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INOSITOL-1,4,5-TRISPHOSPHATE RELEASES CALCIUM FROM SKINNED CULTURED SMOOTH MUSCLE CELLS

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SUMMARY. We examined the effects of inositol-1,4,5-trisphosphate on ^{45}Ca uptake and ^{45}Ca efflux in the saponin skinned primary cultured rat aortic smooth muscle cells. 10 μM inositol-1,4,5-trisphosphate induced a rapid (half time<10 sec) and large quantity of Ca release in both ^{45}Ca uptake and ^{45}Ca efflux in the skinned cells preloaded with 1 μM free Ca. Dose response curves showed that 100 μM inositol-1,4,5-trisphosphate produced a maximal Ca release of 97.3% of the MgATP dependent ^{45}Ca uptake or 289 μ moles/liter cells, which was much greater than the maximal caffeine induced Ca release and would be sufficient to produce maximal tension.

One of the problems in muscle physiology which has been refractory to solution in spite of gargantuan research efforts, has been the link between membrane excitation and release of intracellular Ca. In non-muscle cells inositol-1,4,5-trisphosphate (IP_3) has been recently reported to act as a second messenger responsible for carrying the signal from the surface membranes to the endoplasmic reticulum (1). Last year Kuriyama and co-workers reported IP_3 mediated release of Ca from permeabilized isolated coronary arterial smooth muscle cells (2). However release could only be obtained under very special loading conditions. For this reason, it is of interest that we have now obtained fast release of a large quantity of Ca from an MgATP dependent Ca store in saponin treated primary cultured arterial smooth muscle cells as described below.

MATERIALS AND METHODS

The primary cultured aortic smooth muscle cells from male Wistar rats were obtained according to the method of Chamley et al (3,4). The monolayer

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Abbreviations: IP_3, inositol-1,4,5-trisphosphate; EGTA, ethyleneglycol-bis-(β-aminoethylether) N-N'-tetraacetic acid.

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cells in 35 mm plastic dishes were skinned by saponin treatment under optimal conditions (50 μg/ml, 15 min, at 22° C) as was described for rabbit mesenteric artery (5). All experiments were carried out at room temperature (5). For ^{45}Ca uptake experiments the skinned cells were loaded with ^{45}Ca labelled Ca solution containing 1 x 10^{-6} M free Ca buffered with 0.1 mM EGTA, 65 mM KCL, 65 mM K propionate, 5 mM $MgCl_2$, 3.15 mM Na_2ATP and 20 mM tris-maleate buffer (pH 6.80 at 22° C) for 20 min and then exposed to IP_3 for the desired time periods in the same solution. ^{45}Ca content in the skinned cells was measured using liquid scintillation counting after washing the cells for 7 min with Ca free, 0.1 mM EGTA solution for skinned cells which corresponds to the relaxing solution for skinned smooth muscle fibers. The number of cells was assessed in the two separate dishes cultured at the same time. The data were expressed as n mol/10^6 cells. For ^{45}Ca efflux experiments the skinned cells were loaded with ^{45}Ca labelled 1 x 10^{-6} M free Ca in the presence or absence of 3 mM MgATP for 20 min and incubated in the Ca free, 0.1 mM EGTA solution for skinned cells (efflux solution) which was changed every minute. ^{45}Ca released in the medium was measured and expressed as net Ca loss per minute.

RESULTS AND DISCUSSION

Figure 1 shows the time course of IP_3 induced Ca release from the saponin skinned primary cultured rat aortic smooth muscle cells. Application of 1 x 10^{-5} M IP_3 induced a rapid and large reduction of the Ca content in the skinned cells which were preloaded with 1 x 10^{-6} M free Ca in the presence of MgATP . 87% of the maximal response was induced within 10 sec after application. This rapid response agrees with observations in other cell types (1) and is probably fast enough to produce tension. The same dose of IP_3 could not release Ca from the skinned cells loaded in the absence of MgATP. Thus IP_3 releases Ca from a MgATP dependent Ca store, presumably sarcoplasmic reticulum

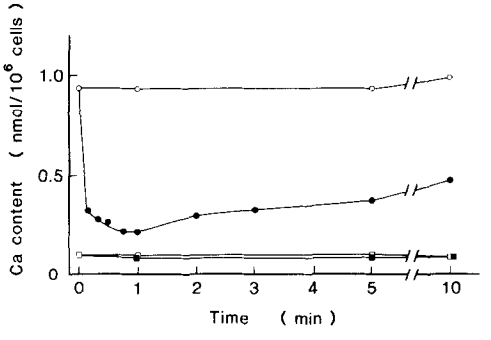

Figure 1 - Time course of IP_3 induced Ca release from the skinned cells. The skinned cells were preloaded with ^{45}Ca labelled 1 x 10^{-6} M free Ca for 20 min and then exposed to 1 x 10^{-5} M IP_3 (●) for various time periods (abscissa) in the same solution. In control (○) the solution was changed to the same Ca solution except of IP_3. The skinned cells preloaded with 1 x 10^{-6} M free Ca in the absence of MgATP (squares) were also treated in the same manner (□ , control; ■ , 1 x 10^{-5} M IP_3). Ca content at time 0 means Ca content after Ca loading for 20 min.

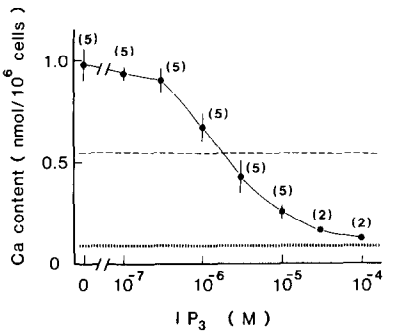

Figure 2 - Dose response curves of IP_3 induced Ca release from the skinned cells. The skinned cells were preloaded with ^{45}Ca labelled 1 x 10^{-6} M free Ca for 20 min in the presence of 3 mM MgATP and exposed to various concentrations of IP_3 for 1 min. Ca content after exposure was plotted as a function of IP_3 concentration (solid line). Points and bars are mean ± standard deviation. The value in parentheses represents the number of observations. The dashed and dotted lines indicate Ca content after 25 mM caffeine application for 1 min and MgATP free Ca loading respectively.

(6). Figure 1 also shows reuptake of Ca into the skinned cells in the presence of IP_3. This phenomenon is probably due to the hydrolysis of IP_3 by IP_3 phosphatase as has been suggested in other cell types.

The extent and sensitivity of Ca release by IP_3 is further shown in the dose response curve (Figure 2). 1 x 10^{-4} M IP_3 produced a maximal Ca release of 97.3% of the MgATP dependent ^{45}Ca uptake which was much greater than the maximal caffeine induced Ca release. Using a cellular volume of 2.94 $\mu l/10^6$ cells as determined by the cytocrit method the IP_3 induced Ca release amounts to 289 μmoles/liter cells. Somlyo and co-workers reported that the increase in cytoplasmic Ca during maintained maximal contractions was 1.0 ± 0.2 mmol Ca/kg dry cytoplasm or approximately 235 μmoles/liter cell water (7). Since agonist induced intracellular Ca release contributes part of the activating Ca (8,9) we may conclude from the above figures that micromolar IP_3 could function as a second messenger in this process. In the intact vascular smooth muscle neurotransmitter releasable Ca store size has been reported to be equal to or smaller than caffeine sensitive Ca store size (5,10). Thus the intracellular IP_3 level during maximal stimulation by neurotransmitters might be near 1.6 μM (ED_{50}) which releases almost the same amount of Ca as 25 mM caffeine does in the skinned cells.

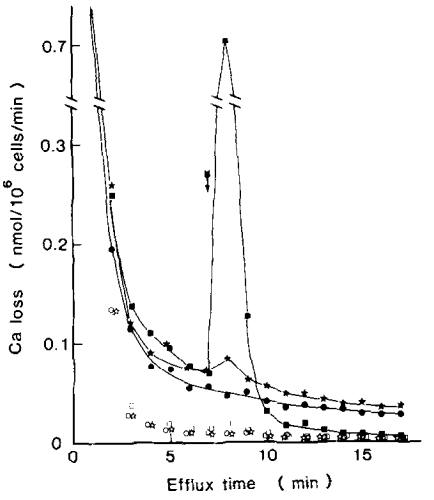

Figure 3 - Effect of IP_3 and caffeine on ^{45}Ca efflux from the skinned cells. The skinned cells were loaded with ^{45}Ca labelled 1 x 10^{-6} M free Ca in the presence of 3 mM MgATP for 20 min and incubated in the Ca free, 0.1 mM EGTA solution for skinned cells (efflux solution) which was changed every minute. ^{45}Ca released in the medium was measured and expressed as net Ca loss per minute (ordinate). 1 x 10^{-5} M IP_3 (■) or 25 mM caffeine (✿) was added at 7 min of efflux time (arrow). ● , control. The skinned cells preloaded with ^{45}Ca labelled 1 x 10^{-6} M free Ca in the absence of MgATP (open symbols) were also treated in the same manner (○, control; □ , 1 x 10^{-5} M IP_3; ☆,25 mM caffeine).

Figure 3 shows the effect of 1 x 10^{-5} M IP_3 on ^{45}Ca efflux from the skinned cells into Ca free solution containing 0.1 mM EGTA. Since there could be no concomitant significant ^{45}Ca uptake under these conditions the rapid and large increase of ^{45}Ca efflux shown here again confirms that IP_3 is a potent Ca releaser in smooth muscle. Moreover, the amount of Ca released by 1 x 10^{-5} M IP_3 was approximately 0.73 n moles/10^6 cells which agrees with the decrease in Ca content induced by 1 x 10^{-5} M IP_3 in the presence of 1 x 10^{-6} M free Ca (0.71 nmoles/10^6 cells, Figure 1). Thus it is suggested that Ca release by IP_3 is independent on Ca outside of the IP_3 sensitive Ca store. On the other hand, 25 mM caffeine induced a significant but small and variable increase in ^{45}Ca efflux. A typical response was shown in Figure 3. This caffeine induced ^{45}Ca release into the Ca free efflux solution always was much less than the decrease in Ca content induced by 25 mM caffeine in the presence of 1 x 10^{-6} M free Ca (Figure 1) suggesting that caffeine requires Ca on the outside of the sarcoplasmic reticulum for its Ca releasing action.

In conclusion our results suggest that IP_3 could be the messenger linking receptor activation to intracellular Ca release in smooth muscle. However, so far, tension development by IP_3 in smooth muscle has not been reported. It remains as an important question which should be resolved soon.

ACKNOWLEDGEMENT

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