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Spatial microenvironment defines Ca^{2+} entry and Ca^{2+} release in salivary gland cells

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Abstract

The difference of Ca^{2+} mobilization induced by muscarinic receptor activation between parotid acinar and duct cells was examined. Oxotremorine, a muscarinic–cholinergic agonist, induced intracellular Ca^{2+} release and extracellular Ca^{2+} entry through store-operated Ca^{2+} entry (SOC) and non-SOC channels in acinar cells, but it activated only Ca^{2+} entry from non-SOC channels in duct cells. RT-PCR experiments showed that both types of cells expressed the same muscarinic receptor, M3. Given that ATP activated the intracellular Ca^{2+} stores, the machinery for intracellular Ca^{2+} release was intact in the duct cells. By immunocytochemical experiments, IP_3R2 colocalized with M3 receptors in the plasma membrane area of acinar cells; in duct cells, IP_3R2 resided in the region on the opposite side of the M3 receptors. On the other hand, purinergic P2Y2 receptors were found in the apical area of duct cells where they colocalized with IP_3R2. These results suggest that the expression of the IP_3Rs near G-protein-coupled receptors is necessary for the activation of intracellular Ca^{2+} stores. Therefore, the microenvironment probably affects intracellular Ca^{2+} release and Ca^{2+} entry.

Keywords: Acinar and duct cells; Ca^{2+} entry; Ca^{2+} release; IP_3 receptor; M3 receptor; P2Y receptor; Microenvironment

Increases in the cytoplasmic Ca^{2+} concentration ($[Ca^{2+}]_i$) regulate many cellular functions such as proliferation, transcription, metabolism, contraction, and exocytosis [1]. The stimulation of G-protein Gq-coupled receptors activates phospholipase Cβ (PLCβ), resulting in the hydrolysis of phosphatidylinositol 4,5-bisphosphate and the production of inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG). IP_3 binds its receptors on the intracellular Ca^{2+} store and releases Ca^{2+} from the store, causing the first phase of an increase in $[Ca^{2+}]_i$. The second phase is Ca^{2+} entry from the extracellular medium into the cell; this phase maintains a high level of $[Ca^{2+}]_i$. Extracellular Ca^{2+} entry after Gq-coupled receptor stimulation is mediated by two mechanisms. One is store-operated Ca^{2+} entry (SOC) and the other is non-store-operated Ca^{2+} entry (non-SOC). SOC channels, which are activated by the depletion of intracellular Ca^{2+} stores, are assumed to provide a major pathway for such Ca^{2+} entry [2]. Many cells also express non-SOC channels, which are activated by Gq-coupled receptor stimulation and support Ca^{2+} entry from the extracellular space. Non-SOC channels cannot be activated by store depletion, but some of them are opened by DAG [3,4]. Electrophysiological and pharmacological experiments have shown that there are multiple SOC and non-SOC channels that are expressed in various types of cells.

The salivary gland is an exocrine organ capable of secreting fluid and enzymes, and is regulated by autonomic nerves. It is made of tubular epithelia that are divided into two major domains. The distal end is the acinar unit, which produces the primary saliva, including the fluid and macromolecules. The proximal area is a duct that is thought to modify the primary saliva by absorbing and/or secreting electrolytes such as Na^+, Cl^-, and HCO_3^- [5,6]. The secretion and modification of saliva in the acini and ducts are triggered by the elevation of $[Ca^{2+}]_i$. Acetylcholine (ACh)

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is one of the most potent physiological stimulators of salivary glands. Muscarinic receptor stimulation induces the elevation of $[Ca^{2+}]_i$ in both acinar and duct cells. Using isolated rat parotid acini and ducts we found previously that carbachol, a muscarinic receptor agonist, induced Ca^{2+} release from intracellular stores and extracellular Ca^{2+} entry in acinar cells, but it activated only Ca^{2+} entry in duct cells [7].

In the present study, we examined and compared the difference in the $[Ca^{2+}]_i$ increase resulting from the stimulation of muscarinic receptors in parotid acinar and duct cells. Oxotremorine (OXO), a muscarinic–cholinergic agonist, induced intracellular Ca^{2+} release and extracellular Ca^{2+} entry through SOC and non-SOC channels in acinar cells, but it activated only Ca^{2+} entry from non-SOC channels in duct cells. In contrast, ATP released Ca^{2+} from the stores and activated both the SOC and non-SOC channels of duct cells. RT-PCR experiments showed that both types of cells expressed the same muscarinic receptor, M3. By immunocytochemical experiments, IP_3R2 colocalized with M3 receptors near the plasma membranes of acinar cells; in duct cells, IP_3R2 resided near the membrane opposite the M3 receptors. On the other hand, purinergic P2Y2 receptors were found in the apical area of duct cells where they colocalized with IP_3R2. These results indicate that G-protein-coupled receptors and the Ca^{2+} release machinery may need to be close to one another for Ca^{2+} to be released from the stores or enter from the extracellular space.

Materials and methods

Reagents. Fluo-3/AM and anti-goat Alexa 488 were obtained from Molecular Probes. Collagenase (type 4) was from Worthington. Anti-M3 receptor antibody, anti-mouse FITC, and anti-rabbit FITC were from Santa Cruz. Anti-IP3R2 mAb was a gift from Dr. K. Mikoshiba. Anti-P2Y2 antibody was from Alomone. Other chemicals were from Sigma.

Isolation of acini and ducts. The Animal Welfare Guidelines of Sapporo Medical University were followed in all the studies and experiments. Parotid acini and ducts were isolated from male Wistar rats (4–5 weeks old) as described previously [7,8]. Briefly, the parotid gland was removed, trimmed of the connective tissue, and cut into small pieces. They were digested with collagenase (2 mg/ml) in serum-free Dulbecco's modified Eagle's medium (DMEM) for 20 min at 37 °C with constant shaking and gentle pipetting. The isolated acini and ducts were incubated with Fluo-3/AM (10 μM) for 20 min in DMEM at 37 °C. After washing, the acini and ducts were resuspended in Krebs–Ringer–Hepes medium (KRH) containing 0.2% BSA. The composition of KRH was as follows: 120 mM NaCl, 5.4 mM KCl, 1.0 mM $CaCl_2$, 0.8 mM $MgCl_2$, 11.1 mM glucose, and 20 mM Hepes (pH 7.4). In the experiments performed in the absence of extracellular Ca^{2+}, Ca^{2+}-free KRH containing 0.2 mM EGTA was used.

RT-PCR. To purify total RNA from acinar and duct cells, several clusters of acini and several small duct fragments were collected in glass micropipettes under microscopic examination. Typical microscopic images of a cluster of acini and a duct fragment are shown in Figs. 1A and B, respectively. Collected acini and ducts were transferred to clean microcentrifuge tubes and broken up by three freeze-thaw cycles. Total RNA was purified from these acini and ducts using an RNeasy Mini Kit (Qiagen). cDNAs were synthesized for 60 min at 42 °C using 200 U of Superscript II RNase H^- Reverse Transcriptase (Invitrogen).

The primers to amplify α-amylase, kallikrein, GAPDH, and M1–M5 were used (see Supplemental information for sequences). PCR was carried out in a GeneAmp PCR System 9700 (Applied Biosystems). The thirty amplification cycles for α-amylase, kallikrein, and GAPDH were conducted with denaturation at 94 °C for 30 s, annealing at 55 °C for 1 min, and extension at 72 °C for 1 min, followed by a final extension at 72 °C for 5 min. The cycling conditions for M1–M5 were 35 cycles of 94 °C for 1 min, 55 °C for 1 min, and 72 °C for 1 min. A second set of nested primers, which immediately followed the first set of primers in their sequences, was used. For the reaction, 1 μl of the PCR product from the first round of amplification (20 μl) was used as a template for the second round of amplification. The PCR conditions were the same as in the first round. The resulting PCR mixtures were then analyzed by gel electro-

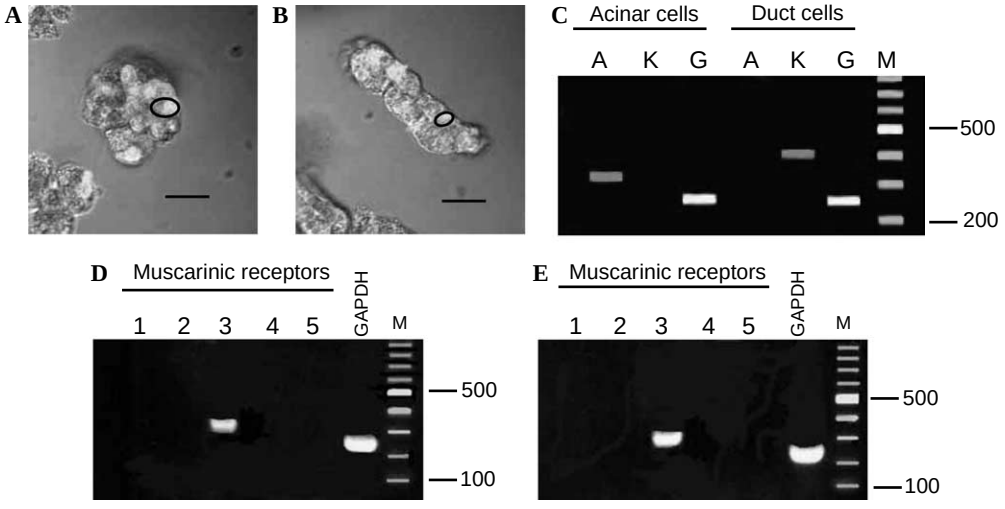

Fig. 1. Acini and ducts were isolated from the parotid gland by digestion with collagenase. (A,B) Typical images of an acinar cluster and a duct fragment loaded with Fluo-3, respectively. Circles show single cells whose Ca^{2+} responses are shown in Figs. 2A and B. Bar: 20 μm. (C) RT-PCR of α-amylase and kallikrein. α-Amylase is a marker enzyme for acinar cells and kallikrein is a marker for duct cells. Lane A, α-amylase. Lane K, kallikrein. Lane G, glyceraldehyde 3-phosphate dehydrogenase (GAPDH). (D,E) RT-PCR of muscarinic receptors. Muscarinic receptor subtype 3 was detected in both acinar (D) and duct (E) cells.