A224

Preliminary studies on mini-glucagon receptor(s) from rat hepatocytes

Mini - glucagon, the C-terminal (19-29) fragment processed from glucagon, exerts a biphasic effect on liver plasma membrane Ca^{+} pumps, evokes positive ionotropic effect in cardiac cells, and modulates insulin release from beta cells via a G-protein coupled receptor, which is not yet isolated and characterized. Here we report the solubilization and purification of mini-glucagon receptor(s) by affinity chromatography. The plsma membranes from Spraque-Dawley rats hepatocytes were solubilized with a buffer containing 1% Triton X-100 at pH7.5. A preliminary affinity chromatography protocol was developed to purify mini -glucagon receptor(s). mini Glucagon (2mg) was coupled to EAH-Sepharose 4B gel in 1-cyclonexy 1-3-(2-morpholinoethy10 carbodiimide metho-p Toluenesulfonate reaction. Mini - glucagon couple dgel was packed into a column. After removing uncoupled proteins from the colums, mini-glucagon receptor(s) proteins were eluted and the eluted proteins were analyzed by denaturing and native SDS-PAGE in mini PROTEIN II dual slab cell and by UV absorbances at 280nm. Uncoupled mini-glucagon was not detected in the eluent and analysis of affinity purified material revealed four distinct silver stained bands.

The results indicate that liver plasma membrane preparations can be efficiently solubilized at pH 7.5 using Triton X-100 (0.125mg/ml) at 4Co for 16 h to isolate mini-glucagon receptor(s) and that there may be several types of mini -glucagon receptor(s)