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Crosstalk between purinergic receptors and canonical signaling pathways in the mouse salivary gland

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ABSTRACT

Isolated clusters of mouse parotid acinar cells in combination with live cell imaging were used to explore the crosstalk in molecular signaling between purinergic, cholinergic and adrenergic pathways that integrate to control fluid and protein secretion. This crosstalk was manifested by (1) β-adrenergic receptor activation and amplification of P2X4R evoked Ca^{2+} signals, (2) β-adrenergic-induced amplification of P2X7R-evoked Ca^{2+} signals and (3) muscarinic receptor induced activation of P2X7Rs via exocytotic activity. The findings from our study reveal that purinoceptor-mediated Ca^{2+} signaling is modulated by crosstalk with canonical signaling pathways in parotid acinar cells. Integration of these signals are likely important for dynamic control of saliva secretion to match physiological demand in the parotid gland.

1. Introduction

Saliva production is positively regulated by cholinergic and adrenergic autonomic neural input and crosstalk between these signaling pathways to facilitate secretory activity has been demonstrated extensively [1–5]. In addition to these canonical pathways, a non-cholinergic non-adrenergic (NANC) pathway regulating protein and fluid secretion [6–9] has also been implicated. One of the primary candidates for NANC input is via activation of ionotropic purinergic receptors [10–16]. Moreover, we recently demonstrated that ionotropic P2X4 and P2X7 receptor (P2X4R and P2X7R) activation can contribute to Ca^{2+} dynamics and protein exocytosis in acinar cells isolated from the mouse parotid salivary gland [17].

In the current study we investigated whether there was crosstalk between the canonical Ca^{2+} signaling pathways and the NANC/purinergic signaling pathway. Enhancement of P2X4R- or P2X7R-mediated intracellular calcium ($[Ca^{2+}]_i$) rises was revealed following ß-adrenergic receptor (β-AR) activation. Although this signaling was largely mediated by receptor activation and a PKA-dependent mechanism, direct activation of PKA or Epac using pharmacological compounds could also enhance P2X-mediated Ca^{2+} signals. Additionally, we assessed whether there was crosstalk between muscarinic receptor mediated and P2X receptor mediated signaling. In these experiments a selective P2X7R blocker was used [18,19] to investigate whether exocytotic activity evoked by muscarinic receptor activation induced luminal ATP release and P2X7R activation. The current study furthers our understanding of how canonical Ca^{2+} pathways in conjunction with NANC Ca^{2+} signaling pathways may integrate to regulate $[Ca^{2+}]_i$ levels and secretory activity in the parotid gland.

2. Materials and methods

2.1. Animal use

Male NIH Swiss Webster mice (18–25 g; Charles River Laboratories International, Inc., Wilmington, MA, USA) were euthanized by CO_2 asphyxiation and puncture of the heart. All experiments using animals were approved by and carried out in strict accordance with the policies of the University of Toledo Institutional Animal Care and Use Committee and conformed to the Guide for the Care and Use of Laboratory Animals, National Institutes of Health Publication No. 85-23 (National Research Council, National Academy Press, Washington, DC, 1996).

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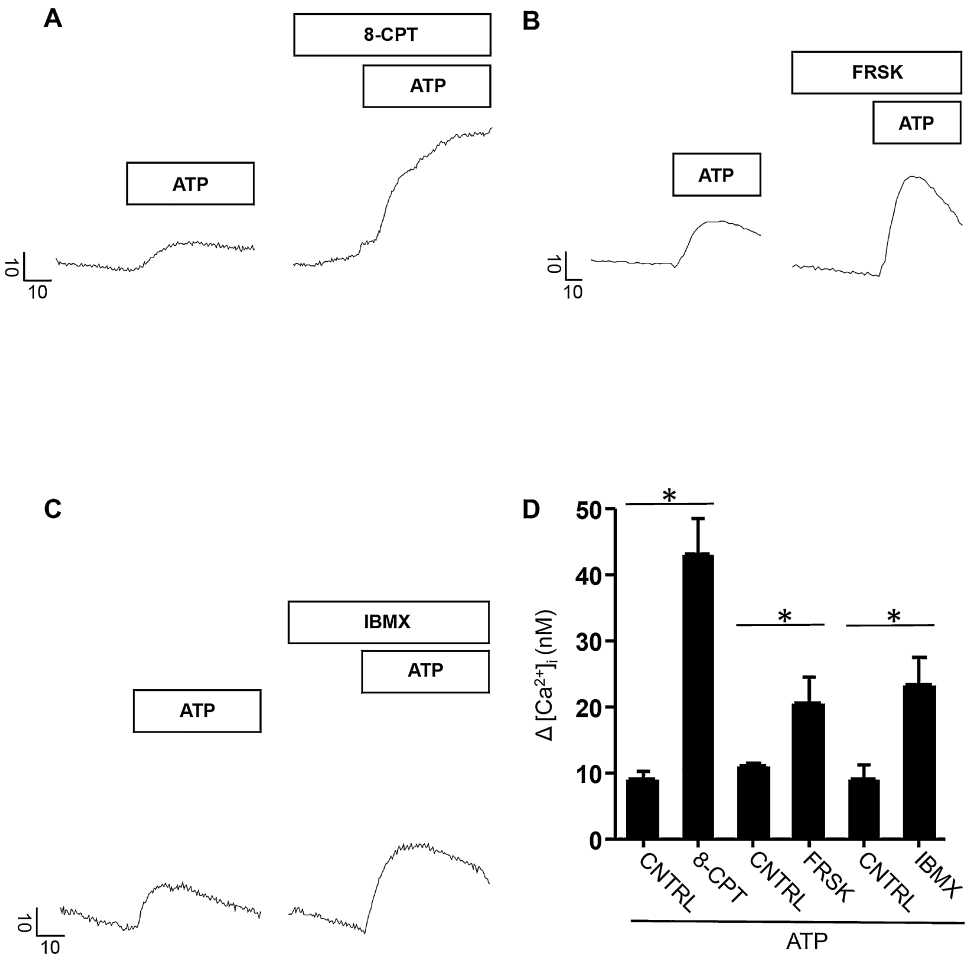

Fig. 1. Pharmacological elevations of cAMP enhanced P2X4R evoked changes in $[Ca^{2+}]_i$ levels. (A–C) Representative line traces showing maximal $\Delta[Ca^{2+}]_i$ evoked by 10 μM ATP alone or following treatment with 1 mM 8-CPT, 10 μM FRSK and 100 μM IBMX, respectively. Bars above line traces indicate application of compounds. Vertical scale bar indicates $\Delta[Ca^{2+}]_i$ (nM) and horizontal scale bar shows time in seconds (s). Prestimulus $[Ca^{2+}]_i$ values for representative control cell (CNTRL, ATP only) and treated cell traces were 155.3 nM and 293.5 nM (ATP + 8-CPT), 82.7 nM and 118 nM (ATP + FRSK), 188.7 nM and 167.9 nM (ATP + IBMX), respectively. (D) Bar graph depicts maximal $\Delta[Ca^{2+}]_i$ following ATP application in standard recording saline, ($3 \leq n \leq 5$) or co-application with either 8-CPT ($n = 4$) or FRSK ($n = 3$) or IBMX ($n = 5$), respectively. Changes in $[Ca^{2+}]_i$ levels were significantly greater during co-treatment with ATP and 8-CPT ($p < 0.0001$), FRSK ($p < 0.003$) or IBMX ($p < 0.002$). Significant difference from control values as indicated by asterisk (*). Prestimulus $[Ca^{2+}]_i$ values for control and treated bars were 147.4 ± 5.3 nM and 181.4 ± 46 nM (ATP + 8-CPT), 91.8 ± 3.5 nM and 117.9 ± 10.2 nM (ATP + FRSK) and 175.7 ± 6.1 nM and 187.8 ± 13.4 nM (ATP + IBMX), respectively. Parotid acinar cells were pretreated with 8-CPT, FRSK, and IBMX for 10–15 min in the above experiments prior to ATP challenge.

2.2. Chemicals

Adenosine 5′-triphosphate disodium salt hydrate (ATP), 2′(3′)-O-(4-Benzoylbenzoyl)adenosine 5′-triphosphate triethylammonium salt (Bz-ATP), 3-Isobutyl-1-methylxanthine (IBMX), carbamoylcholine chloride (CCh), isoproterenol hydrochloride (ISO) and forskolin (FRSK) were obtained from Sigma Chemicals (St Louis, MO, USA). 2-Aminoethoxydiphenylborate (2-APB) was purchased from Calbiochem (San Diego, CA, USA). 8-(4-Chlorophenylthio) adenosine 3′,5′-cyclic monophosphate (8-CPT) and cAMP-dependent protein kinase inhibitor peptide (myristoylated-14-22) amide (PKI) were obtained from Enzo Life Sciences (Farmingdale, NY, USA). 1-(2,3-Dichlorophenyl)-*N*-[[2-(2-pyridinyloxy) phenyl]methyl]-1*H*-tetrazol-5-amine (A839977), a potent P2X7R antagonist and 8-pCPT-2-O-Me-cAMP-AM were obtained from Tocris Bioscience (Bristol, UK).

2.3. Isolation of parotid acini

Dispersed mouse parotid acini were obtained by collagenase digestion [20]. Dissected parotid glands were minced with scissors. The minced glands were subjected to step-wise digestion by suspension in 20 ml of Eagle's minimal essential medium containing collagenase-P (0.04 mg/ml; Roche Applied Science, Indianapolis, IN, USA), 0.01% glutamine and 1% bovine serum albumin (BSA). Parotid gland pieces were subjected to 20 min incubation. This step was followed by two 15 min incubations in collagenase digestion solution with shaking (65 rpm) at 37 °C. After the first incubation, centrifugation was performed at 210 × *g* for 1 min. The pellet was resuspended in a fresh aliquot of oxygenated solution. Next, parotid tissue pieces were passed through glass fire-polished pipettes with progressively smaller tips. Following digestion, dispersed parotid acini were rinsed in basal Eagle's medium (BME) without BSA and centrifuged at 70 × *g* for 1 min. The pellet was resuspended in 20 ml of attachment BME solution containing 0.01% glutamine, 0.02% penicillin–streptomycin (Sigma Aldrich, St. Louis, MO, USA) and 1% BSA. Parotid acinar clumps were loaded with 5 μM fura-2AM (Calbiochem, San Diego, CA, USA) for 30–40 min for live cell imaging.

2.4. Live cell fluorescence and time differentiated imaging

Live cell imaging was performed using a Polychrome IV monochromator-based high-speed digital imaging system (TILL