PKA and Epac mediate cyclic AMP responses in pancreatic acinar cells

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Background: Acute pancreatitis is associated with aberrant intracellular activation of zymogens and the retention of these enzymes within the acinar cell. Supraphysiologic concentrations of cholecystokinin (or its analog caerulein) and the muscarinic agonist carbachol cause intracellular activation and retention of zymogens, thereby damaging the acinar cell and providing an in vitro model by which to study acute pancreatitis. Physiologic doses of these secretagogues cause increased enzyme secretion. Previous studies have shown that increased intracellular cAMP can potentiate zymogen activation and enzyme secretion. There are two potential downstream mediators of the effect of cAMP: the well-characterized PKA pathway and the newly discovered Epac (exchange protein activated by cAMP) pathway. Epac is a cAMP binding protein that activates the small G protein, RAP1, by promoting the exchange of GDP for GTP. The effects of the Epac pathway in the pancreatic acinar cell are unknown.

Aim: To elucidate the contributions of PKA and Epac on zymogen activation and enzyme secretion in the pancreatic acinar cell.

Hypothesis: Both PKA and Epac mediate the effects of cAMP on zymogen activation and secretion.

Methods: Isolated acinar cells were stimulated with supraphysiologic doses of carbachol in the presence and absence of the cAMP analog, 8-Br-cAMP and assayed for trypsin and chymotrypsin activation and amylase secretion. The specific PKA inhibitors, PKI and KT-5729, were added to the above conditions to elucidate the contribution of the PKA pathway. Acinar cells were also stimulated with the Epac agonist, 8-pCPT-2’-O-Me-cAMP in the presence and absence of carbachol and specific PKA inhibitors. These experiments were repeated with a second Epac agonist, 8-pHPT-2’-O-ME-cAMP, to confirm the effects of Epac stimulation. The effects of 8-Br-cAMP and 8-pCPT-2’-O-ME-cAMP on PKA dependent CREB phosphorylation were studied to confirm the specificity of the effects of Epac stimulation. To determine whether Epac affects responses under physiologic conditions, acini were costimulated with physiologic doses of carbachol or caerulein and 8-pCPT-2’-O-Me-cAMP.

Results: PKA inhibitors reduced, but did not eliminate the effects of 8-Br-cAMP on carbachol-induced zymogen activation and enzyme secretion, suggesting that a PKA independent pathway may also mediate the effects of cAMP. The Epac agonist, 8-pCPT-2’-O-Me-cAMP, caused a concentration dependent increase in carbachol-induced trypsin and chymotrypsin activation and amylase secretion. Stimulation by 8-pHPT-2’-O-ME-cAMP had similar effects. The effects of the Epac agonists were not affected by PKA inhibitors and did not affect CREB phosphorylation, suggesting that the effects of Epac agonists are do not involve PKA. Epac stimulation also enhanced zymogen activation and amylase secretion induced by physiologic doses of carbachol and caerulein.

Conclusion: Both the PKA and Epac pathways can stimulate cAMP mediated effects on zymogen activation and enzyme secretion. The redundancy of the two pathways may provide backup mechanisms for important cellular actions. Alternatively, PKA and Epac might act on different cellular pools that are not distinguishable by the assays used in this study. The molecular targets of PKA and Epac in the acinar cell remain unclear and warrant further study.