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INVOLVEMENT OF CYCLIC NUCLEOTIDE PHOSPHODIESTERASE 4B IN AMYLASE RELEASE IN PAROTID ACINAR CELLS

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In salivary parotid acinar cells, stimulation of b-adrenergic receptors provokes exocytotic release of amylase via an increase in cellular cAMP level. Therefore, regulation of cellular cAMP level is important. The cellular cAMP level is regulated by activities of adenylate cyclase and cyclic nucleotide phosphodiesterase (PDE), synthesis and hydrolysis enzymes of cAMP, respectively. At present 11 kinds of families of PDE including 6 cAMP-PDE families have been identified by amino-acid sequence similarities, biochemical properties and inhibitor profiles. We here investigated the role of cAMP-PDE in exocytotic release of amylase in rabbit parotid acinar cells. Rabbit parotid acinar cells were prepared using trypsin and collagenase. Cellular cAMP level was determined by a radioimmunoassay kit. Amylase activity was assayed by the method of Bernfeld (1955). Assay of PDE activity was carried out using [^{3}H]cAMP as a substrate. When effects of PDE inhibitors on amylase release in rabbit parotid acinar cells were examined, the b-adrenergic agonist isoproterenol-induced amylase release was clearly enhanced in the presence of rolipram, a PDE4 inhibitor. Isoproterenol-induced cAMP formation was also enhanced in the presence of rolipram. When the cytosol of rabbit parotid acinar cells was applied on a DEAE ion-exchange column, a fraction containing cAMP-PDE activity was eluted. Ca^{2+}/calmodulin and cGMP had no effect on cAMP-PDE activity in the fraction. A band cross-reacted to anti-PDE4B antibody was detected in the cAMP-PDE activity fraction by immunoblotting, but no band cross-reacted to anti-PDE4A, PDE4C or PDE4D antibodies. When the fraction containing cAMP-PDE activity was suspended with protein A-sepharose 4FF conjugated with anti-PDE4B antibody, incubated and centrifuged, cAMP-PDE activity was not detected in the supernatant. When the cAMP-PDE activity fraction eluted from the DEAE ion-exchange column was further applied on a MonoQ ion-exchange column, three cAMP-PDE activity fractions were isolated. The cAMP-PDE activities in the three fractions were clearly inhibited by rolipram. These results suggest that PDE4B isoforms contribute to regulation of cAMP level and regulatory amylase secretion.

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THE ROLE OF PROTEIN TYROSINE PHOSPHATASE 1B IN HORMONE-STIMULATED Ca^{2+} INFLUX IN PANCREATIC ACINAR CELLS

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Depletion of inositol 1,4,5-trisphosphate (IP_3)-sensitive Ca^{2+} stores generates a yet unknown signal, which leads to increase in Ca^{2+} influx in different cell types (J.W. Putney, 1986). Here we describe a mechanism that modulates this store-operated Ca^{2+} entry (SOCE) by tyrosine phosphorylation/dephosphorylation in HEK293 cells. Since the molecular identity of the SOCE channel is not yet known, we have used TRPV6, a Ca^{2+} channel of the TRP superfamily, to study the effect of tyrosine phosphorylation/dephosphorylation in TRPV6-transfected HEK293 cells and to identify the site of tyrosine phosphorylation in TRPV6. We show that Ca^{2+} influx following store-depletion by acetylcholine (via IP_3) or by thapsigargin (via inhibition of Ca^{2+} uptake into the store) leads to inhibition of the tyrosine phosphatase PTP1B activity in both, untransfected and TRPV6-transfected, HEK293 cells. Since Ca^{2+} does not directly inhibit PTP1B, we assumed an intermediate signal, which links the rise in cytosolic Ca^{2+} concentration and PTP1B inhibition. We could show that Ca^{2+} influx is followed by generation of reactive oxygen species (ROS) and could be reduced in the presence of catalase. H_2O_2 directly added to HEK293 cells inhibited PTP1B activity and led to increase in Ca^{2+} influx. PP1, an inhibitor of the Src family tyrosine kinases, prevented H_2O_2-induced Ca^{2+} influx. These data indicate that ROS act as fine tuning modulators of Ca^{2+} entry. Similar as with H_2O_2 Ca^{2+} influx was increased following addition of the tyrosine phosphatase inhibitor N, N-dimethyl-hydroxoamido hydroxovanadat (DMHV) in both untransfected and TRPV6-transfected cells. This effect of DMHV was further increased by co-transfection with PTP1B and Src. PTP1B interacts with the N-terminal domain of TRPV6 within a region of amino acids 1-191 as shown by co-immunoprecipitation and "bimolecular fluorescence complementation". Point mutation of tyrosines 161 and 162 in the TRPV6 protein abolished the DMHV effect on Ca^{2+} influx and the susceptibility of TRPV6 to be tyrosine phosphorylated by Src. We conclude that phosphorylation/dephosphorylation of tyrosines in position 161/162 is essential for modulation of Ca^{2+} influx through TRPV6 Ca^{2+} channels in HEK293 cells. Since the protein structure of the SOCE channel has not yet been identified it remains open, whether or not direct tyrosine phosphorylation/dephosphorylation of this channel is also involved in the regulation of Ca^{2+} influx.