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Metabotropic glutamate and $GABA_B$ receptors contribute to the modulation of glucose-stimulated insulin secretion in pancreatic beta cells

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Abstract

Aims/hypothesis. The neurotransmitters glutamate and γ-aminobutyric acid (GABA) could participate in the regulation of the endocrine functions of islets of Langerhans. We investigated the role of the metabotropic glutamate (mGluRs) and $GABA_B$ ($GABA_BRs$) receptors in this process.
Methods. We studied the expression of *mGluRs* and *$GABA_BR$s* in rat and human islets of Langerhans and in pancreatic α-cell and beta-cell lines using RT-PCR and immunoblot analysis. Effects of *mGluR* and *$GABA_BR$* agonists on insulin secretion were determined by radioimmunoassays and enzyme-linked immunoadsorbent assays (ELISAs).
Results. We detected *mGluR3* and *mGluR5* (but not *mGluR1*, 6 and 7) mRNAs in all of the samples examined. Trace amount of *mGluR2* was found in MIN6 beta cells; *mGluR4* was identified in rat islets; and *mGluR8* expression was detected in rat islets, RINm5F and MIN6 cells. *$GABA_BR$*1 a/b and 2 mRNAs were identified in islets of Langerhans and MIN6 cells. The expression of mGluR3, mGluR5, $GABA_BR1$ a/b and $GABA_BR2$ proteins was confirmed using specific antibodies. Group I (mGluR1/5) and group II (mGluR2/3) specific mGluR agonists increased the release of insulin in the presence of 3 to 10 mmol/l or 3 to 25 mmol/l glucose, respectively, whereas a group III (mGluR4/6–8) specific agonist inhibited insulin release at high (10–25 mmol/l) glucose concentrations. Baclofen, a $GABA_BR$ agonist, also inhibited the release of insulin but only in the presence of 25 mmol/l glucose.
Conclusion/interpretation. These data suggest that mGluRs and $GABA_BRs$ play a role in the regulation of the endocrine pancreas with mechanisms probably involving direct activation or inhibition of voltage dependent Ca^{2+}-channels, cAMP generation and G-protein-mediated modulation of K_{ATP} channels. [Diabetologia (2002) 45: 242–252]

Keywords Islets, insulin, glutamate, metabotropic glutamate receptor, GABA, $GABA_B$ receptor.

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Abbreviations: Δ Ψ, Membrane depolarization; GABA, γ-aminobutyric acid; mGluR, metabotropic glutamate receptor; $GABA_BR$, type B γ-aminobutyric acid receptor; CNS, central nervous system; iGluR, ionotropic glutamate receptor; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; NMDA, N-methyl-D-aspartate; DHPG, (S)-3,5-dihydroxyphenylglycine; L-CCG-1, (2S, 1'S, 2'S)-2-(carboxycyclopropyl)glycine; ER, endoplasmic reticulum; L-AP4, L-2-amino-4-phosphonobutyrate; RT-PCR, reverse transcription polymerase chain reaction; VDCC, voltage-dependent Ca^{2+}-channels; HIL, human islets of Langerhans; RIL, rat islets of Langerhans; α-TC α-TC cells; RIN, RINm5F beta cells; MIN, MIN6 beta cells; CER, cerebellum; AC, adenylyl cyclase; GAD, glutamic acid decarboxylase; GLT, glutamate transporter; GLUT2, glucose transporter 2; RB, rat brain; $K_{ATP}C$, ATP-dependent K^+channel; PLC, phospholipase C; PKA, protein kinase A

Pancreatic islet cells share some common features with neurones including the expression of proteins specialized for synaptic transmission and the sensitivity of islet-cell hormone secretion to neurotransmitters [1]. Glutamate and γ-aminobutyric acid (GABA) are the major excitatory and inhibitory neurotransmitters in the central nervous system (CNS). These neurotransmitters are also present in islets of Langerhans and can be shown to alter hormone secretion [1–7] but their precise physiological roles in islet function and their cellular mechanisms of action are not clear. It is possible that GABA and glutamate mediate a paracrine-signalling pathway whereby α and beta cells communicate within the islets [1, 3, 6, 7].

In neurones, the transmitter actions of glutamate are mediated by different types of receptors categorised as ionotropic (iGluR) and metabotropic (mGluR) [8, 9]. The iGluRs are multimeric, cation-specific ion channels that are classified into three families on the basis of their pharmacology, electrophysiology and sequence homology; namely the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), kainate, and *N*-methyl-D-aspartate (NMDA) receptors [8]. Within an iGluR subclass, the subunit composition strongly influences the pharmacological and biophysical properties of the receptors [8]. The mGluRs are G-protein coupled receptors and they mediate relatively slow responses to glutamate [9]. To date, the family of mGluRs comprises eight different subtypes (mGluR1–8) classified into three groups on the basis of sequence similarities, pharmacological properties and intracellular signal transduction mechanisms [9, 10]. In group I, the mGluR1 and mGluR5 are coupled to inositol (1, 4, 5) tris phosphate (IP3)/Ca^{2+} and are activated by (S)-3,5-dihydroxyphenylglycine (DHPG). In group II, the mGluR2 and mGluR3 are typically linked to inhibition of cAMP formation and react effectively with (2S, 1'S, 2'S)-2-(carboxycyclopropyl)glycine (L-CCG-1) [9, 10]. However, there are examples of group II mGluR agonists that can also potentiate cAMP responses in some cells [9, 11]. In group III, the mGluR4, mGluR6, mGluR7 and mGluR8 are linked to inhibition of cAMP formation and respond effectively to L-2-amino-4-phosphonobutyrate (L-AP4) [9, 10]. Until now the expression of mGluRs has not been identified in pancreatic islet cells, but the presence of iGluRs is well documented [12–20]. However, the precise physiological role of these iGluRs is not clear [1, 6, 7].

In the CNS, GABA acts at two distinct types of receptors, ligand-gated ionotropic $GABA_A$ ($GABA_A$Rs)and $GABA_C$ receptors, and G protein-linked metabotropic $GABA_B$ receptors ($GABA_B$Rs), thus mediating both fast and slow inhibition of excitability at central synapses [21, 22]. To date, two major $GABA_B$R isoforms ($GABA_B$R1 and $GABA_B$R2) and various splice variants have been described [23–29]. In CNS neurones $GABA_B$R1 and $GABA_B$R2 are widely co-expressed and, a novelty for heptahelical receptors, were found to generate fully functional receptors only when linked by their C-terminal tails in a heterodimeric assembly [22, 24–26]. In short-term signalling, pre-synaptically located $GABA_B$Rs suppress neurotransmitter release by inhibiting voltage-sensitive P, N, and L-type Ca^{2+} channels [30]. Post-synaptically, $GABA_B$R-stimulation generally causes inhibition of adenylyl cyclase through $G_{\alpha i}$ subunits, as well as activation of Kir3 type potassium channels by liberated $G_{\beta\gamma}$ subunits, thereby hyperpolarizing the post-synaptic membrane [23, 31]. $GABA_B$Rs are also capable of directly interacting with transcription factors and could thus use a mechanism for gene transcription regulation upon stimulation [32–34]. In islets of Langerhans, GABA is released from beta cells and inhibits the release of glucagon from α cells [2–4]. This inhibition is believed to be mediated through $GABA_A$Rs [1, 3, 4]. However, it has been suggested that $GABA_B$Rs could be in the endocrine pancreas [35], but this has not been closely examined.

In this study our aims were to determine whether mGluR and $GABA_B$R mediated signalling mechanisms exist in islets of Langerhans and to investigate the contribution of these receptors to the modulation of glucose-stimulated insulin secretion. We investigated which of the *mGluR* and *$GABA_B$*R isoforms are present in rat and human islets of Langerhans and pancreatic α-cell and beta-cell lines using reverse transcription polymerase chain reaction (RT-PCR) and immunochemical detection of the corresponding proteins. Furthermore, we examined and showed the functional roles of these newly identified receptors in insulin secretion.

Materials and methods

Materials. Male Wistar rats (200–250 g) were from Harlan UK (Bicester, UK). TRI-Reagent and all tissue culture materials were obtained from Sigma (Poole, Dorset, UK). DNA oligonucleotide primers were purchased from Cruachem (Glasgow, Scotland). Restriction endonucleases were from Roche Diagnostics (Lewes, UK). The mGluR and $GABA_B$R agonists and antagonists were obtained from Tocris Cookson (Bristol, UK). All other chemicals were of analytical grade.

Isolation of islets of Langerhans and rat brain membranes. Rat islets of Langerhans were obtained by collagenase digestion of the pancreas [36]. The isolated islets were washed four times in HEPES-Krebs buffer (pH 7.4) containing 2.54 mmol/l $CaCl_2$, 1.20 mmol/l $MgSO_4$, 4.75 mmol/l KCl, 1.18 mmol/l K_2HPO_4, 119 mmol/l NaCl, 5 mmol/l NaH_2PO_4, 3 mmol/l D-glucose, 20 mmol/l HEPES, 0.5% (weight/volume; w/v) BSA and selected under a dissecting microscope. Human islets of Langerhans (HIL) were supplied by Dr. R. James, Department of Surgery, University of Leicester, UK and isolated [37]. For RNA preparation, all solutions were prepared using diethyl

pyrocarbonate-(DEPC)-treated water. The preparation of membrane fractions from whole brain or dissected cerebellum was carried out as previously described [38].

Cell culture. MIN6 (MIN) and RINm5F (RIN) pancreatic beta cells and α-TC pancreatic α-cells (α-TC) were maintained in DMEM tissue-culture medium containing fetal calf serum (10 % v/v), penicillin (100 units/ml) and streptomycin (0.1 mg/ml) at 37 °C in an atmosphere of humidified air (95 %) and CO_2 (5 %) [39]. Cells were passaged weekly and harvested using trypsin-EDTA. They were cultured for 3–5 days before RNA or membrane preparation.

RNA preparation, reverse transcription and PCR. Total RNA was extracted from rat and human islets of Langerhans, rat cerebral cortex (RB), cerebellum (CER), and pancreatic α and beta-cell lines using TRI-Reagent according to the manufacturer's protocol and the final RNA pellet was dissolved in DEPC-treated water. The total RNA (10 μg) was reverse-transcribed at 42 °C for 60 min in 50 μl of reaction mixture containing 1 x reverse transcripts buffer (40 mmol/l KCl, 1 mmol/l dithiothreitol (DTT), 6 mmol/l $MgCl_2$ and 50 mmol/l Tris-HCl; pH 8.3), 200 pmol of random primers, 0.5 mmol/l of each dNTP, 10 mmol/l dithiothreitol, 40 units of rRNasin and 500 units of M-MLV reverse transcriptase (Gibco BRL, Uxbridge, Middlesex, UK). One tenth of the cDNA was subjected to a PCR in 50 μl reaction mixture containing 1 x PCR buffer (50 mmol/l KCl, 5 mmol/l dithiothreitol and 10 mmol/l Tris-HCl; pH 9.0), 1.5–3 mmol/l $MgCl_2$, 0.1 mmol/l of each dNTP, 40 pmol of each primer and 1 unit of Amplitaq Gold Polymerase (Perkin Elmer, Norwalk, CT, USA). PCR was carried out at 95 °C for 10 min, followed by 35 cycles at 95 °C for 1 min, at the appropriate annealing temperature for 1 min (Table 1) and 72 °C for 1 min. The last cycle was followed by a final extension step at 72 °C for 10 min. The PCR products were subjected to electrophoresis in 1.5 % (w/v) agarose gels. For DNA sequencing and restriction enzyme digestion, 100 μl PCR samples were fractionated by electrophoresis in 1.3 % (w/v) low-melting-point agarose gels. The separated bands were extracted from the gel slices by Qiagen PCR purification Kit (Qiagen, Venlo, Netherlands). Restriction enzyme digestion and sequencing of the PCR products were carried out as previously described [37, 40]. The following controls were used to check for possible amplification of contaminant DNA and RNA by PCR: RNA blanks taken throughout the cDNA synthesis step in the absence of reverse transcriptase; samples without templates were run for every primer pair for each PCR experiment; rat brain RNA was used as a positive control for each experiment; and, finally, primers for beta actin were used to test the viability of each cDNA sample.

Immunochemical analysis. MIN6 and COS-7 cells were washed three times with PBS then lysed in ice cold RIPA buffer (1 x PBS, 1.0 % (v/v) Nonidet P40, 0.5 % (w/v) sodium-deoxycholate and 0.1 % (w/v) SDS) containing protease inhibitors (1 mmol/l PMSF, 1 mmol/l iodoacetamide, 1 mmol/l benzamidine, 2 mmol/l DTT, 1 mmol/l EDTA, 0.1 mg/ml soybean trypsin inhibitor and 10 μmol/l leupeptin) and left on ice for 1 h. The cell lysate was centrifuged at $15\,000 \cdot g_{max}$ for 20 min at 4 °C. Rat and human islets of Langerhans and rat brain samples were prepared as described above. The protein concentration of the supernatant was determined with BCA Protein Assay Kit (Pierce, Rockford, Ill., USA) using bovine serum albumin (BSA) as standard. Proteins were separated on 9 % (w/v) SDS polyacrylamide gels and transferred to Immobilon membranes (Millipore, Bedford, Mass., USA) using a discontinuous buffer system [39]. Blots were probed with 0.2–1 mg/ml immunoaffinity purified rabbit anti-mGluR2/3, anti-mGluR5 (Chemicon International, Temecula, Calif., USA), guinea pig $GABA_BR1$ a/b and $GABA_BR2$ (Oncogene Research Products, Cambridge, Mass., USA) antibodies. Following overnight incubation with the primary antibodies at 4 °C, immunostaining was revealed with horseradish-peroxidase conjugated anti-rabbit IgG (1:40 000 dilution) using an enhanced chemiluminescence (ECL) detection system (Roche Diagnostics, Lewes, UK).

Assay of insulin secretion. MIN6 cells were plated at a density of $0.5 \cdot 10^6$ cells/well and cultured for 24 h. The cells were washed three times with PBS then incubated with HEPES-Krebs buffer (119 mmol/l NaCl, 4.75 mmol/l KCl, 5 mmol/l $NaHCO_3$, 2.54 mmol/l $CaCl_2$, 1.2 mmol/l $MgSO_4$, 1.18 mmol/l KH_2PO_4 and 20 mmol/l HEPES pH 7.4) containing 2 mg/ml BSA and 0.3 mmol/l glucose for 2 h at 37 °C. This medium was replaced with fresh HEPES-Krebs buffer containing 2 mg/ml BSA and the test reagents. After a further 30 min incubation 100 μl medium was removed and added to 900 μl buffer containing 7.8 mmol/l NaH_2PO_4, 32.2 mmol/l Na_2HPO_4, 0.25 % (w/v) thiomersal and 0.1 % (w/v) BSA. Insulin secretion was measured either by radioimmunoassay [8] or by rat insulin ELISA

Table 1. Oligonucleotides used as PCR primers. The primers were based on rat or human (labelled with *) cDNA sequences. The EMBL/GenBank database accession numbers are indicated in the first column

Isoform specificity of primers	Primer Region		Length of PCR product (bp)	Annealing Temp. (°C)	$MgCl_2$ (mmol/l)
	Forward	Reverse			
mGluR1 (M61099)	1708–1727	2126–2145	437	55	3.0
mGluR2 (M92075)	2301–2319	2743–2763	462	55	1.5
mGluR2 (L35318)*	1594–1613	2336–2356	762	54	1.5
mGluR3 (M92076)	1963–1983	2514–2534	571	55	1.5
mGluR4 (M92077)	2214–2233	2718–2739	525	60	1.5
mGluR5 (D10891)	3318–3337	3759–3778	460	60	1.5
mGluR5 (D28538)*	1986–2004	2784–2802	816	55	1.5
mGluR6 (D13963)	2272–2291	2753–2772	500	58	3.0
mGluR7 (U06832)	1058–1076	1654–1672	614	55	3.0
mGluR8 (U17252)	2269–2287	2688–2708	439	60	1.5
mGluR8 (U92459)*	1779–1799	2223–2241	462	53	1.5
$GABA_B$R1a-d (Y10369)	1941–1960	2448–2466	525 (a, b, d) 618 (c)	58	3.0
$GABA_B$R1c (AB016160)	2081–2099	2733–2751	671	52	1.5
$GABA_B$R1d (AB016161)	2522–2540	3009–3028	506	52	1.5
$GABA_B$R2 (AF109405)	2172–2190	2510–2529	357	55	1.5

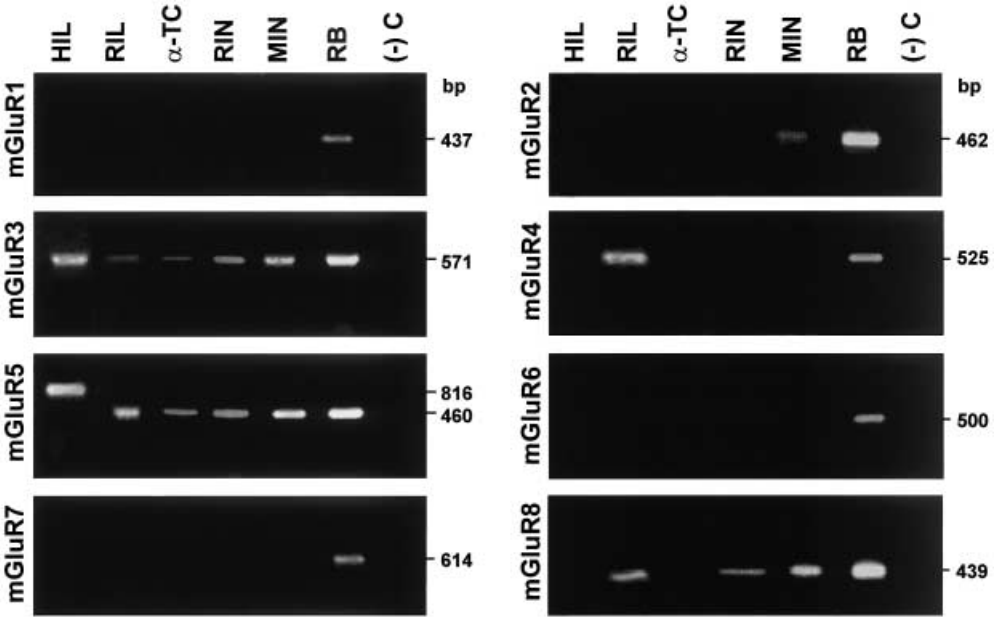


Fig. 1. Detection of mGluR transcripts in endocrine pancreatic cells. Receptor-specific primers for *mGluR1–8* (Table 1) were used to detect mRNAs for each receptor subtype in human islets of Langerhans (HIL), rat islets of Langerhans (RIL), α-TC cells (α-TC), and RINm5F (RIN) and MIN6 beta cells (MIN). The positive and negative controls were rat brain mRNA (RB) and PCR reaction without template ((–) C) respectively. Primers for human *mGluR5* amplified a different region than the rat *mGluR5*, hence the different sizes in the PCR product (see Table 1 for details). PCR products were verified by sequencing or by their restriction enzyme digestion patterns

(Mercodia, Uppsala, Sweden). As described previously [41, 42], the glucose-stimulated insulin secretion from MIN6-cells increased two- to four-fold when the glucose concentration was raised from 3 mmol/l to 25 mmol/l. For statistical analysis independent group *t* tests were used. A $p < 0.05$ (*) and $p < 0.01$ (**) were considered to be statistically significant with relevant control samples.

Results

Identification of mGluR isoforms in pancreatic islet cells using RT-PCR and immunoblotting. To determine whether mRNA encoding the mGluRs is present in pancreatic beta cells and rat and human islets of Langerhans, we designed isoform-specific primers (Table 1) for RT-PCR. We found that mRNAs for at least one of the mGluRs from each of the three groups could be detected in the majority of the clonal-islet cell lines and islets of Langerhans (Fig. 1). From group I (*mGluR1*/5) [9], mRNA for mGluR5, rat and human islets of Langerhans, α-TC, RINm5F and MIN6 cell lines could be detected in all samples. In contrast, *mGluR1* mRNA was not found in any of the samples other than in the positive controls (Fig. 1). The group II (*mGluR2/3*) [9] receptor *mGluR3* was also identified in all the samples examined, whereas very low amounts of *mGluR2* were detected at very low levels in MIN6 cells only (Fig. 1). The group III mGluRs (*mGluR4/6–8*) [9] show more limited expression patterns: *mGluR4* was identified in rat islets of Langerhans but could not be detected in the cell lines or in human islets of Langerhans (Fig. 1). The *mGluR8* mRNA was detected in rat islets of Langerhans, RINm5F and MIN6 cells but not in α-TC or human islet cells (Fig. 1). No mRNA could be detected for *mGluR6* and *mGluR7* in any of the samples tested but both isoforms were present in the positive controls (Fig. 1). In order to confirm that the amplified PCR products correspond to the correct mGluR sequences we used restriction enzyme digestion or DNA sequencing (data not shown). All the above experiments were done in parallel with negative and positive controls using water or cDNAs from rat or human cerebral cortex or cerebellum as appropriate. The integrity of each cDNA sample was confirmed by the detection of beta-actin mRNA [40] (data not shown).

To confirm the expression of mGluR proteins, membrane fractions were prepared from rat and human islets of Langerhans and MIN6 cells. In immunoblots of both brain and islet cells, an immunoaffinity purified antibody against the common C-terminus sequence of rat mGluR2 and mGluR3 (Chemicon International) identified a dominant 100 kDa band. The molecular weight of this band corresponds well with the molecular weight of mGluR2/3 proteins identified in brain samples (Fig. 2, left panel). Because the mGluR2 mRNA was not detected in human and rat islets (Fig. 1), the identified protein probably corresponds to mGluR3. In rat brain mem-

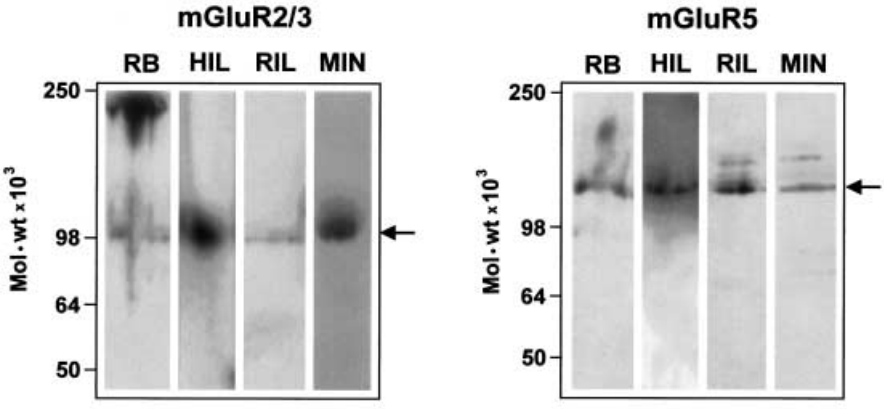
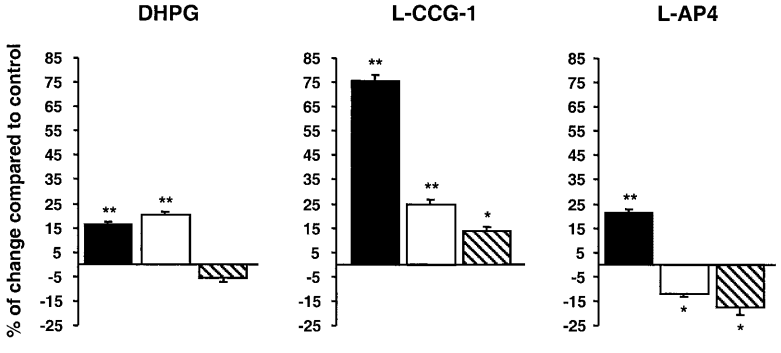


Fig. 2. Detection of mGluR proteins in islets of Langerhans and MIN6 beta cells. Antibodies against mGluR2/3 and mGluR5 were used for immunoblotting of rat brain (RB), human (HIL) and rat islets of Langerhans (RIL), and MIN6 cell (MIN) membrane fractions. Immunoreactive bands with estimated molecular weights of 100 kDa and 145 kDa were observed after immunostaining with anti-mGluR2/3 and anti-GluR5 antibodies, respectively. In rat brain membrane samples, the anti-mGluR2/3 antibody also identified a larger band (~ 190 kDa), thought to be a dimer of the 100 kDa protein. None of the antibodies reacted with COS-7 cell membranes (not shown)

branes, in addition to the 100 kDa protein, the anti-mGluR2/3 antibody also identified a larger band (~ 190 kDa), which was thought to be a dimer of the smaller band [43]. An antibody against the C-terminal 13 amino acids of mGluR5 revealed a densely labelled band of 145 kDa in both human and rat islets of Langerhans, MIN6 and rat brain membrane fractions (Fig. 2, right panel). After prolonged film exposure an additional weaker band of about ~ 150–160 kDa was also visible. These apparent molecular weights are consistent with the predicted size of the glycosylated forms of mGluR5 isoforms and previous studies identified proteins with similar molecular weight in rat brain membranes using mGluR5 selective antibodies [43, 44]. Neither of the antibodies labelled COS-7 cell membrane fractions used as negative controls (not shown). Normal (pre-immune) rabbit and guinea pig sera produced negative results with all samples examined on immunoblots (not shown).

Effects of mGluR agonists on insulin secretion from MIN6 cells. To determine whether the mGluRs identified in the RT-PCR experiments and on immunoblots affect insulin secretion, we examined changes in insulin secretion from MIN6 cells in the presence of mGluR agonists at 3, 10 or 25 mmol/l glucose concentrations. The effect of the group I receptor specific agonist DHPG was studied at a concentration of 10 μmol/l (reported EC_{50} 2 μmol/l (mGluR5), 6.6 μmol/l (mGluR1) [9, 10]). DHPG caused a significant ($p < 0.01$) increase in the release of insulin from MIN6 cells at lower glucose concentrations (3–10 mmol/l). However, it did not have any effect in the presence 25 mmol/l glucose (Fig. 3). L-CCG-1 was used as the specific group II agonist, (reported EC_{50} value for mGluR2 0.3–4 μmol/l and for mGluR3 1 μmol/l [9, 10]). At 3–25 mmol/l glucose concentra-

Fig. 3. The effects of mGluR stimulation on insulin secretion. MIN6 cells were pre-incubated with 0.3 mmol/l glucose for 2 h and then incubated for 30 min with either 3 (■), 10 (□) or 25 (▨) mmol/l glucose. Parallel determinations were done in the presence of the group I (mGluR1/5) agonist, DHPG; the group II (mGluR2/3) agonist, L-CCG-1; or the group III (mGluR4/6–8) agonist, L-AP4 as indicated. Results are expressed as a percentage change in insulin secretion to corresponding control values at 3, 10 or 25 mmol/l glucose in the absence of agonists. For each experiment, a minimum of three parallel samples was used for each condition in three independent tests ($n \geq 9$). * Indicates $p < 0.05$; ** indicates $p < 0.01$ compared with control samples



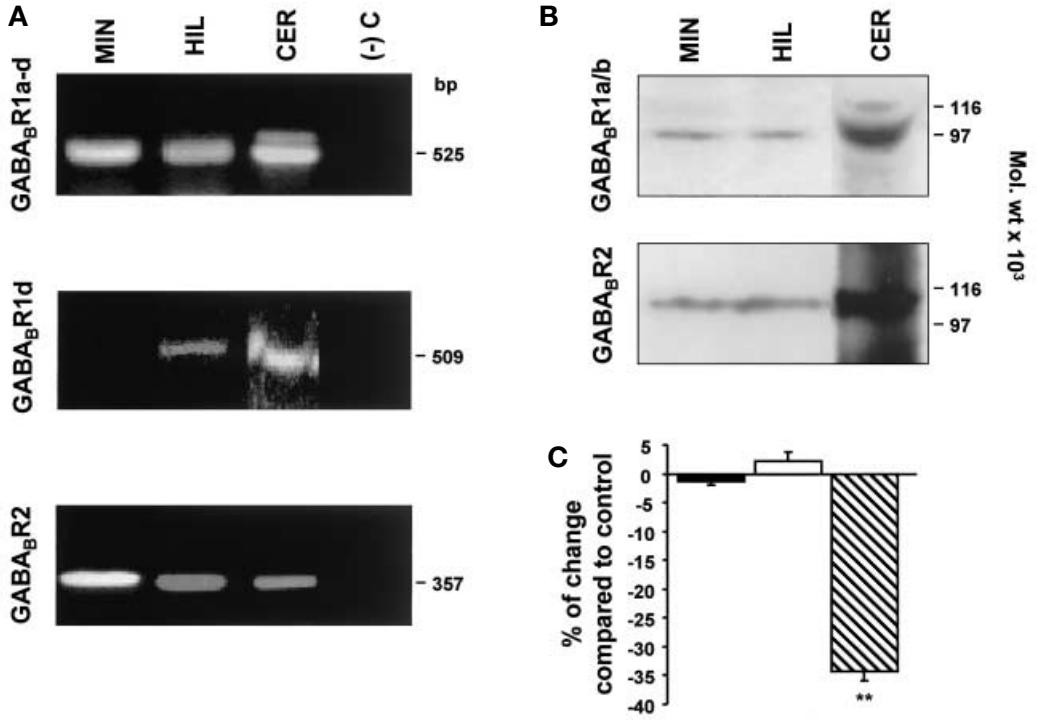


Fig. 4. Functional $GABA_BRs$ are expressed in islet cells. **A** $GABA_BR1$ and $GABA_BR2$ receptor mRNAs were detected in MIN6 pancreatic beta-cell line (MIN) and human islets of Langerhans (HIL). Various $GABA_BR$ isoform- and splice variant-specific primer pairs (Table 1) identified $GABA_BR1a/b$ and $GABA_BR2$ in MIN and HIL while $GABA_BR1d$ was only detected in HIL (see text for details). Rat cerebellar cDNA (CER) was used as a positive, and water as a negative control ((–) C). **B** Immunoblot analysis of crude membrane fractions prepared from MIN6 pancreatic beta-cell line (MIN), human islets of Langerhans (HIL) or rat cerebellum (CER). The anti-$GABA_B$ R1 a/b antibody identified two bands of about 130 kDa and 100 kDa corresponding to the splice variants $GABA_B$ R1 a and $GABA_B$R1 b respectively. The $GABA_BR2$ specific antibody identified a band with an estimated molecular weight of 110 kDa. Positions of molecular weight markers are indicated on the right. **C** Effect of the $GABA_BR$ agonist, baclofen, on insulin secretion. MIN6 cells were incubated at either 3 (■), 10 (□) or 25 (▨) mmol/l glucose concentrations (see Fig. 3 and Methods) in the absence or presence of 10 μmol/l baclofen. ** Indicates $p < 0.01$ compared with control samples

tions 3 μmol/l L-CCG-1 significantly ($p < 0.01$–0.05) increased the release of insulin from MIN6 cells, which was particularly prominent at 3 mmol/l glucose (Fig. 3). The effect of L-CCG-1 required the presence of 3 mmol/l glucose because there was no noticeable increase at very low (0.3 mmol/l) glucose concentration (not shown). Group III receptors were examined using 3 μmol/l L-AP4, a specific agonist for this group with a reported EC_{50} of between 0.4 and 1.2 μmol/l for mGluR4, 6 and 8 [9, 10]. The effect of L-AP4 was different at various glucose concentrations: At 3 mmol/l, glucose L-AP4 significantly ($p < 0.01$) increased the insulin release but at 10 mmol/l and 25 mmol/l glucose L-AP4 caused a significant ($p < 0.05$) inhibition compared to controls at the same glucose concentrations.

Identification of $GABA_BR$ isoforms in pancreatic islet cells using RT-PCR and immunoblotting. The expression of mRNAs encoding for $GABA_BR1$ and $GABA_BR2$ isoforms was also examined. Primers designed to amplify $GABA_BR1a$-b (Table 1) produced a single 525 bp product in MIN6 cells and human islets of Langerhans (Fig 4A). This fragment could correspond to the a, b or d $GABA_BR1$ splice variants [29]. In rat cerebellum, an additional (618 bp) PCR fragment was also amplified (Fig. 4A, CER) which corresponds to $GABA_BR1c$. The absence of this product in MIN6 cells and human islets of Langerhans suggests that $GABA_BR1c$ is not expressed in these samples (Fig. 4A). This result was further confirmed by $GABA_BR1c$ specific primers (Table 1), which did not produce a PCR product in either MIN6 cells or human islets of Langerhans but amplified a 671 bp band in brain (not shown). A $GABA_BR1d$ specific primer pair (Table 1) amplified a 509 bp fragment in both human (Fig. 4A) and rat (not shown) islets of Langerhans but not in MIN6 cells (Fig. 4A). $GABA_BR2$ specific primers (Table 1) amplified a single 357 bp product in all samples tested (Fig. 4A). Taking these data together, beta cells express $GABA_BR1a$ or $GABA_BR1b$ or both together with $GABA_BR2$, while $GABA_BR1d$ is probably expressed in an other islet cell types. Neither $GABA_BR$ subtype mRNA could be detected in the α-cell line, α-TC9 cells (not shown).

Immunoblotting of MIN6 cells, human islets of Langerhans and cerebellar membranes with an anti-

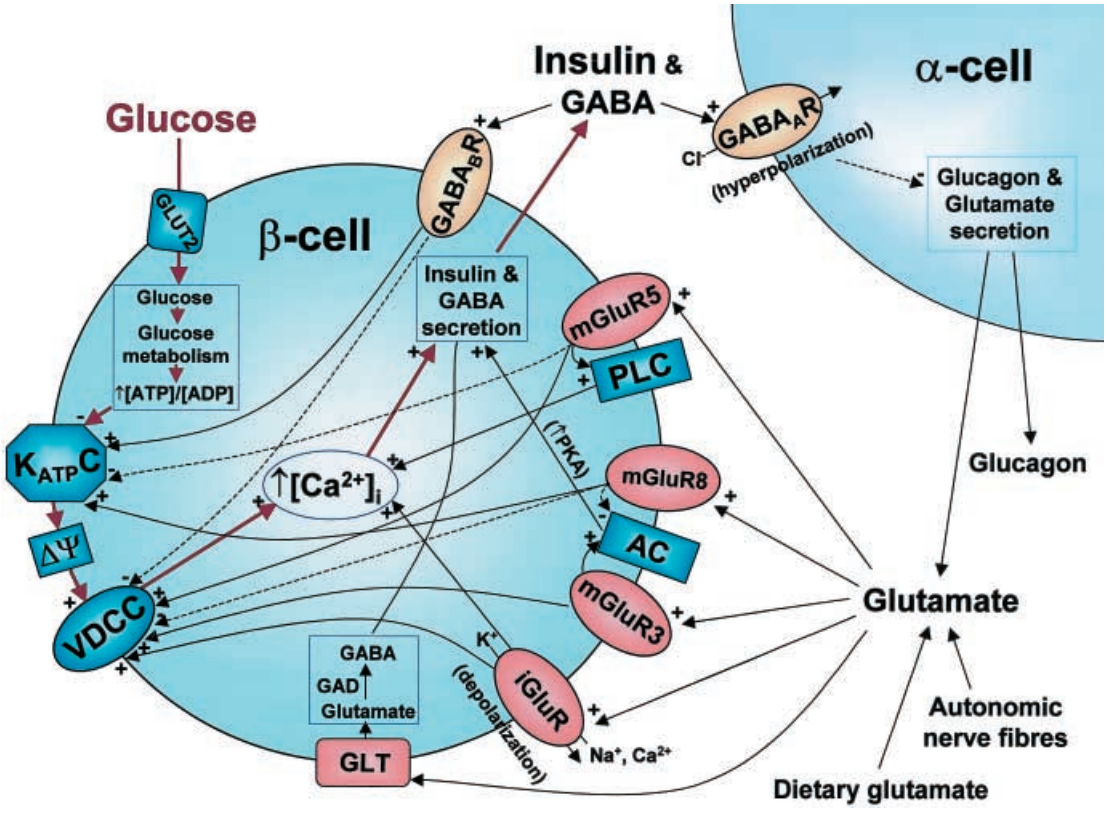


Fig. 5. Hypothetical model of glutamate and GABA mediated signalling between insulin secreting beta cells and glucagon secreting α cells. In this model, glutamate is released from α cells, whereas GABA is co-released with insulin from beta cells. The model illustrates the possible roles of the newly identified mGluRs and $GABA_BRs$ together with iGluRs and $GABA_ARs$ in the control of insulin release. The contributions of glutamate transporters (GLT) [64–66] and protein kinase A (PKA) [67] are described elsewhere

body raised against the common C-terminus sequence of the $GABA_BR1$ a and $GABA_BR1$ b splice variants produced a strong band of 100 kDa and a weaker band at 130 kDa approximately. The 100 kDa was predominant, in agreement with the more intense labelling of the 100 kDa ($GABA_BR1$ b) protein compared to the 130 kDa ($GABA_BR1$ a) protein by [^{125}I]CGP 71 872 in rat cerebellum [23]. The $GABA_BR2$ selective immunoaffinity purified antibody (Chemicon International) revealed a single band of 110 kDa, which is consistent with the predicted size of $GABA_BR2$ and with the observed molecular weights in brain homogenates [23] (Fig. 4C).

The effect of $GABA_BR$ selective agonist baclofen on insulin secretion. To examine the effects of $GABA_BR$ stimulation in MIN6 cells, we used the specific agonist baclofen (10 μmol/l [45]) in conjunction with either 3, 10 or 25 mmol/l glucose. We found that at the lower concentrations of glucose (3 and 10 mmol/l) there was no noticeable difference in the amount of insulin released from MIN6 cells in the presence or absence of baclofen. However at 25 mmol/l glucose, baclofen significantly ($p < 0.01$) inhibited the release of insulin by ~ 34 % compared to controls at the same glucose concentration (Fig. 4C).

Discussion

Functional metabotropic glutamate and GABA receptors are expressed in the endocrine pancreas and their effects on insulin secretion is glucose concentration dependent. The data described here indicate that pancreatic islet cells express mGluRs and $GABA_BRs$ identical with receptors expressed in the CNS [9, 10, 29]. We found mRNAs for the *mGluR3* and *mGluR5* in all the cell types examined (rat and human islets of Langerhans and MIN6, RINm5F, α-TC clonal cell lines). A trace amount of *mGluR2* was found in MIN6 cells but not in any other cell types. *mGluR4* was identified in rat islets only. The expression of *mGluR8* was detected in rat islets, RINm5F and MIN6 cells. The variations in the expression patterns of *mGluR2* could be due to differences in species, cell types or altered gene expression in transformed cell lines compared to the host cells. Additionally, *mGluR4* could be expressed in rat δ cells but not in α or beta cells, hence the absence of *mGluR4* mRNA in α- and beta-cell lines. mRNAs encoding *mGluR1*, *mGluR6* and *mGluR7* were not found in

any of the cell types examined. This is consistent with a previous study [19], where neurones and MIN6 cells were used to study AMPA receptor-mediated regulation of G_i-proteins. RT-PCR analysis with *mGluR6* specific primers and immunoblots indicated that mGluR6 is not expressed in MIN6 cells [19]. These authors have not reported the expression of the other mGluR isoforms identified in our study [19]. However, some caution is needed in interpretation of the results regarding the presence of mRNAs in a cell line because this does not necessarily indicate the presence of functional receptors [15, 46]. Therefore, it is important to investigate whether the mGluR mRNAs found in beta cells are actually translated into proteins. In this study, we confirmed the expression of mGluR3 and mGluR5 proteins using selective antibodies (Fig. 2).

In the CNS glutamate concentration decreases sharply away from the release site [47]. Therefore, it is interesting to note that the mGluRs shown to be present in beta cells, are those that have the highest affinity for the endogenous ligand, glutamate [9, 10]. The expression of these high affinity mGluR isoforms could be due to the cellular organisation of islets [48], where these receptors are exposed to lower glutamate concentrations compared with neuronal synapses.

We used the MIN6 beta-cell line to study the functional role of the identified mGluR proteins using group I (mGluR1/5), group II (mGluR2/3) and group III (mGluR4/6–8) specific agonists DHPG, L-CCG-1 and L-AP4 respectively [9, 10]. We studied the effects of these agonists on secretion of insulin at various glucose concentrations (3, 10 and 25 mmol/l). The group II agonist L-CCG-1 caused a ~ 1.8-fold higher increase in insulin secretion than in controls in the presence of 3 mmol/l glucose; there was relatively smaller L-CCG-1-induced increase in insulin secretion at higher glucose concentrations (Fig. 3). These results indicate that mGluR3 can improve the release of insulin in the presence of glucose. The concentration of glucose required for the potentiating effects of L-CCG-1 in MIN6 cells is low (3 mmol/l). DHPG, the group I agonist [9, 10], stimulated the release of insulin from MIN6 cells at 3 and 10 mmol/l glucose. Interestingly, this stimulation by DHPG was abolished by high concentrations of glucose (Fig. 3). L-AP4, a group III agonist [9, 10], produced moderate activation at 3 mmol/l glucose and caused an inhibition at high glucose concentrations (10–25 mmol/l). These results indicate the functional presence of at least one member of each mGluR group in MIN6 cells and that each group has a different effect on insulin secretion. In the case of the group I receptors this is likely to be mGluR5 as mGluR1 mRNA could not be detected in MIN6 cells (Fig. 1). The identified mGluR3 is an L-CCG-1 sensitive group II receptor. Of the group III receptors, mGluR8 is the only mGluR detected in MIN6 cells from this group (Fig. 1) and is probably mediating the L-AP4-stimulated effect on insulin secretion (Fig. 3).

$GABA_BR1$ and $GABA_BR2$ mRNAs were identified in human and rat islets and MIN6 cells (Fig. 4A). Their expression was confirmed by immunoblot analysis (Fig. 4B). Splice variant specific primers revealed that $GABA_BR1a$ and $GABA_BR1b$ are present in beta cells, whereas $GABA_BR1d$ is only present in islets but not in the MIN6 beta-cell line. This suggests that $GABA_BR1d$ is probably found in pancreatic polypeptide cells or δ-cells or both. Neither $GABA_B$R subtype mRNA could be detected in the α-cell line, α-TC9 cells, indicating that $GABA_B$Rs are not present in α cells. The observed distribution of the $GABA_BR$ is in contrast to the distribution of the $GABA_A$Rs, which are located on α cells [1, 3]. Recent evidence has shown that $GABA_BRs$ must exist as a heterodimer to form a functional receptor at the plasma membrane [22, 24–26, 29]. The presence of both isoforms in beta cells suggests that they can assemble to functional $GABA_BRs$ heterodimers. We showed that in the presence of 25 mmol/l glucose the $GABA_BR$ agonist, baclofen [45], inhibited the secretion of insulin from MIN6 cells, thus showing that a functional $GABA_BR$ is expressed in this cell line (Fig. 4C).

Different transduction pathways could be involved in the glutamate and GABA mediated modulation of insulin secretion. The metabolism of glucose leads to the formation of ATP in beta-cells (Fig. 5). The increased [ATP]/[ADP] closes the ATP-dependent K^+-channels ($K_{ATP}C$), resulting in plasma membrane depolarization, opening of voltage-dependent Ca^{2+}-channels (VDCC) and increase in $[Ca^{2+}]_i$ (Fig. 5) [48]. The increased intracellular $[Ca^{2+}]_i$ stimulates the release of insulin and GABA from beta cells (Fig. 5) [1, 3, 48]. Glutamate, which could be released from α cells, autonomic nerve fibres or the dietary glutamate, could affect insulin secretion [1, 6, 7, 13]. There are glutamatergic neurones and glutamate-mediated neurotransmission in the enteric nervous system [49]; therefore glutamate could be released from autonomic nerve fibres terminating within the pancreatic islet. It has been suggested that glutamate is also released from mitochondria and acts as a messenger in beta cells in glucose-induced insulin exocytosis [50]. However, new studies raised doubts on the proposed intracellular messenger role of glutamate in glucose-induced insulin secretion [51, 52].

In our study, the selective activation of each mGluR group produced a characteristic effect on insulin secretion (Fig. 3). This is probably a reflection of the different transduction pathways that couple to these receptors [9] (Fig. 5). Group I receptors (Fig. 5, mGluR5) can potentiate the release of Ca^{2+} from intracellular stores by activation of phospholipase C (PLC) [10] which in turn could lead to the release of insulin (Fig. 5) as observed with the agonist DHPG

(Fig. 3). Indeed, stimulation of mGluR5 in MIN6 cells with DHPG caused a considerable reduction in the endoplasmic reticulum (ER) Ca^{2+} concentration monitored by ER-targeted aequorin [53] and an increase in intracellular $[Ca^{2+}]_i$ using fura-2 (Varadi A., unpublished data). In neurones group I mGluR agonists inhibit K_{ATP} channels by selective actions of the mGluR1/5 activated Gα subunits [54]. This provides an additional mechanism for the stimulation of insulin release observed after the activation of mGluR5 by DHPG (Fig. 3). Group III receptors (Fig. 5, mGluR8) inhibit adenylyl cyclase (AC) by the G-protein G_i [9]. This pathway has been shown to enhance K_{ATP} channel activity and to inhibit L-type VDCCs [55, 56], which would inhibit the release of insulin as observed following the activation of mGluR8 using L-AP4 at 10 and 25 mmol/l glucose (Fig 3 and 5). In neurones, group II mGluRs are usually linked to the inhibition of cAMP formation but there are examples of group II mGluR agonists potentiating cAMP responses in some cells [9, 11], which would explain the stimulatory effects of L-CCG-1 on beta-cell insulin secretion (Fig. 3 and 5). Glutamate can also modulate insulin secretion via iGluRs by increasing their Na^+, K^+ and possibly Ca^{2+} conductance [1, 6, 7] (Fig. 5).

It is known that beta cells can produce and release GABA in response to glucose [1, 2, 4] (Fig. 5). It has been suggested that the released GABA hyperpolarizes α-cells through $GABA_A$Rs [3] and inhibits the release of glucagon in the presence of high glucose [57] (Fig. 5). However, our present study also shows a $GABA_B$R mediated inhibition of insulin release in the presence of high glucose concentrations. $GABA_B$R stimulates the G-protein G_o, which is known to inhibit N-, P- and R- type VDCCs [58, 59] all of which are expressed in pancreatic beta cells [60–62]. This suggests that $GABA_B$Rs could act as a negative feedback mechanism, possibly through G-protein inhibition of VDCC to prevent dangerously high concentrations of Ca^{2+} entering the cell in hyperglycaemic conditions (Fig. 5). In neurones, K_{ATP} channels are activated by a $GABA_B$R agonist [54, 63], which could also contribute to the $GABA_B$R mediated inhibition of insulin secretion (Fig. 4C, 5). Interestingly, treatment of non-obese diabetic mice with the $GABA_B$R agonist baclofen [45] delayed the onset of diabetes [35].

This work clearly shows the presence of functional mGluRs and $GABA_B$Rs in pancreatic endocrine cells and their ability to modulate secretion of insulin from beta cells. The presence of these receptors in the endocrine pancreas should be also considered during the development of new mGluR and $GABA_B$R specific pharmacological agents for novel treatment strategies of psychiatric and neurological disorders to avoid potential side effects.

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References

1. Satin LS, Kinard TA (1998) Neurotransmitters and their receptors in the islets of Langerhans of the pancreas. Endocrine 8: 213–223
2. Okada Y, Taniguchi H, Shimada S (1976) High concentration of GABA and high glutamate decarboxylase activity in rat pancreatic islets and human insulinoma. Science 194: 620–622
3. Rorsman P, Berggren P-O, Bokvist K et al. (1989) Glucose-inhibition of glucagons secretion involves activation of GABA-receptor chloride channels. Nature 341: 233–236
4. Gilon P, Bertrand G, Loubatieresmariani MM, Remacle C, Henquin JC (1991) The influence of gamma-aminobutyric-acid on hormone-release by the mouse and rat endocrine pancreas. Endocrinology 129: 2521–2529
5. Bertrand G, Gross R, Puech R, Loubatieres-Mariani MM, Bockaert J (1992) Evidence for a glutamate receptor of the AMPA subtype which mediates insulin release from rat perfused pancreas. Br J Pharmacol 106: 354–359
6. Skerry TM, Genever PG (2001) Glutamate signalling in non-neuronal tissues. Trends Pharmacol Sci 22: 174–181
7. Gill SS, Pulido OM (2001) Glutamate receptors in peripheral tissues: Current knowledge, future research, and implications for toxicology. Toxicol Pathol 29: 208–223
8. Dingledine R, Borges K, Bowie D, Traynelis SF (1999) The glutamate receptor ion channels. Pharmacol Rev 51: 7–61
9. Conn PJ, Pin JP (1997) Pharmacology and functions of metabotropic glutamate receptors. Annu Rev Pharmacol Toxicol 37: 205–237
10. Schoepp DD, Jane DE, Monn JA (1999) Pharmacological agents acting at subtypes of metabotropic glutamate receptors. Neuropharmacology 38: 1431–1476
11. Winder DG, Conn PJ (1995) Metabotropic glutamate receptor (mGluR)-mediated potentiation of cyclic AMP responses does not require phosphoinositide hydrolysis: mediation by a group II-like mGluR. J Neurochem 64: 592–599
12. Gonoi T, Mizuno N, Inagaki N et al. (1994) Functional neuronal ionotropic glutamate receptors are expressed in the non-neuronal cell line MIN6. J Biol Chem 269: 16989–16992
13. Bertrand G, Puech R, Loubatieres-Mariani MM, Bockaert J (1995) Glutamate stimulates insulin secretion and improves glucose tolerance in rats. Am J Physiol 269: E551–E556
14. Molnar E, Varadi A, McIlhinney RAJ, Ashcroft SJH (1995) Identification of functional ionotropic glutamate receptor proteins in pancreatic beta-cells and in islets of Langerhans. FEBS Lett 371: 253–257
15. Inagaki N, Kuromi H, Gonoi T et al. (1995) Expression and role of ionotropic glutamate receptors in pancreatic islet cells. FASEB J 9: 686–691
16. Weaver CD, Yao TL, Powers AC, Verdoorn TA (1996) Differential expression of glutamate receptor subtypes in rat pancreatic islets. J Biol Chem 271: 12977–12984
17. Weaver CD, Partridge JG, Yao TL, Moates JM, Magnuson MA, Verdoorn TA (1998) Activation of glycine and glutamate receptors increases intracellular calcium in cells derived from the endocrine pancreas. Mol Pharmacol 54: 639–646

18. Yoshioka A, Ikegaki N, Williams M, Pleasure D (1996) Expression of N-methyl-D-aspartate (NMDA) and non-NMDA glutamate receptor genes in neuroblastoma, medulloblastoma, and other cell lines. J Neurosci Res 46: 164–172
19. Wang Y, Small DL, Stanimirovic DB, Morley P, Durkin JP (1997) AMPA receptor-mediated regulation of a G_i-protein in cortical neurons. Nature 389: 502–504
20. Morley P, MacLean S, Gendron TF et al. (2000) Pharmacological and molecular characterization of glutamate receptors in the MIND6 pancreatic β-cell line. Neurol Res 22: 379–385
21. Vicini S (1999) New perspectives in the functional role of GABA(A) channel heterogeneity. Mol Neurobiol 19: 97–110
22. Marshall FH, Jones KA, Kaupmann K, Bettler B (1999) $GABA_B$ receptors – the first 7TM heterodimers. Trends Pharmacol Sci 20: 396–399
23. Kaupmann K, Huggel K, Heid J et al. (1997) Expression cloning of GABA(B) receptors uncovers similarity to metabotropic glutamate receptors. Nature 386: 239–246
24. Kaupmann K, Malitschek B, Schuler V et al. (1998) $GABA_B$-receptor subtypes assemble into functional heteromeric complexes. Nature 396: 683–687
25. Jones KA, Borowsky B, Tamm JA et al. (1999) GABA(B) receptors function as a heteromeric assembly of the subunits GABA(B)R1 and GABA(B)R2. Nature 396: 674–679
26. White JH, Wise A, Main MJ et al. (1998) Heterodimerization is required for the formation of a functional $GABA_B$ receptor. Nature 396: 679–682
27. Isomoto S, Kaibara M, Sakurai-Yamashita Y et al. (1998) Cloning and tissue distribution of novel splice variants of the rat $GABA_B$ receptor. Biochem Biophys Res Commun 253: 10–15
28. Schwarz DA, Barry G, Eliasof SD, Petroski RE, Conlon PJ, Maki RA (2000) Characterization of γ-aminobutyric acid receptor $GABA_B$(1e), a $GABA_B$(1) splice variant encoding a truncated receptor. J Biol Chem 275: 32174–32181
29. Billinton A, Ige AO, Bolam JP, White JH, Marshall FH, Emson PC (2001) Advances in the molecular understanding of GABAB receptors. Trends Neurosci 24: 277–282
30. Huston E, Cullen GP, Burley JR, Dolphin AC (1995) The involvement of multiple calcium channel sub-types in glutamate release from cerebellar granule cells and its modulation by $GABA_B$ receptor activation. Neuroscience 68: 465–478
31. Kaupmann K, Schuler V, Mosbacher J et al. (1998) Human gamma-aminobutyric acid type B receptors are differentially expressed and regulate inwardly rectifying K^+ channels. Proc Natl Acad Sci USA 95: 14991–14996
32. Nehring RB, Horikawa HPM, Fars OE et al. (2000) The metabotropic $GABA_B$ receptor directly interacts with the activating transcription factor 4. J Biol Chem 275: 35185–35191
33. White JH, McIlhinney RAJ, Wise A et al. (2000) The $GABA_B$ receptor interacts directly with the related transcription factors CREB2 and ATFx. Proc Natl Acad Sci USA 25: 13967–13972
34. Vernon E, Meyer G, Pickard L et al. (2001) $GABA_B$ receptors couple directly to the transcription factor ATF4. Mol Cell Neurosci 17: 637–645
35. Beales PE, Hawa M, Williams AJ, Albertini MC, Giorgini A, Pozzilli P (1995) Baclofen, a gamma-aminobutyric acid-b receptor agonist, delays diabetes onset in the non-obese diabetic mouse. Acta Diabetol 32: 53–56
36. Sutton R, Peters M, McShane P, Gray DWR, Morris PJ (1986) Isolation of rat pancreatic-islets by ductal injection of collagenase. Transplantation 42: 689–695
37. Varadi A, Molnar E, Ashcroft SJH (1996) A unique combination of plasma membrane Ca^{2+}-ATPase isoforms is expressed in islets of Langerhans and pancreatic β-cell lines. Biochem J 314: 663–669
38. Molnar E, Baude A, Richmond SA, Patel PB, Somogyi P, McIlhinney RAJ (1993) Biochemical and immunocytochemical characterization of antipeptide antibodies to a cloned GluR1 glutamate receptor subunit: Cellular and subcellular distribution in the rat forebrain. Neuroscience 53: 307–326
39. Varadi A, Molnar E, Ashcroft SJH (1995) Characterisation of endoplasmic reticulum and plasma membrane Ca^{2+}-ATPases in pancreatic β-cells and in islets of Langerhans. Biochim Biophys Acta 1236: 119–127
40. Varadi A, Molnar E, Ostenson C-G, Ashcroft SJH (1996) Isoforms of endoplasmic reticulum Ca^{2+}-ATPase are differentially expressed in normal and diabetic islets of Langerhans. Biochem J 319: 521–527
41. Tian YM, Urquidi V, Ashcroft SJ (1996) Protein kinase C in beta-cells: expression of multiple isoforms and involvement in cholinergic stimulation of insulin secretion. Mol Cell Endocrinol 119: 185–193
42. Ainskow EK, Zhao C, Rutter GA (2000) Acute overexpression of lactate dehydrogenase-A perturbs b-cell mitochondrial metabolism and insulin secretion. Diabetes 49: 1149–1155
43. Pabst H, Redecker P (1999) Interstitial glial cells of the gerbil pineal gland display immunoreactivity for the metabotropic glutamate receptors mGluR2/3 and mGluR5. Brain Res 838: 60–68
44. Shigemoto R, Nomura S, Ohishi H, Sugihara H, Nakanishi S, Mizuno N (1993) Immunohistochemical localization of metabotropic glutamate receptor, mGluR5, in the rat brain. Neurosci Lett 163: 53–57
45. Bowery NG (1993) $GABA_B$ receptor pharmacology. Annu Rev Pharmacol Toxicol 33: 109–147
46. Tyndale RF, Hales TG, Olsen RW, Tobin AJ (1994) Distinctive patterns of $GABA_A$ receptor subunit mRNAs in 13 cell lines. J Neurosci 14: 5417–5428
47. Kleinle J, Vogt K, Luscher HR et al. (1996) Transmitter concentration profiles in the synaptic cleft: an analytical model of release and diffusion. Biophys J 71: 2413–2426
48. Ashcroft FM, Ashcroft SJH (1992) Mechanism of insulin secretion. In Ashcroft FM, Ashcroft SJH (eds) Insulin, Oxford University Press (Oxford), pp 97–150
49. Liu MT, Rothstein JD, Gershon MD, Kirchgessner AL (1997) Glutamatergic enteric neurons. J Neurosci 17: 4764–4784
50. Maechler P, Wollheim CB (1999) Mitochondrial glutamate acts as a messenger in glucose-induced insulin exocytosis. Nature 402: 685–689
51. MacDonald MJ, Fahien LA (2000) Glutamate is not a messenger in insulin secretion. J Biol Chem 275: 34025–34027
52. Yamada S, Komatsu M, Sato Y, Yamauchi K, Aizawa T, Hashizume K (2001) Glutamate is not a major conveyer of ATP-sensitive K^+ channel-independent glucose action in pancreatic islet β-cell. Endocr J 48: 391–395
53. Mitchell KJ, Pinton P, Varadi A et al. (2001) Dense core secretory vesicles revealed as a dynamic Ca^{2+} store in neuroendocrine cells with a vesicle-associated membrane protein aequorin chimaera. J Cell Biol 155: 41–51
54. Mironov SL, Richter DW (2000) Intracellular signalling pathways modulate K_{ATP} channels in inspiratory brainstem neurones and their hypoxic activation: involvement of metabotropic receptors, G-proteins and cytoskeleton. Brain Res 853: 60–67

55. Seaquist ER, Neal AR, Shoger KD, Walseth TF, Robertson RP (1992) G-proteins and hormonal inhibition of insulin-secretion from HIT-T15-cells and isolated rat islets. Diabetes 41: 1390–1399
56. Dolphin AC (1995) Voltage-dependent calcium channels and their modulation by neurotransmitters and G-proteins. Exp Physiol 80: 1–36
57. Gaskins HR, Baldeon ME, Selassie L, Beverly JL (1995) Glucose modulates gamma-aminobutyric acid release from the pancreatic beta TC6 cell line. J Biol Chem 270: 30286–30289
58. Guyon A, Leresche N (1995) Modulation by different $GABA_B$ receptor types of voltage-activated calcium currents in rat thalamocortical neurones. J Physiol (Lond) 485: 29–42
59. Harayama N, Shibuya I, Tanaka K, Kabashima N, Ueta Y, Yamashita H (1998) Inhibition of N- and P/Q-type calcium channels by post-synaptic $GABA_B$ receptor activation in rat supraoptic neurones. J Physiol (Lond) 509: 371–383
60. Ligon B, Boyd AE 3rd, Dunlap K (1998) Class A calcium channel variants in pancreatic islets and their role in insulin secretion. J Biol Chem 273: 13905–13911
61. Seino S, Chen L, Seino M et al. (1992) Cloning of the alpha 1 subunit of a voltage-dependent calcium channel expressed in pancreatic beta cells. Proc Natl Acad Sci USA 89: 584–588
62. Vajna R, Schramm M, Pereverzev A et al. (1998) New isoform of the neuronal Ca^{2+} channel alpha1E subunit in islets of Langerhans and kidney – distribution of voltage-gated Ca^{2+} channel alpha1 subunits in cell lines and tissues. Eur J Biochem 257: 274–285
63. Ocana M, Baeyens, JM (1993) Differential effects of K^+ channel blockers on antinociception induced by alpha 2-adrenoceptor, $GABA_B$ and kappa-opioid receptor agonists. Br J Pharmacol 110: 1049–1054
64. Manfras BJ, Rudert WA, Trucco M, Boehm BO (1994) Cloning and characterization of a glutamate transporter cDNA from human brain and pancreas. Biochim Biophys Acta 1195: 185–188
65. Manfras BJ, Boehm BO (1995) Expression of a glutamate transporter cDNA in human pancreatic islets. Exp Clin Endocrinol Diabetes 103: 95–98
66. Weaver CD, Gundersen V, Versoon TA (1998) A high affinity glutamate/aspartate transport system in pancreatic islets of Langerhans modulates glucose-stimulated insulin secretion. J Biol Chem 273: 1647–1653
67. McDermott AM, Sharp GWG (1994) Multiple roles of cyclic AMP in the pancreatic B-cell. In: Flatt P, Lenzen S (eds) Frontiers of Insulin Secretion and Pancreatic B-Cell Research, Smith-Gordon, London, pp 243–249