PROCEEDING

Spontaneous Ca^{2+} oscillations *via* purinergic receptors elicit transient cell swelling in rat parotid ducts

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Abstract : Rat parotid ductal cells were found to exhibit spontaneous Ca^{2+} oscillations. These oscillatory Ca^{2+} responses were observed during continuous perfusion with physiological salt solution at 37℃ in the absence of calcium mobilizing agonist stimulation. These Ca^{2+} oscillations were completely blocked by the purinergic receptor inhibitors, pyridoxal phosphate-6-azo (benzene-2,4-disulfonic acid) (PPADS) and suramin, but were not blocked by the muscarinic antagonist, atropine, nor the α-adrenergic antagonist, phentolamine. Simultaneous observation with fura-2 fluorescence and differential interference contrast (DIC) images showed that the spontaneous elevations of $[Ca^{2+}]i$ were well correlated with the shape changes of the ductal cells. Using a plasma membrane fluorescence probe, we found that the changes in DIC images reflected spontaneous cell swelling of ductal cells. Electron microscopic analysis after Ca^{2+} imaging indicated that the spontaneously oscillating duct cells contained numerous granules at the luminal side, which is characteristic of the granular duct cells. These results indicate that the spontaneous $[Ca^{2+}]i$ increase occurs through purinergic receptors, and activates Ca^{2+}-dependent ion transporters and/or channels. Our findings present the possibility that spontaneous Ca^{2+} oscillations *via* purinergic receptors are involved in the regulation of the electrolyte composition of saliva in resting states. J. Med. Invest. 56 Suppl. : 377-380, December, 2009

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INTRODUCTION

The salivary gland consists of specialized epithelial cells which are divided into two major domains, acini and ducts. The acinar cells make up the secretory endpiece and secrete primary saliva, which is a plasma-like and isotonic fluid. The ductal cells reabsorb Na^+ and Cl^- from the primary saliva and excrete K^+ and HCO_3^-, to produce the final saliva. The salivary duct system consists of intercalated, granular, striated, and excretory ducts. The granular duct is a ductal segment between the striated duct and the intercalated duct of rodent submandibular glands. The granular duct cell is characterized by numerous granules in the apical pole, and is known to synthesize and secrete several growth factors (*e.g.*, EGF and NGF) and kallikrein (1).

The salivary secretion in acini and modification of the electrolyte composition in ducts are regulated by the elevation of intracellular Ca^{2+} concentration ($[Ca^{2+}]i$) through activations of various Ca^{2+}-dependent ion channels and transporters (2). We previously showed that purinergic stimulation caused an increase in $[Ca^{2+}]i$ in rat parotid ducts (3). The

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purinergic receptor family comprises G-protein coupled receptors (GPCRs) and ionotropic receptors, termed as P2Y and P2X, respectively. Salivary glands express at least four isoforms of purinergic receptors P2Y1, P2Y2, P2X4, and P2X7 (4). Immunohistochemical studies show that P2Y2 and P2X7 are expressed at the luminal membrane of salivary ducts (5, 6). However, the physiological roles of these purinergic receptors have not yet to be clarified.

In the present study, we found that rat parotid ductal cells exhibit spontaneous $[Ca^{2+}]i$ oscillations via purinergic receptor. Further, we showed that spontaneous Ca^{2+} oscillations were accompanied with transient cell swelling. These findings present the possibility that the purinergic receptor-mediated spontaneous Ca^{2+} oscillations are involved in the regulation of electrolyte absorption by the parotid duct at the resting state.

RESULTS

Spontaneous Ca^{2+} oscillation in parotid ductal cells

We monitored the changes in fluorescence intensity of fura-2-loaded parotid ducts using multiphoton microscopy and found that parotid ducts show oscillatory Ca^{2+} responses during continuous perfusion with a physiological salt solutions at 37°C in the absence of agonist stimulation (Fig. 1). The timing and patterns of these spontaneous $[Ca^{2+}]i$ increases varied in most of the individual ductal cells. A small number of synchronized spontaneous Ca^{2+} responses were observed in adjacent cells or in cells opposite each other across the ductal lumen (7). Time-dependent changes in fluorescence in ductal cells showed that the rise in $[Ca^{2+}]i$ was relatively rapid, and responses reached a peak within 20 s and then returned to basal levels usually in 20 to 120 s. During the 10-min recording period, approximately 60% of responding ductal cells exhibited more than two Ca^{2+} transients, and the average number of Ca^{2+} responses in 10 min was 2.1 (7). Electron microscopic analysis after Ca^{2+} imaging indicated that spontaneously oscillating ducts contained numerous granules at the luminal side, which is characteristic of granular ducts (7).

Involvement of purinergic receptors in the spontaneous Ca^{2+} response in parotid ductal cells

We next examined the mechanism of spontaneous Ca^{2+} response by monitoring fura-2 fluorescence in the presence of various receptor antagonists. Neither atropine (muscarinic receptor antagonist) nor phentolamine (α-adrenergic receptor antagonist) blocked the spontaneous Ca^{2+} response, while purinergic receptor antagonists, pyridoxal phosphate-6-azo (benzene-2,4-disulfonic acid) (PPADS) and suramin, blocked the spontaneous Ca^{2+} response almost completely (7). These results clearly indicate

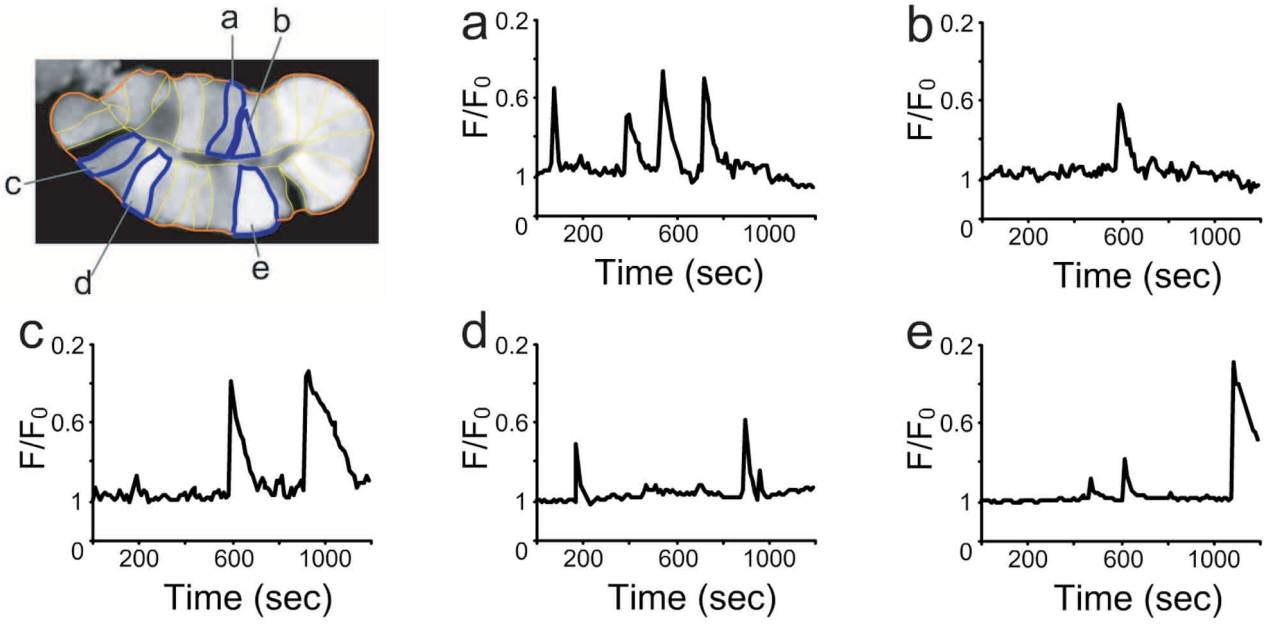

Figure 1. Spontaneous Ca^{2+} responses in rat parotid ducts.
Fura-2 fluorescence was monitored at 37°C in the absence of agonist stimulation. The time course of spontaneous $[Ca^{2+}]i$ changes. Traces are the relative changes in fluorescence intensity in five representative cells indicated by blue lines and marked with corresponding letters (a-e) in the fluorescence image. Reproduced from ref. 7.

the involvement of purinergic receptors in the spontaneous Ca^{2+} response in parotid ductal cells. In addition, these ductal fragments contained numerous vesicles that accumulated quinacrine, a marker for ATP-containing vesicles (7). We therefore speculate that the spontaneous Ca^{2+} responses are triggered by the release of ATP from ductal cells.

Transient cell swelling in spontaneously oscillating ductal fragments

It is known that Ca^{2+} elevation in salivary acinar and ductal cells induces cell shrinkage due to activation of ion transport activities, which signals changes in intracellular solute content (8, 9). Thus, we further examined changes in ductal cell shape by DIC images and simultaneous fura-2 fluorescence. In this experiment, we observed transient changes in cell shape of the ductal cells exhibiting spontaneous Ca^{2+} oscillations.

Subtracted DIC images (Fig. 2) obtained by subtracting each DIC images from the corresponding previous image, allowed us to visualize changes in cell shape clearly (Fig. 2Aa, Ab). By comparison between fura-2 ratio images and subtracted DIC images, the spontaneous Ca^{2+} responses were revealed clearly associated with the changes in cell shape (Fig. 2B). To further clarify the changes in cell shape, we visualized ductal cell membranes using a plasma membrane fluorescence probe, synaptogreen C4. Interestingly, we observed that the cell swelling associated with the changes of the subtracted DIC images (7). These results suggest that spontaneous Ca^{2+} responses contribute to the absorption of electrolytes by parotid ductal cells at the resting state.

DISCUSSION

The present study demonstrated for the first time that the parotid ductal cells exhibit Ca^{2+} oscillation via purinergic receptor in the absence of exogenous stimulation. We also demonstrated that a spontaneous elevation of $[Ca^{2+}]i$ was accompanied by cell swelling. In contrast, it has previously been reported that CCh-induced $[Ca^{2+}]i$ increases in parotid ducts enlarge the luminal space, due to the shrinkage of ductal cells (9). Therefore, the swelling of parotid ductal cells in association with spontaneous Ca^{2+} oscillations was an unexpected result. The cell swelling is thought to reflect the absorption of electrolytes, which would increase cellular osmolality and lead to water inflow. Although the mechanism underlying electrolyte absorption in ductal cells appears to differ markedly depending on the species, gland, or type of ductal cells, the accepted model predicts that electrolyte absorption is primarily mediated by the combination of Na^+ and Cl^- channels in the apical membrane. Two types of Cl^- channels, the Ca^{2+}-activated Cl^--channel (CLCA) and the

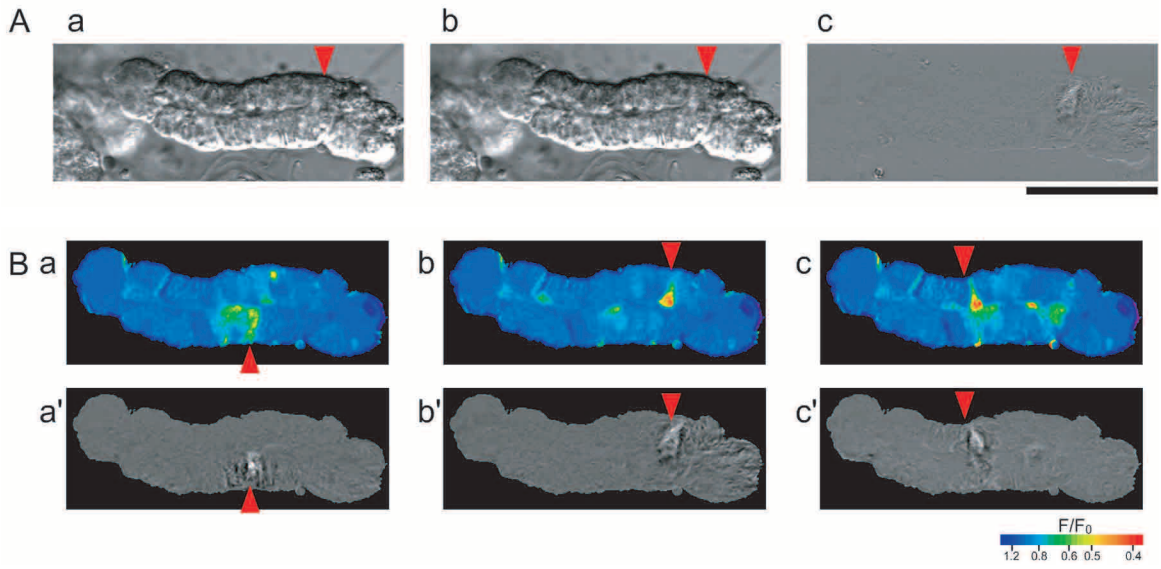

Figure 2. Spontaneous Ca^{2+} responses and associated changes in cell shape.
The fura-2 fluorescence image and the DIC image were taken simultaneously by the multiphoton microscopy system. A : Two DIC images (a) before and (b) after the change in cell shape, and (c) the subtracted DIC image that was created by image subtraction (image b-image a). Scale bar : 50 μm. B : The fura-2 ratio images (a-c) and the subtracted DIC images (a'-c') at 30 s (a and a'), 90 s (b and b'), and 180 s (c and c') after the start of the recording. Arrowheads : spontaneously responding cells. Reproduced from ref. 7.

cystic fibrosis transmembrane conductance regulator (CFTR), and a Na^+ channel, EnaC, are most likely the primary NaCl uptake mechanisms in the majority of salivary gland ducts. In contrast, NaCl efflux across the basolateral membrane of salivary ductal cells is primarily driven by Na^+, K^+ ATPase and by the activation of Cl^- and K^+ channels. Therefore, the cell volume would reflect the balance between the influx and efflux of these electrolytes. The time course of the enlargement of the luminal space in parotid ducts is much slower than that of cell swelling. Thus, the spontaneous Ca^{2+} oscillations implies that the concentration rises and declines periodically within a relatively short duration, which might favor cell swelling.

Our findings provide new insight into the function of purinergic receptors and highlight the possibility that the purinergic receptor-mediated spontaneous $[Ca^{2+}]i$ increase activates electrolyte absorption by ductal cells in the resting state. Further studies are needed to clarify the molecular mechanisms underlying ATP release and cell swelling, and also to determine the physiological relevance of spontaneous Ca^{2+} oscillations.

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REFERENCES

1. Barka T : Biologically active polypeptides in submandibular glands. J Histochem Cytochem 28 : 836-859, 1980
2. Melvin JE, Yule D, Shuttleworth T, Begenisich T : Regulation of fluid and electrolyte secretion in salivary gland acinar cells. Annu Rev Physiol 67 : 445-469, 2005
3. Shitara A, Tanimura A, Nezu A, Morita T, Tojyo Y : Multi-photon microscopic imaging of rat parotid ducts demonstrates cellular heterogeneity in Ca^{2+} responsiveness. Arch Oral Biol 52 : 1072-1078, 2007
4. Turner JT, Landon LA, Gibbons SJ, Talamo BR : Salivary gland P2 nucleotide receptors. Crit Rev Oral Biol Med 10 : 210-224, 1999
5. Takemura H, Horio Y : Spatial microenvironment defines Ca^{2+} entry and Ca^{2+} release in salivary gland cells. Biochem Biophys Res Commun 336 : 223-231, 2005
6. Li Q, Luo X, Zeng W, Muallem S : Cell-specific behavior of $P2X_7$ receptors in mouse parotid acinar and duct cells. J Biol Chem 278 : 47554-47561, 2003
7. Shitara A, Tanimura A, Sato A, Tojyo Y : Spontaneous oscillations in intracellular Ca^{2+} concentration via purinergic receptors elicit transient cell swelling in rat parotid ducts. Am J Physiol 297 : G1198-G1205, 2009
8. Foskett JK, Melvin JE : Activation of salivary secretion : coupling of cell volume and $[Ca^{2+}]_i$ in single cells. Science 244 : 1582-1585, 1989
9. Ohshima K, Shiba Y, Hirono C, Sugita M, Iwasa Y, Shintani H : Luminal space enlargement by carbachol in rat parotid intralobular ducts. Eur J Oral Sci 111 : 405-409, 2003