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CCK ACTIVATES PAK2 THROUGH RAC1 AND BETA PIX IN RAT PANCREATIC ACINAR CELLS

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Cholecystokinin activates small G proteins of the Rho family and Rac1 plays a role in regulating secretion, and the actin cytoskeleton in acinar cells. However, little is known of its upstream regulators and downstream effectors. We used a GST-Rac1 fusion protein loaded with GDP or GTPγS as an affinity reagent to pull out interacting proteins from rat pancreatic lysate, which were then separated by 1D gel electrophoresis. Two protein bands binding uniquely to Rac loaded with GTPγS were identified by MALDI TOF tandem mass spectrophotometer as p21 activated kinase (PAK) 2 and β2Pix. Western blotting identified PAK2 as the major form of PAK present in rat pancreatic acini; β1Pix and β2Pix were both present, and level of β2Pix but not β1Pix was increased by CCK stimulation. CCK activated PAK2 activity, 3 fold at 2 min followed by a decrease; a maximal increase was seen at 100 pM CCK. Both time course and dose response for PAK2 paralleled the activation of Rac1. βPix antisense but not sense oligonucleotide treatment led to a reduction in activation of both PAK2 and Rac following stimulation with CCK, and significantly decreased CCK-induced amylase secretion. Dominant negative Rac1 (RacN17) and PAK interacting domain (PID) also decreased PAK2 activation and CCK-induced amylase secretion. Furthermore, βPix and PAK antisense oligonucleotide as well as RacN17 and PID significantly reduced SRE-mediated gene transcription stimulated by CCK. These results suggests that βPIX is a regulator for both PAK and Rac activation and that PAK2 may mediate some of the actions of Rac in rat pancreatic acinar cells.

Key Words: Cholecystokinin, Pancreatic acinar cells, Beta pix, PAK2