Evidence That Zymogen Granules Are Not a Physiologically Relevant Calcium Pool

DEFINING THE DISTRIBUTION OF INOSITOL 1,4,5-TRISPHOSPHATE RECEPTORS IN PANCREATIC ACINAR CELLS*

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A key event leading to exocytosis of pancreatic acinar cell zymogen granules is the inositol 1,4,5-trisphosphate (InsP₃)-mediated release of Ca²⁺ from intracellular stores. Studies using digital imaging microscopy and laser-scanning confocal microscopy have indicated that the initial release of Ca²⁺ is localized to the apical region of the acinar cell, an area of the cell dominated by secretory granules. Moreover, a recent study has shown that InsP₃ is capable of releasing Ca²⁺ from a preparation enriched in secretory granules (Gerasimenko, O., Gerasimenko, J., Belan, P., and Petersen, O. H. (1996) Cell 84, 473–480). In the present study, we have investigated the possibility that zymogen granules express InsP₃ receptors and are thus Ca²⁺ release sites. Immunofluorescence staining, obtained with antisera specific to types I, II, or III InsP₃ receptors and analyzed by confocal fluorescence microscopy revealed that all InsP₃ receptor types were present in acinar cells. The type II receptor localized exclusively to an area close to or at the luminal plasma membrane. While types I and III InsP₃ receptors displayed a similar luminal distribution, these receptors were also present at low levels in nuclei. The localization of InsP₃ receptor was in marked contrast to the distribution of amylase, a zymogen granule content protein. In a zymogen granule fraction prepared in an identical manner to the aforementioned report demonstrating InsP₃-induced Ca²⁺ release, immunoblotting demonstrated the presence of types I, II, and III InsP₃ receptors. Ca²⁺ release from this preparation in response to InsP₃, but not thapsigargin, could also be demonstrated. In contrast, when the zymogen granules were further purified on a Percoll gradient, InsP₃ receptors were undetectable, and InsP₃ failed to release Ca²⁺. Transmission electron microscopy performed on both preparations showed that the Percoll-purified granule preparation consisted of essentially pure zymogen granules, whereas the granules prepared without this step were enriched in granules but also contained significant contamination by mitochondria, endoplasmic reticulum, and nuclei. It is concluded that zymogen granules do not express InsP₃ receptors and thus are not a site of Ca²⁺ release relevant to the secretory process in the pancreatic acinar cell.

The pancreatic acinar cell is a polarized epithelial cell whose major function is to synthesize digestive enzymes, package them into secretory granules, and then release the granule contents by regulated exocytosis (1). A key event underlying secretagogue-stimulated digestive enzyme secretion is the inositol 1,4,5-trisphosphate (InsP₃)-mediated release of Ca²⁺ from intracellular storage sites. Recently, utilizing digital imaging and confocal microscopy, it has become established that stimulation with the gut hormone cholecystokinin or the neurotransmitter acetylcholine results in a distinct spatial pattern of Ca²⁺ release, such that the release of Ca²⁺ is paradoxically initially observed at the luminal pole of the acinus and proceeds in a “wave” toward the basolateral (presumably receptor bearing) pole of the cell (2–4). At physiological concentrations of secretagogue, it has been shown that Ca²⁺ release can actually be confined to this luminal region without spreading throughout the whole cell (5). In addition to the overwhelming evidence for a role of InsP₃ to mediate release of Ca²⁺, studies have also indicated that release can be initiated through a process of Ca²⁺-induced Ca²⁺ release (3, 5, 8). The expression of ryanodine receptors has, however, not been physically demonstrated in the pancreatic acinar cell. It has been proposed that this pattern of Ca²⁺ release has significance for secretion since zymogen granules are confined to the luminal pole of the acinar cell and thus would provide a mechanism that increases [Ca²⁺]i directly at the site of its projected action.

Although original cell fractionation studies reported the InsP₃-induced Ca²⁺ release site to be located in the endoplasmic reticulum (6, 7), a recent report has suggested that zymogen granules themselves fulfill this role (8). This latter conclusion was primarily based on the observation that InsP₃ was capable of releasing Ca²⁺ from a preparation enriched in granules (8). This proposal, however, remains controversial since the preparation was not extensively characterized, thus raising the possibility that it contained subcellular contaminants (8). In addition, immunolocalization of type III InsP₃ receptors in pancreatic acinar cells indicates that although receptors were indeed expressed in the luminal region of the acinar cell, these receptors do not appear to co-localize with a known zymogen granule protein (secretory carrier membrane protein, SCAMP).

The abbreviations used are: InsP₃, inositol 1,4,5-trisphosphate; MOPS, 4-morpholinepropanesulfonic acid; MES, 4-morpholineethanesulfonic acid.

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In contrast to SCAMP, the type III receptor appeared to be distributed on structures very close to the luminal membrane. These data do not, however, exclude the possibility that an InsP$_3$ receptor other than the type III subtype is present on zymogen granules. Recently, the expression of InsP$_3$ receptors on granules from other secretory cells has also been questioned since it appears that an earlier report of type III InsP$_3$ receptors on secretory granules isolated from pancreatic $\beta$ cells (10) can now be explained by nonspecific binding of the antibody to insulin (11).

In the present study, we have investigated the possibility that InsP$_3$ receptors are expressed on zymogen granules using two preparations, the first prepared by simple centrifugation and identical to that used to previously demonstrate InsP$_3$-induced release (8) and the second involving purification on a Percoll gradient by a protocol that has been used extensively to induce release (8) and the second involving purification on a Percoll gradient (15). Briefly, pancreata were excised from male rats and rinsed in an ice-cold buffer containing 10 mM Hepes, 150 mM NaCl, 5 mM KCl, 1 mM MgCl$_2$, 1 mM CaCl$_2$, 5 mM glucose, 1 mM phenylmethylsulfonyl fluoride, and 0.5% bovine serum albumin (fraction V) from ICN Immunobiologicals (Lisle, IL); ECL detection system from Amersham Corp. (Arlington Heights, IL); isoinitol 1,4,5-trisphosphate, and all other materials were obtained from Sigma or Bio-Rad.

Immunochemistry—Pancreatic lobules were prepared and frozen in Tissue-Tek embedding medium (Miles, Elkhart, IN) with isopentane cooled in liquid nitrogen. Cryostat sections (5–10 m thick) were placed on gelatin-coated slides, air dried, and then fixed in methanol at −20 °C for 10 min prior to immunofluorescence staining. Procedures for immunofluorescence localization of InsP$_3$ receptor isoforms followed methods previously described in detail (15, 20). Immunofluorescence staining of amylase was performed in an identical manner to that previously published (15). Specificity of immunofluorescence was determined by preincubating primary antisera at 4 °C overnight with 10 μg/ml of each of the two preparations of granules were fixed overnight at 4 °C in a mixture of 2.5% glutaraldehyde and 2% paraformaldehyde in 0.13 M sodium cacodylate buffer (pH 7.4). Subsequently, granule fractions were rinsed in cacodylate buffer, post-fixed for 1 h with 1% OsO$_4$ in 0.13 M cacodylate buffer (pH 7.4), dehydrated in ethanol, and embedded in Spurr’s embedding resin (Electron Microscopy Sciences, Fort Washington, PA). Ultrathin sections (60–80 nm thick) were prepared with a Reichert-Jung Ultracut E ultramicrotome, stained with uranyl acetate and lead citrate, and viewed and photographed with a Philips CM100 electron microscope. Digitized images were processed using Adobe™ Photoshop 3.0 software.

RESULTS AND DISCUSSION

InsP$_3$ Receptors Are Expressed at the Secretory Pole of Acinar Cells—The localization of type III receptors in the secretory "trigger zone" of acinar cells has been reported (9); however, the expression and possible localization of types I and II receptors in acinar cells are unknown. Thus the presence and subcellular distribution of each of the InsP$_3$ receptors were determined in cryostat sections of fresh frozen pancreatic lobules (23) fixed in methanol followed by detection using an fluorescein isothiocyanate-labeled secondary antibody and laser scanning confocal microscopy. Immunofluorescence staining was evident in acinar cells with the antibodies APCT-1, -2, and -3 or TL-3 for the specific detection of types I, II, and III InsP$_3$ receptors, respectively. Immunoreactivity was visualized using peroxidase-conjugated secondary antibodies followed by detection using the ECL system. In preliminary experiments, the dilution of antibody was adjusted so that equal amounts of purified types I, II, or III receptor standards produced bands of approximately equal intensity as described previously (19).

Electron Microscopy—Immediately after their preparation, partially purified and purified zymogen granules were fixed overnight at 4 °C in a mixture of 2.5% glutaraldehyde and 2% paraformaldehyde in 0.13 M sodium cacodylate buffer (pH 7.4). Subsequently, granule fractions were rinsed in cacodylate buffer, post-fixed for 1 h with 1% OsO$_4$ in 0.13 M cacodylate buffer (pH 7.4), dehydrated in ethanol, and embedded in Spurr’s embedding resin (Electron Microscopy Sciences, Fort Washington, PA). Ultrathin sections (60–80 nm thick) were prepared with a Reichert-Jung Ultracut E ultramicrotome, stained with uranyl acetate and lead citrate, and viewed and photographed with a Philips CM100 electron microscope. Digitized images were processed using Adobe™ Photoshop 3.0 software.

Granule Preparation—Secretory granules were prepared from rat pancreases by one of two procedures, either a protocol essentially identical to Gerasimenko et al. (8) or by further purifying this preparation by centrifugation on a Percoll gradient (15). Briefly, pancreata were excised from male rats and rinsed in an ice-cold buffer containing 10 mM MOPS-Tris (pH 6.8), 0.1 mM MgSO$_4$, 3 mM ATP, 250 mM sucrose, 0.5 mM Mg$_2$ATP, 3 μM soybean trypsin inhibitor, 1 mg/ml bovine serum albumin, 0.5 mM soybean trypsin inhibitor, and 100 μM Fura-2-free acid), which had been treated with Chelex-100 resin (Bio-Rad) to remove excess dialyzed cations. 25 μl aliquots of granules were placed in a small vial, vortexed on the stage of an ATTOFLOR digital imaging system. Addition of the chamber were made either through a micropipette connected to a pressure ejection positioned in the chamber or directly into the chamber with a pipette. InsP$_3$ and all test agents were reconstituted into an identical buffer but also containing 25 μM sulfohydroxide (excitation 580 nm, emission 630 nm) to confirm the pressure injection and to estimate by dye dilution the amount of agent applied to the bath. Ca$_{2+}$ measurements were performed as described previously (21, 22) with increases in Ca$_{2+}$ described by an increase in 340/380 nm emission ratio.

Immunoblotting—Whole cell lysates from pancreases, preparations of pancreatic acinar cells, together with the two preparations of granules were subjected to electrophoresis and subsequently transferred to nitrocellulose. The blots were incubated with either APCT-1, -2, -3, or TL-3 for the specific detection of types I, II, and III InsP$_3$ receptors, respectively. Immunoreactivity was visualized using peroxidase-conjugated secondary antibodies followed by detection using the ECL system. In preliminary experiments, the dilution of antibody was adjusted so that equal amounts of purified types I, II, or III receptor standards produced bands of approximately equal intensity as described previously (19).

Calcium Release Measurements—Ca$_{2+}$ release was measured from each preparation by procedures similar to those used previously for the measurement of Ca$_{2+}$ release from permeabilized cells (21, 22). The granule pellet was resuspended at a protein concentration of 0.25–1 mg/ml in release media (135 mM KCl, 10 mM MOPS-Tris, 1 mM MgSO$_4$, 3 mM ATP, 25 mM creatinine phosphate, 25 units/ml creatinine phosphokinase, 1 mg/ml bovine serum albumin, 0.5 mg/ml soybean trypsin inhibitor, and 100 μM Fura-2-free acid), which had been treated with Chelex-100 resin (Bio-Rad) to remove excess dialyzed cations. 25 μl aliquots of granules were placed in a small vial, vortexed on the stage of an ATTOFLOR digital imaging system. Addition of the chamber were made either through a micropipette connected to a pressure ejection positioned in the chamber or directly into the chamber with a pipette. InsP$_3$ and all test agents were reconstituted into an identical buffer but also containing 25 μM sulfohydroxide (excitation 580 nm, emission 630 nm) to confirm the pressure injection and to estimate by dye dilution the amount of agent applied to the bath. Ca$_{2+}$ measurements were performed as described previously (21, 22) with increases in Ca$_{2+}$ described by an increase in 340/380 nm emission ratio.

InsP$_3$ Receptors Are Expressed at the Secretory Pole of Acinar Cells—The localization of type III receptors in the secretory "trigger zone" of acinar cells has been reported (9); however, the expression and possible localization of types I and II receptors in acinar cells are unknown. Thus the presence and subcellular distribution of each of the InsP$_3$ receptors were determined in cryostat sections of fresh frozen pancreatic lobules (23) fixed in methanol followed by detection using an fluorescein isothiocyanate-labeled secondary antibody and laser scanning confocal microscopy. Immunofluorescence staining was evident in acinar cells with the antibodies APCT-1, -2, and -3 or TL-3 designed to recognize types I, II, and III InsP$_3$ receptor subtypes, respectively. These data indicate that all three InsP$_3$ receptor subtypes are expressed in this tissue (Fig. 1, A-C, for polyclonal antibodies). Each of the receptors was localized most strikingly to regions close to or at the luminal membrane in the apical pole of the acinar cell (arrows in Fig. 1, A-C). This is a similar localization to that reported previously for the type III receptor (9). While type II receptors were exclusively distributed to the luminal region in the acinar cell, types I and III InsP$_3$ receptors appeared to be weakly expressed in nuclei (arrowheads in Fig. 1). For comparison, sections were stained with amy-1, a polyclonal antisera directed against the secretory protein amylase, known to specifically stain zymogen granules and acinar pancreatic cells. This antisera co-localizes with other zymogen granule proteins such as Rab3D and GP-2 (15). Figs. 1D and 1C (at high power) illustrate the clear difference between anti-InsP$_3$ receptor antibody staining associated with luminal membrane profiles and zymogen granule staining evident with the amylase antisera.

The fluorescence associated with the luminal distribution of type I InsP$_3$ receptor appeared subtly different from that asso-
associated with types II and III receptors. Whereas the types II and III receptor localizations in close proximity to the luminal membrane often appeared punctate (Fig. 1, B and C, and Fig. 2 for high magnification), type I receptor immunofluorescence appeared to be tightly and continuously localized to luminal membrane profiles (compare Figs. 1A, B, and C and see Fig. 2A for high magnification). For comparison, the granule localization of amylase is shown in Fig. 2C. The resolution obtainable with immunofluorescence does not allow a definitive identification of cellular structures associated with fluorescence staining. The staining pattern observed for types I and III receptors may simply reflect “clustering” of receptor expression at discrete sites at the luminal membrane. Alternatively, the punctate nature of the staining, consistently observed with types II and III antibodies is suggestive of a sub-luminal vesicular distribution. In contrast, the continuous staining of type I receptor is more consistent with localization of this receptor to the luminal plasma membrane (compare Fig. 2, A and B).

Staining with each polyclonal antisera was completely competed by overnight incubation of antibody at 4 °C with 10 μg/ml of the respective peptide used to raise the antibody (Fig. 3A, shown for APCT-2 incubated with APCT-2 peptide; Fig. 3B shows the corresponding Nomarski image for this field). In preliminary experiments, using high concentrations of APCT-3, diffuse staining was sometimes evident in the granular region (24). This staining is in all probability nonspecific since low level staining of the granular area was also observed after competition with APCT-3 peptide. Furthermore, monoclonal antibody TL-3, directed against the type III receptor also localized to the luminal membrane area and did not, even at high concentrations, localize to zymogen granules (Fig. 2B).

Although InsP₃ receptors were found not to be obviously associated with secretory granules in the acinar cells, the luminal distribution of InsP₃ receptor subtypes appears entirely consistent with the spatial pattern of Ca²⁺ signaling observed in these cells (2–4). That the Ca²⁺ signal is initially observed in

Fig. 1. The distribution of InsP₃ receptors in pancreatic acinar cells. Immunofluorescence localizations in pancreatic lobules of InsP₃ receptors by confocal microscopy with antibodies APCT-1, -2, and -3 are shown in panels A, B, and C, respectively, and localization of amylase with polyclonal antibody amy-1 is shown in panel D. Types I, II, and III InsP₃ receptors were predominately localized to the luminal region of the acinar cells in close association with the luminal membrane (arrows in panels A-C). The types I and III receptors also appeared to be present in the nuclei (arrowheads in panels A and C). In contrast, amylase is clearly localized to zymogen granules (D). Luminal profiles are indicated by arrows. Scale bar = 10 μm.

Fig. 2. Localization of types I and III receptors. The luminal region of pancreatic acini is shown at high magnification. A, confocal fluorescence staining with APCT-1 shows localization of the type I receptor; the receptor is distributed in a continuous fashion, consistent with a luminal plasma membrane localization. B, staining with anti-type III monoclonal antibody TL-3 shows punctate staining, suggesting localization to a population of sub-luminal vesicles. In contrast, panel C shows amy-1 staining of zymogen granules. Scale bar = 10 μm.
the secretory pole of the cell and can be confined to this region even though the production of InsP₃ presumably occurs in a distal region may reflect the abundance of InsP₃ receptors in this area. The apparent presence of InsP₃ receptors in pancreatic acinar nuclei is also consistent with reports of Ca²⁺ signaling events in other non-excitable cell-types (25).

Are InsP₃ Receptors Expressed on Zymogen Granules?—Since the above morphological data indicate that all InsP₃ receptors are expressed in a region of the acinar cell that is intimately involved in the secretory process, the possibility that zymogen granules themselves are the site of this expression was further investigated. Immunoblots were performed on granule preparations together with lysates generated from whole pancreas and pancreatic acinar cells. Consistent with the immunofluorescence data, each of the InsP₃ receptor antibodies recognized proteins of molecular weight of ~260 kDa in whole pancreatic lysate together with an acinar cell lysate (Fig. 4). In addition, types I, II, and III InsP₃ receptors could also be detected in granule fractions prepared by simple centrifugation in an identical manner to the study demonstrating release of Ca²⁺ from zymogen granules (8). The association of InsP₃ receptors with zymogen granules is not consistent with either immunofluorescence data reported in this study or data reported by Nathanson et al. (9), who demonstrated that type III receptor immunofluorescence did not co-localize with a known secretory granule protein.

To determine whether the InsP₃ receptors detected in this granule preparation reflected the presence of receptors on zymogen granules or was due to the presence of contaminants, zymogen granule fractions were further purified by centrifugation on a Percoll gradient. This procedure generates several distinct fractions, one of which has been shown to be an essentially pure zymogen granule preparation (12, 13). In contrast to granules prepared by simple centrifugation, InsP₃ receptor immunoreactivity could not be detected in the purified granule preparation (Fig. 4). Furthermore, when the pure granules were lysed by treatment with nigericin resulting in a preparation of granule membranes, a procedure which has been shown to enrich granule membrane proteins by up to 50-fold (15, 16), still no signal was observed with the InsP₃-specific antibodies (data not shown).

A possibility exists that InsP₃ receptor antigenicity was in some way altered by purification of granules on a Percoll gradient. This appears somewhat remote based on the widespread use of Percoll to prepare functionally intact cells or subcellular fractions (26–28). Indeed, this preparation of granules has been used in a variety of studies demonstrating the presence of integral zymogen granule membrane proteins by Western blotting techniques (14, 15), the existence of kinase activity on the granules (13), and for the demonstration of a granule ionic conductance (17, 18). Taken together, immunofluorescence and immunoblotting data indicate that InsP₃ receptors are unlikely to be present on zymogen granule membranes prepared from rat pancreatic acinar cells.

Ca²⁺ Release Measurements in Granule Preparations—The above data do not exclude the possibility that zymogen granules express a novel InsP₃ receptor subtype not recognized by the battery of antisera used or that InsP₃ receptors are present on granule membranes in insufficient quantity to be resolved by immunofluorescence and immunoblotting. Experiments were therefore performed to determine if zymogen granules release Ca²⁺ in response to InsP₃. Granules prepared by simple centrifugation (8) consistently released Ca²⁺ in response to InsP₃ as shown by an increase of Ca²⁺ in the medium bathing the preparation (Fig. 5A) (12 experiments, 3 preparations). Interestingly, the release of Ca²⁺ in response to InsP₃ was always sustained, an observation which may be related to the scarcity of InsP₃ metabolizing enzymes or relatively poor Ca²⁺ reuptake back into the releasable pool. Addition of an equal volume of the solution used to dissolve the InsP₃ never increased Ca²⁺, indicating that the increase was not the result of contaminating Ca²⁺ in this solution. Addition of 0.1 μM thapsigargin to this preparation did not result in any release of Ca²⁺ (Fig. 5A; 5 experiments, 3 preparations). However, addition of 0.1 μM thapsigargin to a pancreatic homogenate or a suspension of the 1000 × g pellet discarded in the initial preparation of granules resulted in an increase in Ca²⁺ released in the media, indicating that thapsigargin is capable of releasing Ca²⁺ from these subcellular organelle preparations (1000 × g pellet is shown in Fig. 5C).

These data are consistent with the report by Gerasimenko et al. (8), who showed InsP₃-induced release of Ca²⁺ from both a suspension of organelles and from what was reported to be a single isolated granule. In contrast, when InsP₃ was added to preparations of granules that had been further purified by Percoll centrifugation, no release of Ca²⁺ was observed (Fig. 5B) (8 experiments, 3 preparations). Addition of ionomycin or nigericin resulted in an increase in medium Ca²⁺, indicating that the granules do indeed store Ca²⁺. Addition of thapsigargin did not release Ca²⁺ from this preparation (data not shown). To demonstrate that Percoll itself does not interfere with the binding of InsP₃ to its receptors and the subsequent release process, the granules prepared by simple centrifugation were incubated for 90 min in the release buffer containing 50% Percoll followed by the measurement of Ca²⁺ release after addition of InsP₃. Ca²⁺ release could always be elicited by InsP₃ in these preparations (Fig. 5D) (4 experiments), although the Ca²⁺ release in Percoll-containing samples when compared with paired non-Percoll-treated controls was slower to peak, and the magnitude of the release was reduced by 41 ± 5% (Fig. 5D; compare with 5A). These data show that Percoll, even when included in the Ca²⁺ mobilization buffer, does not interfere with the Ca²⁺ release process to an extent that InsP₃-induced

**Fig. 3. Specificity of immunofluorescence staining.** Specific immunofluorescence staining for type II receptor is abolished by competition with APCT-2 peptide (A). The corresponding Nomarski image is shown in panel B. Arrows indicate luminal membrane, and arrowheads identify nuclei. The conventional epifluorescence data shown are from the same experiment shown in Fig. 1. Types I and III immunofluorescence (both luminal and nuclear) were abolished by incubation with their respective peptide. Scale bar = 20 μm.
release becomes refractory. In total, these data suggest that the source of InsP₃ releasable Ca²⁺ in granule fractions prepared by simple centrifugation is apparently lost when the granules are further purified by Percoll gradient centrifugation.

Granules Prepared by Simple Centrifugation Are Contaminated with Subcellular Organelles—Little obvious differences between the two preparations could be seen viewing the preparations at the light-level with phase contrast microscopy. Both preparations appeared to consist of small vesicular structures less than 1 μm in diameter. Therefore, to gain insight into the differences between granules prepared by both methods, the morphology of the preparations was examined using transmission electron microscopy. As described previously, the preparation produced by purification on a Percoll gradient is dominated, almost to the exclusion of any contamination by zymogen granules (Fig. 6A, and see Refs. 11 and 12). The only non-granular organelle (estimated at less than 1% of the preparation) contamination consisted of mitochondria. In stark contrast, although the preparation produced by simple centrifugation (8) was enriched in zymogen granules, significant contamination was observed in each ultrathin section (Fig. 6B). In addition to zymogen granules, mitochondria, rough endoplasmic reticulum, together with nuclei were readily recognizable in each section of granules prepared in this fashion.

Contamination of the granule preparation prepared by simple centrifugation with InsP₃ receptor-expressing organelles...
apparently underlies the release of Ca\(^{2+}\) from this preparation. However, the nature of the contaminant is presently unclear. Since Ca\(^{2+}\) release from either preparation could not be initiated by treatment with thapsigargin, the zymogen granules, and the site of InsP\(_3\) releasable Ca\(^{2+}\) isolated with the partially purified granules do not accumulate Ca\(^{2+}\) via a SERCA type Ca\(^{2+}\)-ATPase. In addition, it follows that a Ca\(^{2+}\) pool, which is undoubtedly present in pancreatic acinar cells that is thapsigargin releasable (29), has been removed from this preparation.

In conclusion, immunocytochemical data regarding InsP\(_3\) receptor distribution presented in this report is consistent with the view that the site of the physiologically important InsP\(_3\)-releasable Ca\(^{2+}\) pool is localized within the secretory “trigger zone” of the pancreatic acinar cell. This putative Ca\(^{2+}\) release/storage site appears not to be associated with zymogen granules but in all probability is situated in close association with the apical membrane.

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