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Inositol 1,4,5-trisphosphate: A possible chemical link in excitation–contraction coupling in muscle

(skinned muscle fibers/inositol phosphates/calcium signals/neomycin)

JULIO VERGARA*, ROGER Y. TSIEN†, AND MICHAEL DELAY*

*Department of Physiology, University of California, Los Angeles, CA 90024; and †Department of Physiology-Anatomy, University of California, Berkeley, CA 94720

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ABSTRACT **The role of inositol 1,4,5-trisphosphate ($Ins P_3$) in excitation–contraction coupling in skeletal muscle was investigated by several methods. The following results were obtained. $Ins P_3$ is released by electrical stimulation of muscles. Exogenous $Ins P_3$ releases calcium from skinned muscle fibers at relatively high doses under normal conditions but does so at very low concentrations when blockers of the $Ins P_3$ 5-phosphatase are present. Blockers of $Ins P_3$ release are effective blockers of calcium transients elicited by electrical stimulation of muscle fibers. It is proposed that $Ins P_3$ acts as a chemical second messenger between transverse (T)-tubular membrane depolarization and calcium release from the sarcoplasmic reticulum in skeletal muscle.**

One of the outstanding questions in muscle physiology is the mechanism of excitation–contraction coupling in vertebrate skeletal muscle. Electrical depolarization of the transverse (T) tubular system membranes somehow triggers release of Ca^{2+} ions from the sarcoplasmic reticulum (SR), which in turn activates the generation of tension (1–3). Though the T-tubule and SR are only some 100–200 Å apart at the T/SR junctions, their membranes and luminal spaces do not seem to be physically continuous (4). How is the excitatory signal communicated from one structure to the other? The major present hypothesis assumes that intramembrane charge movement in the T-tubule membrane is mechanically linked to the SR membrane, causing the opening of Ca^{2+} channels (5, 6). No direct evidence for such remote-control valves has been obtained. We now wish to present evidence for a biochemical coupling mechanism, in which inositol 1,4,5-trisphosphate ($Ins P_3$) acts as a soluble internal transmitter. This hypothesis was first suggested by two analogies. The first is the homology between SR and endoplasmic reticulum, from which $Ins P_3$ is known to release Ca^{2+} in other tissues (7, 8). Agonist stimulation of many such tissues causes the breakdown of plasma membrane phosphatidylinositol 4,5-bisphosphate [PtdIns(4,5)P_2] to release $Ins P_3$ (8). The water-soluble $Ins P_3$ releases Ca^{2+} from ER but is eventually degraded to Ins(1,4)P_2 by a phosphomonoesterase, $Ins P_3$ 5-phosphatase (9, 10). The second analogy is between the T/SR junction (11) and the neuromuscular junction (12), where acetylcholine is accepted to be the chemical messenger (13). By analogy with synaptic transmitters, criteria for $Ins P_3$ as an internal messenger should include the following. (*i*) Electrical stimulation of muscle should cause the release of $Ins P_3$. (*ii*) Application of $Ins P_3$ onto skinned muscle fibers should cause Ca^{2+} release and tension development. (*iii*) A powerful breakdown system for $Ins P_3$ should be endogenous to the muscle; pharmacological inhibitors of the breakdown enzyme should potentiate and prolong the effectiveness of exogenous $Ins P_3$. (*iv*) Pharmacological inhibitors of $Ins P_3$ formation and activity should block excitation–contraction coupling; i.e., prevent T-system depolarization from releasing Ca^{2+}.

We report here the results of experiments that suggest that $Ins P_3$ may meet all four criteria.

MATERIALS AND METHODS

The experiments on tension development were performed on segments of mechanically or chemically skinned skeletal muscle fibers isolated from the semitendinosus muscle of the frog *Rana catesbeiana*. Chemical skinning was performed by exposing muscle fibers for 10–20 min to saponin (20–50 μg/ml) (14) added to the relaxing solution described below. Mechanical skinning consisted of carefully splitting a segment of muscle fiber immersed in relaxing solution. Short segments (5–8 mm long) of skinned fibers were attached to a fixed hook and to a high sensitivity capacitive tension transducer. Tension was recorded on both chart paper and FM tape. The relaxing solution contained 110 mM potassium aspartate, 20 mM potassium Mops, 5 mM phosphocreatine, 100 μM EGTA, 3 mM ATP, and 3 mM $MgSO_4$. The ATP and Mg^{2+} concentrations were adjusted in order to set the concentration of free Mg^{2+} at chosen levels. The values of the free Mg^{2+} concentration given in the text and figure legends were calculated assuming an effective K_d of 100 μM (15). The pH of the relaxing solution was adjusted to 7.05, and the pCa to 7 by adding $CaCl_2$ and measuring pCa with Ca^{2+}-selective electrodes (16). The fibers were mounted in a chamber that consisted of a trough 25 mm long and 2 mm by 2 mm in cross-section. Solutions could be exchanged to within 95% in about 2 sec. The inositol phosphates used in these experiments were obtained as follows: Ins(1,4,5)P_3, Ins(1,4)P_2, and Ins(4,5)P_2, from C. Ballou (University of California, Berkeley); $Ins P_3$ (probably a mixture of the 1,4,5 and 1,3,4 isomers) from Sigma; and $Ins P_3$ (probably mostly 1,4,5 isomer) from erythrocyte ghosts by following a procedure described in ref. 17. The calcium contamination of the $Ins P_3$ primarily used in these experiments (from C. Ballou) was less than 10 μM for a 3 mM stock solution in water.

Biochemical measurements of the production of inositol phosphates were made on intact pairs of frog sartorius and semitendinosus muscles with wet weights from 0.5 to 1.2 g. A symmetrical pair of muscles was incubated for 12–24 hr at 4°C or 3–4 hr at 27°C in Ringer's solution containing *myo*-[2-^{3}H]inositol (Amersham; 14 Ci/mmol; 1 Ci = 37 GBq) at a total concentration of 5–12 μM and then were washed three times in Ringer's solution at 4°C for up to 1 hr. The control muscle was frozen in liquid Freon at −100°C, and the other was mounted on a rigid-rod assembly with one tendon attached to a tension transducer and the other fixed to a hook, allowing adjustment of the muscle length to its slack value.

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Abbreviations: $Ins P_3$, inositol 1,4,5-trisphosphate; PtdIns, phosphatidylinositol; SR, sarcoplasmic reticulum.

The muscle could be stimulated by passing current between two Pt wires touching its surface. The amplitude of a 4-msec stimulus pulse was adjusted until maximal twitch tension was observed; only a few stimulations were used during this process. The muscle was then tetanically stimulated with a frequency and duration chosen for the experiment. At the end of the stimulation period, the muscle was rapidly frozen, while still being stimulated, by immersing the rod assembly in liquid Freon at −100°C. The tension records demonstrate that there was no mechanical relaxation during the freezing process. The frozen muscles were later transferred to liquid N_2, individually ground to a fine powder, and homogenized in 3 ml of an ice-cold solution containing 10% trichloroacetic acid, 1 mM $CdCl_2$, and 6 mM EGTA. The homogenate was neutralized with KOH, diluted to 12 ml with water, and centrifuged at 12,000 × *g* for 20 min, and the supernatant was passed through a 1.5-ml column of Dowex 1X8 anion-exchange resin (400 mesh; formate form) to separate and quantify inositol phosphates, following standard column separation and scintillation counting procedures (17–19).

Calcium transients were recorded from cut semitendinosus fibers diffused with the Ca indicator dye arsenazo III in a Vaseline gap chamber modified for optical recordings (20, 21). The dye and the drugs used in this study were diffused into the myoplasm from the cut ends of the fiber in pools E and C of the Vaseline gap chamber (20, 21).

RESULTS

Electrical Stimulation Releases InsP_3. The first line of evidence for a role of InsP_3 in muscle is that electrical stimulation does increase the myoplasmic concentration of inositol phosphates. Fig. 1 shows the results from a stimulated muscle compared with its unstimulated control. Water-soluble inositol phosphates extracted from both muscles were assayed by the usual protocol of stepwise elution from an anion-exchange resin (see *Materials and Methods*). It can be seen that much larger amounts of Ins$P$, InsP_2, and InsP_3 were obtained from the stimulated muscle (continuous trace, solid circles) than from the control (dotted trace, open squares). In eight experiments, tetanic stimulation lasting 10 sec or more always produced at least a doubling of InsP_3 content. InsP_2 and Ins$P$ tended to show larger increases for longer tetanic stimulations, whereas InsP_3 showed no consistent increase beyond those attained at 10 sec of stimulation. In two experiments with less than 5 sec of stimulation, InsP_3 elevation was, respectively, less marked and undetectable. Control experiments verified that the inositol phosphate elution profiles were quantitatively the same when both muscles were not stimulated, demonstrating that differences produced by electrical stimulation are significant.

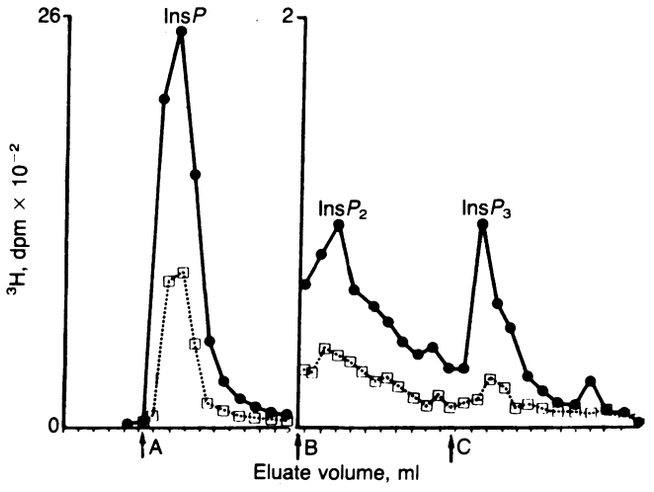

FIG. 1. Separation of radioactive inositol phosphates by Dowex 1 column chromatography. After a pair of frog muscles had been incubated with [^{3}H]inositol, the experimental muscle was stimulated tetanically at 50 Hz for two periods of 5 sec each (separated by a rest period of 5 sec), whereas the control muscle was not stimulated. Inositol phosphates then were extracted and chromatographed. Fractions of 1 ml were collected and their levels of radioactivity were determined by scintillation counting with a confidence interval (2 SD) of less than 7%. Arrows indicate step changes in the elution solutions: 0.1 M formic acid/0.2 M ammonium formate (arrow A); 0.1 M formic acid/0.4 M ammonium formate (arrow B); 0.1 M formic acid/1 M ammonium formate (arrow C). ●, Experimental muscle; □, control muscle.

InsP_3 Causes Contractures in Skinned Muscle Fibers. Fig. 2 illustrates the effects of InsP_3 on a fiber that was chemically skinned with saponin at 50 μg/ml. This concentration of saponin preserves the ability of the SR to release calcium but renders the surface membrane permeable to large solutes (14). Application of 150 μM InsP_3 in relaxing solution containing 0.5 mM free Mg^{2+} resulted in no tension development. However, increasing the InsP_3 concentration to 300 or 450 μM gave appreciable tension development, eventually reaching 80% of that elicited by a subsequent exposure to 5 mM caffeine. Tension development in response to InsP_3 was much slower than both the response to caffeine and the relaxation upon withdrawal of InsP_3 or caffeine. This asymmetry of time courses and the relative insensitivity of the InsP_3 responses might be explained by degradation of InsP_3 in conjunction with its diffusion into the fiber (see below).

Inhibitors of InsP_3 5-Phosphatase Increase Sensitivity to InsP_3. The concentrations of InsP_3 required to produce contractures are somewhat variable from fiber to fiber. However, they are always higher than the micromolar or submicromolar doses effective in other tissue preparations in which the Ca stores are more directly exposed to the externally applied InsP_3 (7, 8). We believe that the differences in sensitivities can be attributed at least in part to diffusional barriers and to a powerful enzymatic system for breaking down InsP_3 in muscle. The enzyme responsible for the first step in deactivation, InsP_3 5-phosphatase, has been studied in other preparations and has been found to be inhibited by lowering the Mg^{2+} concentration or adding inhibitors such as 2,3-bisphosphoglycerate, Cd^{2+}, Zn^{2+}, or Ag^+ (9, 10). We have tested the first three of these manipulations and have found that all of them have a distinct potentiating effect on the mechanical response of skinned fibers following application of InsP_3. Fig. 3 shows the effect of lowering the Mg^{2+} concentration on the responses to different doses of InsP_3. At 1.4 mM Mg^{2+}, 60 μM and 120 μM InsP_3 were unable to elicit significant tension, but the same fiber at 0.4 mM Mg^{2+} subsequently gave a large contracture with only 30 μM InsP_3. The effect of lowering Mg^{2+} concentration seen in Fig. 3 is too dramatic to be explained solely by the known effect of Mg^{2+} on the sensitivity of the contractile proteins to Ca^{2+} (22, 23).

Cd^{2+}, another inhibitor of the InsP_3 5-phosphatase, is also quite effective at potentiating InsP_3 contractures, especially in combination with lowered Mg^{2+} concentration (Fig. 4). At 0.04 mM Mg^{2+}, 0.1 mM Cd^{2+} in the presence of 0.1 mM EGTA did not release Ca^{2+} by itself but enabled just 30 μM InsP_3 to give a large contracture with a fast-rising phase. Tension then decayed spontaneously, probably due to an inhibition of further Ca^{2+} release from the SR, since a subsequent exposure to 60 μM InsP_3 did not result in tension development and since the fiber was able to generate a large amount of tension when exposed to a pCa 5 solution (T_{max} in Fig. 4). We found that Ni^{2+} is comparable to Cd^{2+} in effectiveness. In two trials using 0.2 mM Ni^{2+} and 0.2 mM Mg^{2+}, InsP_3 concentrations as low as 10 μM caused signif-

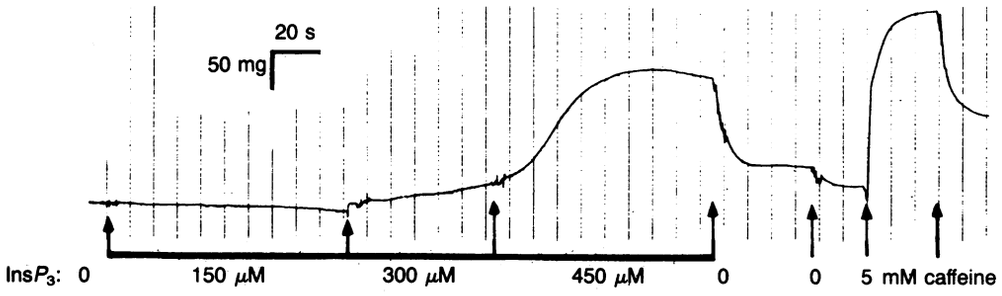

FIG. 2. Isometric tension record from a chemically skinned skeletal muscle fiber in relaxing solution containing 3 mM ATP and 3 mM Mg^{2+} (free Mg^{2+} concentration = 0.5 mM). Arrows denote the time at which the indicated addition was made to the superfusing solution.

icant contractures (24). It was also observed that high doses of $InsP_3$ 5-phosphatase inhibitors always resulted in contractures without exogenous application of $InsP_3$.

In several experiments, Ins(4,5)P_2 was unable to elicit contractures even at millimolar concentrations. Ins(1,4)P_2, however, gave contractures when applied at concentrations >300 μM but was found to be less effective than $InsP_3$ in experiments in which both inositol phosphates were tested in the same fiber. In skinned muscle fibers with the SR depleted of Ca^{2+} or overtreated with saponin to destroy the SR (14), we verified that $InsP_3$ at millimolar concentrations did not elicit contractures although the fibers responded normally to direct Ca^{2+} applications.

Neomycin Blocks Excitation–Contraction Coupling. The observations that stimulated muscles produce $InsP_3$ and that exogenous $InsP_3$ can cause contractures are not sufficient to determine whether this agent is an essential link in excitation–contraction coupling or an inessential adjunct. In several preparations, the polyamine antibiotic neomycin has been shown to bind tightly to phosphatidylinositol phosphates (25) and to prevent their enzymatic degradation (17, 26). Moreover, the Ca^{2+}-stimulated release of $InsP_3$ from erythrocyte membranes has been shown to be severely blocked by this drug (17). If $InsP_3$ were essential in muscle, such blocking agents would be expected to disable excitation–contraction coupling. Fig. 5 shows that neomycin indeed blocks the Ca^{2+} transients elicited by action potential stimulation in skeletal muscle fibers into which the Ca indicator arsenazo III had diffused. Here the peak absorbance change of the Ca signal, recorded at 660 nm, is given as a function of time. The initial steady rise in the absorbance changes is due to the diffusion of the indicator into the fiber. At the times indicated by the arrows, different concentrations of neomycin were added to the solutions diffusing into the myoplasm. The signal amplitudes subsequently diminished toward a lower steady level in response to neomycin at 0.15 mM (Fig. 5*A*) and virtually disappeared in response to higher doses (0.3 mM, Fig. 5*A*, and 0.5 mM, Fig. 5*B*). Polylysine and to a lesser extent spermine also induced blockage of the Ca signals. The relative potencies among the different polyamines in blocking Ca transients are similar to those in the inhibition of metabolism of phosphatidylinositol phosphates in other preparations (26). The waveforms of the Ca signals in response to electrical stimulation were the same before and after partial blockage, being superimposable after scaling for the decrease in amplitude. Control experiments *in vitro* verified that neomycin had no effect on the arsenazo III response to Ca^{2+}. These polyamines do not act as local anesthetics such as tetracaine or procaine nor do they grossly alter membrane surface charge, since voltage-clamp experiments showed no change in magnitude, kinetics, or voltage sensitivity of Na^+ and K^+ ionic currents at drug concentrations up to 0.5 mM. The blockage of excitation–contraction coupling by polyamines is not due to depletion of the SR store of calcium. Neomycin (1 mM) applied to a skinned fiber did not cause any tension, even with repeated applications and prolonged exposure. Application of 5 mM caffeine after removal of neomycin showed that the SR still retained Ca^{2+} in sufficient amounts to elicit a large contracture.

DISCUSSION

The results presented in this paper suggest that $InsP_3$ may be a chemical intermediary between the T-system depolarization and the Ca^{2+} release from the SR in skeletal muscle fibers. We have presented evidence from four types of experiments in support of this hypothesis. $InsP_3$ is generated by electrical stimulation of skeletal muscles; $InsP_3$ is able to release Ca^{2+} from SR in skinned muscle fibers; manipulations that inhibit $InsP_3$ 5-phosphatase in other systems potentiate $InsP_3$ in muscle; and drugs that inhibit $InsP_3$ release are effective blockers of excitation–contraction coupling. Although each line of evidence by itself cannot prove that excitation–contraction coupling is mediated by $InsP_3$ as a chemical transmitter, the combination of results does strong-

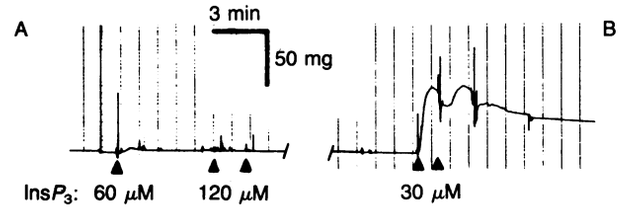

FIG. 3. Isometric tension record from a mechanically skinned muscle fiber superfused with relaxing solution containing 6 mM ATP, 7 mM Mg^{2+} (free Mg^{2+} concentration = 1.4 mM) (*A*) or 8 mM ATP, 7 mM Mg (free Mg^{2+} concentration = 0.4 mM) (*B*). Arrowheads denote the addition of the indicated concentrations of $InsP_3$ to the solution. The tension recording was interrupted between *A* and *B*.

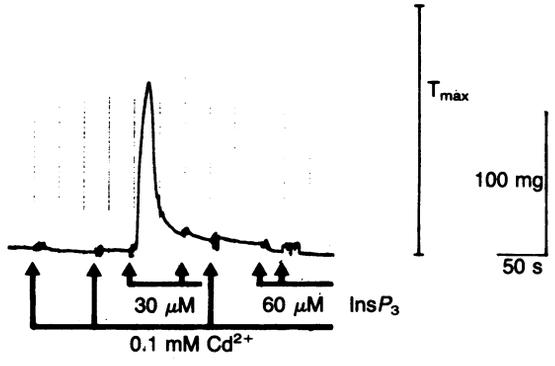

FIG. 4. Isometric tension record from a fiber chemically skinned with saponin (25 μg/ml). The relaxing solution contained 3 mM ATP, 1 mM Mg^{2+} (free Mg^{2+} concentration = 0.04 mM). Long arrows denote additions of 0.1 mM $CdCl_2$, and short arrows, of indicated concentrations of $InsP_3$. At the end of the experiment the fiber was exposed to a pCa 5 relaxing solution, developing a contracture of 170 mg (T_{max}).

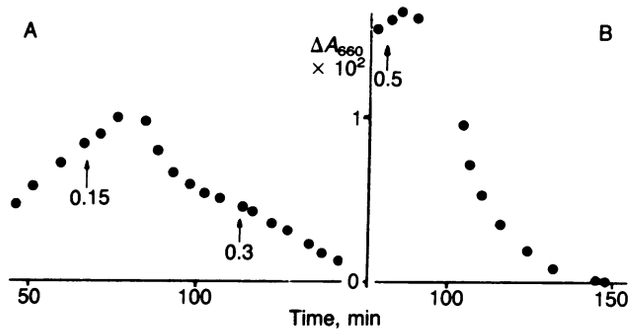

FIG. 5. (*A*) Ca signal amplitudes as a function of the time after the fiber ends were cut in the dye-containing internal solution. Arrows denote the addition of 0.15 and 0.3 mM neomycin to the solution diffusing into the myoplasm. (*B*) A different fiber treated with 0.5 mM neomycin.

ly suggest some major role for InsP_3. A simplified scheme of the major steps proposed to occur at the T/SR junction is presented in Fig. 6. This scheme relies heavily on others described for other tissues (8) but has been modified for the specific case of skeletal muscle in that, instead of agonist binding to a receptor, the voltage change across the T-system membrane is proposed here to have the primary activating role on the hydrolysis of PtdIns(4,5)P_2 by a phosphodiesterase (PDE) to release InsP_3 and diacylglycerol (DG).

Most of the experiments showing generation of InsP_3 in tetanized muscle (e.g., Fig. 1) used stimulation periods of 3 sec or longer. They are therefore not sufficient to decide whether InsP_3 is generated on the millisecond time scale required for a primary role in excitation–contraction coupling. Better quantification of the time course and absolute amount of InsP_3 released will obviously be an important goal of future experiments, especially if the powerful InsP_3 5-phosphatase can be partially arrested. It is worth remembering that acetylcholine is accepted as the transmitter at the neuromuscular junction even though its release there is chemically detectable only with heavy repetitive stimulation and acetylcholinesterase inhibition (27). In addition, the freezing method used here may not be fast enough throughout the whole muscle to completely suppress the InsP_3 phosphatase activity, possibly resulting in larger stimulated-vs.-control differences in the InsP_2 and Ins$P$ peaks relative to the InsP_3 peak. Similar effects are observed in other tissues known to use InsP_3 as a messenger (19). Future studies should compare the release of inositol phosphates with the metabolism of phosphatidylinositol phosphates in control and activated muscles. Such work would extend the experiments of Novotny *et al.* (28), who showed that prolonged K^+ depolarizations increased [^{32}P]phosphate incorporation into phosphatidylinositol in muscle.

Our results show that skinned muscle fibers do contract in response to exogenous InsP_3 and that the response becomes faster and requires less InsP_3 when steps are taken to inhibit the InsP_3 phosphatase (Figs. 2–4). We have observed much faster responses by direct local pressure injection or iontophoresis of InsP_3 from a micropipette pressed against the surface of a muscle fiber (24), in which local sarcomere shortening occurred in well under a second. More sophisticated techniques, such as photochemical release of InsP_3 from a "caged" derivative, will be needed to determine the true kinetics of the response to InsP_3 and to determine whether this is compatible with the speed of excitation–contraction coupling. Use of dyes to measure both the T-system

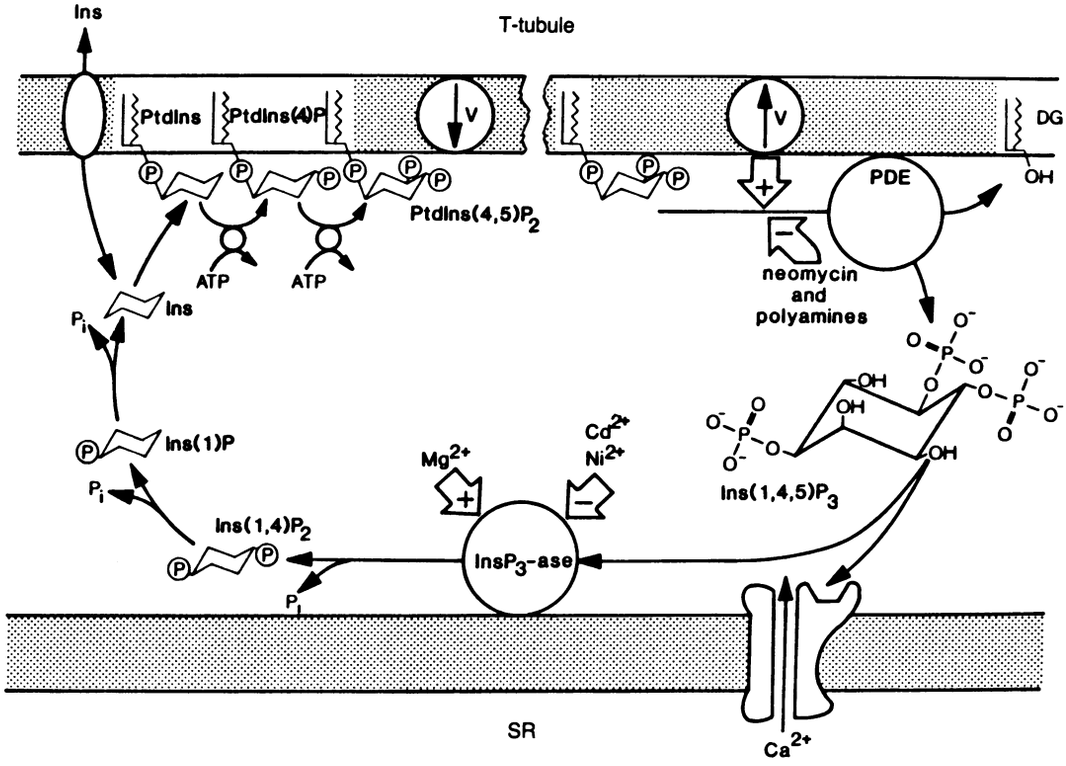

FIG. 6. The proposed role of InsP_3 as a chemical link in excitation–contraction coupling. T-tubular membrane depolarization (V) stimulates the hydrolysis of PtdIns(4,5)P_2 by a phosphodiesterase (PDE) to form diacylglycerol (DG) and InsP_3. The latter binds to a specific receptor on the SR membrane to release calcium. InsP_3 is rapidly deactivated by the InsP_3 5-phosphatase (InsP_3-ase) to form InsP_2. The soluble cycle ends with *myo*-inositol (Ins), which is readily transported across the membrane. Ins is also rapidly incorporated into the lipid phase to form PtdIns, which is phosphorylated twice to form PtdIns(4,5)P_2. The blocking or stimulating effects of tested substances on different parts of the cycle are indicated with unshaded arrows.

depolarization and the sarcoplasmic free Ca^{2+} concentration shows that the upswing of the T-system potential precedes that of the Ca signal by about 2.5 msec at 10°C (ref. 29 and unpublished results). Therefore, excitation–contraction coupling is not necessarily too fast for chemical transmission, since comparable latencies are obtained for the complex chain of events in neuromuscular transmission (30). Though diffusion times are likely to contribute very significantly to the response latency to exogenous InsP_3, they would be negligible for InsP_3 generated in the T/SR junction from endogenous PtdIns(4,5)P_2, since aqueous diffusion over a distance of 100–200 Å requires <1 μsec.

As little as 10–30 μM InsP_3 was found to generate tension with free Mg^{2+} concentration lowered and Cd^{2+} or Ni^{2+} added to inhibit the InsP_3 phosphatase. The true range of local InsP_3 concentration required for Ca^{2+} release from the SR should therefore be <10–30 μM. In other cell types, ED_{50} values for InsP_3 release of Ca^{2+} are in the micromolar or submicromolar range. However, it might be advantageous for muscle to use higher transmitter concentrations and lower receptor affinities to get faster association and dissociation rates for InsP_3 with its receptor. Generating a local concentration of 10 μM InsP_3 in a T/SR junction 100–200 Å wide would require the hydrolysis of about 100 molecules of PtdIns(4,5)P_2 per μm^2 of adjacent T-tubular membrane, a small number compared with the $\approx 10^6$ total phospholipid molecules per μm^2 (31).

Although the results of neomycin blockade of excitation–contraction coupling are consistent with a transmitter role for InsP_3, we have yet to prove that neomycin acts in muscle by blocking InsP_3 formation rather than by blocking Ca^{2+} channels in the SR. However, our results show that neomycin is at least more specific than tetracaine or procaine, known blockers of excitation–contraction coupling (32, 33), since these local anesthetics also affect Na^+ and K^+ conductances (34, 35). It is also not proven that Cd^{2+}, Ni^{2+}, 2,3-bisphosphoglycerate, and lowered Mg^{2+} concentration act in muscle by inhibiting InsP_3 phosphatase, although it is not obvious what other hypothesis could account for the similar effects of such disparate agents. The well-known ability of extracellular Cd^{2+} and Ni^{2+} to block Ca^{2+} channels does not explain the enhancement of skinned-fiber contraction reported here. However, the reported ability of ≥10 μM Cd^{2+} to induce Ca^{2+} release (36) and block ATPase activity (37) in isolated SR preparations might affect our results. Nevertheless, our experiments and those in which the blockage of the InsP_3 phosphatase was studied *in vitro* (10) were both done in the presence of EGTA at concentrations in which the calculated free Cd^{2+} concentration is in the subnanomolar range.

The mechanism by which InsP_3 formation might be controlled by T-tubular membrane potential remains unclear. In other systems, two mechanisms have been proposed by which a chemical receptor can trigger the enzymatic hydrolysis of PtdIns(4,5)P_2: GTP-binding coupling proteins (38) and changes in PtdIns(4,5)P_2 head-group conformation (8). In muscle such mechanisms would have to be activated by the T-tubular membrane depolarization. Conformational changes in transmembrane coupling proteins or PtdIns(4,5)P_2 head group might be responsible for the charge displacements proposed to be associated with excitation–contraction coupling (5). These hypotheses should be experimentally testable.

Obviously, a role for InsP_3 as primary transmitter requires that its formation be independent of any rise in cytoplasmic Ca^{2+} concentration, as has recently been demonstrated in secretory cells (39, 40). In some systems (e.g., human erythrocytes), InsP_3 can also be stimulated by high levels of Ca^{2+} (17). If such a mechanism exists in muscle, it could help to explain the phenomenon of Ca^{2+}-induced Ca^{2+} release, which also requires high levels of Ca^{2+} (41). At present we cannot definitively exclude the possibility that the results shown in Fig. 1 reflect Ca^{2+}-triggered instead of voltage-triggered InsP_3 formation. The results of Novotny *et al.* (28) would seem to indirectly favor the voltage-triggered InsP_3 release in muscle at physiological conditions.

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