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Comparison of agonist-induced Ca^{2+} responses in rat submandibular acini and ducts

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Summary Changes in intracellular Ca^{2+} concentration ($[Ca^{2+}]i$) induced by agonists were simultaneously monitored in rat submandibular acini and ducts using a Ca^{2+} imaging system. Substance P (SP) elicited marked increases in $[Ca^{2+}]i$ in acini but not in ducts. Carbachol (CCh) increased $[Ca^{2+}]i$ in both acini and ducts, but the maximal level was higher in acini than in ducts. In contrast, epinephrine (Epi) also induced an increase in $[Ca^{2+}]i$ in acini and ducts, but to a greater extent in ducts than in acini. Isoproterenol (ISO) caused a small but significant increase in $[Ca^{2+}]i$ in ducts but not acini. Reverse transcriptase-polymerase chain reaction (RT-PCR) analysis using total RNA extracted from highly purified acinar and ductal cells showed that substance P receptor mRNA was present in acini at higher levels than in ducts. In contrast, α_{1a}-adrenoceptor mRNA was more strongly expressed in ducts than in acini. The muscarinic receptors (M_3 and M_5) and β-adrenoceptors (β_1 and β_2) were expressed at equivalent levels in both cell types. These results confirm that acini and ducts exhibit significant differences in agonist-induced Ca^{2+} responses. Furthermore, substance P- and epinephrine-induced Ca^{2+} responses were consistent with receptor mRNA expression in acini and ducts, but carbachol- and isoproterenol-induced $[Ca^{2+}]i$ increases were not.

Introduction

Salivary gland parenchyma consists of secretory endpieces, which are mostly acinar, and a branching ductal system. Saliva is initially produced as an isotonic primary secretion in the salivary acini. The primary fluid is modified by ductal reabsorption of sodium chloride and secretion of potassium and bicarbonate as it flows through the salivary ducts.[1] The saliva is finally discharged as a hypotonic fluid into the oral cavity. The different physiological

Abbreviations: $[Ca^{2+}]i$, intracellular calcium concentration; CCh, carbachol; Epi, epinephrine; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HEPES, 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid; IP_3, inositol 1,4,S-trisphosphate; ISO, isoproterenol; Prop, propranolol; Pt, phentolamine; RT-PCR, reverse transcriptase-polymerase chain reaction; SP, substance P

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functions of the salivary acinar and ductal cells suggest that these two cell types differ in the distribution of membrane receptors or in agonist sensitivity.

In rat salivary acinar cells, stimulation of muscarinic, α-adrenergic and substance P (SP) receptors causes breakdown of phosphoinositide, resulting in the formation of inositol phosphates and changes in intracellular Ca^{2+} concentration ($[Ca^{2+}]i$).[1,2] β-Adrenergic stimulation does not change the $[Ca^{2+}]i$ in salivary acinar cells.[3] However, due to the technical difficulties of isolating ductal cells, there is much less information about Ca^{2+} signalling in ductal cells than in acinar cells. Several studies have reported that ductal cells are less sensitive than acinar cells to muscarinic agonists and SP.[4–6] Also, β-adrenergic stimulation has been demonstrated to cause a small but significant increase in $[Ca^{2+}]i$ in ductal cells.[6–8] These findings suggest that agonist-induced Ca^{2+} signals in salivary ductal cells are not necessarily identical with those in acinar cells.

In the present study, we used a Ca^{2+}-imaging system to simultaneously monitor changes in $[Ca^{2+}]i$ induced by agonists in individual acini and ducts prepared from rat submandibular glands. Moreover, we performed reverse transcriptase-polymerase chain reaction (RT-PCR) analysis using highly purified acinar and ductal preparations to compare the expression of surface receptors in the two cell types.

Materials and methods

Chemicals

SP, carbachol (CCh), isoproterenol (ISO), epinephrine (Epi), propranolol (Prop) and bovine serum albumin (BSA) were purchased from Sigma (St. Louis, MO, USA). Phentolamine (Pt) was obtained from Nippon Chiba-Geigy (Hyogo, Japan). Fura-2 [acetoxymethyl ester (AM)] and HEPES were from Dojin Laboratories (Kumamoto, Japan). Collagenase P was from Boehringer Mannheim (Darmstadt, Germany). Restriction enzymes were from Toyobo (Osaka, Japan), Roche (Basel, Switzerland), New England Biolabs (Beverly, MA, USA) and Boehringer Mannheim. All other reagents were of analytical grade.

Preparation of rat submandibular gland cells

Male rats of the Wistar strain (7–12 weeks) were anaesthetized with diethyl ether and killed by cardiac puncture. The two submandibular glands removed from each rat were finely minced and incubated for 20 min at 37 °C in 10 mL Dulbecco's modified eagle medium (D-MEM) containing collagenase P (0.24 mg/mL), 2 mM L-glutamine, 10 mM HEPES–NaOH (pH 7.4) and 0.2% BSA. After dispersion, the cell suspension was passed through a nylon mesh, and the filtrate was centrifuged (400 × *g* for 5 s). The resulting pellet was resuspended in 20 mL fresh modified Hanks' balanced salt solution (Sigma) buffered with 20 mM HEPES–NaOH, pH 7.4 (HBSS-H) and washed by 1-min centrifugation at 100 × *g*. The cells were washed again and finally resuspend in fresh HBSS-H. This preparation, consisting of dispersed acini and ductal fragments, was employed for monitoring $[Ca^{2+}]i$.

Determination of $[Ca^{2+}]i$

The mixture of acini and ducts was incubated with 2 μM fura-2/AM for 45 min at room temperature and washed twice with fresh HBSS-H without BSA. The fura-2-loaded cells were attached to a Cell-Tak- (BD Biosciences, Bedford, MA, USA) coated glass coverslip at the bottom of a small recording chamber, which was mounted on the stage of an inverted microscope (Diaphot; Nikon, Tokyo, Japan) equipped with a 40×, 1.3 numeric aperture, oil immersion objective (Fluor 40, Nikon). Fura-2 fluorescence images were acquired as described elsewhere[9] using an ARGUS HiSCA imaging system (Hamamatsu Photonics, Shizuoka, Japan). $[Ca^{2+}]i$ was calculated separately in ducts and acini from the fura-2 fluorescence ratio (340/380 nm), as described by Grynkiewicz et al.[10]

Purification of submandibular ducts and acini

The mixture of ducts and acini prepared by collagenase digestion was centrifuged on an isotonic 40% Percoll solution, as described previously,[4,8] which separated the cells into two distinct populations, one at the interphase of the tube, consisting predominantly of ducts, and the other at the bottom, an acini-rich population. Each population was collected, washed and resuspended in cold fresh HBSS-H. To obtain highly purified cells, duct- and acini-rich populations were transferred into cooled glass dishes, and each cell type was collected from the appropriate enriched population using an Eppendorf micro pipetter under a stereomicroscope (Olympus, Tokyo, Japan). The purified ducts and acini were quickly suspended in cold RNA later™ (Takara, Kyoto, Japan) to prevent denaturation of RNA and subjected to RT-PCR analysis of membrane receptors.