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Abstracts

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GLIBENCLAMIDE IMPAIRMENT OF β CELL FUNCTION INVOLVES ALTERATIONS OF CYTOPLASMIC CA^{2+} REGULATION.

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Chronic treatment with glibenclamide alters the β cell response to various insulin secretagogues. The possible mechanisms have been studied with islets cultured for 18h in the presence of 10 mmol/l glucose, with (test) or without (control) a therapeutical concentration of glibenclamide (10 nmol/l). Acute responses of the islets were then determined in the absence of glibenclamide. Insulin release from test islets was unaffected by sulphonylureas or diazoxide because the drugs failed to increase and decrease $[Ca^{2+}]_i$ that was already elevated in the absence of any stimulus. The responsiveness to glucose was preserved but modified in several respects. As compared with controls, insulin release by test islets was increased in the absence of glucose, and mainly stimulated between 0-10 instead of 7-20 mmol/l glucose. The maximum response was halved, but this difference disappeared after correction for the decrease in insulin content. The first phase of glucose-induced insulin secretion was abrogated because of a paradoxical decrease of the high basal $[Ca^{2+}]_i$ in β cells. The second phase was preserved but occurred with little rise of $[Ca^{2+}]_i$. These abnormalities did not result from alterations of glucose metabolism (NADPH fluorescence). In islets cultured with tolbutamide, glucose-induced $[Ca^{2+}]_i$ and insulin changes were normal, except for a lower maximal secretion rate due to the fall in insulin content. These islets, however, behaved like those cultured with glibenclamide if tolbutamide remained present during the acute functional tests. In conclusion, treatment with a low glibenclamide concentration perturbs β cell function by permanently blocking K^+-ATP channels and raising $[Ca^{2+}]_i$. Glucose stimulation of insulin secretion occurs at lower concentrations, is delayed and is largely mediated by a modulation of Ca^{2+} action on exocytosis.

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AMINO ACID TRANSFORMATION OF OSCILLATORY Ca^{2+} SIGNALS IN MOUSE PANCREATIC B-CELLS AND INTACT ISLETS

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Glucose-induced increase of cytoplasmic Ca^{2+} in pancreatic B-cells is usually manifested as slow oscillations from the basal level. The significance of this rhythmicity for maintaining normal B-cell function with periodic variations of circulating insulin made it of interest to investigate how the oscillatory Ca^{2+} signal was affected by various amino acids. Cytoplasmic Ca^{2+} was measured in individual B-cells and islets from ob/ob mice using dual wavelength microfluorometry and the indicator fura-2. The individual B-cells were very sensitive to alanine, glycine and arginine. When added at concentrations as low as 0.1 mM and 0.5 mM, each of these amino acids transformed the oscillations into sustained elevation of cytoplasmic Ca^{2+} in 30 and 60 % respectively of the B-cells. Stimulation of the entry of Ca^{2+}, obtained either by raising the extracellular concentration or by prolonging the open state of the voltage-dependent Ca^{2+} channels with BAY K 8644, resulted in reappearance of the rhythmic activity. Oscillatory Ca^{2+} signals in intact islets were more resistant to transformation by amino acids than those of individual B-cells. Even at 10 mM of glycine, alanine or arginine sustained elevation was observed in only about 55% of the islets. It is suggested that signals from the adjacent cells make it possible for B-cells situated in islets to overcome a suppression of the oscillatory activity otherwise seen in the presence of amino acids.

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ENGINEERED PEPTIDES FROM THE H3 DOMAIN OF SYNTAXIN INHIBIT INSULIN RELEASE IN INTACT MOUSE PANCREATIC β CELLS.

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Recent studies show that syntaxin-1 is implicated in the calcium-dependent secretory pathway in pancreatic endocrine β-cells. Moreover, the functional role of two 23^{er} segments (Syn A and SynB) of the H3 domain of syntaxin-1 have been demonstrated. In the present study, we have stablished in intact β-cells a minimal functional active zone of 17 residues of the H3 domain of syntaxin-1, as the very effective uncoupling element of Syn A segment. 13^{er} peptides (Syn-1 to Syn-6) from peptides Syn A, Syn I and one additional peptide, with the same amino acid composition than Syn I but in random sequence to be used as controls (Syn I_c) were synthesized by t-boc chemistries using an authomated solid-phase peptide synthesizer. Peptides were purified by RP-HPLC. The second portion of 13^{er} peptides Syn I and Syn I_c were subjected to myristoylation. After deprotection of the amino terminus of the peptides, myristic acid was activated "in situ" with BOP. Functional experiments were carried out in digitonin-permeabilized and intact cells incubated for 10 and 30 min respectively, in the presence of the different synthetic peptides. Insulin was determined by RIA. Basal or stimulated secretion were measured in media containing 5 mmol/l EGTA, 10 μmmol/l Ca^{2+} or 22 mmol/l glucose. 200 μmol/l of peptides Syn-2, Syn-3 and Syn-4 inhibited Ca^{2+}-dependent insulin release ($p< 0.0001$, n= 6) in permeabilized cells. No additive effects were observed when Syn-2 to Syn-4 were incubated together (n= 4). myr-Syn I provoked a dose-dependent inhibition of 22 mmol/l glucose-induced insulin release (IC_{50} ~ 23 mmol/l) in intact cells (n= 4). myr-Syn I_c had not inhibitory effect at 300 μmmol/l (n= 4). These data demonstrated for the first time, that nutrient-induced secretory process can be uncoupled in intact β-cells by using specific peptides and support previous studies concerning the direct role of specific regions of syntaxin-1 in the insulin exocytotic process.

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COUPLING OF THE α-LATROTOXIN RECEPTOR LATROPHILIN TO INSULIN EXOCYTOSIS IN CLONAL β-CELLS

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The black-widow spider venom component α-latrotoxin (α-LTX) induces massive exocytosis in neurons and evokes exocytotic insulin release in primary β-cells as well as in the clonal cell lines MIN6 and INS-1, but not in HIT-T15 or RINm5F cells. The effect of α-LTX in insulin secreting cells is mediated by latrophilin (LPH), a class II G-protein coupled receptor.

We have investigated the coupling of latrophilin to exocytosis in HIT-T15 cells. Transient transfection of full-length cDNA coding for LPH induced dose dependent insulin release with an EC_{50} of 0.5 nM for its agonist α-LTX. LPH was targeted to discrete areas of the plasma membrane as evidenced by confocal immunocytochemistry. The agonist dependent insulin release is of vesicular origin as it was blocked by co-expression of the light chains of the botulinum neurotoxins C1 or E known to cleave syntaxin and SNAP-25, respectively. Coupling of LPH to insulin release was specifically reproduced in streptolysin-O permeabilized cells by a receptor-mimetic peptide corresponding to an intracellular loop of LPH. Therefore it involves most likely heterotrimeric G-proteins. However, the effect of α-LTX was insensitive to pertussis toxin. Moreover, the effect of α-LTX on insulin release was not altered by concomitant overexpression of the Regulators of G-protein Signalling RGS3 and RGS3-t, which impeded stimulation of hormone secretion by carbachol and PACAP.

We conclude that latrophilin is capable of mediating the effects of α-LTX on insulin exocytosis and induces vesicular release of insulin by coupling to a heterotrimeric G-protein distinct from $G_{i/o}$, $G\alpha_s$ or $G\alpha_q$.