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INOSITOL 1,4,5-TRISPHOSPHATE RELEASES Ca^{2+} FROM INTRACELLULAR STORE SITES IN SKINNED SINGLE CELLS OF PORCINE CORONARY ARTERY

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Summary : Effects of inositol 1,4,5-trisphosphate, extracted from human erythrocyte ghosts, on Ca^{2+} release from intracellular store sites were studied in saponin-treated single muscle cells of the porcine coronary artery. Application of micromolar concentrations of inositol 1,4,5-trisphosphate released Ca^{2+} from the intracellular non-mitochondrial store sites, within 1 min. However, when the concentrations of free Ca^{2+} were over 1.5×10^{-6} M, the release of Ca^{2+} by this agent was inhibited. The Ca^{2+} releasing mechanism differed from that seen with A23187, therefore this release of Ca^{2+} from store sites was not due to Ca^{2+} ionophore actions. This agent may play the role of messenger in increasing the cytosolic Ca^{2+}, provoking pharmaco-mechanical coupling, and thus producing the contraction.

Increases of free Ca^{2+} in the cytosol play a key role in the contraction of vascular smooth muscles. Free Ca^{2+} in concentrations over 10^{-7} M activates the contractile proteins, and sources of the Ca^{2+} are either the release from intracellular store sites, presumably sarcoplasmic reticulum, or influx through the plasma membrane (1). In particular, the former plays an important role in regulating the contraction-relaxation cycle in vascular smooth muscles (1). A receptor-activated contraction occurring with no change in the membrane property has been termed "pharmaco-mechanical coupling" (2). In the porcine coronary artery, contraction occurs in the presence of acetylcholine, but this agent has no effect on membrane potential and resistance. The acetylcholine-induced contraction does occur in Ca^{2+}-free 2 mM ethylenglycol-bis-(ß-amino-ethylether)-N,N,N'N'-tetraacetate (EGTA) containing solution but this contraction is blocked by procaine, an inhibitor of Ca^{2+}-release from intracellular store sites (3).

Agonists rapidly induce hydrolysis of membrane phosphatidylinositol 4,5-bisphosphate and phosphatidylinositol 4-phosphate to yield inositol 1,4,5-

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trisphosphate (Ins-P_3) and inositol 1,4-bisphosphate (Ins-P_2), respectively, in rat brain slices, parotid glands and insect salivary glands (4). Streb et al. (5) reported that Ins-P_3 released Ca^{2+} from the non-mitochondrial intracellular stores in rat pancreatic acinar cells.

We now report that Ins-P_3 releases Ca^{2+} from the intracellular store site in smooth muscle cells of the porcine coronary artery treated with saponin.

Materials and Methods

Single smooth muscle cells from porcine coronary arteries were prepared as described (6). Cell viability, as assessed by trypan blue exclusion test, was over 85 %. To obtain the release of Ca^{2+} from intracellular stores, saponin-treatment was carried out using essentially the same method as applied to macrophages (7-9). In brief, 3×10^6 cells were incubated in the solution (6 ml) containing 100 mM KCl, 20 mM Tris-maleate (pH 6.8), 2 mM $MgCl_2$, 1 mM ATP, 1 mM EGTA and 25 μg/ml saponin (I.C.N. Corp.) for 10 min at 37°C. Saponin treatment of cells resulted in a selective destruction of plasma membrane, while the contractile apparatus and intracellular organelles remained intact (1, 7-9). Ca^{2+} uptake and release were assayed by a filtration method using ^{45}Ca (for details : see the legend of Fig.1). Ins-P_3 and Ins-P_2 were prepared from human erythrocyte ghosts by the method of Downes and Michell (10).

Results and Discussion

In the skinned muscle from the porcine coronary artery, contraction was evoked in concentrations of Ca^{2+} over 3×10^{-7} M and an almost maximum amplitude of contraction was obtained by application of 1×10^{-6} M Ca^{2+}. After a brief accumulation of Ca^{2+} (10^{-6} M) into store sites, the Ca^{2+} is released by caffeine, but not by treatment with acetylcholine or norepinephrine, i.e. after saponin treatment, the Ca^{2+} store sites and the contractile proteins are preserved and agonists release Ca^{2+} from store sites through productions of intermediate substances (1). In the presence of 10 mM NaN_3, saponin-treated smooth muscle cells of the porcine coronary artery accumulate Ca^{2+} at concentrations ranging from 10^{-8} M to 10^{-5} M, and the maximal uptake (capacity) is about 0.25 nmol/10^5 saponin-treated cells. Half maximal Ca^{2+} uptake was obtained at about 3×10^{-7} M Ca^{2+}, that is much the same as in the case of macrophages (7,8). Therefore, non-mitochondrial store sites accumulate Ca^{2+} in saponin-treated single smooth muscle cells, in the presence of NaN_3. Using these saponin-treated cells, the effects of Ins-P_3 on the Ca^{2+} release from the non-mitochondrial store site were examined.

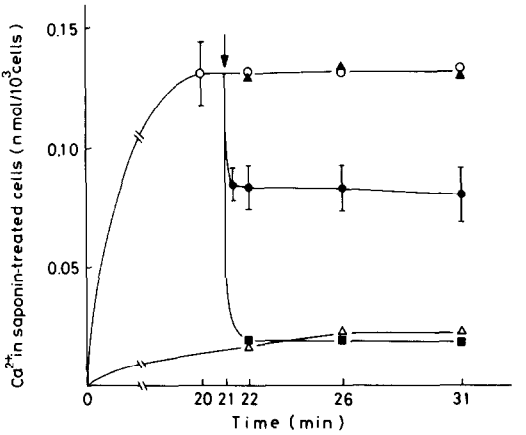

Fig. 1. Time course of Ca^{2+} release induced by Ins-P_3 from intracellular store site of saponin-treated single smooth muscle cells. The Ca^{2+} was accumulated in these cells in the solution (5 ml) containing 100 mM KCl, 20 mM Tris-maleate (pH 6.8), 5 mM $MgCl_2$, 5 mM ATP, 10 mM NaN_3, 0.12 mM $CaCl_2$ (containing 1 μCi/ml ^{45}Ca), 0.44 mM EGTA (free Ca^{2+} concentration was 3.7×10^{-7} M), and 1×10^5 saponin-treated cells/ml at 37°C. At 20 min, 1 ml of the above mixture was passed through a glass fiber filter (Whatman GF/C; pore size: 1.2 μ), and the filter was washed twice with 2 ml of the above solution without ^{45}Ca and cells. Thus, the amount of Ca^{2+} uptake was determined. At 21 min, 1/100 volumes of reagents were added, and at indicated times, the Ca^{2+} in saponin-treated cells was determined as described above. o: control; ●: 5 μM Ins-P_3; ▲: 1 μM Ins-P_2; ■: 5 μM A23187; △: ATP-free. The vertical bars represent the S.E. for five independent experiments.

Fig. 1 shows the time course of Ca^{2+} release from the store site by application of Ins-P_3. Ins-P_3 (5 μM) released Ca^{2+} within 20 sec and this released Ca^{2+} was about 40 % of the accumulated Ca^{2+}, and was not taken up again. Ca^{2+} ionophore, A23187 (5 μM) released all the accumulated Ca^{2+}, while Ins-P_2 (1 μM) had no effect on the release of Ca^{2+}. The lack of re-uptake of the released Ca^{2+} from store sites seen in the present experiments differs from the data of Streb et al. (5). This discrepancy may be due to different procedures used to prepare the permeabilized cells (nominally Ca^{2+}-free solution and saponin), or tissue differences. Their preparation may have contained trisphosphatase to produce the Ins-P_2 while our preparation did not.

Fig. 2 shows the relationship between the Ca^{2+} release and Ins-P_3 concentration after accumulation into the store site in saponin-treated cells. The near-maximal and half-maximal release of Ca^{2+} from store sites was obtained at 3 μM and 0.7 μM, respectively, such being comparable to data of Streb et al. (5).

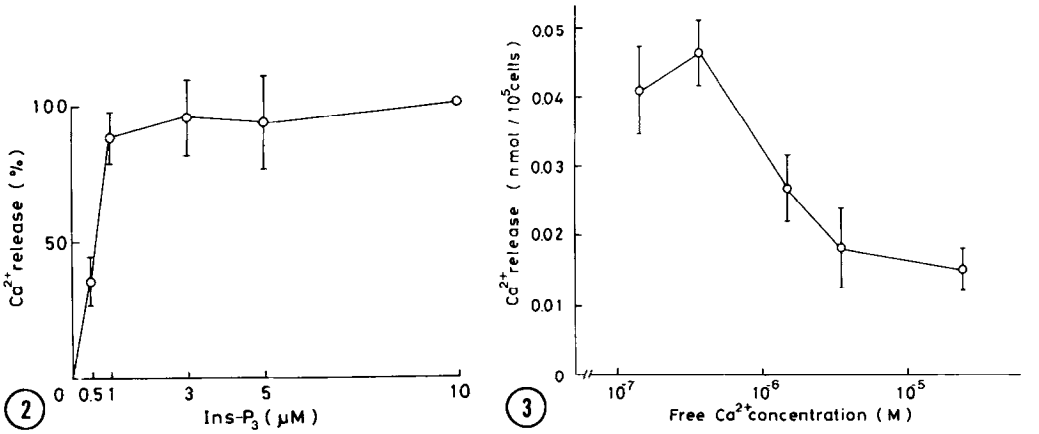

Fig. 2. A dose-response relationship of Ins-P_3-induced Ca^{2+} release. The Ca^{2+} release was measured using procedures described in Fig. 1 legend. The Ca^{2+} release was relative to that 1 min after the addition of 10 μM Ins-P_3. The vertical bars represent the S.E. for four independent experiments.

Fig. 3. Effects of free Ca^{2+} concentrations on the Ca^{2+} release induced by 5 μM Ins-P_3. The Ca^{2+} release was measured by essentially the same procedure as described in Fig. 1 legend. Various concentrations of free Ca^{2+} were prepared by addition of 0.12 mM $CaCl_2$ and appropriate concentrations of EGTA. The apparent affinity constant of EGTA for Ca^{2+} was assumed to be 1×10^6 M^{-1} at pH 6.8 (7). The vertical bars represent the S.E. for five independent experiments.

We also examined the effects of free Ca^{2+} concentrations on the Ins-P_3-induced Ca^{2+} release (Fig. 3). The maximal Ca^{2+} release induced by Ins-P_3 was obtained when Ca^{2+} uptake was assayed at 3.7×10^{-7} M free Ca^{2+}, and the Ca^{2+} release was inhibited when Ca^{2+} was loaded in the presence of free Ca^{2+} in concentrations over 1.5×10^{-6} M. This may be due to the inhibitory effects of either higher concentrations of extra-vesicular Ca^{2+} or to a larger accumulation of Ca^{2+} into the store site, because the Ca^{2+} was accumulated in proportion to concentrations of free Ca^{2+}.

Our results show that micromolar concentrations of Ins-P_3 do release Ca^{2+} from non-mitochondrial Ca^{2+} store sites of vascular smooth muscles, as was observed in pancreatic acinar cells (5). Thus, Ins-P_3 may be "initial messenger" for the pharmaco-mechanical coupling in the contraction of coronary artery muscles by acetylcholine. However, Akhtar and Abdel-Latif (11) reported that the hydrolysis of phosphatidylinositol 4,5-bisphosphate to yield Ins-P_3 induced by acetylcholine was dependent on Ca^{2+}, in smooth muscles of the rabbit iris. Thus, the production of Ins-P_3 seems to be a result of an

increase in cytosolic Ca^{2+}. Egawa et al (12) found that in the rabbit vas deferens, the hydrolysis by acetylcholine did not require Ca^{2+}. Thus, requirement of Ca^{2+} in the hydrolysis of phosphoinositides seems to differ with the tissue (13). Further experiments are underway to clarify whether or not acetylcholine or other agonists increase the production of Ins-P_3, through activations of phosphoinositide hydrolysis.

Acknowledgments

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