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Activation of β-adrenoceptors does not cause any change in cytosolic Ca^{2+} distribution in rat parotid acinar cells

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Abstract

The effects of the β-adrenoceptor agonist isoproterenol on the distribution of cytosolic Ca^{2+} concentrations were studied with digital imaging microscopy in fura-2-loaded rat parotid acinar cells. At concentrations < 10 μM, isoproterenol did not cause any measurable change in cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$). Monitoring of $[Ca^{2+}]_i$ in selected areas of the acinar cells failed to show that stimulation with isoproterenol causes a localized rise in $[Ca^{2+}]_i$ at the apical region close to the lumen. As the maximum response of amylase exocytosis is observed at 0.1 or 1 μM isoproterenol [Tanimura, A., Matsumoto, Y., Tojyo, Y., 1990. Evidence that isoproterenol-induced Ca^{2+}-mobilization in rat parotid acinar cells is not mediated by activation of β-adrenoceptors. Biochim. Biophys. Acta, 1055, pp. 273–277], the data obtained here indicate that the isoproterenol-induced amylase exocytosis is not accompanied by Ca^{2+} mobilization. The high concentration (100 μM) of isoproterenol caused a small but significant increase in $[Ca^{2+}]_i$, particularly in the apical region. This response was completely attenuated by the α-adrenoceptor antagonist phentolamine, but not by the β-adrenoceptor antagonist propranolol, indicating that the isoproterenol-induced increase in $[Ca^{2+}]_i$ resulted from an activation of α-adrenoceptors. Further, the effect of cyclic AMP on Ca^{2+} release from intracellular Ca^{2+} stores was studied in saponin-permeabilized acinar cells using the lipophilic Ca^{2+} indicator Calcium Green C_{18}. Cyclic AMP had no effect on the Ca^{2+} release, while the same acinar cells responded strongly to inositol 1,4,5-trisphosphate. This result does not support the hypothesis that cyclic AMP directly stimulates Ca^{2+} mobilization in rat parotid acinar cells.

Keywords: Parotid acinar cell; Ca^{2+} concentration, cytosolic; Ca^{2+} imaging; β-Adrenoceptor; Isoproterenol; cAMP

1. Introduction

Amylase release from rat parotid acinar cells is mainly induced by activation of β-adrenoceptors which leads to an accumulation of cyclic AMP (cAMP). Although exocytosis is generally considered to be triggered by a rise in the cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$), it is rather controversial whether the cAMP-mediated amylase exocytosis in parotid acinar cells is accompanied by Ca^{2+}-mobilization from intracellular Ca^{2+} stores. Several studies (Hughes et al., 1989; Tanimura et al., 1990) have shown that stimulation with 1 μM isoproterenol, a concentration producing the maximum amylase release, has no effect on $[Ca^{2+}]_i$ in a suspension of fura-2-loaded rat parotid acinar cells, suggesting that the cAMP-mediated amylase exocytosis may be relatively independent of changes in $[Ca^{2+}]_i$. However, a more recent study (Rubin and Adolf, 1994) suggests that cAMP directly elicits Ca^{2+} mobilization from intracellular Ca^{2+} stores in permeabilized rat parotid acinar cells. Also, there are some reports indicating that β-adrenoceptor agonists and cAMP derivatives stimulate unidirectional ^{45}Ca efflux from parotid and submandibular slices and cells (Kanagasuntheram and Randle, 1976; Putney et al., 1977; Butcher, 1980; McPherson and Dormer, 1984; Argent and Arkle, 1985). This ^{45}Ca efflux has been considered to be the result of Ca^{2+} release from intracellular Ca^{2+} stores.

Amylase exocytosis occurs exclusively at the luminal membrane. If β-adrenoceptor stimulation results in only a small rise in $[Ca^{2+}]_i$ at the area close to the lumen, it may be difficult to detect the response in the cell suspensions of fura-2-loaded cells. Digital imaging analysis allows a visualization of the spatial and temporal distribution of $[Ca^{2+}]_i$ in individual cells. In the present study, we assessed the possibility that the cAMP-mediated amylase exocytosis is

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associated with the localized and compartmentalized changes in $[Ca^{2+}]_i$, and the effects of isoproterenol on $[Ca^{2+}]_i$ were studied with digital imaging microscopy in fura-2-loaded rat parotid acinar cells. In addition, using the membrane-bound Ca^{2+} fluorescence indicator Calcium Green C_{18} (Lloyd et al., 1995), it was examined whether cAMP directly induces Ca^{2+} release from intracellular Ca^{2+} stores in permeabilized parotid acinar cells.

2. Materials and methods

2.1. Materials

The isoproterenol, phenylephrine, carbachol, atropine, cAMP, collagenase (type II), trypsin (type III), trypsin inhibitor (type II-S) and bovine serum albumin were purchased from Sigma (St. Louis, MO, USA). Fura-2 acetoxymethyl ester (fura-2/AM) and Hepes were from Dogin Laboratories (Kumamoto, Japan). Phentolamine was from Nippon Ciba-Geigy (Hyogo, Japan). Calcium Green C_{18}, Calcium Sponge S and inositol 1,4,5-trisphosphate (IP_3) were from Molecular Probes (Engene, OR, USA).

2.2. Cell preparation

Male Wistar-strain rats (about 300 g) were anesthetized with diethyl ether and killed by cardiac puncture. Dispersed acinar cells were prepared by the same enzymatic digestion procedure described previously (Tanimura et al., 1990). After dispersion, cells were washed and resuspended in a Hanks' balanced salt solution containing 137 mM NaCl, 5.4 mM KCl, 1.3 mM $CaCl_2$, 1.03 mM $MgSO_4$, 0.34 mM Na_2HPO_4, 0.44 mM KH_2PO_4, 5.6 mM glucose, 0.2% bovine serum albumin, and 20 mM Hepes (HBSS-H). The medium was adjusted to pH 7.4 with NaOH.

2.3. Fura-2 fluorescence measurements

Dispersed acinar cells were incubated with 2 μM fura-2/AM for 40 min at room temperature and washed twice with fresh HBSS-H without bovine serum albumin. The fura-2-loaded cells were transferred to a small sample chamber and attached to the Cell-Tak-coated glass coverslip which formed the bottom of the chamber. The sample chamber was mounted on the stage of an inverted microscope (Diaphot: Nikon, Tokyo, Japan) equipped with a Nikon CF Fluor 40 × 1.3 NA or 100 × 1.3 NA oil immersion objective. The cells were kept at 32°C using a thermostatically controlled stage (Warner Instrument, CT, USA). Excitation wavelengths were set at 340 and 380 nm. Emitted light was passed through a 400 nm dichroic mirror, filtered at 520 nm and collected by a cooled CCD camera (Hamamatsu Photonics, Hamamatsu, Japan). The fluorescence images were digitized and stored in an ARGUS HiSCA imaging system (Hamamatsu Photonics). The $[Ca^{2+}]_i$ was calculated in individual cells from the fura-2 fluorescence ratios (340:380) as described by Grynkiewicz et al. (1985). The fluorescence ratios at saturating and zero free Ca^{2+} concentration required for these calculations were determined from 6 μM fura-2 solution containing 5 mM $Ca^{2+}Cl_2$ or 5 mM EGTA, respectively.

The volume of solution was maintained at ~40 μM by placing a vacuum line in the sample chamber. Solution changes were accomplished by quickly adding 200 μl of fresh solution to the chamber.

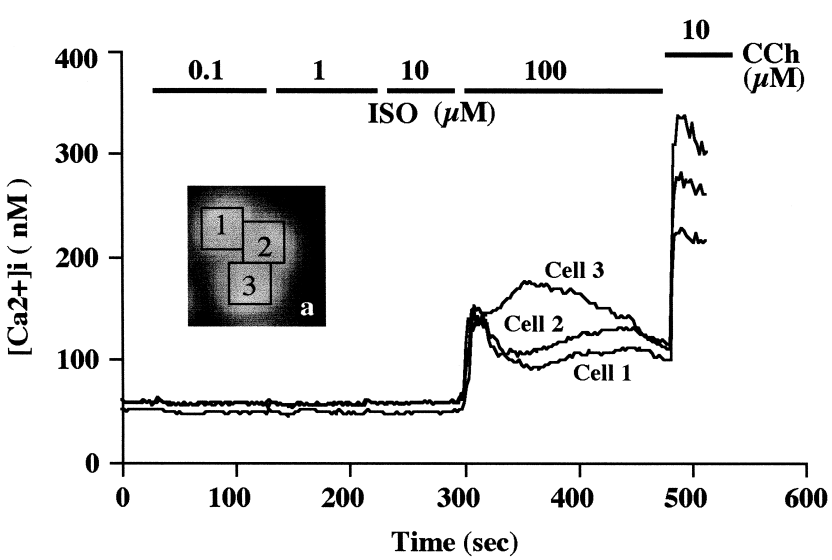

Fig. 1. Effects of different concentrations of isoproterenol (ISO) in rat parotid acinar cells. The fura-2-loaded cluster of three acinar cells was successively exposed to the indicated concentrations of isoproterenol and then to 10 μM carbachol (CCh). The fluorescence ratio of each acinar cell was captured every 2 s. The time-courses of changes in $[Ca^{2+}]_i$ represents the averaged $[Ca^{2+}]_i$ within the squares depicted in the fluorescence image (a). The horizontal bars at the top of the figure indicates the periods when the cells were exposed to the indicated concentrations of isoproterenol or carbachol.

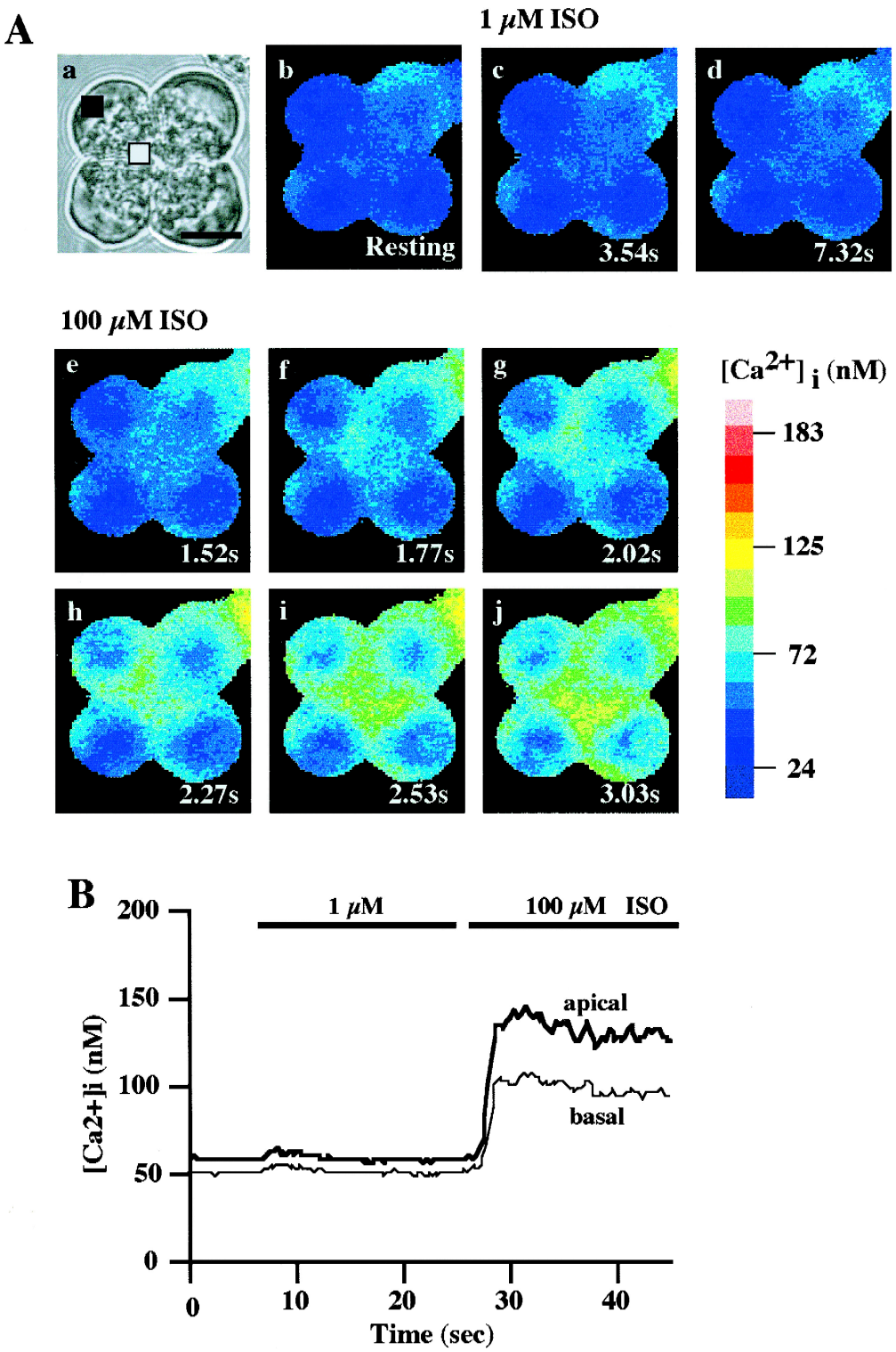

Fig. 2. Images of changes in $[Ca^{2+}]_i$ evoked by isoproterenol (ISO) in rat parotid acinar cells. (A) The fura-2-loaded cluster of four acinar cells was stimulated with 1 and 100 μM isoproterenol. (a) Transmission image; scale bar 10 μm, (b) Ca^{2+} image before stimulation expressed as pseudocolour, (c and d) images after stimulation with 1 μM isoproterenol, (e–j) images after stimulation with 100 μM isoproterenol. The time after addition of 1 or 100 μM isoproterenol is shown in each panel. (B) Time-courses of changes in $[Ca^{2+}]_i$ at the apical and basal poles. The averaged $[Ca^{2+}]_i$ within the two small squares depicted in (A–a) were measured. The horizontal bars at the top of (B) indicate the period when the cells were exposed to the indicated concentrations of isoproterenol.

2.4. Loading of permeabilized cells with Calcium Green C_{18}

Dispersed acinar cells settled on a Cell-Tak-coated coverslip were permeabilized with saponin as described previously (Tojyo et al., 1997a) and then incubated with intracellular-like medium (ICM) containing 10 μM Calcium Green C_{18} for 3 min. The ICM contained 125 mM KCl, 19 mM NaCl, 10 mM Hepes (pH 7.3 with KOH), 3 mM ATP, 1.4 mM $MgCl_2$, 0.33 mM $CaCl_2$, and 1 mM EGTA. Following washing with dye-free ICM, the solution was switched to calcium sponge-treated medium (CaS). The CaS was prepared as described elsewhere (Tanimura and Turner, 1996a). Briefly, 60 ml of a solution containing 125 mM KCl, 19 mM NaCl, 10 mM Hepes (pH 7.3), and 3 mM ATP was passed twice over a column packed with 0.3 g of Calcium Sponge S to remove residual contaminating Ca^{2+}. Following the Ca^{2+} removal, 1.0 mM $MgCl_2$ was added to the solution. The final Ca^{2+} concentration of CaS was determined to be ~50 nM from the florescence using fura-2.

2.5. Measurement of Calcium Green C_{18} fluorescence

Calcium Green C_{18} fluorescence from the permeabilized cells was detected with a confocal laser scanning

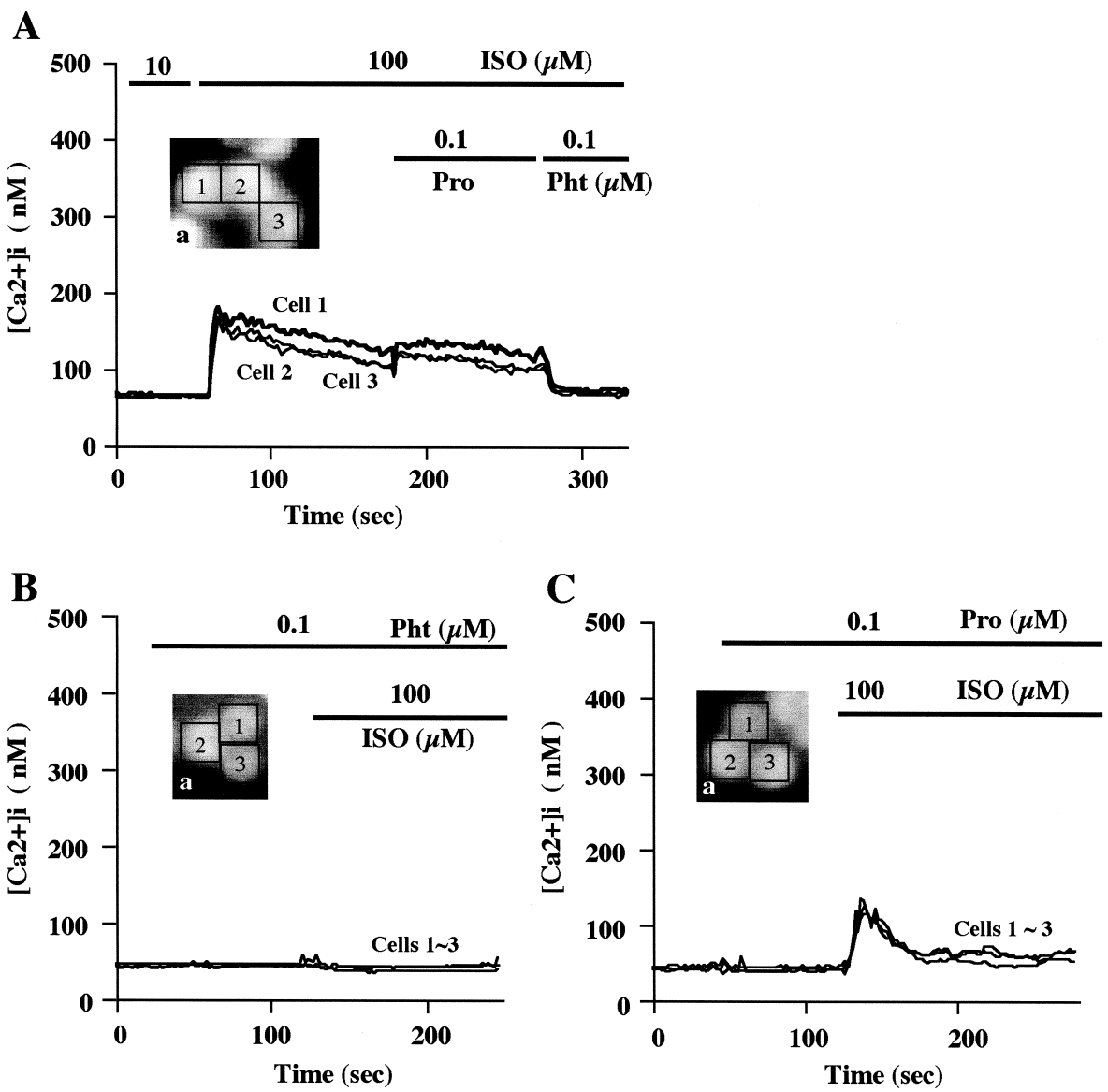

Fig. 3. Effects of propranolol (Pro) and phentolamine (Pht) on the increase in $[Ca^{2+}]_i$ induced by isoproterenol (ISO). (A) The fura-2-loaded cluster of three acinar cells was successively exposed to 10 and 100 μM isoproterenol and then to 100 μM isoproterenol plus 0.1 μM propranolol or 0.1 μM phentolamine. (B and C) The fura-2-loaded cells were preincubated for 1 min with 0.1 μM phentolamine or 0.1 μM propranolol and then stimulated with 100 μM isoproterenol in the presence of the antagonists. The fluorescence ratio of each acinar cell was captured every 2 s. The time-courses of changes in $[Ca^{2+}]_i$ represents the averaged $[Ca^{2+}]_i$ within the squares depicted in the fluorescence image (a). The horizontal bars at the top of the figure show the periods when the cells were exposed to the indicated concentrations of agents.

microscope system (Leica TCS 4D, Leica, Heidelberg, Germany) equipped with a 40 × PL Fluotar objective. Confocal images (128 × 128 pixels) of Calcium Green C_{18} fluorescence were obtained at 488 nm excitation and > 515 nm emission using a 170 μm pinhole.

3. Results

3.1. Effect of isoproterenol on $[Ca^{2+}]i$

The fura-2-loaded acinar cells were stimulated at 1–2 min intervals with different concentrations of isoproterenol in the presence of extracellular Ca^{2+}, and the fluorescence ratio in individual acinar cells was monitored every 2 s with the ARGUS HiSCA imaging system. The averaged resting $[Ca^{2+}]_i$ of 121 acinar cells before stimulation was 78.4 ± 2.0 nM (mean ± S.E.). Fig. 1 shows typical changes in $[Ca^{2+}]_i$ obtained in a cluster of three acinar cells. At concentrations < 10 μM, isoproterenol has little or no effect on $[Ca^{2+}]_i$, but a subsequent addition of 100 μM isoproterenol caused a significant increase in $[Ca^{2+}]_i$ in 115 out of the 121 acinar cells examined. The maximum $[Ca^{2+}]_i$ elevated by 100 μM isoproterenol was 192.0 ± 7.8 nM ($P < 0.01$, as compared with the resting level). At the end of each experiment, the same acinar cells were stimulated with 10 μM of the muscarinic receptor agonist carbachol. As shown in Fig. 1, carbachol caused a rapid and marked increase in $[Ca^{2+}]_i$ (515.0 ± 22.2 nM) in all cells examined, indicating that the ability of isoproterenol to induce the increase in $[Ca^{2+}]_i$ is much lower than that of muscarinic receptor agonists.

3.2. Spatio-temporal distribution of $[Ca^{2+}]i$ following stimulation with isoproterenol

The subcellular regional change in $[Ca^{2+}]_i$ induced by isoproterenol was studied at higher magnifications using a 100 × oil immersion objective. Fig. 2A shows pseudocolor images of $[Ca^{2+}]_i$ in a cluster of four acinar cells. Stimulation with 1 μM isoproterenol did not change the image of $[Ca^{2+}]_i$ in any region of the acinar cells (Fig. 2A, b–d), and the absence of an effect of 1 μM isoproterenol on $[Ca^{2+}]_i$ was confirmed by monitoring the changes in $[Ca^{2+}]_i$ within the small areas depicted at the apical and basal poles of the acinar cells (Fig. 2B). Subsequent stimulation with 100 μM isoproterenol caused a significant increase in $[Ca^{2+}]_i$ throughout the acinar cells (Fig. 2A, e–j). The response was particularly strong at the apical rather than at the basolateral regions of the cells (Fig. 2A,B). Similar spatial changes in $[Ca^{2+}]_i$ in response to 100 μM isoproterenol were observed in 24 out of the 26 acinar cells.

3.3. Effects of propranolol and phentolamine on isoproterenol-induced increases in $[Ca^{2+}]i$

To show that the increase in $[Ca^{2+}]_i$ induced by the high concentration of isoproterenol was not due to activation of β-adrenoceptors, the effects of two receptor antagonists (propranolol and phentolamine) on the increase in $[Ca^{2+}]_i$ were examined. Fig. 3A shows the typical effects of the antagonists on the sustained increase in $[Ca^{2+}]_i$ elevated by 100 μM isoproterenol. The increase in $[Ca^{2+}]_i$ was not attenuated by addition of 0.1 μM propranolol, a β-adrenoceptor antagonist, but subsequent addition of 0.1 μM phentolamine, an α-adrenoceptor antagonist, decreased $[Ca^{2+}]_i$ to nearly the resting level. Similar results were obtained in all acinar cells examined ($n = 79$). Further, acinar cells were preincubated for 1 min with the antagonists and then stimulated with 100 μM isoproterenol in the presence of the antagonists. The preincubation with 0.1 μM phentolamine completely blocked the isoproterenol-induced rise in $[Ca^{2+}]_i$ ($n = 39$) (Fig. 3B), while 0.1 μM propranolol did not inhibit the $[Ca^{2+}]_i$ response ($n = 33$) (Fig. 3C).

3.4. Effects of cAMP and IP_3 on Ca^{2+} release in permeabilized acinar cells

Rubin and Adolf (1994) have suggested that cAMP directly stimulates Ca^{2+} mobilization from intracellular Ca^{2+} stores in permeabilized rat parotid acinar cells. To test this possibility, the effect of cAMP on the Ca^{2+} release was studied in saponin-permeabilized acinar cells using the lipophilic Ca^{2+} indicator Calcium Green C_{18}. This dye strongly labels the outer-surface of intracellular compartments in permeabilized cells and allows a detection of the changes in Ca^{2+} concentration adjacent to the

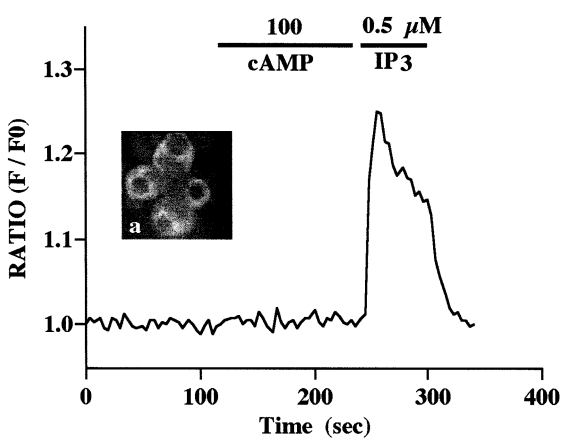

Fig. 4. Effects of cyclic AMP (cAMP) and inositol 1,4,5-triphosphate (IP_3) on Calcium Green C_{18} fluorescence in permeabilized acinar cells. The saponin-permeabilized acinar cells were labelled with Calcium Green C_{18} and exposed to 100 μM cAMP and then to 0.5 μM IP_3. (a) Confocal image of the fluorescence. Confocal images of the whole cluster were obtained every 4 s. The presence of cAMP and IP_3 in the medium is indicated by the horizontal bars at the top of the figure. The relative changes in fluorescence were determined by dividing the fluorescence intensity at each time by that at time 0.

membrane of intracellular organelles (Tanimura and Turner, 1996b; Tojyo et al., 1997a). Fig. 4 shows typical changes in fluorescence in a whole cluster of four permeabilized acinar cells monitored with confocal laser microscopy. Exposure of the cells to 100 μM cAMP did not increase the fluorescence in Calcium Green C_{18}, while the same acinar cells showed a rapid and marked increase in fluorescence following addition of 0.5 μM IP_3. Similar results were obtained in all cell clusters examined ($n = 5$).

Forskolin, a direct activator of adenylate cyclase, stimulates an accumulation of cAMP in rat parotid acinar cells (Takuma, 1990), and the images of $[Ca^{2+}]_i$ following addition of forskolin was monitored for 1–5 min in intact parotid acinar cells loaded with fura-2. The addition of 10 or 20 μM forskolin did not alter the distribution of $[Ca^{2+}]_i$ in all acinar cells ($n = 15$) examined (data not shown).

4. Discussion

The β-adrenoceptor agonist isoproterenol is the most potent secretagogue in amylase release from rat parotid acinar cells. At concentrations > 1 nM, isoproterenol stimulates amylase release in a dose-dependent manner, and the maximum response is observed at 0.1 or 1 μM (Tanimura et al., 1990; Tojyo et al., 1991). The present study has shown that isoproterenol up to 10 μM does not cause any measurable change in the distribution of $[Ca^{2+}]_i$ in rat parotid acinar cells. As the absence of changes in $[Ca^{2+}]_i$ was also confirmed by monitoring a small part of the apical region, it is unlikely that the concentrations of isoproterenol elicited a localized rise in $[Ca^{2+}]_i$ in areas close to the luminal membrane. These results strongly suggest that the isoproterenol-induced amylase release is not accompanied by Ca^{2+} mobilization. Although stimulation with a high concentration (100 μM) of isoproterenol resulted in a small but significant rise in $[Ca^{2+}]_i$, this response was easily attenuated by addition of the α-adrenoceptor antagonist phentolamine. Therefore, the isoproterenol-induced Ca^{2+} mobilization probably resulted from an activation of α-adrenoceptors rather than of β-adrenoceptors, as also suggested in earlier papers (Hughes et al., 1989; Tanimura et al., 1990). The Ca^{2+} mobilization in response to 100 μM isoproterenol was predominantly elicited in the apical region of acinar cells, and the pattern was similar to the Ca^{2+} signal in the parotid acinar cells evoked by low concentrations of the muscarinic receptor agonist carbachol (Tojyo et al., 1997b). As the intracellular Ca^{2+} stores localized in the apical region are more sensitive to IP_3 than those in the basolateral region (Tanimura et al., 1998), the finding that the rise in $[Ca^{2+}]_i$ was predominant in the apical region is compatible with the idea that the Ca^{2+} signal induced by high concentrations of isoproterenol is mediated by a formation of IP_3 via activation of α-adrenoceptors.

It is known that isoproterenol and cAMP derivatives accelerate the ^{45}Ca efflux from parotid and submandibular slices or cells (Kanagasuntheram and Randle, 1976; Putney et al., 1977; Butcher, 1980; McPherson and Dormer, 1984; Argent and Arkle, 1985). This ^{45}Ca efflux has been considered as indirect evidence for Ca^{2+} mobilization evoked by β-adrenoceptor stimulation. However, a recent study of rat submandibular acinar cells showed that substantial amounts of Ca^{2+}, which are sequestered in secretory granules, are released into extracellular medium via exocytosis (Belan et al., 1998), strongly suggesting that the isoproterenol-induced ^{45}Ca efflux occurs as a consequence of exocytosis.

It has been reported that isoproterenol stimulates Ca^{2+} mobilization in quin-2-loaded parotid and submandibular cells (Helman et al., 1987; Horn et al., 1988). However, the concentrations (> 5 μM) of isoproterenol required to cause a rise in $[Ca^{2+}]_i$ are much higher than those for inducing amylase and mucin release (McPherson and Dormer, 1984; Tanimura et al., 1990), suggesting that this $[Ca^{2+}]_i$ response was due to the activation of α-adrenoceptors. In rat submandibular cells, a relatively low concentration (1 or 2 μM) of isoproterenol has been demonstrated to increase the Ca^{2+}-dependent Cl^- and K^+ currents (Cook et al., 1988), and the authors considered that β-adrenoceptor stimulation activated Cl^- and K^+ channels by raising $[Ca^{2+}]_i$. However, there is no direct evidence showing that $[Ca^{2+}]_i$ increased during the isoproterenol-induced Cl^- and K^+ currents.

Rubin and Adolf (1994) found that application of cAMP changed the fluorescence of fura-2 in a suspension of permeabilized rat parotid acinar cells and suggested that cAMP stimulates Ca^{2+} release by acting directly on intracellular Ca^{2+} store. Further, the same group reported that other adenine nucleotides, such as ADP and cyclic ADP-ribose, and forskolin were also able to induce the Ca^{2+} mobilization (Zhang et al., 1997). These results do not agree with our data and an earlier report (Takuma and Ichida, 1986) showing that IP_3, but not cAMP, evoked ^{45}Ca release from saponin-permeabilized rat parotid cells. Although there is no ready explanation for the discrepancy, it is possible that Ca^{2+} contamination of the applied agents results in the changes in fura-2 fluorescence. Also, cAMP induces amylase release in permeabilized rat parotid cells (Takuma and Ichida, 1988), suggesting that the apparent cAMP-induced Ca^{2+} mobilization may be due to Ca^{2+} release from secretory granules. It is surprising that ADP and cyclic ADP-ribose were more potent in stimulating Ca^{2+} release in permeabilized rat parotid acinar cells than IP_3 (Zhang et al., 1997), as this leads to the assumption that ADP and cyclic ADP-ribose may be the major messengers for Ca^{2+} mobilization in this cell type. The operation of intracellular Ca^{2+} stores in permeabilized rat parotid acinar cells was recently studied by monitoring the Ca^{2+} concentration within organelles loaded with the low affinity Ca^{2+} indicator Mag-fura-2 (Tojyo et al., 1997a), and

the results showed that cyclic ADP-ribose had no effect on Ca^{2+} release from intracellular Ca^{2+} stores.

In conclusion, the present study does not provide evidence suggesting that activation of β-adrenoceptors causes an increase in $[Ca^{2+}]_i$ in rat parotid acinar cells, indicating that the cAMP-mediated amylase exocytosis occurs without Ca^{2+} mobilization.

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References

Argent, B.E., Arkle, S., 1985. Mechanism of action of extracellular calcium on isoprenaline-evoked amylase secretion from isolated rat parotid glands. J. Physiol. 369, 337–353.

Belan, P., Gardner, J., Gerasimenko, O., Gerasimenko, J., Mills, C.L., Petersen, O.H., Tepikin, A.V., 1998. Isoproterenol evokes extracellular Ca^{2+} spikes due to secretory events in salivary gland cells. J. Biol. Chem. 273, 4106–4111.

Butcher, F.R., 1980. Regulation of calcium efflux from isolated rat parotid cells. Biochim. Biophys. Acta 630, 254–260.

Cook, D.I., Day, M.L., Champion, M.P., Young, J.A., 1988. Ca^{2+} not cyclic AMP mediates the fluid secretory response to isoproterenol in the rat mandibular salivary gland: whole-cell patch-clamp studies. Pflüg. Arch. 413, 67–76.

Grynkiewicz, G., Poenie, M., Tsien, R.Y., 1985. A new generation of Ca^{2+} indicators with greatly improved fluorescence properties. J. Biol. Chem. 260, 3440–3450.

Helman, J., Ambudkar, I.S., Baum, B.J., 1987. Adrenoreceptor mobilization of calcium in rat submandibular cells. Eur. J. Pharmacol. 143, 65–72.

Horn, V.J., Baum, B.J., Ambudkar, I.S., 1988. β-Adrenergic receptor stimulation induces inositol trisphosphate production and Ca^{2+} mobilization in rat parotid acinar cells. J. Biol. Chem. 263, 12454–12460.

Hughes, A.R., Takemura, H., Putney Jr., J.W., 1989. Does β-adrenoceptor activation stimulate Ca^{2+} mobilization and inositol trisphosphate formation in parotid acinar cells?. Cell Calcium 10, 519–525.

Kanagasuntheram, P., Randle, P.J., 1976. Calcium metabolism and amylase release in rat parotid acinar cells. Biochem. J. 160, 547–564.

Lloyd, Q.P., Kuhn, M.A., Gay, C.V., 1995. Characterization of calcium translocation across the plasma membrane of primary osteoblasts using a lipophilic calcium-sensitive fluorescent dye, Calcium Green C_{18}. J. Biol. Chem. 270, 22445–22451.

McPherson, M.A., Dormer, R.L., 1984. Mucin release and calcium fluxes in isolated rat submandibular acini. Biochem. J. 224, 473–481.

Putney Jr., J.W., Weiss, S.J., Leslie, B.A., Marier, S.H., 1977. Is calcium the final mediator of exocytosis in the rat parotid gland?. J. Pharmacol. Exp. Ther. 203, 144–155.

Rubin, R.P., Adolf, M.A., 1994. Cyclic AMP regulation of calcium mobilization and amylase release from isolated permeabilized rat parotid cells. J. Pharmacol. Exp. Ther. 268, 600–606.

Takuma, T., 1990. Propranolol inhibits cyclic AMP accumulation and amylase secretion in parotid acinar cells stimulated by isobutylmethylxanthine and forskolin. Biochim. Biophys. Acta 1052, 461–466.

Takuma, T., Ichida, T., 1986. Does cyclic AMP mobilize Ca^{2+} for amylase secretion from rat parotid cells?. Biochim. Biophys. Acta 887, 113–117.

Takuma, T., Ichida, T., 1988. Amylase secretion from saponin-permeabilized parotid cells evoked by cyclic AMP. J. Biochem. 103, 95–98.

Tanimura, A., Turner, R.J., 1996a. Calcium release in HSY cells conforms to a steady-state mechanism involving regulation of the inositol 1,4,5-trisphosphate receptor Ca^{2+} channel by luminal $[Ca^{2+}]$. J. Cell Biol. 132, 607–616.

Tanimura, A., Turner, R.J., 1996b. Inositol 1,4,5-trisphosphate-dependent oscillations of luminal $[Ca^{2+}]$ in permeabilized HSY cells. J. Biol. Chem. 271, 30904–30908.

Tanimura, A., Matsumoto, Y., Tojyo, Y., 1990. Evidence that isoproterenol-induced Ca^{2+}-mobilization in rat parotid acinar cells is not mediated by activation of β-adrenoceptors. Biochim. Biophys. Acta 1055, 273–277.

Tanimura, A., Matsumoto, Y., Tojyo, Y., 1998. Polarized Ca^{2+} release in saponin-permeabilized parotid acinar cells evoked by flash photolysis of 'caged' inositol 1,4,5-trisphosphate. Biochem. J. 332, 769–772.

Tojyo, Y., Tanimura, A., Matsui, S., Matsumoto, Y., Sugiya, H., Furuyama, S., 1991. NaF-induced amylase release from rat parotid cells is mediated by PI breakdown leading to Ca^{2+} mobilization. Am. J. Physiol. 260, C194–C200.

Tojyo, Y., Tanimura, A., Matsumoto, Y., 1997a. Monitoring of Ca^{2+} release from intracellular stores in permeabilized rat parotid acinar cells using the fluorescent indicators Mag-fura-2 and Calcium Green C_{18}. Biochem. Biophys. Res. Commun. 240, 189–195.

Tojyo, Y., Tanimura, A., Matsumoto, Y., 1997b. Imaging of intracellular Ca^{2+} waves induced by muscarinic receptor stimulation in rat parotid acinar cells. Cell Calcium 22, 455–462.

Zhang, X., Wen, J., Bidasee, K.R., Besch Jr., H.R., Rubin, R.P., 1997. Ryanodine receptor expression is associated with intracellular Ca^{2+} release in rat parotid acinar cells. Am. J. Physiol. 273, C1306–C1314.