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.

P600

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).

P601

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

technique, we have now compared the patterns of GIEA in collagenase-isolated rat and mouse islets. Resting membrane potential of rat and mouse β-cells was ~ -50 and -60 mV, respectively. Both rat and mouse β-cells displayed prompt membrane depolarizations in response to glucose. However, while the latter exhibited a bursting pattern consisting of alternating hyperpolarized and depolarized active phases, rat β-cells fired action potentials from a non-oscillating membrane potential at all glucose concentrations (8.4-22 mM). This was mirrored by changes in cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_i$), which was oscillatory in mouse and non-oscillatory in rat islets. Stimulated rat β-cells were strongly hyperpolarized by diazoxide, an activator of ATP-dependent K^+ channels. Glucose evoked dose-dependent depolarizations and $[Ca^{2+}]_i$ rises both in rat (EC_{50} = 5.9-6.9 mM) and mouse islets (EC_{50} = 8.3-9.5 mM), albeit without affecting the burst plateau potential in the latter case. We conclude that there are important differences between β-cells from both species with respect to early steps in the stimulus-secretion coupling cascade: 1) mouse β-cells have a larger resting K^+ conductance in 2 mM glucose; 2) rat β-cells lack the compensatory mechanism responsible for generating membrane potential oscillations and holding the depolarized plateau potential in mouse β-cells; and, 3) the electrical and $[Ca^{2+}]_i$ dose-response curves in rat β-cells are shifted towards lower glucose concentrations. Exploring the molecular basis of these differences may clarify several *a priori* assumptions on the electrophysiological properties of rat β-cells and foster the development of new working models of pancreatic β-cell function.

P602

Lack of Induction of 8-Oxoguanine Following Cytokine Treatment of Wistar Rat Islets

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In IDDM, cytokines generate nitric oxide (NO) within the β-cell leading to oxidative stress and DNA damage. We have used single cell gel electrophoresis to identify the reactive species responsible for cytokine-induced DNA damage and possible repair processes in rat islets. Exposure to 3 cytokines for 22h (IL-1β 20U/ml + TNFα 100 U/ml +IFNγ 5U/ml) causes DNA damage (mean comet length 33±2.1 to 51.7±2.4μm ($P<0.001$), which is prevented by the nitric oxide synthase inhibitor, L-NMMA (1mM). Of potential reactive species, peroxynitrite and hydroxyl radical are thought to form 8oxoguanine (8-oxoG) which we can detect with the repair enzyme, formamidopyrimidine glycosylase. Cultured rat islets showed significant 8-oxoG (increase in comet length 15.3±1.3μm vs control, $P=0.05$). When treated with cytokines for 18h, no additional 8-oxoG lesions were found. Cytokine treatment (18h) followed by 4h incubation with NMMA to prevent further NO formation, allowed significant repair (mean comet length from 50.9±1.6 to 40.6±1.6μm, $P<0.001$; significantly damaged nuclei (>40μm) reduced from 66.2 to 40.5%, $P<0.001$; highly damaged (>70μm) reduced from 14.1 to 6.1%, $P<0.001$). We conclude that peroxynitrite and hydroxyl radical may not contribute to the cytokine-mediated DNA damage in rat islets and that 22h nitric oxide-induced DNA damage can be repaired.

P603

Tumor Necrosis Factor-α Induces Apoptosis in an Insulin-Secreting β-Cell Line

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Tumor necrosis factor-α (TNF-α) exhibits a cytotoxic effect on insulin secreting β-cells. The mechanisms behind this effect are only partially characterized at present.

Purpose: To detect the TNF receptor 1 (TNFR1) and to characterize the effects of TNF-α on cell proliferation, DNA-fragmentation and caspase-3 activation in an insulin-secreting β-cell line (β-TC3).

Methods: Expression of TNFR1 was determined using a reverse transcriptase-polymerase chain reaction method. Cytotoxicity induced by TNF-α (12 ng/ml) was assessed by a cell proliferation MTT (thiazolyl blue) assay. Induction of apoptosis was detected by measurements of cytoplasmic caspase-3 activity using a colorimetric assay, and by a DNA-fragmentation ELISA using an antibody against 5'-bromo-2'-deoxy-uridine-labeled cytoplasmic DNA fragments.

Results: TNFR1 was expressed in the β-TC3 cells. After a 48-h incubation, TNF-α reduced the cell proliferation by 50% and increased the caspase-3 activity by 75% when compared to the controls ($p<0.01$). Interestingly, TNF-α caused a DNA fragmentation in β-TC3 cells as early as after 20 min with a gradual increase of the DNA fragmentation to 40% at 6 h ($p<0.03$).

Conclusion: The TNF-α induced cytotoxic effects in an insulin secreting β-cell line, as demonstrated by activation of caspase-3 and early DNA fragmentation, indicate an induction of apoptosis.

P604

The Porcine Rubulavirus LPMV Infects Rat Pancreatic Islets Resulting in an Impairment of Glucose Stimulated Insulin Release

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Abstract In 1980, a new porcine virus disease emerged in Mexico with high mortality and morbidity rate in young piglets. The major clinical symptoms were pneumonia, encephalomyelitis and corneal opacity. A virus was subsequently isolated from the brain of a piglet and named La Piedad Michoachan Mexico Virus (LPMV). It was classified as a member of the family paramyxoviridae; genus rubulavirus. Studies of the biology of the virus and comparative DNA sequence analysis revealed 45-55% amino acid homology to other paramyxoviruses with the highest degree of identity to human mumps virus. After an experimental infection the LPMV was isolated also from the pancreas (Alan et al. 1996). Previous studies have suggested that autoimmune destruction of pancreatic β-cells in Type-1 diabetes may be caused by mumps virus. The homology between LPMV and mumps virus therefore enabled us to exploit the possibility of using insulin producing pancreatic islet cells (β-cells) as an in vitro model for the study of LPMV infection at the cellular level.

Pancreatic islets isolated from rats were maintained in 1640 RPMI medium either in suspension or adherent to the bottom of the culture dishes. In a few days a monolayer of fibroblastic cells formed around islets attached to the culture dishes. Infection with LPMV of the cultured cells caused cytopathic effects (CPE) as evidenced by granulation and formation of syncytia within 3 days post infection. After altogether 90 passages the cultured cells showed evidence of persistent infection. Double immunofluorescence studies for insulin and LPMV indicated that the virus replicated both in insulin producing β-cells and in non-insulin producing cells in the culture of islets. Studies of insulin release from the cultured islets showed that their response to an increased glucose concentration was markedly diminished.

The results suggest that islet β-cells are susceptible to LPMV infection and that the present in vitro model system is suitable for both morphological and functional studies of the cellular effects of infection with this virus.