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Inositol trisphosphate-induced calcium release and contraction in vascular smooth muscle

(pharmacomechanical coupling/sarcoplasmic reticulum/norepinephrine)

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ABSTRACT **Inositol 1,4,5-trisphosphate ($InsP_3$) caused Ca release and tension development in rabbit main pulmonary artery smooth muscle permeabilized with saponin or digitonin. Both of these responses to single additions of $InsP_3$ (0.5–30 μM) were repeatable and occurred in the presence of 0.0–1.9 mM free Mg^{2+}. Sustained contractions were induced by $InsP_3$. The amount of Ca released by $InsP_3$, measured with a Ca^{2+}-selective electrode, was also estimated to be sufficient to stimulate contraction in intact smooth muscle. Ca release was not influenced by inhibitors of mitochondrial oxidative phosphorylation. The uptake of Ca^{2+} from the medium into the $InsP_3$-sensitive pool was ATP-dependent. The present results support the hypothesis that, in smooth muscle, $InsP_3$ is the messenger, or one of the messengers, involved in transmitter-induced (pharmacomechanical) Ca release from the sarcoplasmic reticulum, which is the intracellular Ca store identified previously as the source of Ca released by norepinephrine in main pulmonary artery.**

Activation of smooth muscle by transmitters and drugs involves, at least in part, the release of intracellular Ca (1–3), followed by Ca activation of the calmodulin-regulated myosin light chain kinase (4). The release of intracellular Ca does not require influx of extracellular Ca (5–7) and can be triggered by pharmacomechanical coupling, a process independent of changes in surface membrane potential (8). Recent electron probe analytic studies have directly demonstrated that the sarcoplasmic reticulum (SR) is the source of intracellular Ca released by norepinephrine in rabbit main pulmonary artery (MPA) (1), portal vein (7), and, probably, other smooth muscles. However, until very recently, the mechanism through which drugs and transmitters released Ca from the SR remained unknown.

The recognition, in nonmuscle cells, that stimulation of phosphatidylinositol turnover, stimulated by cholinergic agents and other secretogogues (9, 10), is associated with the production of a metabolite, inositol 1,4,5-trisphosphate ($InsP_3$), that can release Ca from the endoplasmic reticulum (11, 12) indicated that $InsP_3$ may also function as an excitatory messenger in smooth muscle. In this study, we demonstrate that $InsP_3$ can, indeed, release Ca from smooth muscle cells of the rabbit MPA, as indicated by Ca^{2+}-selective electrode measurements and by $InsP_3$-induced contraction of vascular strips permeabilized with saponin or digitonin. The quantity of Ca^{2+} released by $InsP_3$, like that released from the SR by norepinephrine in this tissue (1), is sufficient for the activation of contraction. These and other recent studies of phosphatidylinositol turnover (including its stimulation by norepinephrine) and of the effects of $InsP_3$ in smooth muscle (13–17) provide further evidence for the possibility that $InsP_3$ is a physiological messenger mediating agonist-induced Ca release in smooth muscle.

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MATERIALS AND METHODS

Tissue Preparation. Male New Zealand rabbits (1–2 kg of body weight) were killed by a blow on the back of the head. The MPA was excised up to about 5 mm distal to the bifurcation, and the adventitia was removed.

For Ca^{2+} electrode measurements, the entire vessel was cut into a fringe of strips, 200–500 μm wide, parallel to the circular smooth muscle bundles. An average of 37 mg of wet weight of dissected strips was used in each experiment.

For tension recordings, single strips of MPA (150–250 μm wide), with the adventitia and endothelium removed, were used.

Skinning Procedure. Strips of MPA were permeabilized at room temperature by the following procedure: (*i*) 5 min in solution 1; (*ii*) 15 min in solution 2 containing 300 μg of saponin per ml or, alternatively, 10 min in solution 2 containing 0.005% digitonin (18); (*iii*) 5 min in solution 3; and (*iv*) three 5-min rinses in 0 EGTA/0 Ca solution (solution 4 or 5). In some experiments, skinning in solution 2 and subsequent steps were carried out in the presence of 2 mM KCN with 0.5 nmol of oligomycin per mg of tissue wet weight.

Ca^{2+} Measurements. Ca^{2+} movements were measured kinetically, with a Ca^{2+}-selective electrode, as the change in free Ca^{2+} in the bath medium surrounding the strips. The Ca^{2+} electrode and amplifier were designed and built by the Biomedical Instrumentation Group, University of Pennsylvania. The Ca^{2+} electrode was constructed with an ETH 1001 ligand (19), and the reference electrode was a silver/silver chloride pellet with porcelain filled with saturated KCl. Both electrodes (2 mm each, measuring tip diameter) were inserted horizontally at a 100° angle in a Lucite cylindrical vessel of 6 mm internal diameter containing a microstirring bar. The minimal operating volume was 0.28 ml and the volume occupied by bath medium and strips in most of the experiments was 0.4 ml. A potential of 2 mV usually gave a full-scale deflection in the recorder and the response time of the electrode was 1 sec. Unless specifically indicated, the bath medium was either solution 4 or 5. All of the additions were made as aliquots of 0.5–5 μl.

Relative Ca^{2+} calibrations were obtained by adding known aliquots of $CaCl_2$ to the bath medium during and after the experiment. Absolute calibrations were obtained by adding to the vessel Ca^{2+} concentrations ranging from 10 μM to 100 mM and noting the resulting increments in mV. Such additions resulted in a linear slope between the logarithm of added $CaCl_2$ and the electrode response in mV. Since, with all of the electrodes used, the response to Ca^{2+} varied linearly (29.5

Abbreviations: $InsP_3$, inositol 1,4,5-trisphosphate; SR, sarcoplasmic reticulum; MPA, main pulmonary artery.

mV·decade^{-1}) with logarithmic Ca^{2+} concentration down to 0.1 μM or lower, the absolute level of free Ca^{2+} (in the experiments shown) could be extrapolated to the level of 0.1–1 μM Ca^{2+}. The values (extrapolated back to either 0.1 or 1 μM) were within 15% of those obtained by adding CaEGTA buffer (20) of known composition.

Tension Measurements. Single strips of MPA were tied with silk thread to one end of a Plexiglas trough (0.8 ml in volume), and the other end was tied to a force transducer (AM 801, AME, Horten, Norway) at 22–24°C. The strips were stretched to 1.35× resting length. Solutions were exchanged by rapid perfusion or, to conserve InsP_3, by rapidly draining the bath and immediately adding the test solution. In some experiments, InsP_3 was added topically.

Estimation of Proportion of Skinned Cells. The proportion of skinned cells in the saponin- or digitonin-treated MPA preparations was determined by three different procedures. The first, a qualitative estimate of the extent of skinning, made use of the brilliant fluorescence of cell nuclei (21) in the presence of ethidium bromide (M_r 300). Small strips of MPA skinned with digitonin or saponin after brief (up to 5 min) incubation in 50 μM ethidium bromide revealed a massive number of fluorescent nuclei throughout the preparation, in contrast to the control preparations that displayed nuclear fluorescence only at the cut edges. The second procedure utilized normally impermeant La^{3+} (20 mM) (7) as a marker for hyperpermeable cells used in experiments with Ca^{2+} electrodes. Thirty to 50 min after incubation in 20 mM La^{3+}, the strip was rapidly frozen in supercooled Freon 22 and transversely sectioned on a cryoultramicrotome (7) at −130°C. Cytoplasmic La was detected by electron probe microanalysis of cells sampled across the entire strip, which included more than eight bands of elastic lamellae. La was detected in 70% of the smooth muscle cells analyzed. The low Ca content in the mitochondria (1.6 ± 2.4 mmol/kg of dry weight; mean ± SEM, n = 5) confirmed the effectiveness of the mitochondrial blockers. The third procedure, based on the observation that the contractile responses to excitatory agonists are lost in permeabilized cells (22), utilized the addition of norepinephrine to saponin- or digitonin-treated strips and the monitoring of force development. In four experiments on small-diameter strips, norepinephrine had no effect, indicating complete skinning of the cells in the bundle. The higher proportion of skinned cells estimated by this procedure, compared with the La method, is directly related to the smaller diameter of the strips used for the force measurements than for the Ca^{2+} electrode studies.

Solutions. Solution 1 (0.1 mM EGTA relaxing solution) contained 5.5 mM Na_2ATP, 7.9 mM $MgCl_2$, 0.1 mM EGTA, 49.5 mM disodium creatine-*P*, and 30 mM Pipes. The concentrations of free Mg^{2+} (1.5 mM) and MgATP (5.0 mM) were determined by a computer program (20) that solved the equations for multiple binding equilibria; the stability constants were obtained from the literature (20). Solution 2 (0.2 mM EGTA relaxing solution) contained 0.2 mM EGTA, but was otherwise identical to solution 1. Solution 3 (0.1 mM EGTA activating solution) contained, in addition to the constituents of solution 1, 0.082 mM $CaCl_2$, giving a free Ca^{2+} concentration of 1 μM. Solution 4 (0 EGTA, high creatine-*P* solution) contained 5.5 mM Na_2ATP, 7.9 mM $MgCl_2$, 49.5 mM disodium creatine-*P*, and 30 mM Pipes. Solution 5 (0 EGTA, low creatine-*P* solution) contained 5.5 Na_2ATP, 7.9 mM $MgCl_2$, 40.0 mM 1,6-diaminohexane-*N,N,N',N'*-tetraacetic acid (dipotassium salt) (K_2HDTA), 9.5 mM disodium creatine-*P*, and 30 mM Pipes (0.9 mM free Mg^{2+} after Chelex treatment and 5.1 mM MgATP). Solution 6 contained 5.5 mM Na_2ATP, 5.7 mM $MgCl_2$, 40.0 mM K_2HDTA, 9.5 mM disodium creatine-*P*, and 30 mM Pipes (0.0 free Mg^{2+} after Chelex treatment and 4.6 mM MgATP). The free Ca^{2+} concentration in solutions 4–6 (contaminating Ca^{2+}) was between 1 and 5 μM (measured by Ca^{2+}-selective electrode). Solution 7 (La^{3+} solution) contained 3.5 mM $MgCl_2$, 30 mM potassium Pipes, and 70 mM methanesulfonate (potassium salt). All solutions were at pH 7.1, 20°C, and 0.2 M ionic strength and contained 1 μM leupeptin. Activating and 0 EGTA solutions also contained 50 units of creatine phosphokinase per ml. One hundred- to 200-mesh Dowex chelating resin (Chelex) (35 mg/ml) was added to all 0 EGTA solutions. The solutions were then centrifuged for 5 min at low speed and the supernatant was removed. Since Chelex also complexes with Mg^{2+}, the free Mg^{2+} in the Chelex-treated solution was measured with 30 μM antipyralazo at 600 nm.

Materials. ETH 1001 exchanger was from W. Simon (Switzerland); K_2HDTA was from Fluka Chemical, Hauppauge, NY; saponin was from ICN (lot 13399); and InsP_3, *myo*-inositol, phosphatidylinositol 4,5-bisphosphate, and phosphatidylinositol 4-phosphate were from Sigma. The InsP_3 is stated by the manufacturer to be "predominantly the 1,4,5-isomer with some 2,4,5-isomer present." In view of this and the possible presence of other contaminants comigrating with inositol bisphospate and with glycerophosphoinositol bisphosphate (ref. 23 and B. W. Agranoff, personal communication), the InsP_3 concentrations given must be considered an upper limit of InsP_3. A control experiment was performed in which the tension response to 5 μM InsP_3 from Sigma was compared with a 5 μM dose of a second preparation of InsP_3 from Robin Irvine (kindly donated by Marc Prentki): contractile responses of similar magnitude were obtained with both preparations. Stock solutions of InsP_3 were made up either in 100 mM potassium aspartate buffer (pH 7.0) or in H_2O, mixed with Chelex resin (100–200 mesh), and centrifuged for 5 min at low speed and the supernatant was removed. The stability of the InsP_3 stock solutions was indicated by the fact that similar results could be obtained

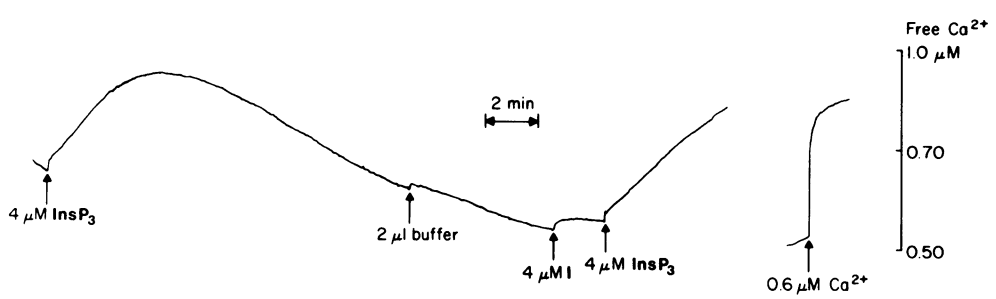

FIG. 1. Changes in free Ca^{2+} measured by a Ca^{2+}-selective electrode in a cuvette (0.5 ml in volume) containing solution 4 and saponin-permeabilized strips of rabbit MPA (21 mg of wet weight), which had been preequilibrated with solution 4. One- to 2-μl additions of InsP_3, its diluent buffer alone (100 mM potassium aspartate), or *myo*-inositol were made. The two calibrations, relative and "absolute," are shown on the right: under these conditions, approximately one-half of the total $CaCl_2$ added is recorded as free Ca^{2+} by the electrode.

over a period of 2–3 days with the same stock solution, which was frozen and thawed between each experiment.

RESULTS

Ca^{2+}-Selective Electrode Measurements. Figs. 1 and 2 illustrate experiments in which the net movement of Ca^{2+} from intracellular Ca^{2+} pools of permeabilized strips was measured by kinetically recording the changes in free Ca^{2+} in the bathing solution. Fig. 1 shows the result of an experiment carried out with saponin-skinned strips of MPA. The free Ca^{2+} concentration in the reaction mixture was 3.4 μM. The addition of the bundles of MPA strips resulted in a time-dependent (≈10 min) decrease of free Ca^{2+} in the medium to a steady-state level at which free Ca^{2+} was ≈0.5 μM (not shown). This decrease in free Ca^{2+} in the medium was dependent on the presence of ATP and an ATP-regenerating system, indicating that it was the result of ATP-dependent sequestration in a cellular compartment. The addition of 4 μM InsP_3 caused a transient increase in the Ca^{2+} concentration in the reaction mixture, indicating the release of Ca^{2+} from an intracellular Ca^{2+} pool, followed by its uptake. The total amount released by the first addition of InsP_3 was ≈520 nM, corresponding to an increase in free Ca^{2+} concentration of 300 nM, relative Ca^{2+} calibrations being determined by pulsing the tissue in the cuvette with known amounts of Ca^{2+} (see *Materials and Methods* and the legend to Fig. 1). Ca^{2+} releases of similar magnitude (equivalent to a total release of 100–300 nM Ca^{2+}) were obtained with multiple InsP_3 additions in two other experiments in which relative Ca^{2+} calibrations were obtained (e.g., Fig. 2*A*). Based on the tissue-to-medium volume ratio (≈1:11) and with cells occupying about 50% of the tissue volume, it could be estimated from these three experiments that the Ca^{2+} released by InsP_3 would increase the free Ca^{2+} concentration in the cell by 2–10 μM. These calculations are subject to several assumptions and approximations. Thus, smaller rises in free Ca^{2+} would occur in the cell *in situ*, in which such a large Ca^{2+} release could lead to the occupation of cellular binding sites or accelerate uptake by the SR (or both). In contrast, the measured release of free Ca^{2+} is probably less than the total released, due to buffering of Ca^{2+} by the skinned strips, including the extracellular matrix, and by ATP and would also be an underestimate of the releasable Ca^{2+} if a fraction of the strip was functionally impaired or not skinned. The addition of 2 μl of buffer or 4 μM *myo*-inositol (Fig. 1) caused a negligible increase in free Ca^{2+} concentration, consistent with the contaminant free Ca^{2+}. A second addition of InsP_3 triggered a second release of Ca^{2+}.

Fig. 2 *A* and *B* show the results of other experiments carried out with strips skinned with saponin and digitonin, respectively. In Fig. 2*A*, InsP_3 released Ca^{2+} from a cellular store, and this was followed by Ca^{2+} removal. A second addition of InsP_3 caused a rather large increase in Ca^{2+} concentration. This effect is consistent with the combined effects of InsP_3 and the unfavorable ATP/ADP ratio (measured in other experiments), the latter due to the ATPase activity of the relatively large amounts of tissue required. The addition of ATP resulted in further accumulation of Ca^{2+} by the strip, and a subsequent addition of InsP_3 stimulated further release.

Fig. 2*B* shows an experiment in which the addition of ATP to a digitonin-skinned strip produced a time-dependent decrease of Ca^{2+} concentration in the bathing solution. This accumulation into cellular sites was reversed by high concentrations of InsP_3. By changing the bathing medium surrounding the strips, the Ca^{2+}-releasing effects of lower and higher concentrations of InsP_3 could be further demonstrated.

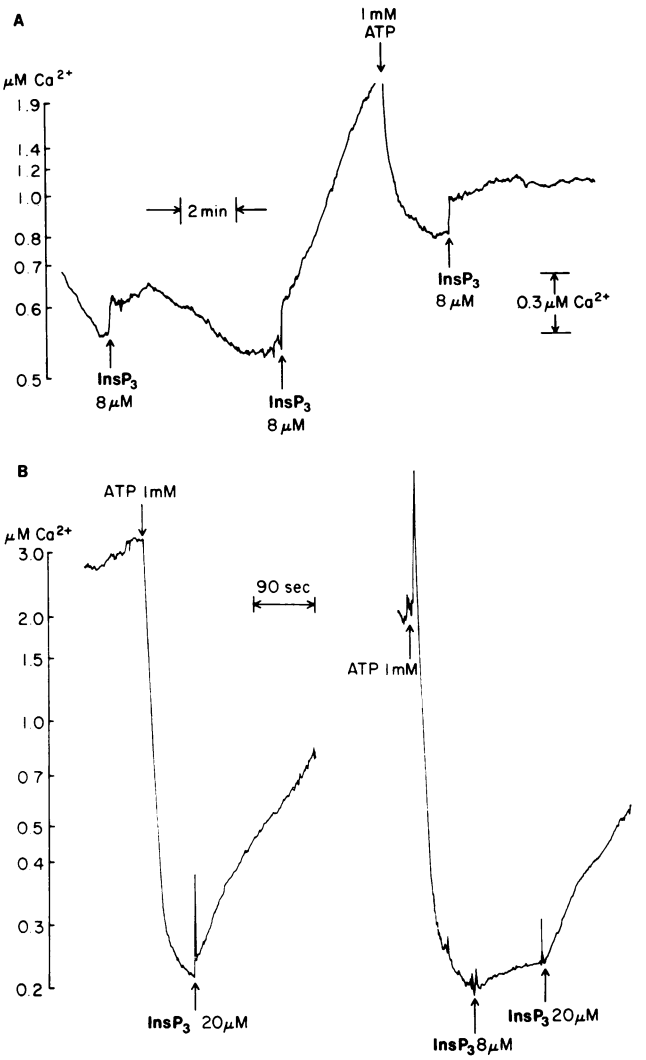

FIG. 2. (*A*) Ca^{2+} electrode measurements of InsP_3 and ATP responses of saponin-skinned strips of MPA (300 μg/ml, 15 min). Following saponin skinning, incubations in solution 3 and then in solution 5 were made in the presence of 2 mM KCN with 0.5 nmol of oligomycin per mg of tissue wet weight. Reaction mixture was solution 5 with KCN and oligomycin. The calibration scale on the right indicates that at a free Ca^{2+} in the range of 0.6–0.7 μM, the addition of 0.3 μM Ca^{2+} gives a 0.15 μM increase in free Ca^{2+}. (*B*) Ca^{2+} electrode measurements of InsP_3 and ATP-dependent responses of digitonin-treated strips of MPA (0.005%, 10 min). The skinned strips of tissue were incubated in solution 3 and then preequilibrated with solution 5 (0 EGTA, low creatine-*P*) containing 2 mM KCN and 0.5 nmol of oligomycin per mg of tissue. Reaction mixture was solution 5 (total volume, 0.4 ml).

Similar experiments were carried out with >20 sets of strips. Although InsP_3 released Ca^{2+} in virtually all of the strips, the kinetics and the total amount of Ca^{2+} released were variable. A maximal release was observed when the sequestration of Ca^{2+} by the strip reached a steady state (low ATP/ADP ratio), and a minimal and very transient release was observed when the strips were accumulating Ca^{2+} at a fast rate (high Ca^{2+}, high ATP/ADP, or both). If the rate of pumping is fast, the role of ATP-dependent uptake probably masks the InsP_3 effect. A larger effect (not shown) was observed when InsP_3 was added in the presence of 2 mM vanadate. The effect of the InsP_3 was specific and could not be duplicated by adding higher concentrations of *myo*-inositol, phosphatidylinositol 4,5-bisphosphate, or phosphatidylinositol 4-phosphate.

The addition of Chelex-treated InsP_3 to the bathing medi-

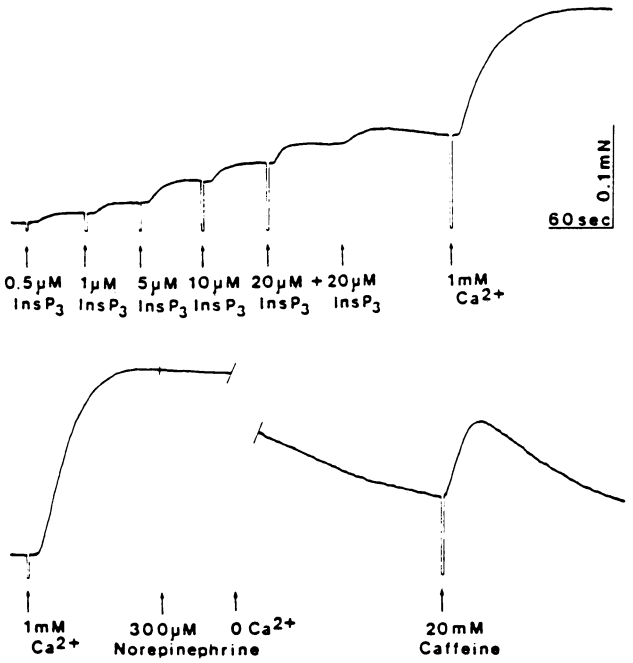

FIG. 3. Force record from a 180-μm (diameter) strip of saponin-skinned MPA illustrating the InsP_3 concentration dependence of force development, the maximal Ca^{2+}-dependent produced force, and the size of the caffeine-releasable Ca store. Maximal skinning is indicated by the absence of a norepinephrine-induced contraction in the presence of 1 mM Ca^{2+}. The downward deflections represent exchanges of the bathing solution. The last InsP_3 addition was added topically, resulting in a much higher transient local concentration of InsP_3.

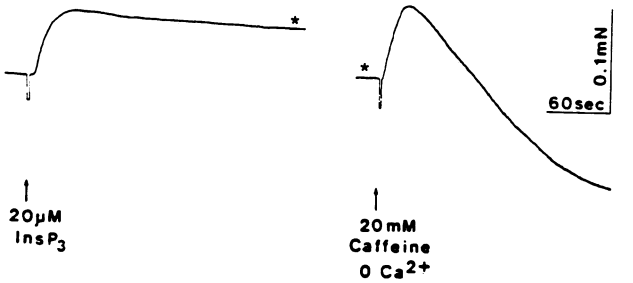

FIG. 4. Force record from a 160-μm (diameter) strip of saponin-skinned MPA in which the sizes of the InsP_3- and caffeine-releasable Ca stores are compared. Both additions were made at the same baseline tensions. An interval of 9 min in solution 5 to load the Ca store was permitted prior to addition of either InsP_3 or caffeine. The asterisks indicate the times when the free Ca^{2+} concentration of the bathing solution was measured with the Ca^{2+}-selective electrode (3 μM).

um, after the strips were removed and the concentration of free Ca^{2+} was either 0.2 or 0.5 μM, did not produce detectable changes in Ca^{2+}. Similarly, no effect was seen by adding InsP_3 to equal amounts of unskinned strips.

Contractile Responses. Contractions were evoked in each of 10 strips stimulated with InsP_3 (0.5–30 μM). A cumulative dose–response curve of a saponin-permeabilized strip (Fig. 3) indicates that the response was both graded and sustained at each of the InsP_3 concentrations tested. The contractions induced by InsP_3 were superimposed on a baseline tension (≈30% of the maximal, Ca-activated tension). In one experiment (Fig. 4) the free Ca^{2+} concentration in the bathing solution containing the strip was measured with the Ca^{2+}-selective electrode and was 2.9 μM. Reducing the free Mg^{2+} from 0.9 to 0.0 mM (solution 6) or increasing it to 1.9 mM did not change the sensitivity or the magnitude of the contractions. The maximal response to InsP_3 was approximately equal to the size of the contracture obtained with 20 mM caffeine, and each of these responses was about 50% of the maximal Ca-activated tension (in two completely permeabilized strips, as indicated by the absence of their response to norepinephrine). The sustained nature of the InsP_3-induced tension contrasted with the caffeine-induced contractures in the same preparations (Figs. 3 and 4) that, following an initial contraction, rapidly relaxed below the initial baseline. After destruction of the SR with Triton X-100, only the relaxant effect of caffeine remained. The relaxation of the caffeine contracture represents a secondary, inhibitory effect independent of Ca release and is also observed in intact smooth muscle (8, 24). Caffeine-induced Ca^{2+} release could not be measured with the Ca^{2+}-electrode because caffeine interacts directly with the Ca-selective membrane of the electrode. Arterial strips that were prepared identically, but not permeabilized with saponin, did not respond to InsP_3 (up to 30 μM). In skinned strips, no contraction was observed upon addition of *myo*-inositol (120 μM).

In comparing the Ca^{2+} electrode results with the tension measurements, it should be noted that much larger amounts of tissue (smaller bath volume-to-tissue ratio) were required for the former. Therefore, the enzyme concentrations per unit volume (i.e., tissue ATPases, including the SR Ca pump, and inositol phosphatases) were higher in the Ca^{2+} electrode experiments, leading (respectively) to lower average ATP/ADP ratios and more rapid hydrolysis of InsP_3.

DISCUSSION

The release of Ca and the contractions induced by InsP_3 are consistent with the hypothesis that InsP_3 is the second messenger mediating Ca^{2+} release in response to activation of receptors in smooth muscle. If so, this mechanism parallels that initially demonstrated in nonmuscle cells (11) in which considerable evidence now suggests that a variety of transmitters act via InsP_3-mediated release of Ca from the endoplasmic reticulum (for review, see refs. 12 and 25). We emphasize, however, that these effects of InsP_3 provide no information about the mechanism of Ca release induced by action potentials (electromechanical coupling) or about transmitter-induced Ca^{2+} influx (3). The results of these experiments differ from the InsP_3-dependent Ca^{2+} release observed recently in isolated coronary artery cells (17) in which ^{45}Ca reuptake was *not* observed following InsP_3-induced ^{45}Ca release. In addition, Ca^{2+} release was inhibited above 1.5 μM free Ca^{2+} concentration in the previous study, whereas we observed InsP_3-induced contractions at 3 μM free Ca^{2+} concentrations and higher.

The Ca^{2+} releases and contractions evoked by single additions of InsP_3 were more sustained (lasting several minutes) in MPA smooth muscle (this study) than reported previously to occur in nonmuscle cells (e.g., ref. 26), except when InsP_3 was superfused through the preparation (27). This finding suggests that inactivation of InsP_3 was relatively slow (27), even in the presence of millimolar Mg^{2+}, and the SR did not become rapidly desensitized to the Ca-releasing action of InsP_3. The important physiological implication is that tonic contractions in smooth muscle can occur in response to intracellular Ca release mediated by InsP_3 and, even in intact smooth muscle, may not require continuous Ca influx. The repeatable and sustained contractions and Ca releases observed in our experiments in the presence of millimolar Mg^{2+} resemble the sustained drug-induced contractions elicited in normal rabbit MPAs (8, 28) and indicate that InsP_3-induced Ca release occurs even at the highest

Mg^{2+} concentrations likely to be present *in vivo*. The quantity of Ca released into the bath by InsP_3 and the magnitude of the InsP_3-induced contractions suggest that the amount of Ca released, if it were not diluted into a large volume of bathing medium, could be sufficient to activate a maximal contraction. This is also borne out by the equal size of the InsP_3-induced and caffeine-induced contractures. We have observed the Ca-releasing effect of InsP_3 in the presence of mitochondrial blockers (present study) and demonstrated previously (1) that the SR is the source of intracellular Ca released by norepinephrine in rabbit MPA smooth muscle. Therefore, it appears that both InsP_3 and norepinephrine (1, 7) can release sufficient Ca from the SR of smooth muscle to activate contraction. The mechanism by which InsP_3 releases Ca from the SR remains to be determined. However, the fact that this release occurs in the presence of vanadate, a known inhibitor of the Ca^{2+}-ATPase of SR and endoplasmic reticulum (29), suggests that InsP_3 acts by increasing the Ca permeability of the SR membrane rather than by inhibition or reversal of the Ca^{2+}-ATPase (26).

The presence of an ATP-dependent Ca pump that, in the absence of InsP_3, removed Ca^{2+} from the bath solution is compatible with the operation of the ATP-dependent Ca pump in the SR of smooth muscle (2, 30–32). We emphasize that, in this as in previous studies of smooth muscle made permeable with saponin (31, 32), the ATP-dependent Ca pump could not be located in the (hyperpermeable) plasma membrane.

Smooth muscle can be activated by depolarization and action potentials (electromechanical coupling) or by pharmacomechanical coupling (8, 24, 33, 34). In trachealis smooth muscle, phosphatidylinositol turnover is increased by cholinergic stimulation but not by sustained depolarization with high K alone, suggesting that pharmacomechanical coupling is mediated through some step in phospholipid metabolism (15). The effect of InsP_3 on rabbit MPA, a tonic smooth muscle that responds in a graded fashion to stimulation (8, 28) similar to the trachealis (35) and is readily activated by pharmacomechanical coupling (6), provides further support for such a mechanism. Ca can be released from the central SR in this tissue (1); thus, if InsP_3 is a messenger, it may have to diffuse to the central SR from the surface membrane where it is produced in response to the action of agonists on plasmalemmal receptors. Ca may also diffuse within the lumen of the SR to peripheral release sites or stimulation of the junctional SR at the surface couplings may, by some unknown mechanism, increase the Ca permeability of the central SR. Our study provides a strong link for considering the phosphatidylinositol pathway, and, specifically, InsP_3, in transmitter-induced release of Ca^{2+} from the SR. Several criteria (e.g., time-resolved measurements of InsP_3, effects of inhibitors, InsP_3 free of isomers and other compounds, etc.) will have to be met before it can be asserted with greater certainty that, as now appears likely, InsP_3 is the major physiological mediator of pharmacomechanical coupling.

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1. Kowarski, D., Shuman, H., Somlyo, A. P. & Somlyo, A. V. (1985) *J. Physiol. (London)*, in press.
2. Johansson, B. & Somlyo, A. P. (1980) in *Handbook of Physiology—The Cardiovascular System: Vascular Smooth Muscle*, eds. Bohr, D. F., Somlyo, A. P. & Sparks, H. V. (American Physiological Society, Bethesda, MD), Vol. 2, pp. 301–324.
3. Somlyo, A. P. (1985) *Circ. Res.*, in press.
4. Hartshorne, D. J. & Gorecka, A. (1980) in *Handbook of Physiology—The Cardiovascular System: Vascular Smooth Muscle*, eds. Bohr, D. F., Somlyo, A. P. & Sparks, H. V. (American Physiological Society, Bethesda, MD), Vol. 2, pp. 93–120.
5. Somlyo, A. P., Devine, C. E., Somlyo, A. V. & North, S. R. (1971) *J. Cell Biol.* **51**, 722–741.
6. Devine, C. E., Somlyo, A. V. & Somlyo, A. P. (1972) *J. Cell Biol.* **52**, 690–718.
7. Bond, M., Kitazawa, T., Somlyo, A. P. & Somlyo, A. V. (1984) *J. Physiol. (London)* **355**, 677–695.
8. Somlyo, A. V. & Somlyo, A. P. (1968) *J. Pharmacol. Exp. Ther.* **159**, 129–145.
9. Hokin, M. R. & Hokin, L. E. (1953) *J. Biol. Chem.* **203**, 967–977.
10. Jones, L. M. & Michell, R. H. (1975) *Biochem. J.* **148**, 479–485.
11. Streb, H., Irvine, R. F., Berridge, M. J. & Schulz, I. (1983) *Nature (London)* **306**, 67–69.
12. Berridge, M. J. & Irvine, R. F. (1984) *Nature (London)* **312**, 315–321.
13. Takenawa, T. (1982) *Cell Calcium* **3**, 359–368.
14. Akhtar, R. A. & Abdel-latif, A. A. (1984) *Biochem. J.* **224**, 291–300.
15. Baron, C. B., Cunningham, M., Strauss, J. F. & Coburn, R. F. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 6899–6903.
16. Lapetina, E. G., Briley, P. A. & De Robertis, E. (1976) *Biochim. Biophys. Acta* **431**, 624–630.
17. Suematsu, E., Hirata, M., Hashimoto, T. & Kuriyama, H. (1984) *Biochem. Biophys. Res. Commun.* **120**, 481–485.
18. Fiskum, G., Craig, S. W., Decker, G. L. & Lehninger, A. L. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 3430–3434.
19. Simon, W., Ammann, D., Oehme, M. & Morf, W. E. (1978) *N.Y. Acad. Sci.* **307**, 52–70.
20. Fabiato, A. & Fabiato, F. (1979) *J. Physiol. (Paris)* **75**, 463–505.
21. Gomperts, B. D. (1983) *Nature (London)* **306**, 64–66.
22. Itoh, T., Kuriyama, H. & Suzuki, H. (1983) *J. Physiol. (London)* **337**, 609–629.
23. Irvine, R. F., Letcher, A. J., Lander, D. J. & Downes, C. P. (1984) *Biochem. J.* **223**, 237–243.
24. Leijten, P. A. A. & van Breemen, C. (1984) *J. Physiol. (London)* **357**, 327–339.
25. Somlyo, A. P. (1984) *Nature (London)* **309**, 516–517.
26. Prentki, M., Wollheim, C. B. & Lew, P. D. (1984) *J. Biol. Chem.* **259**, 13777–13782.
27. Prentki, M., Corkey, B. E. & Matschinsky, F. M. (1985) *J. Biol. Chem.*, in press.
28. Somlyo, A. V., Vinall, P. & Somlyo, A. P. (1969) *Microvasc. Res.* **1**, 354–373.
29. Martonosi, A. N. (1984) *Physiol. Rev.* **64**, 1240–1320.
30. Wuytack, F. & Casteels, R. (1980) *Biochim. Biophys. Acta* **595**, 257–263.
31. Endo, M., Yagi, S. & Iino, M. (1982) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **41**, 2245–2250.
32. Somlyo, A. P., Somlyo, A. V., Shuman, H. & Endo, M. (1982) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **41**, 2883–2890.
33. Coburn, R. F. & Yamaguchi, T. (1977) *J. Pharmacol. Exp. Ther.* **201**, 276–284.
34. Casteels, R. (1980) *Chest* **78**, 150–156.
35. Somlyo, A. P. & Somlyo, A. V. (1970) in *Airway Dynamics*, ed. Bouhuys, A. (Thomas, Springfield, IL), pp. 209–228.