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High Extracellular Ca^{2+} Stimulates Ca^{2+}-Activated Cl^- Currents in Frog Parathyroid Cells through the Mediation of Arachidonic Acid Cascade

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Abstract

Elevation of extracellular Ca^{2+} concentration induces intracellular Ca^{2+} signaling in parathyroid cells. The response is due to stimulation of the phospholipase C/Ca^{2+} pathways, but the direct mechanism responsible for the rise of intracellular Ca^{2+} concentration has remained elusive. Here, we describe the electrophysiological property associated with intracellular Ca^{2+} signaling in frog parathyroid cells and show that Ca^{2+}-activated Cl^- channels are activated by intracellular Ca^{2+} increase through an inositol 1,4,5-trisphophate (IP_3)-independent pathway. High extracellular Ca^{2+} induced an outwardly-rectifying conductance in a dose-dependent manner (EC_{50}~6 mM). The conductance was composed of an instantaneous time-independent component and a slowly activating time-dependent component and displayed a deactivating inward tail current. Extracellular Ca^{2+}-induced and Ca^{2+} dialysis-induced currents reversed at the equilibrium potential of Cl^- and were inhibited by niflumic acid (a specific blocker of Ca^{2+}-activated Cl^- channel). Gramicidin-perforated whole-cell recording displayed the shift of the reversal potential in extracellular Ca^{2+}-induced current, suggesting the change of intracellular Cl^- concentration in a few minutes. Extracellular Ca^{2+}-induced currents displayed a moderate dependency on guanosine triphosphate (GTP). All blockers for phospholipase C, diacylglycerol (DAG) lipase, monoacylglycerol (MAG) lipase and lipoxygenase inhibited extracellular Ca^{2+}-induced current. IP_3 dialysis failed to induce conductance increase, but 2-arachidonoylglycerol (2-AG), arachidonic acid and 12S-hydroperoxy-5Z,8Z,10E,14Z-eicosatetraenoic acid (12(S)-HPETE) dialysis increased the conductance identical to extracellular Ca^{2+}-induced conductance. These results indicate that high extracellular Ca^{2+} raises intracellular Ca^{2+} concentration through the DAG lipase/lipoxygenase pathway, resulting in the activation of Cl^- conductance.

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Introduction

Parathyroid hormone (PTH) regulates extracellular free Ca^{2+} concentration ($[Ca^{2+}]_o$) in cooperation with 1,25-dihydroxycholecalciferol (1,25-$(OH)_2D_3$)and calcitonin. On the other hand, $[Ca^{2+}]_o$ regulates the secretion of PTH from parathyroid cells through an extracellular Ca^{2+}-sensing receptor (CaR) [1,2]. High $[Ca^{2+}]_o$ inhibits the secretion, whereas low $[Ca^{2+}]_o$ enhances the secretion. It is believed that extracellular Ca^{2+} binds to CaR, and as a consequence inhibits the secretion of PTH via intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$). However, the molecular mechanism by which $[Ca^{2+}]_i$ regulates the secretion is not well elucidated.

The CaR belongs to the family C of G protein-coupled receptors (GPCRs) and has a large extracellular domain that binds external Ca^{2+} and other CaR agonists. The CaR controls various signaling pathways [3–5]. Calcium binding to the receptor results in G protein-dependent activation of phosphatidylinositol-specific phospholipase C (PI-PLC) causing accumulation of inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG) and promoting rapid release of Ca^{2+} from its intracellular stores [6,7]. The CaR-mediated activation of PI-PLC in parathyroid cells is a direct G protein-mediated process, while activation of phospholipase A_2 (PLA_2) and D by high $[Ca^{2+}]_o$ are probably indirect, through the mediation of PLC-dependent activation of protein kinase C [4].

DAG can be utilized for 2-arachidonoylglycerol (2-AG) generation [8]. PLC hydrolyzes phosphatidylinositol and produces arachidonic acid-containing DAG. Then, DAG is converted into 2-AG by the action of DAG lipase. Next, 2-AG is hydrolyzed by monoacylglycerol (MAG) lipase and yields arachidonic acid. Finally, arachidonic acid is oxidized by cycloxygenase (COX), lipoxygenase (LO) or epoxygenase (cytochrome P450).

The mitogen-activated protein kinase (MAP kinase) pathways are found in bovine parathyroid cells [9]. MAP kinase is activated by dual tyrosine and threonine phosphorylation [10]. Phosphorylated MAP kinase can phosphorylate cytosolic phospholipase A_2 ($cPLA_2$) [11]. In bovine parathyroid cells, the MAP kinase is activated by CaR [9]. There are several mechanisms by which