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Cyclic AMP accelerates calcium waves in pancreatic acinar cells

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Abstract

Cytosolic Ca^{2+} (Ca_i^{2+}) flux within the pancreatic acinar cell is important both physiologically and pathologically. We examined the role of cAMP in shaping the apical-to-basal Ca^{2+} wave generated by the Ca^{2+}-activating agonist carbachol. We hypothesized that cAMP modulates intra-acinar Ca^{2+} channel opening by affecting either cAMP-dependent protein kinase (PKA) or exchange protein directly activated by cAMP (Epac). Isolated pancreatic acinar cells from rats were stimulated with carbachol (1 μM) with or without vasoactive intestinal polypeptide (VIP) or 8-bromo-cAMP (8-Br-cAMP), and then Ca_i^{2+} was monitored by confocal laser-scanning microscopy. The apical-to-basal carbachol (1 μM)-stimulated Ca^{2+} wave was 8.63 ± 0.68 μm/s; it increased to 19.66 ± 2.22 μm/s (*$P < 0.0005$) with VIP (100 nM), and similar increases were observed with 8-Br-cAMP (100 μM). The Ca^{2+} rise time after carbachol stimulation was reduced in both regions but to a greater degree in the basal. Lag time and maximal Ca^{2+} elevation were not significantly affected by cAMP. The effect of cAMP on Ca^{2+} waves also did not appear to depend on extracellular Ca^{2+}. However, the ryanodine receptor (RyR) inhibitor dantrolene (100 μM) reduced the cAMP-enhancement of wave speed. It was also reduced by the PKA inhibitor PKI (1 μM). 8-(4-chloro-phenylthio)-2′-*O*-Me-cAMP, a specific agonist of Epac, caused a similar increase as 8-Br-cAMP or VIP. These data suggest that cAMP accelerates the speed of the Ca^{2+} wave in pancreatic acinar cells. A likely target of this modulation is the RyR, and these effects are mediated independently by PKA and Epac pathways.

Keywords

calcium signaling; pancreatitis; zymogen activation; exocrine pancreas

In most living cells, cytosolic Ca^{2+} Ca_i^{2+} flux mediates multiple cellular responses (1). The spatial and temporal characteristics of the Ca^{2+} signal determines how that information is encoded. In the pancreatic acinar cell, the spatial components of the Ca^{2+} signal are important in both normal physiology and disease. A rise in apical Ca_i^{2+} mediates the apical exocytosis of pancreatic enzymes (27). This Ca^{2+} is released via the inositol 1,4,5-trisphosphate (InsP3) receptor (InsP3R), one of the two major intracellular Ca^{2+} channels. With increasing concentrations of Ca^{2+}-activating agonists (35), the Ca^{2+} signal propagates as a wave from the apical to the basal region, and this Ca^{2+} wave has been associated with fluid and electrolyte secretion (18). The pathological intra-acinar activation of zymogens, an early event in the pathogenesis of pancreatitis (24), is associated with the release of Ca^{2+}

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from basally distributed ryanodine receptors (RyR), the second major intracellular Ca^{2+} channel (14). Thus factors that regulate Ca_i^{2+} flux in acinar cells are relevant to disease. There is increasing recognition that cAMP has multiple points of interaction with the Ca^{2+} signal (4). In particular, both the InsP3R and RyR have sites for phosphorylation by cAMP-dependent protein kinase (PKA). They can also be modulated by a second major cAMP pathway, the exchange protein directly activated by cAMP (Epac; Ref. 3). In this study, we examined the effects of cAMP on the speed of the apical-to-basal Ca^{2+} wave generated by the muscarinic agonist carbachol using isolated pancreatic acini and confocal imaging of Ca_i^{2+} signals. Our findings suggest that cAMP accelerates Ca^{2+} wave speed in acinar cells by acting on the RyR through both PKA and Epac-dependent mechanisms.

METHODS

Preparation of pancreatic acini

Groups of pancreatic acinar cells were isolated as previously described (14) with minor modifications. Briefly, Sprague-Dawley rats weighing 50–100 g (Charles River Laboratories, Wilmington, MA) were euthanized by a protocol approved by the Animal Care and Use Committee. The pancreas was removed and minced for 5 min in buffer containing 20 mM HEPES (pH 7.4), 95 mM NaCl, 4.7 mM KCl, 0.6 mM $MgCl_2$, 1.3 mM $CaCl_2$, 10 mM glucose, and 2 mM glutamine, plus 1.0% BSA, 1× MEM nonessential amino acids (GIBCO/BRL), 200 units/ml type-4 colla-genase (Worthington, Freehold, NJ), and 1 mg/ml soybean trypsin inhibitor. The tissue was incubated for 30 min at 37°C with shaking (120 rpm). The digest was transferred to a 15 ml conical tube and washed several times with collagenase-free and BSA-free buffer. The cells were vigorously shaken and then filtered through a 300-μm mesh (Sefar American, Depew, NY) to separate the cells into smaller clusters.

Detection of cellular Ca^{2+} signals

Acinar cells were loaded with either the high-affinity (K_{Ca} = 345 nM) Ca^{2+}-sensing dye fluo-4/AM or the low-affinity variant fluo-5F/AM (K_{Ca} = 8 μM; both from Molecular Probes) with or without 8-Br-cAMP (100 μM) or the Epac agonist 8-(4-chloro-phenylthio) (pCPT)-2′-*O*-Me-cAMP (8-pCPT-cAMP; 100 μM). They were plated on acid-washed glass coverslips and then mounted on a perfusion chamber. Thereupon, they were pretreated with vasoactive intestinal polypeptide (VIP) or 8-Br-cAMP and then stimulated with the muscarinic agonist carbachol at the concentrations indicated. A Zeiss LSM510 laser scanning confocal microscope was used with a ×63 1.4-numerical aperture objective. The dye was excited at 488-nm wavelength, and emission signals of >515 nm were collected at frame speeds between 250 and 300 ms with full-screen scanning. Fluorescence from individual acinar cells as well as apical and basal subcellular regions was recorded.

Determination of Ca^{2+} wave speed and rise time

Apical and basal regions of interest in the acinar cell recordings were chosen by use of Zeiss LSM510 software, and mean fluorescence over time in each region was graphed. Ca^{2+} wave speed was calculated by dividing the distance along the long axis of the acinar cell by the time it took for the Ca^{2+} wave to travel from the apical to the basal region. “Rise time” for each Ca^{2+} wave was defined as the difference between the time at 20 and 80% of maximal fluorescence intensity generated by a best-fit trend line (11).

Immunoprecipitation and Western blot analysis

The RyR was immunoprecipitated as previously described (31). Briefly, acinar cell lysate (4 mg) was incubated with anti-RyR antibody (2.5 μl; kind gift of Dr. Andrew Marks) in 0.5 ml

of modified RIPA buffer [50 mM Tris·HCl (pH 7.4), 0.9% NaCl, 5.0 mM NaF, 1.0 mM Na_3VO_4, 1.0% Triton X-100, and protease inhibitors] overnight at 4°C. The samples were incubated with protein A-Sepharose beads (Amersham Pharmacia Biotech, Piscataway, NJ) at 4°C for 1 h, after which the beads were washed three times with RIPA buffer. Samples were resuspended in 15 μl of sample buffer, heated to 95°C, and size fractionated on 4–20% gradient polyacrylamide gel. Immunoblots were developed by use of anti-RyR (1:5,000 dilution; from Andrew R. Marks and Steve R. Reikon) or anti-phospho-RyR2 (P2809, kind gift of Dr. Andrew Marks; 1:10,000). The P2809 phosphoepitope-specific anti-RyR antibody is an affinity-purified polyclonal rabbit antibody custom made by Zymed Laboratories (San Francisco, CA) using the peptide CRTRRI-(pS)-QTSQ.

Statistics

Data represent means ± SE of at least three individual experiments with multiple cells from each experiment. Statistical significance was determined by Student's *t*-test analysis.

RESULTS

The Ca^{2+} activating agonist VIP increases the apical-to-basal Ca^{2+} wave speed generated by carbachol

Ca^{2+}-activating agonists cause an apical-to-basal Ca^{2+} wave in pancreatic acinar cells (18,29). Stimulation of isolated acinar cells with the muscarinic agonist carbachol (1 μM) resulted in an apical-to-basal Ca^{2+} wave of speed 8.63 ± 0.68 μm/s (n = 18 cells; Fig. 1). We used carbachol in our studies because, unlike other common agonists such as caerulein, it does not lead to elevations in cellular cAMP or activation of PKA, irrespective of concentration applied (26). To determine the effects of cAMP addition on Ca^{2+} wave speed, we pretreated acinar cells for 30 min with the cAMP agonist VIP (100 nM). VIP binding to its G protein-coupled receptor causes cAMP elevations by activating adenylate cyclase (39). The Ca^{2+} wave speed increased greater than twofold to 19.66 ± 2.22 μm/s (n = 22 cells; P < 0.0005). The rise in wave speed was entirely due to a reduction in time between start of the apical and basal Ca^{2+} rise. There was no difference in the size of the cells between control and VIP treatments in which the Ca^{2+} wave propagated (data not shown).

To confirm that the increase in wave speed was due to cAMP elevation, we used the cell permeant cAMP analog 8-Br-cAMP (100 μM; Fig. 2). Pretreatment enhanced carbachol-stimulated apical-to-basal Ca^{2+} wave speed to a similar extent as VIP (carbachol alone 11.01 ± 0.98 μm/s, n = 22 cells; with 8-Br-cAMP pretreatment 21.39 ± 3.04 μm/s, n = 19 cells; P < 0.01). We also examined the effects of cAMP on a range of carbachol concentrations (0.1–1,000 μM) and found that 8-Br-cAMP pretreatment significantly enhanced the speed of the apical-to-basal Ca^{2+} wave at each of the concentrations (Fig. 3). This shows that cAMP accelerates Ca^{2+} waves in pancreatic acinar cells.

We next looked at additional parameters in the Ca^{2+} transient generated by carbachol. They include rise time, lag time, and maximal Ca^{2+} rise (Fig. 4*A*). Consistent with our observation that cAMP pretreatment caused an increase in Ca^{2+} wave speed in pancreatic acinar cells, Ca^{2+} rise time was also reduced but to a greater degree in the basal region than the apical [reduction of 25.0% (P < 0.0005) and 44.8% (P < 0.00001) in apical and basal rise times, respectively; n = 16–21 cells in each group; Fig. 4*B*]. Of the two major intracellular Ca^{2+} channels, the InsP3R is thought to primarily mediate the latency of Ca^{2+} rise between the time of carbachol administration and first rise in Ca_i^{2+}, also known as the lag time. Thus, to determine whether InsP3Rs were modulated by the cAMP treatments, lag time was measured; no significant difference was observed with 8-Br-cAMP pretreatment (carbachol alone 12.33 ± 0.46 s, n = 31 cells; with 8-Br-cAMP pretreatment 11.23 ± 0.80 s, n = 31

cells; $P = 0.24$; Fig. 4*C*). These data suggest that our cAMP conditions do not modulate apical InsP3R triggering of Ca^{2+} release. We next examined whether cAMP affected the maximal Ca^{2+} elevation in the apical or basal regions. Because cytosolic Ca^{2+} levels are known to approach low micromolar concentrations in pancreatic acinar cells after agonist stimulation (16), we used the low-affinity Ca^{2+} dye fluo-5F (5 μM) to monitor Ca_i^{2+} flux. Importantly, by using this dye the baseline speed of the carbachol-stimulated Ca^{2+} wave and the degree of enhancement of wave speed by 8-Br-cAMP pretreatment were similar to those seen with fluo-4 (data not shown). There was a modest trend toward an increase in the maximal Ca^{2+} elevation in each region, but the changes were not statistically significant (Fig. 4*D*).

The effect of cAMP on Ca^{2+} waves does not depend on extracellular Ca^{2+}

The initial rise in Ca^{2+} in the pancreatic acinar after agonist stimulation is generated by the release of Ca^{2+} from intracellular stores (36). To confirm that the wave speed after carbachol stimulation is generated by intracellular Ca^{2+} release and to determine whether extracellular Ca^{2+} contributes to the cAMP enhancement of wave speed, we excluded any contribution of extracellular Ca^{2+} by switching the perfusion buffer to a Ca^{2+}-free media containing EGTA (2 mM) for 1 min prior to carbachol stimulation with or without 8-Br-cAMP pretreatment. Neither the baseline Ca^{2+} wave speed nor its enhancement with cAMP was changed by the Ca^{2+}-free/EGTA media (carbachol alone 10.56 ± 1.29 μm/s, $n = 23$ cells; with 8-Br-cAMP pretreatment 20.00 ± 2.77 μm/s, $n = 16$ cells; $P < 0.01$). These data imply that the cAMP-mediated enhancement of Ca^{2+} wave speed acts only on intracellular Ca^{2+} release in the pancreatic acinar cell.

RyR inhibition reduces cAMP enhancement of Ca^{2+} wave speed

To determine whether the cAMP enhancement of Ca^{2+} wave speed is conducted via the RyR, we pretreated cells with the RyR inhibitor dantrolene (100 μM; Fig. 5). Dantrolene abrogated the 8-Br-cAMP enhancement of wave speed and further reduced the wave speed to near control levels (with 8-Br-cAMP 18.30 ± 1.62 μm/s, $n = 20$ cells; plus dantrolene pretreatment 11.87 ± 0.76 μm/s, $n = 17$ cells; $P < 0.005$). This suggests that the RyR is a target of the cAMP enhancement of Ca^{2+} wave speed. To confirm that the RyR is phosphorylated during the cAMP treatments, phosphoblots of the RyR were performed (Fig. 6). Both 8-Br-cAMP (100 μM) and VIP (100 nM) caused RyR phosphorylation above control levels, although the 8-Br-cAMP band appeared more intense. The results demonstrate RyR phosphorylation in acinar cells by cAMP.

PKA inhibition reduces the cAMP-enhancement of Ca^{2+} wave speed, and an Epac agonist enhances Ca^{2+} wave speed

Two major downstream signaling pathways for cAMP are activation of cAMP dependent protein kinase (PKA) and exchange protein directly activated by cAMP (Epac). To determine whether PKA mediates the cAMP-enhancement of Ca^{2+} wave speed, we pretreated a subset of cells with the PKA inhibitor (PKI; 1 μM). This treatment reduced the effects of the cAMP agonist on wave speed by 66.5% ($n = 12$–27 cells in each group; $P < 0.00005$; Fig. 7). Next, to determine whether Epac might affect the cAMP-enhanced activation of Ca^{2+} wave speed, cells were pretreated with the Epac-specific agonist 8-pCPT-cAMP (100 μM; Fig. 8*A*; Ref. 12). Notably, no Epac antagonist is commercially available for use at this time. 8-pCPT-cAMP caused a similar degree of enhancement in the wave speed as equimolar concentrations of the cAMP analog 8-Br-cAMP (carbachol alone 10.72 ± 0.72 μm/s, $n = 32$ cells; with 8-Br-cAMP pretreatment 20.52 ± 1.25 μm/s, $n = 25$ cells; $P < 0.00000005$). To confirm that the 8-pCPT-cAMP effect on wave speed was not due to PKA activation, cells were cotreated with PKI (1 μM) and the Epac agonist. No reduction in 8-pCPT-cAMP-enhancement of Ca^{2+} wave speed was observed with PKI (with 8-pCPT-

cAMP 17.0 ± 2.40 μm/s, n = 9 cells; plus PKI 18.18 ± 2.23 μm/s, n = 12 cells; P = 0.72; Fig. 8*B*). This lack of effect with PKI confirms that the effects of 8-pCPT-cAMP are through Epac, not PKA. Taken together, these data suggest that both PKA and Epac are downstream targets of cAMP and independently contribute to the enhancement of Ca^{2+} wave speed.

DISCUSSION

In this study, we have shown that in pancreatic acinar cells cAMP enhances the speed of the apical-to-basal Ca^{2+} wave generated by carbachol. The speed increased when cells were pretreated with VIP to induce endogenous cAMP production through G protein-coupled adenylate cyclase or given cAMP exogenously by using 8-Br-cAMP. The mechanism of apical-to-basal Ca^{2+} wave formation in pancreatic acinar cells is dependent on release of Ca^{2+} from intracellular stores (36). This model is consistent with our finding that the Ca^{2+} wave was not affected by chelation of extracellular Ca^{2+}. The Ca^{2+} wave is known to initiate at the apical pole owing to the opening of InsP3Rs that are concentrated along a compartmentalized segment of apical endoplasmic reticulum (ER). Two prevailing models that focus on different ER Ca^{2+} channels have been proposed to explain the mechanism by which the Ca^{2+} wave propagates from the apical to the basal region. One suggests that in contrast to the apical pole, either a lower density of InsP3Rs or lower affinity InsP3Rs are distributed along the basal region and required to propagate the Ca^{2+} wave (15,19). As evidence for this, the release of InsP3 into the acinar cell cytosol by flash photolysis of high concentrations of caged InsP3R could induce apical-to-basal Ca^{2+} waves (15). A second model provides evidence that RyRs are responsible for Ca^{2+} wave propagation (22,29,34). Acinar cells express both InsP3Rs and RyRs (8,23). By immunofluorescence, InsP3Rs are primarily distributed along the apical pole of acinar cells, whereas RyRs are distributed throughout the basal region (8,14,23). Furthermore, release of InsP3 in the cytosol primarily increases Ca_i^{2+} in the apical region whereas the RyR agonist, cyclic adenosine diphosphate ribose (cADPR), increases Ca_i^{2+} basally (22). Propagation of Ca^{2+} waves is reduced by the RyR inhibitor ryanodine (29,34), and the cADPR inhibitor 8-amino-cADPR prevents the Ca^{2+} signal from spreading into the basal region (22). These latter studies suggest that InsP3Rs are critical to the initiation of the Ca^{2+} wave apically but that RyRs are primarily responsible for its propagation to the basal region.

There is no consensus on the effects of cAMP on intracellular Ca^{2+} signaling. In hepatocytes (2,10,17) and parotid acinar cells (5,40), for example, several studies suggest that cAMP positively modulates Ca^{2+} signals by phosphorylating InsP3Rs. In pancreatic acinar cells, studies show both stimulatory and inhibitory effects of cAMP on Ca^{2+} signaling (20). Two studies by the same group showed that pretreatment of cells with dibutyryl-cAMP and forskolin results in slowing Ca_i^{2+} rise upon carbachol stimulation or flash photolysis of caged InsP3 (9,33). These pretreatments were suggested to negatively modulate InsP3R sensitivity. The discrepancy between those results and our findings might be explained by the fact that in our system the lag time between application of agonist and initiation of apical Ca^{2+} signal did not change with VIP or 8-Br-cAMP treatments. This indicates that our cAMP conditions appear to modulate Ca^{2+} release from an InsP3R-insensitive Ca^{2+} pool. For this reason, and because RyRs have been implicated in the agonist-induced propagation of Ca^{2+} waves, we speculated that our observed increase in wave speed with cAMP was due to RyR activation. Indeed, the RyR inhibitor dantrolene blocked the acceleration of wave speed observed with 8-Br-cAMP. Most studies, with notable exceptions (37), assign cAMP an important role in the positive regulation of RyR opening (28). PKA is anchored to the RyR by scaffolding proteins such as A-kinase anchoring proteins (28). PKA-dependent phosphorylation of specific residues on RyR1 and RyR2 isoforms leads to an increased probability of channel opening and has been linked to pathology, including fatal inherited arrhythmias and heart failure (38). Independent of PKA, cAMP also directly interacts with

Epac, a guanine nucleotide exchange factor (3). Epac has been shown to modulate RyR in beta cells (13) as well as in cardiomyocytes (32). Our data suggest that PKA and Epac independently mediate cAMP enhancement of wave speed. The PKA inhibitor PKI reduced the cAMP-dependent enhancement of Ca^{2+} wave speed, whereas treatment with the Epac agonist 8-pCPT-cAMP in place of cAMP induced a similar degree of enhancement. The latter effect was PKA independent, since PKI did not reduce the Epac agonist-induced enhancement. These data imply the presence of redundant pathways for cAMP-induced enhancement of Ca^{2+} wave speed and thereby underscore the importance of cAMP interactions with Ca^{2+} signals. It will be of interest in future studies to determine whether PKA and Epac directly affect the RyR in pancreatic acinar cells.

The functional importance of acinar cell Ca^{2+} waves and specifically modulation by cAMP is not clear. Globalized apical-to-basal Ca^{2+} release in acinar cells is followed by the sequential apical-to-basal activation of Cl^- channels (18). Conditions that cause pancreatitis in vivo and pathological zymogen activation in vitro are associated with activation of high basal Ca^{2+} release from pancreatic acini (14). In a few studies, cAMP increased the sensitivity of Ca_i^{2+} to induce exocytosis (7,21). We and others have reported that cAMP sensitizes acinar cells to the effects of caerulein- and carba-chol-stimulated enzyme secretion as well as zymogen activation (6,25,30). Future studies are required to determine whether these functional enhancements with cAMP are directly related to changes in Ca^{2+} wave speed. It would be of particular interest from a disease-based perspective to determine whether one or a combination of cAMP pathways modulates basally localized RyR-Ca^{2+} release in acinar cells to potentially alter the risk of developing pathological zymogen activation and pancreatitis. In summary, cAMP increases the speed of the apical-to-basal Ca^{2+} wave in pancreatic acinar cells. Intracellular Ca^{2+} dynamics appear to be affected, and a likely target of modulation is the RyR Ca^{2+} channel. Finally, the effects of cAMP on Ca^{2+} wave speed are mediated by both PKA and Epac pathways.

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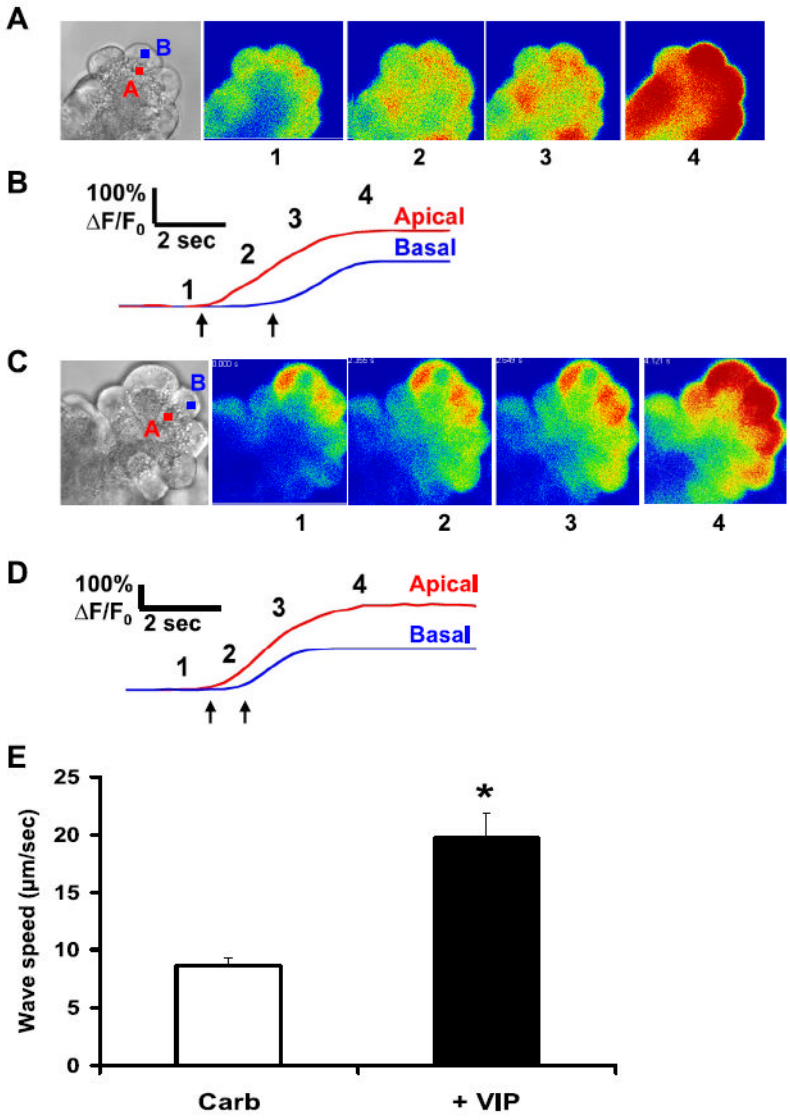

Fig. 1.

The cAMP agonist VIP enhances the speed of the apical-to-basal Ca^{2+} wave generated by carbachol. *A*: phase image of an isolated pancreatic acinar cell. Apical (A, red) and basal (B, blue) regions of interest are labeled. Cells were loaded with the Ca^{2+} indicator fluo-4 (5 μM). Upon stimulation with carbachol (1 μM), subsequent images show the initiation of the Ca^{2+} signal in the apical region followed by propagation to the basal region. *B*: each fluorescent image corresponds to a frame along a representative tracing of change in fluorescence over time for each region of interest. Left and right arrows show time of first Ca^{2+} rise in the apical and basal regions, respectively. *C* and *D*: cells were pretreated with VIP (100 nM) for 30 min prior to carbachol (Carb) stimulation. *E*: quantitation of difference in wave speed between cells receiving carbachol alone (n = 18 cells) and VIP pretreatment (n = 22 cells). *P < 0.0005.

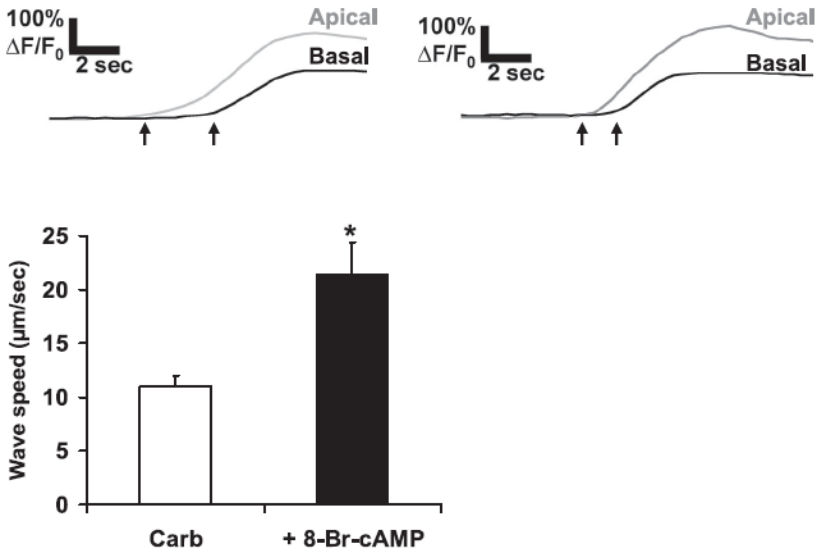

Fig. 2.

The cell-permeant cAMP agonist 8-bromo-cAMP (8-Br-cAMP) increases the speed of the carbachol-stimulated apical-to-basal Ca^{2+} wave. Acinar cells were pretreated with 8-Br-cAMP (100 μM) for 30 min prior to carbachol (1 μM) stimulation. *Insets* above are representative tracings corresponding to each group. Carbachol alone (n = 22 cells); 8-Br-cAMP pretreatment (n = 19 cells). *P < 0.01.

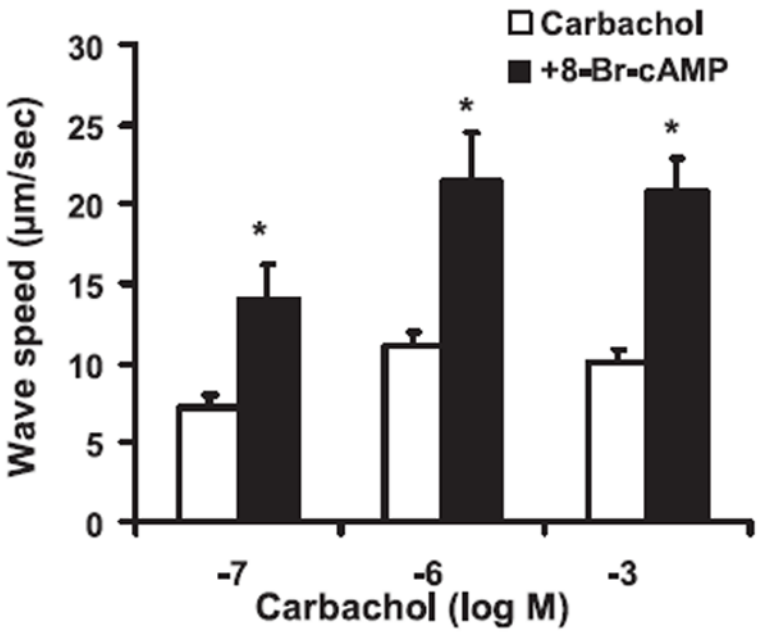

Fig. 3.

cAMP accelerates the apical-to-basal Ca^{2+} wave at a range of carbachol concentrations. Acinar cells were pretreated with 8-Br-cAMP (100 μM) for 30 min prior to carbachol stimulation (n = 7–22 cells). *P<0.05.

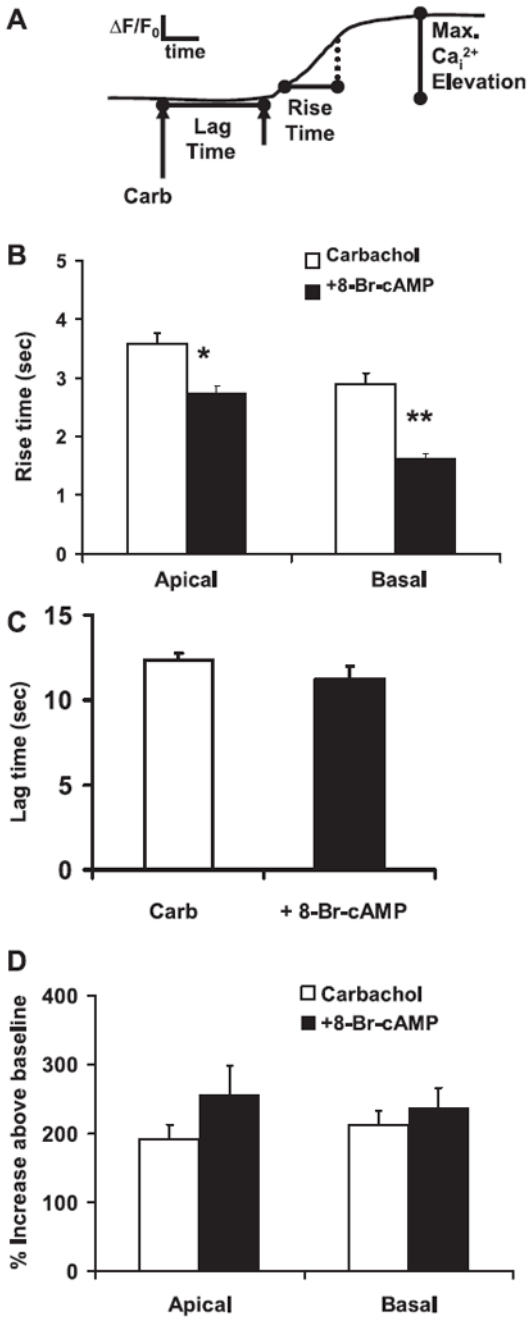

Fig. 4.

Additional effects of cAMP on the carbachol-stimulated Ca^{2+} transient. *A*: schematic tracing of a Ca^{2+} transient observed after carbachol (1 μM) stimulation, in which rise time, lag time, and maximum (Max.) Ca^{2+} elevation are depicted. *B*: a reduction in Ca^{2+} rise time was observed with 8-Br-cAMP (100 μM) pretreatment. The reduction was greater in the basal region than the apical (n = 16–21 cells in each group). *P < 0.0005; **P < 0.00001 for 8-Br-cAMP pretreatment compared with carbachol alone in each region. *C*: however, there was no change in lag time (n = 31 cells in each group; P = 0.24). *D*: when the high-affinity Ca^{2+} dye fluo-5F was used to avoid saturation of dye, there was a trend toward an increase in the

maximal Ca^{2+} elevation in both regions, but the changes were not statistically significant (n = 9–12 cells in each group; P = 0.21, apical; P = 0.516, basal).

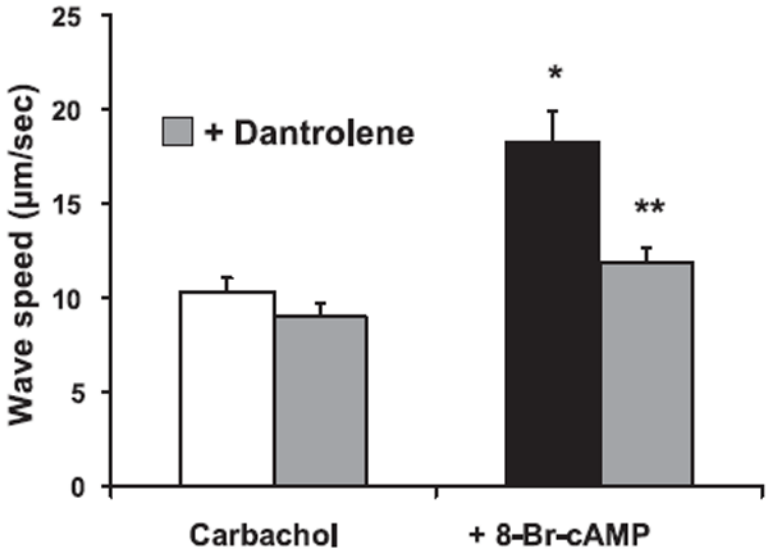

Fig. 5.

Ryanodine receptor (RyR) inhibition reduces cAMP enhancement of Ca^{2+} wave speed. In addition to pretreatment with 8-Br-cAMP (100 μM), a third group of acinar cells was incubated with dantrolene (100 μM) for 30 min prior to carbachol (1 μM) stimulation (n = 17–20 cells in each group). *$P < 0.05$; **$P < 0.005$, with respect to carbachol alone and 8-Br-cAMP pretreatment only, respectively; $P = 0.06$ between carbachol alone and 8-Br-cAMP/ dantrolene pretreatment.

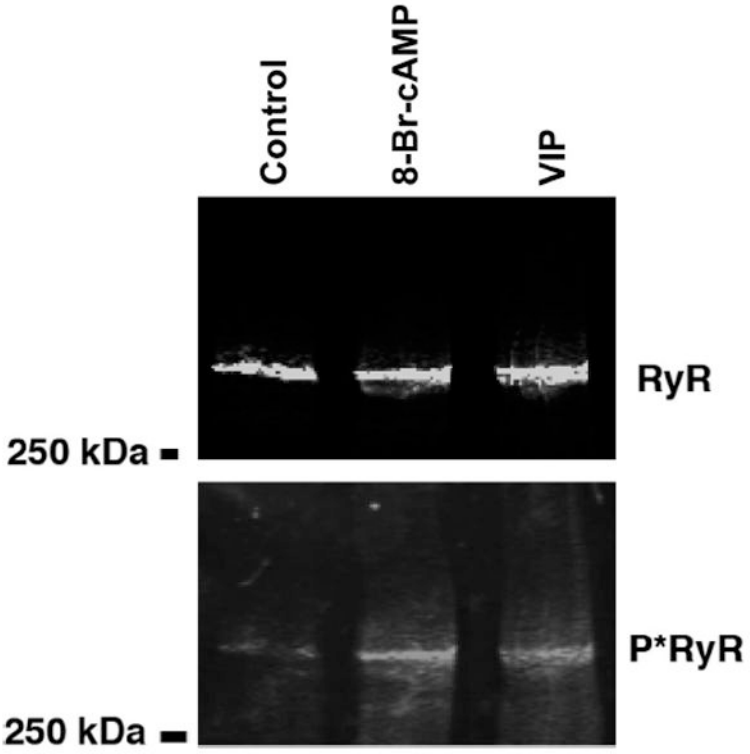

Fig. 6.
cAMP causes RyR phosphorylation. Acinar cells were pretreated with 8-Br-cAMP (100 μM) or VIP (100 nM) for 30 min, then analyzed by immunoprecipitation and immunoblotting for RyR and phospho-RyR (P*RyR), as described in methods.

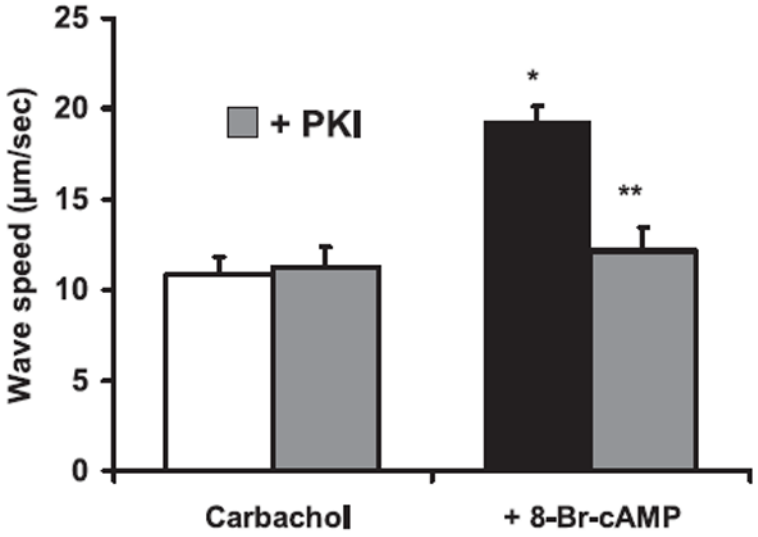

Fig. 7.

PKA inhibition reduces the cAMP-enhancement of Ca^{2+} wave speed. In addition to pretreatment with 8-Br-cAMP (100 μM), a third group of acinar cells was incubated with PKA inhibitor PKI (1 μM) for 30 min prior to carbachol (1 μM) stimulation (n = 12–27 cells in each group). *P < 0.00005; **P < 0.005, with respect to carbachol alone and 8-Br-cAMP pretreatment only, respectively; note P < 0.05 between carbachol alone and the 8-BrcAMP/PKI pretreatment.

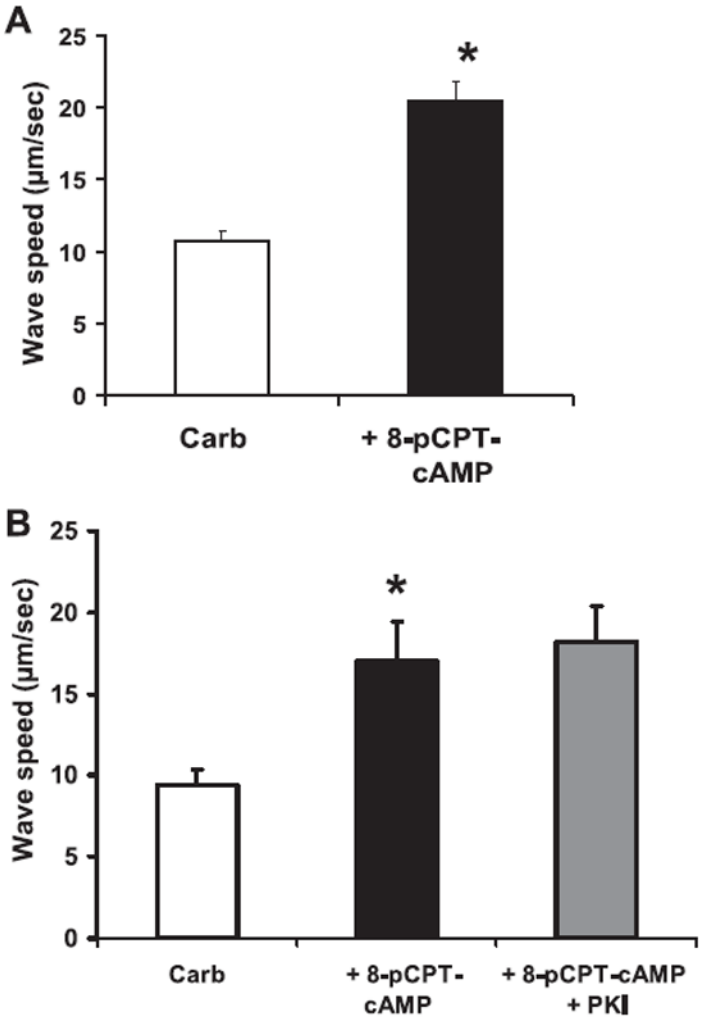

Fig. 8.

The Epac agonist 8-pCPT-2′-*O*-Me-cAMP (8-pCPT-cAMP) enhances the speed of the Ca^{2+} wave. *A*: acinar cells were pretreated with 8-pCPTcAMP (100 μM) for 30 min prior to carbachol (1 μM) stimulation (n = 25–32 cells in each group). *P < 0.00000005, with respect to carbachol alone. *B*: in addition to 8-(4-chloro-phenylthio) (pCPT)-cAMP pretreatment, a third group of acinar cells was incubated with PKI (1 μM) (n = 9–12 cells in each group). *P < 0.05, with respect to carbachol alone; P = 0.72 between 8-pCPT-cAMP and 8-Br-cAMP/PKI pretreatments.