Isradipine as a specific inhibitor of L-Type Ca^{2+}-channels inhibited continuous spiking activity, but was not able to suppress depolarization of the membrane. The same result was seen in the presence of D 600, a less specific Ca^{2+}-channel inhibitor. Using $CoCl_2$ as a Ca^{2+}-antagonist, continuous spiking activity induced by MP (20 mM) stopped immediately and the membrane potential partly repolarized. The depletion of $[Ca^{2+}]_o$ in the presence of MP (20 mM) caused a depolarization of the membrane without spike activity. In contrast, high concentrations of $[Ca^{2+}]_o$ (20 mM) either minimized the spike amplitude or induced a partial repolarization of the membrane potential without spikes. When KCl (15 mM) was used to open Ca^{2+}-channels, MP (20 mM) in the presence of glucose (2.8 mM) induced a sustained depolarization with continuous spike activity. The addition of $[Ca^{2+}]_o$ (20 mM) partly repolarized the membrane potential and provoked oscillations from the plateau level. **Conclusion:** The action of methylpyruvate on electrical activity is dependent on the presence of Ca^{2+}. Obviously, an influx of Ca^{2+} through voltage-dependent Ca^{2+}-channels underlies the spike activity. However other ion channels may contribute to the membrane potential induced by MP, too.

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Functional Effect of Metabotropic Glutamate and GABA Receptors on Insulin Secretion

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The neurotransmitter glutamate is known to potentiate the release of insulin from β cells via the ionotropic subclass of glutamate receptors. Conversely, the transmitter γ-amino butyric acid (GABA) is known to inhibit the release for glucagon from α cells via the ionotropic class of GABA receptors. The characterisation and functional involvement of the metabotropic subclass of glutamate (mGluR) and GABA ($GABA_B$) receptors in insulin secretion has not previously been investigated. Here, we used RT-PCR, Western blots and radioimmunoassays to determine if mGluRs and $GABA_B$ receptors are expressed in the endocrine pancreas and if they can influence insulin secretion from β cells. RT-PCR showed the presence of mRNA encoding mGluR2, 3, 5 and 8 and $GABA_{B2}$ in MIN6 cells and Western blots showed the presence of mGluR5 protein and one or both of mGluR2 and 3 in membrane preparations in this cell line. Incubation of MIN6 cells with 0.3-25mM glucose ± one of the specific mGluR group agonists (S)-3,5-Dihydroxyphenylglycine (DHPG)(10mM) (mGluR 1 and 5 (group I)), (2S, 1'S, 2'S)-2-(carboxycyclopropyl) glycine (L-CCG-1)(3mM) (mGluR 2 and 3 (group II)) or L-2-amino-4-phosphonobutanoate (L-AP4)(3mM) (mGluR 4, 6, 7 and 8 (group III)). L-CCG-1 potentiated insulin secretion 181% (n=4) above control (n=6) in the presence of 3mM glucose, whereas L-AP4 and DHPG caused a 122% (n=4) and 117% (n=4), potentiation in insulin secretion respectively at 3mM glucose. The $GABA_B$ agonist, baclofen, inhibited insulin release in the presence 25mM glucose by 34% (n=4). The results show that mGluRs and $GABA_B$ receptors are expressed in MIN6 cells and that they have a functional relavence in insulin secretion.

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Glucose-Induced Apoptosis in Pancreatic Beta (HIT) Cells

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High glucose concentrations are toxic to pancreatic beta cells and contribute to beta cell dysfunction. It is unclear however, what cellular mechanisms are involved in this process, and how cell death ensues. The objective of this study was to determine whether clonal pancreatic beta (HIT) cells exposed to high glucose levels exhibit apoptosis, and characterize the molecular pathways involved.

HIT cells were grown in culture media containing varying glucose concentrations (5, 10 and 25 mM) for a period of 28 days. Cells were harvested and a systematic search for apoptotic markers was performed. A reproducible increase in the number of cells undergoing apoptosis was correlated to the ambient level of glucose. The percentage of TUNEL-positive HIT cells growing in 5, 10 and 25 mM glucose was 4, 8 and 12.5% respectively. In addition, this was associated with cytosolic translocation of Cytochrome C and cleavage of the apoptotic target PARP (poly-ADP ribose polymerase) uniquely in cells exposed to 25 mM glucose.

These results demonstrate that high glucose-induced toxicity of pancreatic beta cells may be mediated by activation of apoptotic pathways, and could play a role in the beta cell dysfunction associated with type II diabetes.

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Involvement of Protein Kinase C in Cholinergic Potentiation of Glucose-Induced 5-HT/Insulin Secretion

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Pancreatic β-cells release pre-stored insulin in response to extracellular glucose challenges. Glucose-induced insulin release (GIIR) from single islets is pulsatile and ultimately driven by cyclic changes in cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_i$). GIIR can also be amplified following activation of protein kinase C (PKC)-linked receptors (e.g. muscarinic receptors). We have used the PKC activator phorbol 12-myristate 13-acetate (PMA), the muscarinic agonist carbachol (Cch) and single islet techniques (fura-2 microfluorescence and carbon fibre microamperometry for $[Ca^{2+}]_i$ and 5-HT release measurements, respectively) to elucidate the mechanisms underlying PKC- and muscarinic receptor-mediated amplification. Glucose (11 mM) evoked synchronous oscillations of $[Ca^{2+}]_i$ and 5-HT/insulin release. The frequency of these oscillations was markedly increased by 20 min exposures to either PMA (100 nM) or Cch (50μM). PMA and Cch increased the amount of 5-HT released per oscillation while reducing both the duration and amplitude of the underlying $[Ca^{2+}]_i$ oscillations. $[Ca^{2+}]_i$ oscillations of identical duration were more effective to elicit 5-HT/insulin release in the presence of either PMA or Cch. As a result there was a 2- to 5-fold rise in overall 5-HT secretory rate. PMA and Cch did not affect mean amplitude/charge of fast amperometric transients (indicative of quantal release from single β-cells) while augmenting their frequency. Down-regulating PKC by long-term (ca. 20 h) exposures to PMA suppressed the steady-state effects of Cch. It is concluded that muscarinic potentiation of pulsatile insulin release is mediated by phorbol ester-sensitive isoforms of PKC. PKC activation appears to enhance pulsatile insulin release by increasing the effectiveness of Ca^{2+} at pre-exocytotic steps (probably granule translocation).

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Lack of Bursting Electrical Activity and Intracellular Calcium Oscillations in Glucose-Stimulated Single Rat Islets

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Although isolated rat islets are widely used to study *in vitro* insulin secretion and the underlying metabolic and ionic processes, knowledge on the properties of glucose-induced electrical activity (GIEA), a key step in glucose-response coupling, has been gathered almost exclusively from microdissected mouse islets. Using a modifed intracellular recording