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Abstracts

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TIME RELATIONSHIP BETWEEN RISES IN REDUCED PYRIDINE NUCLEOTIDES AND INSULIN RELEASE

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Abnormalities in first phase insulin release is an early indicator of faulty islet function in both Type I and Type II diabetes. To evaluate possible coupling of events for the initiation of insulin release, I have dissected temporal relationships of components of rat pancreatic islet stimulus-secretion coupling. Isolated rat pancreatic islets were perifused under a microscope equipped for microfluorometry. Insulin release, NAD(P)H/NAD(P) ratios, and cytoplasmic calcium were studied in parallel. The rise in insulin release started later in experiments with mannose (60-75 s, $p<0.05$ Mann-Whitney *u* test) when compared with glucose. Insulin release started earlier in overnight cultured islets stimulated with 20 mmol/l glucose (60 s, $P<0.05$) or 3 mmol/l glucose + 17 mmol/l mannose (75-90 s, $P<0.05$) when compared with freshly prepared islets. The protein kinase C activator 100 nmol/l phorbol-12-myristate-13-acetate shortened the lag time (60-75 s, $P<0.01$). There was no difference between 12, 20, or 30 mmol/l glucose with regard to timing of onset of insulin release or rise in NAD(P)H fluorescence. The timing of rise in NAD(P)H was the same in experiments with mannose when compared with equimolar concentrations of glucose but the maximum effect of mannose was lower than that of glucose at all concentrations tested (up to 30 mmol/l). After 4 days' culture the rise in NAD(P)H fluorescence started slightly earlier (15 s, $P<0.01$) then in freshly prepared islets. Increasing mitochondrial metabolism by 20 mmol/l ketoisocaproate induced an earlier rise in NAD(P)H fluorescence and insulin secretion when compared with stimulatory glucose. The time of rise in cytoplasmic calcium was the same for glucose and mannose. Conclusion: It is probably not the time of onset of increased hexose metabolic flux or differences in the initial amount of glycolytic signal(s) for exocytosis that govern the timing of onset of exocytosis. Instead, mitochondrial metabolism (probably ATP production), degree of protein phosphorylation at late steps in the exocytotic process, and kinetic properties of the rate of generating cell messages may be important.

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THEORETICAL STUDY OF THE ROLE OF Na/Ca EXCHANGE IN THE REGULATION OF THE β-CELL ELECTRICAL ACTIVITY.

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Glucose-stimulated insulin secretion from pancreatic β cells is associated with the generation of electrical activity which consists in groups of action potentials superimposed on a plateau potential (bursts) interceded by silent more repolarized intervals. The burst duration is influenced by glucose concentration but the mechanisms involved remain the subject of much debate. The role of a Na/Ca exchange current in the regulation of the electrical activity of the mouse pancreatic β cell was investigated by combining the patch-clamp technique and mathematical modeling. We have recorded electrical activity from freshly isolated islets in the presence of 11 mM glucose before, during and after reducing extracellular Na^+ concentration from 140 mM to 30 mM. This partial removal of extracellular Na^+ results in a transient shortening of the plateau phase (during which action potentials are generated) and a slight (5 mV) hyperpolarization. Moreover, we have extended existing mathematical models of the pancreatic β cell to include the Na/Ca exchange current. Our numerical simulations of β-cell electrical activity reproduce the observed effects of Na^+-removal. Furthermore, the model predicts that the activity of the exchanger is influenced by glucose-induced changes in the cytoplasmic Na^+-concentration and that this could contribute to the effects of the secretagogue on β-cell electrical activity. We have also extended the model to a cluster of heterogeneous cells with gap-junctional coupling. Our results show that the exchanger plays a substantial role in the regulation of cytosolic free Ca^{2+} inside the islet.

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A TOXIN-RESISTANT SNAP-25 FOR STUDYING THE ROLE OF SNAP-25 IN REGULATED INSULIN SECRETION

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The tSNARE SNAP-25 is expressed in pancreatic B-cells and its cleavage by botulinum neurotoxin E (BoNT/E) abolishes stimulated secretion of insulin. Using RT-PCR we have detected both SNAP-25 isoforms a and b in primary pancreatic B-cells as well as in an insulin secreting cell line. For further studies on the role of SNAP-25 and its isoforms in insulin secretion, we wished to generate a SNAP-25 mutant resistant to cleavage by BoNT/E. When insulin secreting cells are treated with BoNT/E the toxin inactivates endogenous SNAP-25 thereby inhibiting stimulated insulin secretion. If the mutant SNAP-25 is expressed it will not be inactivated by toxin and its ability to restore insulin secretion can thus be tested. To this end we have generated and characterised a SNAP-25 molecule in which the sequence around the BoNT/E cleavage site (R^{176}QIDRIM^{182}) has been changed to P^{176}QIKRIT^{182}. This is the sequence of the equivalent region of human SNAP-23 (P^{187}-T^{194}) which has been shown to be resistant to BoNT/E. The mutant SNAP-25 was resistant to BoNT/E in vitro even after treatment with 50 nM toxin for 3h at 37°C, whereas half maximal cleavage of wild type SNAP-25 occurred after only 10 min with a toxin concentration of 10 nM. Mutant SNAP-25 was also toxin resistant in vivo when expressed (transient transfection) in toxin treated HIT cells in which endogenous SNAP-25 is cleaved. In such cells, mutant but not native SNAP-25b fully restored stimulated insulin secretion. In conclusion the SNAP-25 mutant has been shown to be completely resistant against toxin cleavage both in vitro and in vivo and to be functionally active in regulated insulin secretion. It will now be possible to determine whether the two isoforms can reconstitute regulated insulin release to a similar extent and, by introducing additional mutations in the toxin-resistant form, screen SNAP-25 domains for their functional importance in the exocytotic process.

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HIERARCHY OF THE SIGNALS DRIVING PULSATILE INSULIN SECRETION

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Three types of signals occurring in β cells have been proposed to trigger the oscillations of insulin secretion induced by glucose: oscillations of metabolism, cytoplasmic $[Ca^{2+}]_i$ and protein kinase activity. This study was an attempt to establish the hierarchy between these three mechanisms. We followed the standard procedure of repetitive and sustained depolarizations of mouse islets with high $[K]_o$ in the presence of diazoxide. In a medium containing 10 mM glucose, repetitive increases of $[K]_o$ induced rises in $[Ca^{2+}]_i$ and pulses of insulin secretion. Continuous stimulation of PKA by dibutyryl cAMP (or forskolin) or PKC by a phorbol ester (PMA) did not dissociate the $[Ca^{2+}]_i$ and insulin responses from the $[K]_o$ rises. However, the amplitude of the insulin pulses was consistently increased, while that of the Ca^{2+} pulses was either increased (PKA activation) or decreased (PKC activation). Receptor-mediated (acetylcholine + GLP-1) activation of both kinases increased the secretory response without altering the pacing imposed by the $[Ca^{2+}]_i$ oscillations. When $[Ca^{2+}]_i$ was steadily elevated by a sustained rise of $[K]_o$, insulin secretion was stable. Under these conditions, oscillations of metabolism imposed by periodic variations in the glucose concentration resulted in damped oscillations of secretion, much smaller than those occurring when $[Ca^{2+}]_i$ fluctuates and metabolism is stable. These results indicate that oscillations of $[Ca^{2+}]_i$ exert a greater control on the oscillations of insulin secretion than do Ca^{2+}-independent variations of protein kinase activity or of other metabolic signals. That oscillations of metabolism entrain $[Ca^{2+}]_i$ oscillations remains possible.