D-myo-Inositol 1,4,5-trisphosphate phosphatase in skeletal muscle

Daria MILANI*
Centro di Studio delle Biomembrane del CNR, Istituto di Patologia Generale dell’Università di Padova, via Loredan 16, 35131 Padova, Italy,
Pompeo VOLPE†
Centro di Studio per la Biologia e la Fisiopatologia Muscolare del CNR, Istituto di Patologia Generale dell’Università di Padova, via Loredan 16, 35131 Padova, Italy, and Tullio POZZAN
Istituto di Patologia Generale dell’Università di Ferrara, via Borsari, 44100 Ferrara, Italy

The presence and subcellular distribution of D-myo-inositol 1,4,5-trisphosphate phosphatase (InsP3ase) in rabbit fast-twitch skeletal muscle were investigated. A specific InsP3ase was found in both sarcotubular-membrane and soluble fractions. Membrane-bound InsP3ase accounted for 60–65% of total activity. The InsP3ase was detected both on the surface membranes and on the InsP3-sensitive intracellular Ca2+ store, i.e. the sarcoplasmic reticulum. The Km for inositol 1,4,5-trisphosphate (InsP3) ranged between 15 and 18 µM, and the highest Vmax (19.6 nmol of InsP3 hydrolysed/min per mg of protein) was measured in a membrane fraction enriched in transverse tubules. Several known inhibitors of InsP3ase, e.g. 2,3-bisphosphoglycerate, CdCl2 and EDTA, were active on skeletal-muscle InsP3ase. Total InsP3ase activity of both rabbit and frog skeletal muscle was comparable with that of rabbit brain, liver and main pulmonary artery (smooth muscle). The present results are consistent with the hypothesis that InsP3 plays a role in excitation–contraction coupling in skeletal muscle [Volpe, Salvati, Di Virgilio & Pozzan (1985) Nature (London) 316, 347–349].

INTRODUCTION

Ca2+ release from the TC of skeletal-muscle SR is triggered by the depolarization of sarcolemma and T-tubules. It is well established that signal transduction for Ca2+ release occurs at the triad where TC and T-tubules are functionally associated, yet the nature of the excitation–contraction coupling mechanism is still largely unknown (Somlyo, 1985).

Among the hypotheses proposed to explain excitation–contraction coupling, one states that, as a consequence of T-tubule depolarization, a chemical messenger, e.g. InsP3, produced at the level of the T-tubule membrane and released within the triad junction, opens Ca2+ channels localized in the junctional SR membrane (Volpe et al., 1986). This model reproduces with some major kinetics and anatomical modifications what has been previously proposed for non-muscle cells (Berridge & Irvine, 1984): receptor activation at the plasma-membrane level evokes phospholipase C-dependent breakdown of PtdInsP2 to InsP3 and diacylglycerol. InsP3 acts as a second messenger and releases Ca2+ from intracellular non-mitochondrial stores. InsP3 was shown to induce Ca2+ release from the SR of both isolated fractions (Volpe et al., 1985) and skinned fibres (Volpe et al., 1985; Vergara et al., 1985) of skeletal muscle. Other laboratories have subsequently confirmed our findings (Donaldson et al., 1987; Walker et al., 1987; Volpe et al., 1987a; but see Palade, 1987). The model involving InsP3 in excitation–contraction coupling requires that the complex enzymic machinery responsible for the synthesis and hydrolysis of PtdInsP2, the membrane-bound precursor of InsP3 (Berridge & Irvine, 1984), and for the catabolism of InsP3 should be present in skeletal muscle. In this respect, Hidalgo et al. (1986) and Varsanyi et al. (1986) have shown that PtdInsP3 is localized in the T-tubule along with the relevant PtdIns and PtdInsP kinases (Hidalgo et al., 1986).

A major argument against the physiological role of the InsP3 in excitation–contraction coupling has been the relatively high concentrations of InsP3 required to elicit contractions in skinned fibres. The ad hoc explanation has been that high concentrations of InsP3 were needed to overcome a potent InsP3ase (Vergara et al., 1985; Donaldson et al., 1987). However, the skeletal-muscle InsP3ase activity was directly measured in single skinned fibres, and the specific activity appeared to be extremely low (Walker et al., 1987). This finding was considered a blow to the proposed role of InsP3 in excitation–contraction coupling (Walker et al., 1987).

In the present paper we show, instead, that a specific InsP3ase is present in skeletal muscle. The total activity of skeletal-muscle InsP3ase was comparable with that of other tissues, e.g. brain, liver and smooth muscle, where a role for InsP3 in stimulus–activation coupling is undisputed. Here we also demonstrate that skeletal-

Abbreviations used: SR, sarcoplasmic reticulum; LSR, longitudinal SR; TC, terminal cisternae; JFM, junctional SR; T-tubule, transverse tubule; InsP3, D-myo-inositol 1,4,5-trisphosphate; InsP2, D-myo-inositol 1,4-bisphosphate; InsP1, D-myo-inositol 1-phosphate; InsP3ase, D-myo-inositol 1,4,5-trisphosphate phosphatase; PtdInsP3, phosphatidylinositol 4,5-bisphosphate; PtdInsP2, phosphatidylinositol 4-phosphate; PtdIns, phosphatidylinositol.

* Present address: Fidia Research Laboratories, Abano Terme, Padova, Italy.
† To whom correspondence should be addressed. Present address: Department of Physiology and Biophysics, University of Texas Medical Branch, Galveston, TX 77550, U.S.A.
muscle InsP$_3$ase is present not only at the plasma-membrane level and in the myoplasm, as in other tissues, but also at the level of the SR, the intracellular InsP$_3$-sensitive target (Volpe et al., 1985). Thus the skeletal-muscle InsP$_3$ase seems to have a strategic localization at the triad level, on both T-tubules and TC membranes, where signal transduction for muscle activation occurs.

**EXPERIMENTAL**

**Isolation of SR fractions**

SR was isolated from rabbit fast-twitch skeletal muscles and fractionated by isopycnic sucrose-density-gradient centrifugation into R1 (10%–27% sucrose interface), R2 (27%–32% sucrose interface), R3 (32%–38% sucrose interface) and R4 (38%–45% sucrose interface) as previously described (Saito et al., 1984; Zorzato et al., 1985). SR fractions were resuspended in 0.3 M sucrose/5 mM-imidazole, pH 7.4 (buffered sucrose), and stored at −80 °C until used. JFM was obtained from the TC fraction by the procedure developed by Costello et al. (1986), in the presence of either 0.7% Triton X-100 or 1% octaethylene glycol mono-n-dodecyl ether (C$_{12}$E$_8$). Protein concentration was determined by the method of Lowry et al. (1951), with bovine serum albumin as a standard.

**KCl wash of SR fractions**

Samples of fractions R1, R2 and R4 were incubated in buffered sucrose supplemented with 0.6 M-KCl for 10 min at 0 °C and then centrifuged at 150000 g for 60 min. Pellets were resuspended in buffered sucrose, and the supernatants were dialysed at 0 °C for 12 h against buffered sucrose. Both pellets and supernatants were stored at −80 °C until used.

**Isolation of muscle soluble fractions**

Soluble fractions were obtained during purification of SR (Saito et al., 1984). The supernatant obtained from the centrifugation of the first homogenate at 7700 g for 10 min was further centrifuged at 120000 g for 90 min, and the resulting supernatant (soluble fraction A) was collected and stored at −80 °C. A second soluble fraction (fraction B) was obtained by re-homogenizing the 7700 g pellet and treating the supernatant as described above.

**Homogenates of rabbit liver, brain, main pulmonary artery and skeletal muscle and of frog skeletal muscle**

The tissues were homogenized with 10 vol. of 0.3 M-sucrose, 5 mM-imidazole, pH 7.4, containing 200 μg of phenylmethanesulphonyl fluoride/l in a motor-driven Potter–Elvehjem homogenizer. Homogenates were centrifuged at 2500 g for 10 min to remove intact cells, debris and nuclei, and supernatants were frozen in liquid N$_2$ and stored at −80 °C until used. Several main pulmonary arteries were pooled for a single experiment, and their adventitia were removed as indicated by Somlyo et al. (1985)

**Assay for degradation of [³H]InsP$_3$**

For analysis of InsP$_3$ase activity, InsP$_3$ (final concn. 5–400 μM) and [³H]InsP$_3$ (20000 d.p.m.) were diluted into medium (final vol. 0.1 ml) containing 110 mM-KCl, 10 mM-NaCl, 1 mM-KH$_2$PO$_4$, 20 mM-Hepes, pH 7.2, 3 mM-MgSO$_4$ (standard medium at 30 °C) (Joseph & Williams, 1985). The reaction was started by adding an equal volume of the assay medium containing the protein (final concn. 1–0.5 mg/ml). The reaction was stopped by adding 0.2 ml of ice-cold 15%, (v/v) trichloroacetic acid. After centrifugation at 12000 rev./min for 5 min, the supernatants were extracted with 4 x 0.4 ml of diethyl ether, neutralized with 0.1 ml of 0.1 m-sodium tetraborate and loaded on columns containing 0.6 ml of Dowex-1 (formate form). Elution was performed as previously described (Downes & Michell, 1981). 0.6 ml fractions were collected, and the radioactivity was measured by liquid-scintillation spectrometry.

**Materials**

D-myo-[³H]InsP$_3$ and D-myo-InsP$_3$ were obtained from Amersham. One batch of especially purified InsP$_3$ was kindly given by Dr R. F. Irvine. 2,3-Bisphosphoglycerate was obtained from Boehringer, and D-(α)-fructose 1,6-bisphosphate, D-(α)-fructose 2,6-disphosphate and D-(α)-glucose 1,6-bisphosphate were from Sigma. Dowex AG1-X8 (formate form) was purchased from Bio-Rad. All other reagents were of analytical or higher grade.

**RESULTS**

An enzyme that specifically hydrolysates InsP$_3$ to InsP$_2$ has been demonstrated in many cell types (Downes & Michell, 1981; Downes et al., 1982; Storey et al., 1984; Joseph & Williams, 1985; Connolly et al., 1986; Sasaguri et al., 1985; Kukita et al., 1986; Rana et al., 1986; Erneux et al., 1986; Guillemin et al., 1987; Walker et al., 1987). From the data presented in Table 1, it appears that a similar InsP$_3$ase activity is also present in homogenates of both rabbit and frog skeletal muscle. The total InsP$_3$ase activity of skeletal muscle, expressed as nmol of InsP$_3$ produced/g wet wt. of tissue, is slightly lower than that of brain, liver and main pulmonary artery (smooth muscle). If allowance is made for the myofibrillar protein content (more than 80% of total

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Specific activity (nmol of InsP$_3$ produced/min per mg of protein)</th>
<th>Total activity (nmol of InsP$_3$ produced/min per g wet wt.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frog muscle</td>
<td>0.63</td>
<td>77</td>
</tr>
<tr>
<td>Rabbit muscle</td>
<td>1.36</td>
<td>111</td>
</tr>
<tr>
<td>Rabbit liver</td>
<td>1.43</td>
<td>190</td>
</tr>
<tr>
<td>Rabbit brain</td>
<td>4.70</td>
<td>305</td>
</tr>
<tr>
<td>Rabbit MPA</td>
<td>2.95</td>
<td>203</td>
</tr>
</tbody>
</table>
protein), the total Ins$_P_3$ase activity in skeletal muscle is comparable with that of other tissues.

After 5 min incubation at 30 °C, a small amount of radioactivity was found in the fractions attributable to inositol and Ins$_P_3$ for homogenates of skeletal muscles and rabbit liver. On the other hand, a relatively high percentage of radioactivity co-eluted with $[^3H]$Ins$_P_2$ in the case of homogenates of brain and main pulmonary artery (Table 1).

**Membrane-bound Ins$_P_3$ase**

By following the fractionation procedure developed by Saito et al. (1984), we have isolated membrane fractions derived from different portions of the sarcotubular system of rabbit skeletal muscle. As reported by Saito et al. (1984), fraction R1 contains mostly longitudinal cisternae (LSR), with some T-tubules and, probably, sarcolemma; fraction R2 is enriched in LSR; fraction R3 is a mixture of LSR and TC; and fraction R4 is enriched in TC. The JFM corresponds to the junctional SR (Costello et al., 1986). All membrane fractions were tested for their ability to hydrolyse $[^3H]$Ins$_P_2$. Unless otherwise specified, experiments were carried out in the presence of 3 mM-MgCl$_2$ and 50 μM-Ins$_P_3$, a concentration at least double the $K_m$ (see below).

The time course of $[^3H]$Ins$_P_3$ production by R1, R2 and R4 fractions is shown in Fig. 1. The kinetics of formation of the product is linear over at least 2 min for R1, and over 4 min for R2 and R4. Similar results are obtained for R3 and JFM (not shown). The enzyme activity seems to be specific for Ins$_P_3$, since $[^3H]$Ins$_P_2$ production is almost negligible (2–2.5 % of Ins$_P_2$ formed) even after a 5 min incubation.

$[^3H]$Ins$_P_3$ hydrolysis by muscle membranes displays normal Michaelis–Menten kinetics: values of $V_{max}$, $K_m$ and specific activity at 50 μM-Ins$_P_3$ are summarized in Table 2. The $K_m$ for Ins$_P_3$ ranges between 15.5 and 18.7 μM. The enzyme appears to be distributed in LSR as well as in TC and JFM fractions, whereas fraction R1 shows the highest $V_{max}$. Given the membrane composition of R1 (Saito et al., 1984), these results imply that the Ins$_P_3$ase is heavily concentrated in T-tubules and, probably, sarcolemma. It might be argued that the Ins$_P_3$ase activity of R2, R4 and JFM fractions is due to contamination by T-tubules. Although we cannot rule out this possibility for the R2 fraction we think it is unlikely for R4 and JFM, which are devoid of T-tubules as judged by enzyme markers and electron microscopy (Saito et al., 1984; Costello et al., 1986; Volpe et al., 1987b).

In order to establish whether the Ins$_P_3$ase is membrane-bound or only loosely bound to muscle membranes, fractions R1, R2 and R4 were treated with high ionic strength (0.6 M-KCl), and the distribution of the enzyme activity between supernatants and pellets was investigated. The results of this experiment are shown in Table 3. Since only 2–8 % of the Ins$_P_3$ase is found in the supernatants, and more than 90 % is recovered in the particulate fractions, we infer that the enzyme is in a tightly membrane-bound form.

**Soluble Ins$_P_3$ase**

We also looked for the presence of a soluble Ins$_P_3$ase in rabbit skeletal muscle. Two soluble fractions (A and B; see the Experimental section for details) were obtained and Ins$_P_3$ase activity was measured. The soluble Ins$_P_3$ase accounts for 35–40 % of the total Ins$_P_3$ase activity (cf. Table 1). The $K_m$ of the soluble enzyme (fraction A) is about 24 μM, i.e. slightly higher than that of the membrane-bound enzyme. The $V_{max}$ is 1.5 nmol of Ins$_P_3$ produced/min per mg of protein, and the specific activity at 50 μM-Ins$_P_3$ is about 0.6 nmol of Ins$_P_3$ produced/min per mg of protein.
Table 3. Distribution of InsP$_3$ase activity after KCl wash of sarcotubular membrane fractions

InsP$_3$ase activity was measured in the standard medium by using R1, R2 and R4 fractions before and after treatment with 0.6 mM-KCl. The InsP$_3$ concentration was 50 $\mu$M. Determinations were done in duplicate on two different preparations. Abbreviations: sp, supernatant; and pt, pellet after KCl wash (see the Experimental section for details).

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Specific activity (nmol of InsP$_3$ produced/min per mg)</th>
<th>Activity recovered (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1</td>
<td>10.40</td>
<td></td>
</tr>
<tr>
<td>sp R1</td>
<td>1.30</td>
<td>1.9</td>
</tr>
<tr>
<td>pt R1</td>
<td>9.50</td>
<td>98.1</td>
</tr>
<tr>
<td>R2</td>
<td>3.10</td>
<td></td>
</tr>
<tr>
<td>sp R2</td>
<td>2.50</td>
<td>1.7</td>
</tr>
<tr>
<td>pt R2</td>
<td>5.25</td>
<td>98.3</td>
</tr>
<tr>
<td>R4</td>
<td>2.76</td>
<td></td>
</tr>
<tr>
<td>sp R4</td>
<td>2.45</td>
<td>8.0</td>
</tr>
<tr>
<td>pt R4</td>
<td>2.53</td>
<td>92.0</td>
</tr>
</tbody>
</table>

Table 4. Effect of various substances on InsP$_3$ase activity

Various compounds at indicated concentrations were added to the standard incubation medium containing 50 $\mu$M-InsP$_3$. The reaction time was 2 and 5 min for membrane-bound (R1) and soluble (fraction A) InsP$_3$ase respectively. In preliminary studies on R4 fraction at 5 $\mu$M-InsP$_3$, addition of 100 $\mu$M-Ca$^{2+}$ to the standard medium did not change InsP$_3$ase activity. Results are expressed as percentage inhibition, and are means $\pm$ S.D. for triplicate determinations carried out on two different preparations; ND, not determined.

<table>
<thead>
<tr>
<th>Activity (% inhibition)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Membrane-bound</td>
</tr>
<tr>
<td>-----------------</td>
</tr>
<tr>
<td>3 mm-2,3-Bisphosphoglycerate</td>
</tr>
<tr>
<td>5 mm-Fructose 2,6-bisphosphate</td>
</tr>
<tr>
<td>5 mm-Fructose 1,6-bisphosphate</td>
</tr>
<tr>
<td>5 mm-Glucose 1,6-bisphosphate</td>
</tr>
<tr>
<td>0.1 mm-CdCl$_2$</td>
</tr>
<tr>
<td>0.5 mm-EGTA (free [Ca$^{2+}] &lt; 1$ nm)</td>
</tr>
<tr>
<td>10 mm-EDTA</td>
</tr>
<tr>
<td>3 mm-ATP*</td>
</tr>
</tbody>
</table>

* In these experiments the total Mg$^{2+}$ concentration was 6 mm.

InsP$_3$ase inhibitors

Various agents have been reported to inhibit InsP$_3$ase activity, and the effects of some of them are presented in Table 4. The results are very similar for the membrane-bound (R1) and the soluble (fraction A) enzyme. Mg$^{2+}$ is essential for enzymatic activity (Downes et al., 1982), as demonstrated by 96–98% inhibition obtained with 10 mm-EDTA. CdCl$_2$ (0.1 mm) (Downes et al., 1982) is also a potent inhibitor (84–94% inhibition). 2,3-Bis-phosphoglycerate (3 mm), which seems to act as a competitive inhibitor (Downes et al., 1982), decreases the InsP$_3$ase activity by 60%. Three bisphosphorylated glucose metabolites, at 5 mm (Rana et al., 1986), give an inhibition in the range 30–70%. Negligible effects are detected with 0.5 mm-EGTA, i.e. at a nominal free [Ca$^{2+}$] < 1 nm. Interestingly, ATP (3 mm) inhibits by about 50% both membrane-bound and soluble InsP$_3$ase.

DISCUSSION

In this study we show for the first time the occurrence of a specific powerful InsP$_3$ase in skeletal muscle, which shares several biochemical properties with the homologous enzyme from many different tissues (Downes & Michell, 1981; Downes et al., 1982; Storey et al., 1984; Joseph & Williams, 1985; Connolly et al., 1986; Sasaguri et al., 1985; Kukita et al., 1986; Rana et al., 1986; Erneux et al., 1986; Guillemette et al., 1987; Walker et al., 1987). It is present in membrane-bound (60–65%) and soluble form (35–40%), has an absolute requirement for Mg$^{2+}$, has a $K_m$ for InsP$_3$ around 20 $\mu$M, and is inhibited by compounds such as 2,3-bisphosphoglycerate and Cd$^{2+}$. The InsP$_3$ase of the R1 fraction displays the highest $V_{max}$ so far reported for the membrane-bound form from any other tissue.

The existence of a powerful InsP$_3$ase in skeletal muscle has been previously postulated on the basis of experiments where the Ca$^{2+}$-releasing action of InsP$_3$ was enhanced by manipulations capable of inhibiting InsP$_3$ase. Vergara et al. (1985) showed that low Mg$^{2+}$ or Cd$^{2+}$, Ni$^{2+}$ and 2,3-bisphosphoglycerate all potentiate the action of InsP$_3$ on frog skinned fibres. A similar observation was made by Rojas et al. (1987), though Lea et al. (1986) did not. Donaldson et al. (1987) observed that microinjected InsP$_3$ (1 $\mu$M) in rabbit skinned fibres was as effective as 100–300 $\mu$M-InsP$_3$ added to the bathing solution. The explanation for this result was that 'endogenous phosphatases create a radically decreasing concentration gradient' of exogenously added InsP$_3$ (Donaldson et al., 1987). InsP$_3$ase was also implicated in the transient nature of InsP$_3$-induced Ca$^{2+}$ release, since 'microinjection of more InsP$_3$ during the decline of an InsP$_3$-induced tension transient elicits an abrupt increase in fibre tension' (Donaldson et al., 1987).

Our present data and the previous suggestions (Vergara et al., 1985; Donaldson et al., 1987; Rojas et al., 1987) are in conflict with the results of Walker et al. (1987). The latter authors have reported that: (a) InsP$_3$ase activity of frog skinned fibres is 35 times slower than that of skinned stripes of rabbit main pulmonary artery (smooth muscle); (b) there is no diffusible ('soluble') InsP$_3$ase in either skeletal or smooth muscle; and (c) InsP$_3$ase activity was negligible in both types of muscle. They concluded that rapid InsP$_3$ degradation cannot be accounted for by an active InsP$_3$ase in skeletal muscle. On the other hand, we have shown that: (a) total InsP$_3$ase activity of main pulmonary artery is comparable with that of skeletal muscles (Table 1); we also note that the occurrence of rapid InsP$_3$ degradation has been indirectly shown in intact frog skeletal muscles (Vergara et al., 1987); (b) $V_{max}$ of InsP$_3$ase of several different skeletal-muscle fractions (see Table 2 and the Results section) is similar to (or even higher than) that reported for soluble and particulate fractions of pig coronary artery...
(2–2.4 nmol of InsP₃ produced/min per mg of protein in Sasaguri et al., 1985); (c) the existence of InsP₃ase activity is indicated by the recovery of a significant amount of InsP₃ in homogenates of main pulmonary artery after incubation with [³²P]InsP₃ (Table 1; see also Sasaguri et al., 1985); (d) a soluble InsP₃ase exists in skeletal muscle as well as in smooth muscle (Sasaguri et al., 1985). Although the experimental models and protocols of our study are different from those of Walker et al. (1987), the reasons for the discrepancy remain unknown.

In other cell types (e.g., liver, endocrine pancreas) InsP₃ase has been preferentially, but not exclusively, localized in the plasma membrane (Seyfred et al., 1984; Joseph & Williams, 1985; Rana et al., 1986). Likewise, our data also indicate that membrane-bound InsP₃ase activity is higher in a fraction enriched in T-tubules. Moreover, the InsP₃ase is localized on the junctional SR too. This anatomical organization would be ideally suited to dispose rapidly of the InsP₃ being formed at the T-tubule level, and to terminate Ca²⁺ release (Volpe et al., 1988). A rough calculation based on the content of PtdInsP₃ (8 pmol/g of muscle; Vergara et al., 1987) and on the total InsP₃ase activity (1.8 pmol of InsP₃ hydrolysed/ms per g of muscle; Table 1) indicates that skeletal muscle InsP₃ase would be capable of hydrolysing all InsP₃ formed within a few milliseconds, i.e., on the same time scale as a single twitch (provided that the turnover number of the enzyme, which is at present unknown, was compatible).

In conclusion, our data are consistent with the hypothesis that InsP₃ plays a role in excitation–contraction coupling. In fact, not only do all the enzymic steps involved in the generation of the lipid precursors (Hidalgo et al., 1986; Varsanyi et al., 1986) and the InsP₃-sensitivity (Volpe et al., 1985) appear to be strategically localized in the triad, but also the InsP₃ase which hydrolysates the second messenger seems to be present in the T-tubule membrane. However, other key aspects of the InsP₃ hypothesis (Volpe et al., 1986) have to be addressed before giving a definitive appraisal on the role of InsP₃ in excitation–contraction coupling. For instance, it remains to be ascertained whether and how T-tubule depolarization is linked to phospholipase C-dependent PtdInsP₃ hydrolysis (see Di Virgilio et al., 1986), whether PtdInsP₃ turnover is compatible with the time scale of excitation–contraction coupling (~3 ms; Vergara et al., 1987; Maylie et al., 1987), and how fast are rates of InsP₃-induced Ca²⁺ release from SR.

We thank Dr Susan Treves for helping in preliminary experiments, and G. A. Tobaldin and G. Ronconi for excellent technical assistance. This work was supported by Institutional funds from the Consiglio Nazionale delle Ricerche (Italy).

REFERENCES