Cell Side-specific Sensitivities of Intracellular Ca\(^{2+}\) Stores for Inositol 1,4,5-Trisphosphate, Cyclic ADP-ribose, and Nicotinic Acid Adenine Dinucleotide Phosphate in Permeabilized Pancreatic Acinar Cells from Mouse*

In pancreatic acinar cells hormonal stimulation leads to a cytosolic Ca\(^{2+}\) wave that starts in the apical cell pole and subsequently propagates toward the basal cell side. We used permeabilized pancreatic acinar cells from mouse and the mag-fura-2 technique, which allows direct monitoring of changes in [Ca\(^{2+}\)]\(_i\) of intracellular stores. We show here that Ca\(^{2+}\) can be released from stores in all cellular regions by inositol 1,4,5-trisphosphate. Stores at the apical cell pole showed a higher affinity to inositol 1,4,5-trisphosphate (EC\(_{50}\) = 89 nm) than those at the basolateral side (EC\(_{50}\) = 256 nm). In contrast, cADP-ribose, a modifier of Ca\(^{2+}\)-induced Ca\(^{2+}\) release, and nicotinic acid adenine dinucleotide phosphate (NAADP) were able to release Ca\(^{2+}\) exclusively from intracellular stores located at the basolateral cell side. Our data agree with observations that upon stimulation Ca\(^{2+}\) is released initially at the apical cell side and that this is caused by high affinity inositol 1,4,5-trisphosphate receptors. Moreover, our findings allow the conclusion that in Ca\(^{2+}\) wave propagation from the apical to the basolateral cell side observed in pancreatic acinar cells Ca\(^{2+}\)-induced Ca\(^{2+}\) release, modulated by cADP-ribose and/or NAADP, might be involved.

In pancreatic acinar cells regulation of spatio-temporal changes in the free cytosolic Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_i\)) plays an essential role in receptor-mediated stimulation of protein secretion. Hormonal stimulation of the cells leads to production of inositol 1,4,5-trisphosphate (IP\(_3\))\(^1\) (1), which is followed by a cytosolic Ca\(^{2+}\) signal starting in the apical cell pole (2, 3). This cytosolic Ca\(^{2+}\) signal can either remain localized at the apical side in the form of Ca\(^{2+}\) oscillations, or it can spread toward the basolateral cell side in form of a “Ca\(^{2+}\) wave” (3, 4).

The mechanism underlying generation and propagation of the Ca\(^{2+}\) wave is still a matter of debate. Despite IP\(_3\) generation by activation of phospholipase C located in the basolateral plasma membrane, the initial rise in [Ca\(^{2+}\)]\(_i\), takes place at the opposite side of the cell. From results obtained by measurements of the [Ca\(^{2+}\)]\(_i\) it was concluded indirectly that stores in the apical cell pole possess receptors with higher sensitivities for IP\(_3\) than stores in the basolateral cell pole (3, 5–7). A structural basis for the assumption of different IP\(_3\) sensitivities may be the existence of different IP\(_3\) receptor isoforms in pancreatic acinar cells (types I, II, and III) (8, 9). In IP\(_3\) binding studies it has been shown that the type II IP\(_3\) receptors have a higher IP\(_3\) affinity than the type III receptors (10, 11) and that the type II IP\(_3\) receptors are located exclusively in the apical cell pole (12, 13). Taken together, these findings suggest that Ca\(^{2+}\) stores located in the apical region are equipped mainly with the higher affinity type II IP\(_3\) receptors; however, these predicted sensitivity differences between the stores in the two cell poles have not yet been quantified as changes of the [Ca\(^{2+}\)]\(_i\) in the Ca\(^{2+}\) stores itself which allow concentration-response measurements.

Recently, it has been shown that the speed of the Ca\(^{2+}\) wave that propagates from the apical to the basolateral cell side depends on the type of stimulating hormone such as acetylcholine, bombesin, or cholecystokinin (14). Because ryanodine receptors are present in pancreatic acinar cells (15) and participate in the generation of Ca\(^{2+}\) signals (16–18), a potential regulator of hormone-induced Ca\(^{2+}\) signals could be cADP-ribose, which modifies the ability of ryanodine receptors to mediate Ca\(^{2+}\)-induced Ca\(^{2+}\) release in many cell systems (19). Another candidate that may play a role in Ca\(^{2+}\) release is nicotinic acid adenine dinucleotide phosphate (NAADP) (20, 21). It has been shown that both cADP-ribose and NAADP can induce Ca\(^{2+}\) oscillations in pancreatic acinar cells (22, 23). This led to the hypothesis that in addition to IP\(_3\)-induced Ca\(^{2+}\) release, part of stored Ca\(^{2+}\) is released via other mechanisms, probably by hormone-induced elevation of the cellular cADP-ribose and/or NAADP concentration.

Using fluorescence methods we have shown recently that Ca\(^{2+}\) stores in distinct regions of pancreatic acinar cells in primary culture release Ca\(^{2+}\) in response to cADP-ribose and IP\(_3\) from different and in part from common Ca\(^{2+}\) stores (24). Primary cultured acinar cells lack the typical polarized organization of a differentiated acinar cell. We therefore used freshly isolated acinar cells from mouse in the present study to obtain more information about the localization of functionally different Ca\(^{2+}\) stores.

By use of the mag-fura-2 technique, which allows monitoring of changes in [Ca\(^{2+}\)] of intracellular Ca\(^{2+}\) stores ([Ca\(^{2+}\)]\(_{store}\)) from permeabilized cells (25) we demonstrate directly here that IP\(_3\) added to pancreatic acinar cells at low concentrations (0.1–1 μM) releases Ca\(^{2+}\) mainly from stores located in the apical cell pole. In contrast, cADP-ribose and NAADP release

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Ca\(^{2+}\) exclusively from basolateral stores. These findings may help to explain the typical spatio-temporal pattern of the cytoplasmic Ca\(^{2+}\) signal after hormonal stimulation of pancreatic acinar cells.

**EXPERIMENTAL PROCEDURES**

**Cell Preparation**—Adult male CD-1 mice were killed by cervical dislocation, and the pancreas was rapidly removed and placed into preparation buffer (composition in mM: 130 NaCl, 4.7 KCl, 1.3 CaCl\(_2\), 1 MgCl\(_2\), 1.2 KH\(_2\)PO\(_4\), 10 HEPES, 10 glucose, 0.2% (w/v) albumin, and 0.01% (w/v) trypsin inhibitor; pH adjusted to 7.4 with NaOH). After removing adherent blood vessels and fat tissue we injected the pancreas with 1 ml of the preparation buffer supplemented with collagenase type V (30 units/ml) and incubated it for 10 min at 37 °C. After enzymatic digestion the tissue was cut into small pieces of ~1 mm\(^3\) and pipetted gently through tips of decreasing diameter to dissociate the tissue into single cells. Cells were then centrifuged for 2 min at 30 × g, and the pellet was resuspended in preparation buffer without collagenase. With this isolation procedure single cells as well as small clusters consisting of two up to five cells were obtained.

**Mag-fura-2 Loading and Permeabilization**—Cells were loaded with 5 μM mag-fura-2-AM for 20 min at room temperature in preparation buffer. For permeabilization cells were washed with permeabilization buffer (composition in mM: 125 KCl, 25 NaCl, 10 HEPES, pH 7.2 adjusted with KOH) and then incubated with ice-cold permeabilization buffer plus 1 unit/ml streptolysin O for 3–5 min. Finally cells were rinsed with intracellular buffer containing increasing 

**Conical Microscopy**—To check to what extent the subcellular organization of pancreatic acinar cells is perturbed by streptolysin O, permeabilized cells were loaded with the intracellular membrane marker 3,3′-dihexyloxacarbocyanine iodide (DiOC\(_6\), 1 μg/ml for 2 min) in intracellular buffer. After loading, cells were washed in preparation buffer, and confocal pictures of the cells were taken with a confocal microscope (Bio-Rad MRC1024) with an excitation wavelength of 488 nm before and after permeabilization.

**RESULTS**

**Experimental Approach**

We used freshly isolated pancreatic acinar cells from mice loaded with the Ca\(^{2+}\)-sensitive dye mag-fura-2. This dye not only stains the cytoplasm of the cell but is also taken up into intracellular compartments such as the endoplasmic reticulum. To estimate [Ca\(^{2+}\)]\(_{store}\) not influenced by the cytoplasmic dye and to test the effects of IP\(_3\), ADP-ribose, and NAADP on these stores, cells had to be permeabilized with streptolysin O before each experiment as described in “Experimental Procedures.” Using this method about 30% of the cells lost cytoplasmic dye, demonstrating successful permeabilization. The remaining cells that had been permeabilized showed a discrete distribution of fura dye with a high intensity at the apical cell
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Fig. 3. IP$_3$-induced Ca$^{2+}$ release in different cell areas. Panel A, ratio signal from Ca$^{2+}$ stores of different regions of a permeabilized pancreatic acinar cell. Small areas of interest were chosen from both the basolateral and the apical part of the cell (see diagram). Application of 5.0 $\mu$M IP$_3$ (arrow) induces maximal Ca$^{2+}$ release from stores located at both cell sides. Panel B, comparison of the effect of a submaximal [IP$_3$] (0.1 $\mu$M) on Ca$^{2+}$ release from different cell sides. In 10 independent experiments the relative ratio changes ($\Delta$ratio) in percent of the maximal IP$_3$-induced change (at 5 $\mu$M IP$_3$) in response to 0.1 $\mu$M IP$_3$ were determined in both apical and basolateral areas. Both single values and the mean ± S.D. are shown. Sensitivity to IP$_3$ appeared to be higher in the apical cell region, where a higher decrease in [Ca$^{2+}$] store was found than in the basolateral cell region with the same [IP$_3$]. Panel C, dose-response curves for the apical and the basolateral cell side. Data as shown in panel B were collected for different [IP$_3$] ($n = 2–10$ for each [IP$_3$]). The data for each cell side were fit separately by a logistic function. Values for both $K_v$ and the Hill coefficient nearest to the curves are shown. Panel D, the quotient $\Delta$ratio$_{apical}$/ $\Delta$ratio$_{basolateral}$ was calculated and plotted against the [IP$_3$] from data similar to those shown in panel B. The sensitivity of the Ca$^{2+}$ pools in the apical cell region for 0.1 $\mu$M IP$_3$ apparently was 3-fold higher than that of pools at the basolateral cell side.

pool and a low intensity at the basolateral cell side (not shown). Light microscopy with differential interference contrast showed that the nucleus and the zymogen granules of these cells did not redistribute after permeabilization (Fig. 1, A and B). Confocal microscopy of DilO6-stained cells before and after permeabilization verified that even the stained membranes (of mitochondria and endoplasmic reticulum) were largely kept in position (Fig. 1, A and B).

IP$_3$-induced Ca$^{2+}$ Release

Effect of IP$_3$—When permeabilized cells were analyzed we found that a concentration of 5 $\mu$M IP$_3$ induced a maximal Ca$^{2+}$ release in all regions of the cell. Further release of Ca$^{2+}$ could only be achieved by the application of the Ca$^{2+}$ ionophore ionomycin (10 $\mu$M), by which Ca$^{2+}$ stores were completely depleted (Fig. 2A). The maximal IP$_3$-induced Ca$^{2+}$ release as estimated by the decrease in the fluorescence ratio was 91.8 ± 7.0% of the ionomycin effect ($n = 8$ experiments, 43 cells; Fig. 2B).

The so-called “quantal Ca$^{2+}$ release,” which means that an increase in submaximal [IP$_3$] leads to stepwise release of discrete amounts of Ca$^{2+}$, could also be observed by direct monitoring of [Ca$^{2+}$]$_{store}$ (Fig. 2A).

The IP$_3$ effect was reversible. Washing the cells with intracellular buffer, subsequently to IP$_3$-induced Ca$^{2+}$ release, resulted in complete refilling of the stores (data not shown; $n = 8$ experiments, 25 cells).

Effects of IP$_3$ in Different Subcellular Regions—The use of video imaging allowed us to analyze simultaneously Ca$^{2+}$ release from different regions within a single cell. We found that all regions of a cell responded to 5.0 $\mu$M IP$_3$ with a maximal release of Ca$^{2+}$ (Fig. 3A). However, the fluorescence ratio before stimulation was always higher in the basolateral cell side compared with the apical cell side. This does not essentially mean that the [Ca$^{2+}$]$_{store}$ is higher basolaterally. It is more probable that the typical spatial distribution of organelles (high proportion of endoplasmic reticulum at the basolateral side versus high proportion of zymogen granules at the apical cell pole) is responsible for these different fluorescence ratios in the opposite cell poles. Assuming that compartments in the apical cell region take up fluorescence dye but no Ca$^{2+}$, fluorescence from these stores will quench part of the mag-fura-2 signal from functional Ca$^{2+}$ stores in the apical cell pole. This problem of the so-called “silent compartments” has been considered earlier (28). Thus it did not seem appropriate to use the ratio signal itself for estimation of different IP$_3$ sensitivities in different cell regions. We therefore standardized the data for the IP$_3$-induced decrease in the mag-fura-2 ratio by comparison with the maximal ratio decrease induced by ionomycin in the respective cell region (set at 100%). By this procedure it became obvious that a low concentration of IP$_3$ showed different efficiencies in the apical and in the basolateral side of the cell. At a submaximal [IP$_3$] of 0.1 $\mu$M, Ca$^{2+}$ release was 55.3 ± 23.8% of the maximal ionomycin-inducible ratio decrease in the apical side of the cell, whereas it was only 20.5 ± 9.8% (Fig. 3, B–D) in the basolateral cell side.

Based on this result we tested different concentrations of IP$_3$ between 0.1 and 5.0 $\mu$M IP$_3$ and found that the dose-response curve for IP$_3$ was shifted to the left for the apical compared with that for the basolateral cell regions (Fig. 3C). The half-maximal effective concentration for IP$_3$ in the apical cell side was 89 nM compared with 256 nM in the basolateral cell side ($n = 10$).
**cADP-ribose- and NAADP-induced Ca\(^{2+}\) Release**

**Effect of cADP-ribose and NAADP on the Whole Cell**—The distribution pattern of different IP\(_3\)-sensitive stores described in the last paragraph led us to assume that start and propagation of hormone-induced Ca\(^{2+}\) waves from the apical to the basolateral cell sides (14, 17, 29, 30) are related to the functions of Ca\(^{2+}\) stores in these cell regions. In particular we addressed the question of whether cADP-ribose- and NAADP-induced Ca\(^{2+}\) release from stores in pancreatic acinar cells could be involved in propagation of the Ca\(^{2+}\) wave toward the basolateral cell side.

We therefore first superfused permeabilized cells with up to 100 μM cADP-ribose, a concentration known to be supermaximal for Ca\(^{2+}\) release from the stores of rat pancreatic acinar cells in primary culture (24). However, cADP-ribose was ineffective in freshly prepared cells.

The inability of cADP-ribose to release Ca\(^{2+}\) from permeabilized cells could be caused by the loss of a cytoplasmic factor necessary for the releasing process. We therefore tried application of 5 μg/ml calmodulin, described to be a cofactor for the cADP-ribose effect (31). But this did not result in cADP-ribose-induced Ca\(^{2+}\) release from the stores either. A pharmacological tool often used to sensitize the Ca\(^{2+}\)-induced Ca\(^{2+}\) release mechanism is caffeine. We tested cADP-ribose in the presence of caffeine, which alone had no effect on Ca\(^{2+}\) release from intracellular Ca\(^{2+}\) stores (n = 3 experiments, 14 cells). At 50 μM cADP-ribose and in the presence of 10 mM caffeine, Ca\(^{2+}\) was released from the stores (n = 11 experiments, 39 cells; Fig. 4A), and the effect was maximal (33 ± 15% of the ionomycin-induced ratio decrease). Neither an increase in caffeine nor an increase in [cADP-ribose] further induced Ca\(^{2+}\) release. Therefore the following cADP-ribose experiments were performed in the presence of 10 mM caffeine without further mention in this text.

Similar to cADP-ribose, NAADP was able to release Ca\(^{2+}\) from permeabilized pancreatic acinar cells (Fig. 4B). In a concentration of 50 nM the ratio decrease that could be induced by NAADP was 35 ± 18% of the ionomycin-induced ratio decrease (n = 4 experiments, 8 cells). Increasing the NAADP concentration to 100 mM did not change this result (data not shown).

The effects of cADP-ribose- and NAADP-induced Ca\(^{2+}\) release were reversible. Removal of cADP-ribose or NAADP from the intracellular buffer resulted in refilling of the stores (data not shown).

Comparing the effect of cADP-ribose, NAADP, and IP\(_3\) on Ca\(^{2+}\) release it became evident that IP\(_3\) released Ca\(^{2+}\) with higher efficiency than either cADP-ribose or NAADP. After a supermaximal concentration of IP\(_3\) (5.0–10 μM), the addition of 50 μM cADP-ribose or 50 nM NAADP did not result in a further Ca\(^{2+}\) release (Fig. 4C and D). However, the addition of supermaximal doses of IP\(_3\) subsequently to supermaximal effective concentrations of either cADP-ribose or NAADP further decreased the [Ca\(^{2+}\)]\(_{l_{\text{iono}}}\) (Fig. 4, A and B). Therefore, although IP\(_3\) is additive to cADP-ribose and NAADP, these two substances are not additive to each other. As shown in Fig. 4E application of NAADP subsequently to cADP-ribose did not increase Ca\(^{2+}\) release further. This led us to conclude that there are at least two types of Ca\(^{2+}\) stores in pancreatic acinar cells: one type with IP\(_3\) receptors and another one with all three IP\(_3\), cADP-ribose, and NAADP receptors.
Heparin, an IP₃ receptor antagonist, should discriminate between both IP₃- and cADP-ribose-induced Ca²⁺ release. As shown in Fig. 5, IP₃-induced Ca²⁺ release could be completely inhibited by 100 μg/ml heparin (n = 4 experiments, 22 cells; Fig. 5, A–C), whereas cADP-ribose-induced release of Ca²⁺ was unaffected by heparin (n = 3 experiments, 13 cells; Fig. 5, B and C).

**Effect of cADP-ribose and NAADP at the Subcellular Level**—As was shown in the second last paragraph, Ca²⁺ stores highly sensitive to IP₃ are located in the apical cell region, whereas the hormone-induced Ca²⁺ wave starts. We then analyzed discrete regions in a cell with respect to their Ca²⁺ releasing properties in response to cADP-ribose and NAADP. We found that release of Ca²⁺ in response to the application of 50 μM cADP-ribose and 50 nM NAADP occurred only from basolateral Ca²⁺ stores (Fig. 6, A and B). In a series of 10 similar experiments with 37 cells for cADP-ribose and 4 experiments with 8 cells for NAADP, we never observed any Ca²⁺ release from stores at the apical side of the cell. We therefore assume that the distribution of Ca²⁺ stores that are highly sensitive to IP₃ in the apical cell pole and which respond to cADP-ribose and NAADP in the basolateral cell area could explain the start and propagation of Ca²⁺ waves from the apical to the basolateral cell side.

**DISCUSSION**

Ca²⁺ stores play an important role in the regulation of free [Ca²⁺], a trigger for different cellular events. In pancreatic acinar cells an increase in [Ca²⁺], in the apical cell pole is necessary for fusion of zymogen granules with the apical plasma membrane and for opening of Cl⁻ channels, which leads to exocytosis of proteins and secretion of Cl⁻, respectively. An increase in [Ca²⁺], at the basolateral cell side leads to opening of cation channels, which results in Na⁻ influx and in sustained NaCl and water secretion from the cell at the apical side (32). Opening and closing of these Ca²⁺-sensitive channels is coordinated spatially and temporally by the Ca²⁺ signal itself (push-pull model (2)). Hypotheses concerning this phenomenon predict different Ca²⁺ stores (i.e. different affini-


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FIG. 7. Model for the localization of IP\(_3\), NAADP-, and cADP-ribose-sensitive Ca\(^{2+}\) stores in pancreatic acinar cells. Hormone-induced activation of phospholipase C (PLC) results in production of IP\(_3\). This IP\(_3\) diffuses from the basolateral cell pole to the apical cell side where Ca\(^{2+}\) pools most sensitive to IP\(_3\) are located (trigger zone). Ca\(^{2+}\) that is released from this trigger zone diffuses to the opposite cell side (Ca\(^{2+}\) wave). The speed of this wave can be accelerated by different processes. During continuous activation of phospholipase C the [IP\(_3\)] increases and releases Ca\(^{2+}\) also from lower sensitive Ca\(^{2+}\) stores on the way to the basolateral cell pole. Ca\(^{2+}\)-induced Ca\(^{2+}\) release (CICR) occurs when the wave reaches more basolaterally located Ca\(^{2+}\) stores. The magnitude of Ca\(^{2+}\)-induced Ca\(^{2+}\) release and with this also the speed of the Ca\(^{2+}\) wave may be modulated by cADP-ribose and/or NAADP released into the cytoplasm probably during hormonal stimulation. PIP\(_2\), phosphatidylinositol bisphosphate; G, G protein; DAG, diacylglycerol.

In the present study we have characterized different Ca\(^{2+}\) pools at the apical and basolateral cell side of permeabilized pancreatic acinar cells from mouse. Permeabilization of the cells was necessary for two reasons: first, cytoplasmic mag-fura-2 dye had to be removed from the cytoplasm; and second, the cytoplasmic region of the cell had to be accessible for membrane-impermeant compounds such as IP\(_3\), cADP-ribose, NAADP, and heparin. As indicated in Fig. 1 and also suggested by others (34), the permeabilization procedure did not change the distribution of the major compartments of the cell (nucleus, mitochondria, zymogen granules) dramatically. However it is likely to assume that permeabilization favors the loss of some smaller cytoplasmic constituents that are also necessary for a normal Ca\(^{2+}\) release process. This may be why we had to use 50 \(\mu\)M cADP-ribose, which is a high concentration compared with other systems (31) to obtain maximal effects. We cannot exclude the possibility, however, that the integrity of the endoplasmic reticulum had been disturbed by permeabilization. Recently it has been suggested that in pancreatic acinar cells the endoplasmic reticulum is continuous (35, 36). This would make determination of discrete Ca\(^{2+}\)-releasing structures difficult if not impossible because Ca\(^{2+}\) release at any site from a tubular system with a continuous space should finally empty it. This was obviously not the case in our experiments. But even if the endoplasmic reticulum should have been disconnected by permeabilization our main conclusion that in different cell regions the distribution of IP\(_3\), cADP-ribose, and NAADP receptors is different, remains unaffected.

We conclude from our studies that Ca\(^{2+}\) pools highly sensitive for IP\(_3\) but not for cADP-ribose or NAADP are located in the apical cell pole, whereas Ca\(^{2+}\) pools less sensitive for IP\(_3\) and sensitive for cADP-ribose and NAADP are located at the basolateral cell side. In an earlier study on the distribution of IP\(_3\) receptors it had been concluded that Ca\(^{2+}\) release in response to IP\(_3\) is spatially homogeneous in rat pancreatic acinar cells (34). Here we used mouse cells that differ from rat pancreatic acinar cells in some features concerning Ca\(^{2+}\) homoeostasis (37).

Because of limited spatial resolution we could not identify the structural source for Ca\(^{2+}\) release in the apical cell pool. Gerasimenko et al. (38) have demonstrated that isolated zymogen granules can release Ca\(^{2+}\) in response to both IP\(_3\) and cADP-ribose. This result had been questioned by Yule et al. (13), who could not find IP\(_3\) receptors in highly purified granular preparations. Because we could not detect any cADP-ribose-induced Ca\(^{2+}\) release from the apical cell side where zymogen granules are located, the present study does not support the observation of Gerasimenko et al. on cADP-ribose-induced Ca\(^{2+}\) release from zymogen granules (38). Spatial resolution of our technique does not allow a decision as to whether IP\(_3\), cADP-ribose, and NAADP, which are all effective in the basolateral cell side, release Ca\(^{2+}\) from one structural pool or if there are different pools. However, our data, which show that the actions of NAADP and cADP-ribose are not additive, argue for the hypotheses that receptors for both substances are present in the same basolaterally located pools. The finding that IP\(_3\) alone could induce maximal Ca\(^{2+}\) release, which could not be enhanced by either cADP-ribose or NAADP, suggests that in addition to Ca\(^{2+}\) pools only sensitive to IP\(_3\) in the apical pool, basolateral located pools contain all three types of receptor, for IP\(_3\), cADP-ribose, and NAADP.

Previous observations that hormone-induced Ca\(^{2+}\) release starts in the apical cell pole (2, 3, 5, 14, 29, 30) and is followed by spreading of the Ca\(^{2+}\) signal to the basolateral cell pole via Ca\(^{2+}\)-induced Ca\(^{2+}\) release can now be interpreted more substantially by our present data. An arrangement of IP\(_3\)- and cADP-ribose- and/or NAADP-sensitive Ca\(^{2+}\) pools in sequence should explain initiation of the apical Ca\(^{2+}\) signal with low [IP\(_3\)] and the spreading of the Ca\(^{2+}\) wave to the basolateral cell side when [IP\(_3\)] increases and cADP-ribose and/or NAADP is produced probably during hormonal stimulation. IP\(_3\) receptors act as a trigger for the apical Ca\(^{2+}\) signal, whereas cADP-ribose and NAADP receptors are amplifiers in the Ca\(^{2+}\) wave propagation. This view is in part similar to the conclusion of Cancela et al. (39), who claim that acetylcholine initially activates IP\(_3\) receptors and that this signal is modified by cADP-ribose and NAADP. On the other hand these authors conclude from their data that the initial Ca\(^{2+}\) release in the apical cell pole in response to another secretagog, cholecystokinin, is mediated by NAADP and not by IP\(_3\) (39). Even though no data are available to show hormone-induced production of NAADP in pancreatic acinar cells so far, our present study does not support the idea that NAADP receptors are present in the apical cell pole.

In summary we speculate that IP\(_3\) receptors in Ca\(^{2+}\) stores with decreasing sensitivities for IP\(_3\) toward the basolateral cell side lead to a Ca\(^{2+}\) response from pool to pool in form of a Ca\(^{2+}\) wave in the direction opposite that of the IP\(_3\) gradient. This Ca\(^{2+}\) wave is modulated by cADP-ribose- and NAADP-induced Ca\(^{2+}\) release. Because both IP\(_3\) receptors and cADP-ribose receptors are regulated by [Ca\(^{2+}\)]\(_{\text{cyt}}\) (41), local changes in [Ca\(^{2+}\)]\(_{\text{cyt}}\) in both the stores and in the cytoplasm because of IP\(_3\), NAADP- and cADP-ribose-induced Ca\(^{2+}\) release should also contribute...
to the regulation of Ca$^{2+}$ signals in pancreatic acinar cells.

The knowledge of different Ca$^{2+}$ pools in the cell opens the question of whether activation of capacitative Ca$^{2+}$ entry (42) is activated by one (solely IP$_3$-sensitive) or both (IP$_3$-sensitive as well as IP$_3$-Ca$^{2+}$-ADP-ribose- and NAADP-sensitive) types of stores. Further studies on the characteristics of different Ca$^{2+}$ pools in the cell could help to identify and to characterize the Ca$^{2+}$ pool that generates the message to activate capacitative Ca$^{2+}$ entry.

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