Molecular Characterization of Miniglucagon receptor(s) from Rats hepatocytes

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Abstract:

Miniglucagon, the C-terminal (19-29) fragment processed from glucagons, exerts a biphasic effect on liver plasma membranes, evokes positive inotropic effect in cardiac cells, and modulates insulin release from beta cells via a specific miniglucagon receptor (Mgr) which is linked to a pertussis toxin sensitive G-protein. The present study was designed for purification and characterization of mGRs by affinity chromatography on immobilized miniglucagon and optimization of conditions required for solubilization of liver plasma membrane proteins. The plasma membrane pellets prepared from Spraque-Dawley rats hepatocytes were solubilized with a buffer A1 containing 1percent Triton X-100. A preliminary affinity chromatography protocol with simple column apparatus (such as Pasteur pipette, 10 ml disposable syring) was developed to purify the Mgr. 2mg of miniglucagon peptide ligands were coucpled to 1 ml of EAH Sepharose 4B gel by using 1-cyclohexyl-3-(2-morpholinoethyl) carbodiimide meth-p-toluene sulfonae reaction. Then buffer A2 containing 0.1 percent Triton X-100 and 200 mM NaCl, and elution buffer containing 2M urea and 0.4M NaCl were used for ewceptor binding to ligand, removing excess uncoupled proteins, and elution of mGR from affinity column, respectively. The eluted proteins were analyzed by denaturing and native SDS-PAGE in Mini PROTEIN II dual slab cell and by UV absorbencies at 280nm. Furthurmore, conditions for solubilization of plasma membrane proteins were optimized. According to the results obtained it was concluded that, mGR may be comprised of four polypeptide subunits possibly rich in basic amino acids or there may be different types of miniglucagon receptors in liver plasma membranes and it was shown, that plasma membrane preparations with about 1 mg/ml protein content can be effectively solubilized by incubating in 0.125 mg/ml of Triton X-100 detergent at 4o C for 16 h.

Key Words : Peptide Fragments / Glucagon / Glucagon (19-29) / MOLECULAR BIOLOGY