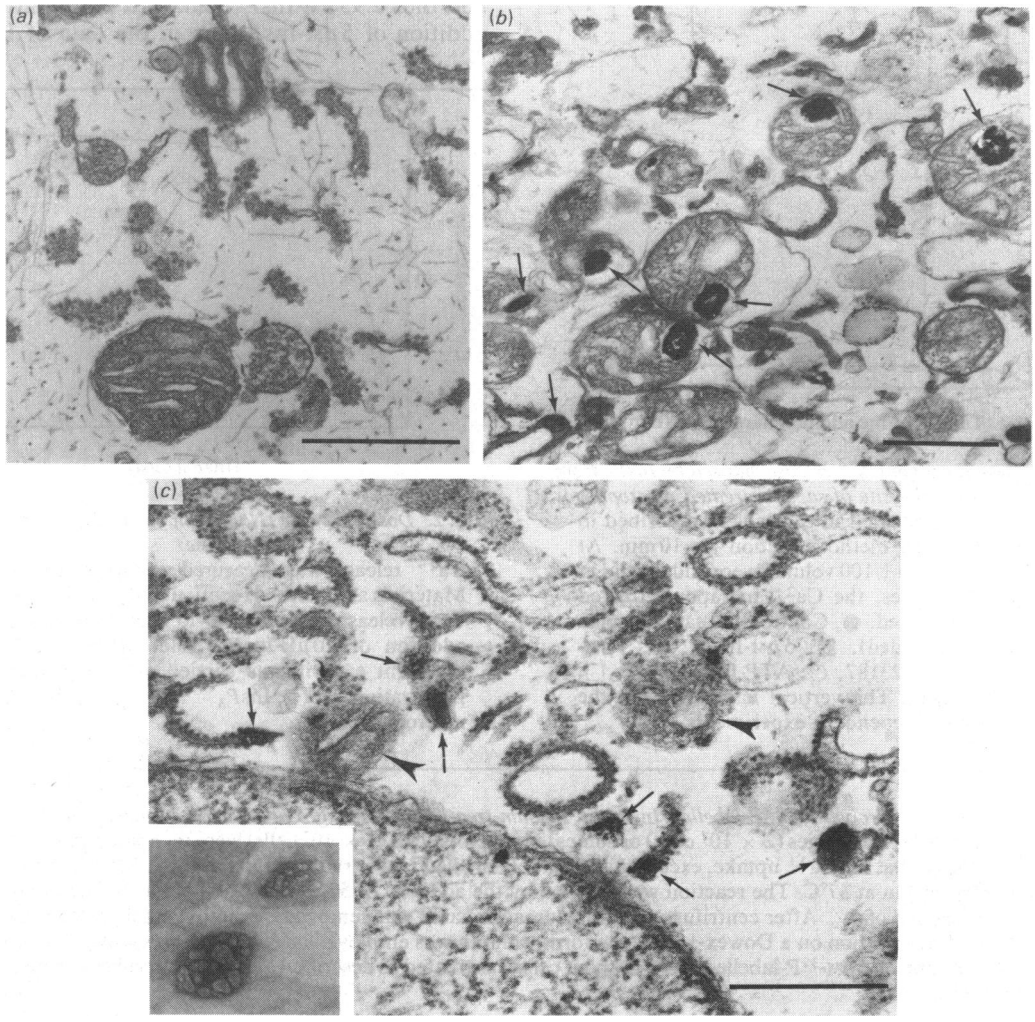

Fig. 3. *Electron micrographs of saponin-treated macrophages that have accumulated Ca^{2+} in the presence of 5 mM-oxalate* Ca^{2+} was accumulated in these cells in the presence (*c*) or absence (*b*) of 10 mM-NaN_3, as marked in the Materials and methods section. The amounts of Ca^{2+} uptake were about 31 or 170 nmol/4 $\times$ 10^6 saponin-treated macrophages in the presence or absence of NaN_3, respectively. (*a*) represents the saponin-treated macrophages which were assayed in the absence of $CaCl_2$. The precipitates of calcium oxalate are indicated by small arrows. The addition of NaN_3 seems to destroy the structure of mitochondria, as indicated by arrow-heads in (*c*). Inset in (*c*): unstained precipitate of calcium oxalate. Magnification: $\times$ 46000 (*a* and *c*); $\times$ 33000 (*b*); $\times$ 77000 (inset in *c*). Bar, 0.5 μm.

show ribosomes on the endoplasmic reticulum, the precipitates of calcium oxalate were seen as totally electron-dense areas. The precipitates which were not stained could be seen as shown in the inset of Fig. 3(*c*).

Characteristics of $InsP_3$-induced Ca^{2+} release

Fig. 4 shows the effects of varying concentrations of free Ca^{2+} and $MgCl_2$ on the Ca^{2+} release induced by $InsP_3$ (5 μM). The maximal Ca^{2+} release induced by $InsP_3$ was obtained when Ca^{2+} uptake was assayed at a free Ca^{2+} concentration less than 1.5×10^{-6} M, and Ca^{2+} release was inhibited when Ca^{2+} was loaded in the presence of free Ca^{2+} at concentrations over 3.5×10^{-6} M. $MgCl_2$ inhibited the $InsP_3$-induced Ca^{2+} release (Fig. 4). Since the amounts of Ca^{2+} uptake were not affected by the concentrations (3–10 mM) of $MgCl_2$ tested, the inhibitory effect may be due to higher concentrations of $MgCl_2$, but not to larger accumulation of Ca^{2+} into the store site.

To examine the inhibitory mechanism of $InsP_3$-induced Ca^{2+} release by higher free Ca^{2+} concentration, we employed the dilution method as described previously (Hirata *et al.*, 1983*b*). Ca^{2+} release seems to be a passive phenomenon because the final ATP concentration was as low as 0.007 mM in the mixture of the Ca^{2+} uptake solution and dilution medium after taking into account the hydrolysis of ATP during Ca^{2+} uptake (70 nmol/10 min per 4 $\times 10^6$ saponin-treated macrophages at 37°C) and no apparent Ca^{2+} uptake was observed in the presence of 0.007 mM-ATP. Fig. 5 shows the passive Ca^{2+} release induced by $InsP_3$. Ca^{2+} uptake into the store site of saponin-treated macrophages was carried out at a free Ca^{2+} concentration of either 1.4×10^{-7} M or 2.4×10^{-5} M, Then, passive Ca^{2+} release in the presence or absence of $InsP_3$ was observed after dilution. Since the dilution medium contained 2 mM-EGTA, the free Ca^{2+} concentration in the mixture of the Ca^{2+} uptake solution and dilution medium became less than 10^{-8} M in both cases. Thus, the result seems to represent the effect of the amount of accumulated Ca^{2+} on the $InsP_3$-induced Ca^{2+} release. At free Ca^{2+} concentrations of 1.4×10^{-7} M and

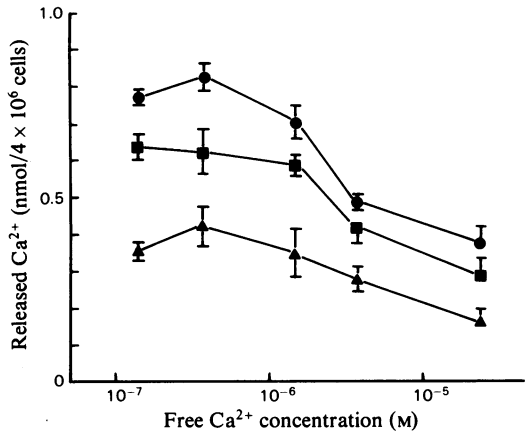

Fig. 4. *Effect of various concentrations of free Ca^{2+} and $MgCl_2$ on the Ca^{2+} release induced by $InsP_3$*
Ca^{2+} was accumulated in saponin-treated macrophages at various free Ca^{2+} concentrations indicated on the abscissa in the presence of 3 mM (●), 5 mM (■) and 10 mM (▲) $MgCl_2$, and 2 mM-ATP. The amounts of accumulated Ca^{2+} at free Ca^{2+} concentrations of 1.4×10^{-7} M, 3.7×10^{-7} M, 1.5×10^{-6} M, 3.5×10^{-6} M and 2.4×10^{-5} M were 2.2, 3.5, 4.2, 4.7 and 4.7 nmol/4 × 10^6 saponin-treated macrophages respectively. The amounts of released Ca^{2+} 1 min after the addition of 5 μM-$InsP_3$ were plotted against free Ca^{2+} concentrations. The vertical bars represent the S.E.M. for four independent experiments.

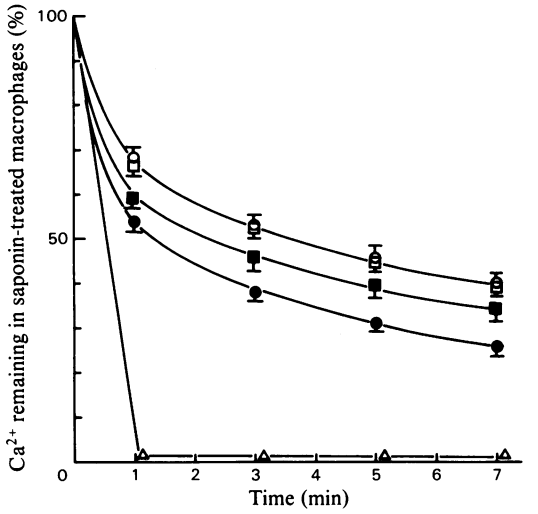

Fig. 5. *Passive Ca^{2+} release by $InsP_3$ in saponin-treated macrophages that had accumulated Ca^{2+} at a free Ca^{2+} concentration of 1.4×10^{-7} M or 2.4×10^{-5} M*
Passive Ca^{2+} release was assayed as described in the Materials and methods section. The Ca^{2+} was accumulated in saponin-treated macrophages at free Ca^{2+} concentrations of either 1.4×10^{-7} M (○, ●) or 2.4×10^{-5} M (□, ■). Since the dilution medium contained 2 mM-EGTA, the free Ca^{2+} concentration became less than 10^{-8} M. Open symbols, control; closed symbols, 5 μM-$InsP_3$; △, 1 μM-A23187. The amount of Ca^{2+} remaining in saponin-treated macrophages in the absence of ATP during Ca^{2+} uptake was subtracted from that in the presence of ATP. The vertical bars represent the S.E.M. for four independent experiments.

2.4×10^{-5}M, the amounts of Ca^{2+} accumulated into the store site in the presence of 0.2mM-ATP were 1.6 ± 0.1 ($n = 4$) and 3.4 ± 0.2 ($n = 4$) nmol/4×10^6 saponin-treated macrophages, which were slightly smaller than those obtained in the presence of 2mM-ATP. Ca^{2+} release induced by InsP_3 was inhibited by the larger amount of accumulated Ca^{2+}. The addition of 1 μM-A23187 released all of the accumulated Ca^{2+} within 1 min.

Fig. 6 shows the passive InsP_3-induced Ca^{2+} release at various extravesicular free Ca^{2+} concentrations. Ca^{2+} uptake into the store site of saponin-treated macrophages was carried out at a free Ca^{2+} concentration of 1.4×10^{-7}M. The dilution medium contained 2mM-EGTA and various concentrations of $CaCl_2$ to obtain free Ca^{2+} concentrations of 1.4×10^{-7}M and 2.4×10^{-5}M. Ca^{2+} cannot be accumulated in spite of the presence of a higher free Ca^{2+} concentration (2.4×10^{-5}M), because the final ATP concentration becomes 0.007mM in the mixture of the Ca^{2+} uptake solution and the dilution medium as described above. Therefore, the result shown in Fig. 6 seems to represent the effect of extravesicular free Ca^{2+} concentration on InsP_3-induced Ca^{2+} release. When the extravesicular Ca^{2+} concentration was higher, the accumulated Ca^{2+} was more easily released. This may be due to Ca^{2+}-induced Ca^{2+} release, as shown previously (Hirata *et al.*, 1983*b*). Ca^{2+} release induced by InsP_3 in the presence of an extravesicular free Ca^{2+} concentration of 2.4×10^{-5}M was smaller than that observed in the presence of less than 10^{-8}M and 1.4×10^{-7}M free Ca^{2+}. Thus, the results shown in Figs. 5 and 6 indicate that both the higher concentrations of extravesicular Ca^{2+}, and larger accumulation of Ca^{2+} into the store site, may have an inhibitory effect on the Ca^{2+} release induced by InsP_3.

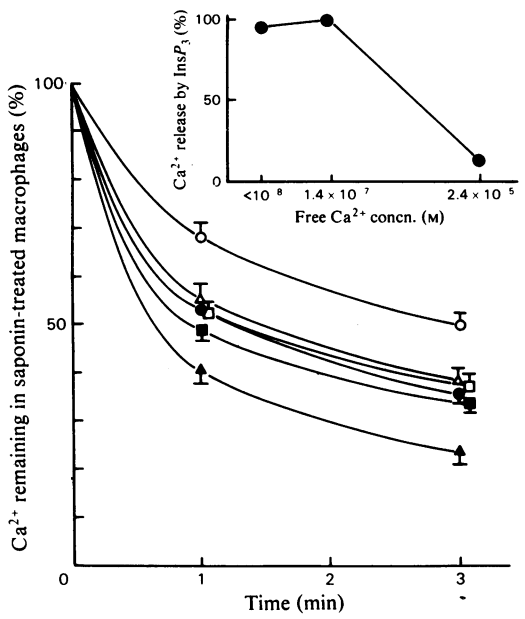

Fig. 6. *Passive Ca^{2+} release by InsP_3 at various free Ca^{2+} concentrations*

Ca^{2+} was accumulated in saponin-treated macrophages at a free Ca^{2+} concentration of 1.4×10^{-7}M. The dilution medium contained 2mM-EGTA and various concentrations of $CaCl_2$ to obtain a free Ca^{2+} concentration of 1.4×10^{-7}M or 2.4×10^{-5}M. ○, ●, free Ca^{2+} concentration less than 10^{-8}M; △, ▲, 1.4×10^{-7}M free Ca^{2+}; □, ■, 2.4×10^{-5}M free Ca^{2+}. Open symbols, control; closed symbols, 5 μM-InsP_3. The amount of Ca^{2+} remaining in saponin-treated macrophages in the absence of ATP during Ca^{2+} uptake was subtracted from that in the presence of ATP. The vertical bars represent the S.E.M. for three independent experiments. The inset represents the Ca^{2+} release induced by InsP_3 1 min after dilution at various free Ca^{2+} concentrations.

InsP_3 releases Ca^{2+} from cardiac sarcoplasmic reticulum, but not from erythrocyte inside-out vesicles

Maximal Ca^{2+} uptake of cardiac sarcoplasmic reticulum was obtained after 10 min. On the other hand, maximal Ca^{2+} uptake of inside-out vesicles was not obtained even after more than 30 min.

Fig. 7 shows the effect of InsP_3 on Ca^{2+} release in cardiac sarcoplasmic reticulum and erythrocyte inside-out vesicles. InsP_3 induced Ca^{2+} release in cardiac sarcoplasmic reticulum. About 19% of the Ca^{2+} accumulated by cardiac sarcoplasmic reticulum in the presence of 3.7×10^{-7}M free Ca^{2+} was released by the application of 5 μM-InsP_3. InsP_3-induced Ca^{2+} release was inhibited when Ca^{2+} uptake into sarcoplasmic reticulum was carried out at a free Ca^{2+} concentration of 2.4×10^{-5}M, the same as for saponin-treated macrophages. On the other hand, InsP_3 (5 μM) did not induce release of Ca^{2+} accumulated by erythrocyte inside-out vesicles in the presence of free Ca^{2+} concentrations of 3.7×10^{-7}M and 2.4×10^{-5}M, while the addition of A23187 (1 μM) released all of the Ca^{2+} accumulated into inside-out vesicles (Fig 7*b*). Passive Ca^{2+} release was also enhanced by the addition of InsP_3 to cardiac sarcoplasmic reticulum but not to erythrocyte inside-out vesicles (results not shown).

Discussion

In this study, we showed that micromolar concentrations of InsP_3 release Ca^{2+} from intracellular store sites of guinea pig peritoneal macrophages treated with saponin, as for rat pancreatic acinar cells as reported by Streb *et al.* (1983). The store sites of Ca^{2+} released by InsP_3 seem to be non-mitochondrial sources, because mitochondria

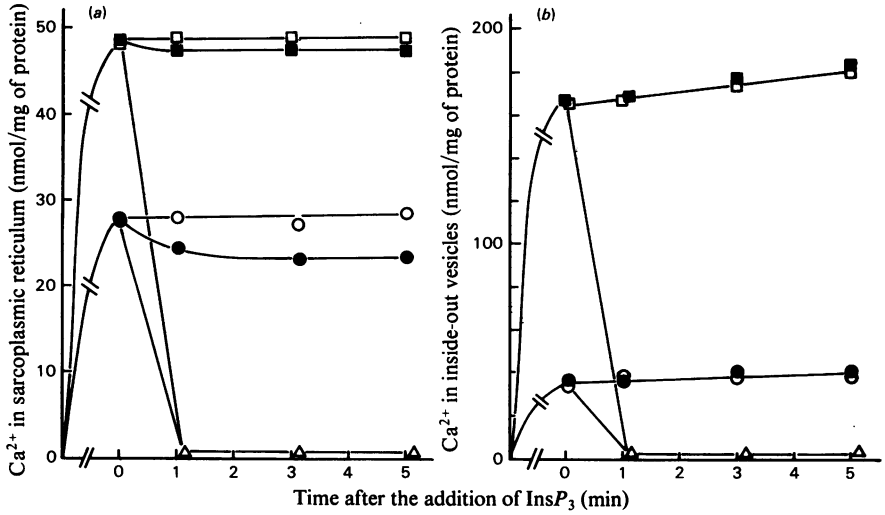

Fig. 7. *Effect of $InsP_3$ on Ca^{2+} release in cardiac sarcoplasmic reticulum and erythrocyte inside-out vesicles*
Ca^{2+} was accumulated in cardiac sarcoplasmic reticulum (*a*) or erythrocyte inside-out vesicles (*b*) at a free Ca^{2+} concentration of either 3.7×10^{-7}M (○, ●) or 2.4×10^{-5}M (□, ■) for 10min (*a*) and 30min (*b*), respectively. At 11min or 31min, 5μM-$InsP_3$ was added to the reaction mixture. Open symbols, control (the same volume of water was added); closed symbols, 5μM-$InsP_3$; △, 1μM-A23187. Ca^{2+} uptake in the absence of ATP was subtracted from the value of Ca^{2+} uptake in the presence of ATP. Each point represents the mean for two independent experiments.

are unable to accumulate Ca^{2+} from a solution containing 1.4×10^{-7}M free Ca^{2+} and 10mM-NaN_3 (Hirata & Koga, 1982; Hirata *et al.*, 1983*a*,*b*) which was routinely used to accumulate Ca^{2+} into store sites throughout this work. The cytoplasmic sides of plasma membrane and endoplasmic reticulum have been proposed as non-mitochondrial Ca^{2+} store sites (Hoffstein, 1979; Hirata & Koga, 1982; Burgess *et al.*, 1983). We directly showed precipitates of calcium oxalate only in the endoplasmic reticulum which accumulated Ca^{2+} in the presence of NaN_3. Although the experiments on Ca^{2+} release by $InsP_3$ were carried out in the absence of oxalate, the store site of Ca^{2+} released by $InsP_3$ may be endoplasmic reticulum. Furthermore, $InsP_3$ also released Ca^{2+} from cardiac sarcoplasmic reticulum, which is muscle endoplasmic reticulum, and we recently found that $InsP_3$ released Ca^{2+} from the sarcoplasmic reticulum of vascular smooth muscle (Suematsu *et al.*, 1984). However, we cannot completely exclude the possibility that the plasma membrane of macrophages may also be the store site of Ca^{2+} released by $InsP_3$, because saponin treatment destroys the plasma membrane but cannot remove the membrane (Hirata *et al.*, 1983*b*).

$InsP_3$-induced Ca^{2+} release is partial, whereas A23187-induced Ca^{2+} release is total. This may be due either to the $InsP_3$-induced release not being Ca^{2+} ionophore action or the presence of endoplasmic reticulum insensitive to $InsP_3$. Since the mechanisms for Ca^{2+} release by $InsP_3$ are unknown at the moment, why a larger accumulation of Ca^{2+} into the store site and higher concentrations of extravesicular Ca^{2+} inhibit the $InsP_3$-induced Ca^{2+} release has not been elucidated.

It is well established that Ca^{2+} is important in regulating several of the basic functions of neutrophils. For example, the intracellular free Ca^{2+} concentration has been implicated in the regulation of chemotaxis, degranulation, and the stimulation of the respiratory burst (Becker *et al.*, 1981). Such may be the case in macrophages. We recently found by using ^{45}Ca and the Ca^{2+} indicator quin2 that *N*-formyl chemotactic peptides increase the intracellular free Ca^{2+} concentration by inducing Ca^{2+} release from the non-mitochondrial store sites in guinea pig peritoneal macrophages (Hirata *et al.*, 1983*c*, 1984). However, the mechanisms by which chemotactic peptides signal the Ca^{2+} release have not been clarified. Takenawa *et al.* (1983) reported that the chemotactic peptide fMet-Leu-Phe rapidly induced phosphatidylinositol hydrolysis in a Ca^{2+}-independent manner in guinea pig peritoneal neutrophils. Thus, $InsP_3$ produced from phosphatidylinositol 4,5-bisphosphate upon chemotactic peptide stimulation of macrophages may be signalling Ca^{2+} release from non-mitochondrial stores (mainly endoplasmic reticulum). Further studies are now required to examine

whether the time course and amount of $InsP_3$ production are correlated with those of the increase in the intracellular free Ca^{2+} concentration in macrophages stimulated with fMet-Leu-Phe.

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