The Cross-Talk between GABA Signalling and Metabolic Hormones in the Brain and Pancreatic Islets

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Abstract

GABA is a well-known neurotransmitter that can be synthesized in the central nervous system (CNS) and, interestingly, also in pancreatic islets. Once released, GABA activates GABA-A channels tonically or transiently resulting in different physiological functions. The pancreatic islets are important micro-organs composed of mainly α, β and δ cells secreting the metabolic hormones, namely insulin, glucagon and somatostatin, respectively. When insulin is secreted from pancreatic β cells, it can enter the blood and travel to the target tissues including the brain where the insulin receptor is prominently expressed such as in the hippocampus. It has been suggested that insulin regulates hippocampal function and, thereby, possibly modulates cognition. However, how this comes about is not understood. On the other hand, GABA secreted from the pancreatic β cells can regulate the islet cells via the para or autocrine loop. Nevertheless, in order to elucidate the details of GABA effects on cellular function, more insight into the pharmacological characteristics of GABA-A receptors, the physiological concentration of GABA and activation types of the GABA-receptors are required. We, therefore, used the whole-cell and single-channel patch-clamp technique to record from cells in the hippocampal slice and pancreatic islets for studying the function of GABA-A receptors and how they are modified by hormones, GABA or drugs. RT-qPCR was utilized to profile the expression of GABA-A receptors in the intact tissues. We also initiated the patch-clamp combined single-cell RT-PCR in the intact rat and human islets to investigate the cell-specific function of GABA-A receptors.

We have shown in acute rat hippocampal slices that 1 nM insulin “turns on” extrasynaptic GABA-A receptors in CA1 pyramidal neurons resulting in decreased frequency of action potential firing. The single-channel current amplitude is related to the GABA concentration resulting in a single-channel GABA affinity in the pM range. The benzodiazepines, flumazenil and zolpidem, are inverse agonists. The results demonstrated an unexpected hormonal control of the inhibitory channel subtype expressed and excitability of hippocampal neurons.

In the intact rat islets, the GABA-evoked tonic currents were present in the α cells and may contribute to keeping the resting membrane potential of α cells population at hyperpolarized membrane potential and, thereby, making it more difficult to depolarize the cells. In the human, the GABA signaling system was compromised in islets from type 2 diabetic individuals, where the expression of genes encoding the α1, α2, β2 and β3 GABA-A receptor subunits were down-regulated. GABA originating within the islets evoked tonic currents in the α, β and δ cells. However, transient current was observed only in δ cells, which implies a rapid regulation of somatostatin secretion by GABA. The effects of SR95531 on hormone release revealed that activation of GABA-A receptors decreased both insulin and glucagon secretion. The data is important for understanding the mechanism underlying GABA regulation of hormones secretion in human islets.

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“...when you have eliminated all which is impossible, then whatever remains, however improbable, must be the truth.”

*Sherlock Holmes*

*The Blanche Soldier*
List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.


IV  Jin Y, Korol SV, Jin Z, Birnir B. In a cell-type specific manner, high-affinity GABA-A receptors participate in autocrine and paracrine GABA signaling in human pancreatic islets. (Manuscript)

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Other papers published:


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<tbody>
<tr>
<td>AC</td>
<td>Associational commissural</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine triphosphate</td>
</tr>
<tr>
<td>ADP</td>
<td>Adenosine diphosphate</td>
</tr>
<tr>
<td>Akt</td>
<td>Protein kinase B</td>
</tr>
<tr>
<td>Best1</td>
<td>Bestrophin 1</td>
</tr>
<tr>
<td>CNS</td>
<td>Central nervous system</td>
</tr>
<tr>
<td>cAMP</td>
<td>Cyclic adenosine monophosphate</td>
</tr>
<tr>
<td>DG</td>
<td>Dentate gyrus</td>
</tr>
<tr>
<td>EC</td>
<td>Entorhinal cortex</td>
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<tr>
<td>EC50</td>
<td>Half maximal effective concentration</td>
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<tr>
<td>GAT</td>
<td>GABA transporter</td>
</tr>
<tr>
<td>GLP-1</td>
<td>Glucagon-like peptide-1</td>
</tr>
<tr>
<td>GAD</td>
<td>Glutamate decarboxylase</td>
</tr>
<tr>
<td>GABA</td>
<td>Gamma-aminobutyric acid</td>
</tr>
<tr>
<td>GABA-T</td>
<td>4-aminobutyrate aminotransferase</td>
</tr>
<tr>
<td>IPSPs</td>
<td>Inhibitory postsynaptic potentials</td>
</tr>
<tr>
<td>KATP</td>
<td>ATP-sensitive potassium channel</td>
</tr>
<tr>
<td>LPP</td>
<td>Lateral perforant path</td>
</tr>
<tr>
<td>MF</td>
<td>Mossy fibres</td>
</tr>
<tr>
<td>MPP</td>
<td>Medial perforant path</td>
</tr>
<tr>
<td>NOD</td>
<td>Non-obese diabetic</td>
</tr>
<tr>
<td>IAA</td>
<td>Autoantibodies to insulin</td>
</tr>
<tr>
<td>RNAs e</td>
<td>Ribonuclease</td>
</tr>
<tr>
<td>Sb</td>
<td>Subiculum</td>
</tr>
<tr>
<td>SC</td>
<td>Schaffer collateral</td>
</tr>
<tr>
<td>SSADH</td>
<td>Succinic semialdehyde dehydrogenase.</td>
</tr>
<tr>
<td>SLMV</td>
<td>Synaptic-like microvesicles</td>
</tr>
<tr>
<td>T1D</td>
<td>Diabetes mellitus type 1</td>
</tr>
<tr>
<td>T2D</td>
<td>Diabetes mellitus type 2</td>
</tr>
<tr>
<td>TCA</td>
<td>Tricarboxylic acid</td>
</tr>
<tr>
<td>THIP</td>
<td>4,5,6,7-tetrahydroisoxazolo(5,4-c)pyridin-3-ol</td>
</tr>
<tr>
<td>VIAAT</td>
<td>Vesicular inhibitory amino acid transporter</td>
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</table>
Introduction

1. Overview

The brain is a complex organ containing billions of cells that communicate, respond and work together in a coherent fashion enabling thoughts, feelings and ultimately life. In other organs, like the pancreatic islets, some of the same extracellular cellular signal molecules are present enabling communications between the cells (Franklin and Wollheim 2004). Interestingly, pancreatic islets cells, like neurons, fire action potentials triggering the release of vesicles in a quantal manner. The vesicles do not only contain hormones, but also neurotransmitters, such as glutamate, gamma-aminobutyric acid (GABA) and somatostatin (Satin and Kinard 1998). It is not yet clear what the function of the neurotransmitters is in the pancreas but emerging evidences indicated that the different types of pancreatic cells utilize neurotransmitters as paracrine signal molecules (Caicedo 2013). Furthermore, in both the brain and the pancreatic islets, hormones, e.g. insulin, alter the neurotransmitters signal transmission. In this thesis, I focus on the GABA signalling that takes place in the brain and the pancreatic islets.

2. GABA and GABA-A receptors in the CNS

2.1 Hippocampus

The hippocampus is an important part of the brains of vertebrates. It belongs to the limbic system and plays an important role in the consolidation of information from short-term memory to long-term memory and spatial navigation (Jarrard 1993). Humans and other mammals have two hippocampi, one in each cerebral hemisphere. The hippocampus is closely associated with the cerebral cortex, and in primates is located in the medial temporal lobe, underneath the cortical surface. It can be divided into several different regions including the CA region and dentate gyrus (Fig. 1)
The hippocampus forms a principally uni-directional network with input from the entorhinal cortex (EC) that forms connections with the dentate gyrus (DG) and CA3 pyramidal neurons via the perforant path (PP-split into lateral and medial). CA3 neurons also receive input from the DG via the mossy fibres (MF). CA3 neurons send axons to CA1 pyramidal cells via the Schaffer collateral pathway (SC), as well as to CA1 cells in the contralateral hippocampus via the associational commissural pathway (AC). CA1 neurons also receive input directly from the perforant path and send axons to the subiculum (Sb). These neurons in turn send the main hippocampal output back to the EC, forming a loop (Bird and Burgess 2008).

### 2.2 GABA synthesis, release and uptake

GABA is the most important inhibitory neurotransmitter in the adult mammalian central nervous system (CNS). It is also found in some peripheral tissues including the pancreas (Rorsman, et al. 1989; Taniguchi, et al. 1979; Taniguchi, et al. 1977). GABA is formed by a metabolic pathway commonly referred to as the “GABA shunt” (Fig 2). The first step in the GABA formation is the transamination of α-ketoglutarate by 4-aminobutyrate aminotransferase (GABA-T) to form glutamate. Glutamate is then decarboxylated by glutamate decarboxylase (GAD) to form GABA. GAD has two isoforms named GAD65 and GAD67, based on their molecular weight. The completion of the GABA shunt, and therefore the catabolism of GABA, again requires GABA-T and α-ketoglutarate to transaminate GABA to form succinate semialdehyde. Succinic semialdehyde is then converted to succinic
acid by succinic semialdehyde dehydrogenase (SSADH) allowing the entry of GABA metabolites into the TCA cycle (Erlander and Tobin 1991).

In the presynaptic terminals of neurons, GABA is packed into synaptic vesicles by the vesicular inhibitory amino acid transporter (VIAAT) (Erlander and Tobin 1991), which is highly dependent on the electrochemical gradient across the vesicle membrane. When the action potential arrives at the presynaptic terminal, the presynaptic cell membrane becomes depolarized which activates voltage-gated calcium channels. The entry of calcium triggers activation of the exocytosis process resulting in release of millimolar concentration of GABA into the synaptic cleft (Glykys and Mody 2007). The synaptic transmission is terminated rapidly as GABA is removed from the synaptic cleft immediately after it is released by the actions of several types of plasma membrane GABA co-transporters located in the presynaptic terminal and in the glial cells (Glykys and Mody 2007). The whole process is over within a few milliseconds from its start.

These GABA transporters, including GAT-1, GAT-2, GAT-3 and BGT-1, require two Na+ and one Cl- co-transported for each GABA molecule. GABA returned to GABAergic nerve terminals can be repacked for release;

**Figure 2.** A diagram of the GABA shunt. GAD, glutamate decarboxylase; GABA-T, 4-aminobutyrate aminotransferase; SSADH, succinic semialdehyde dehydrogenase.
whereas in glia, GABA is metabolized into succinic semialdehyde by the action of GABA-T, transform into glutamate and then glutamine, which is transferred back to the neuron (Gether, et al. 2006). In addition to the classical synaptic GABA release, researchers have found that GABA can be released from glial cells by reverse transport of transporters (Attwell, et al. 1993; Clark, et al. 1992) or through openings of the bestrophin 1 (Best1) anion channel (Lee, et al. 2010).

2.3 GABA-A receptors and composition

GABA receptors are classified according to two main categories: the ionotropic GABA-A receptors and the metabotropic GABA-B receptor. The GABA-A receptors (GABA-A channels) are heteromeric protein complexes that consist of two GABA binding sites coupled to an integral Cl⁻ channel (Olsen and Sieghart 2009). The GABA-B receptor is a heterodimer and belongs to the superfamily of G protein-coupled receptors (Chebib and Johnston 1999).

There are 19 known GABA-A receptor subunits: α1-6, β1-3, γ1-3, δ, ε, θ, π and ρ1-3. All the subunits have similar membrane topology: a long extracellular N-terminal domain, four α-helical transmembrane spanning segments (TM1-TM4), a long intracellular amino acid sequence between TM3 and TM4, which forms a large intracellular domain containing important intracellular regulatory binding sites, and a short extracellular C-terminal loop (Olsen and Sieghart 2009).

The receptor complex is composed of five subunits, usually containing two α and two β subunits plus one another. Although theoretically it is possible to form 195 distinct GABA-A receptors, significantly fewer different subtypes are thought to be formed (Olsen and Sieghart 2009). Heteropentameric assemblies containing proteins from the α, β, γ, δ, ε, θ, π and ρ subunit families are formed, but the ρ subunits can, in addition, form homopentamers (Chebib and Johnston 1999).

2.4 Phasic and tonic activation of GABA-A receptors

Upon activation, GABA-A receptors open and permit chloride to pass through. In the CNS, high concentration of GABA (~mM) is released from the presynaptic terminal and activates postsynaptic GABA-A receptors located close to the release site on the postsynaptic neuron, leading to a rapid influx of anions that hyperpolarizes the postsynaptic terminal generating the inhibitory postsynaptic potentials (IPSPs) (Semyanov, et al. 2004). The synaptic GABA-A receptors quickly desensitize when exposed to high concentrations of GABA (Semyanov et al. 2004). It is, therefore, important that GABA is removed rapidly from the synaptic cleft and this is achieved by reuptake of GABA by co-transporters located on the presynaptic terminals.
and glial cells (Gether et al. 2006). This transient activation of the GABA-A receptors allows the phasic and precise point-to-point transmission of pre-synaptic activity being translated into a postsynaptic signal (Glykys and Mody 2007). In contrast, the low, ambient concentration of GABA (~100 nM) can activate high-affinity, extrasynaptic GABA-A receptors located on neurons outside of synapses (Glykys and Mody 2007). This low concentration of GABA results in persistent or tonic activation of the GABA-A receptors (Glykys and Mody 2007). How the ambient GABA concentration is maintained and regulated is not well understood today but spillover from synapses, reverse transport of co-transporters and opening of non-selective pores are thought to be involved (Clark et al. 1992; Lee et al. 2010; Semyanov et al. 2004). In contrast to phasic activation that involves transmission between individual neurons, during tonic activation the GABA is pooled around a neural network, and may stabilize or even synchronize the resting membrane potential of a population of cells (Semyanov et al. 2004). Furthermore, the magnitude of the tonic conductance effectively alters the input/output gain control of the neuron (Semyanov et al. 2004) and, therefore, has an important physiological function. In a mouse model of temporal lobe epilepsy it has been reported that the GABA-A receptor subunits mediating tonic currents were significantly down-regulated in dentate gyrus neurons, leading to uncontrolled neural firing (Peng, et al. 2004; Zhang, et al. 2007).

Almost any GABA-A subunit expressed in a neuron can be found in the plasma membrane outside of synapses (Olsen and Sieghart 2009). However, depending on the affinity of the GABA-A receptor subtype and the level of the ambient GABA concentration, the receptors may or may not be activated. So far, in a variety of central neurons the most common types of heteropentameric GABA-A receptors evoking tonic currents contain α1, α4, α5, α6, γ2, δ or ε subunits (Olsen and Sieghart 2009). GABA-A receptor subtypes containing α4, α6 together with the δ subunit have been shown to underlie tonic inhibition in cerebellar granule cells whereas in hippocampal neurons α1, α4, α5 together with γ2 or δ subunit are most common (Essrich, et al. 1998b; Lindquist, et al. 2004; Lindquist, et al. 2003; Lindquist, et al. 2005; Nusser and Mody 2002; Nusser, et al. 1998; Stell, et al. 2003). Nevertheless, the exact subunit composition of extrasynaptic GABA-A receptor is not known to-date.

3. GABA-A receptor pharmacology

3.1 GABA binding site

GABA-A receptors are activated by GABA and structural analogues of GABA, such as muscimol, and synthetic analogues, e.g. 4,5,6,7-tetrahydroisoxazolo-pyridin-3-01 (THIP) whereas the receptors are blocked
by the competitive inhibitors gabazine (SR95531) and bicuculline (Olsen and Sieghart 2009). These agonists and antagonist interact with the GABA binding sites that are located at the α / β subunit interface. Picrotoxin, another antagonist, is an open channel blocker at GABA-A receptors and binds within the channel pore (Yoon, et al. 1993). The GABA affinity of the receptor can be modulated by other subunits in the heteromeric ion channel complex. For instance, a channel made of α1, β1 and γ2 has a half-maximal effective concentration (EC50) for GABA activation of the channel of 41 μM, whereas an α3, β1, γ2 channel has an EC50 of 100 μM when studied in heterologous expression system (Olsen and Sieghart 2009).

3.2 Benzodiazepines and the benzodiazepine-binding site

The benzodiazepine-binding site co-purifies with the GABA-binding sites (Ueno, et al. 1997). In addition, it was immunoprecipitated with antibodies that were developed to recognize the protein contributing to the GABA-binding site (Stephenson, et al. 1982). This indicates that the benzodiazepine-binding site is an allosteric modulatory site on the GABA-A receptor complex, however, clearly distinct from the GABA-binding site. The site is formed between the interface of the α and γ2 subunits (Sigel and Buhr 1997). The mechanism of action of benzodiazepine agonists is to enhance GABA-A receptor opening. From electrophysiological studies, it is known that these benzodiazepines increase the frequency of channel opening in response to GABA and often increase the mean open time and conductance of the channel thus accounting for their pharmacological and therapeutic action (Eghbali, et al. 1997; Fritschy and Mohler 1995; MacDonald, et al. 1989).

The benzodiazepine agonists are a part of a group of compounds in the general class of drugs that decrease neuronal excitability and which also includes the barbiturates. The drugs have anticonvulsant, anxiolytic and sedative—hypnotic effects (Sigel and Buhr 1997). Well-known examples include diazepam (valium) and zolpidem, which are coupled allosterically to the barbiturate and picrotoxin sites (Macdonald and Olsen 1994). Benzodiazepine-binding site containing GABA-A receptors are heterogeneous with respect to affinity for certain ligands e.g. zolpidem (Olsen and Sieghart 2009). A wide variety of nonbenzodiazepines, such as the β-carbolines, cyclopyrrolones and imidazopyridines, also bind to the benzodiazepine site (Sigel and Buhr 1997). Flumazenil is an antagonist at the benzodiazepine binding site. It binds to and occupies the benzodiazepine-binding site, but does normally not affect the function of the receptor (Nutt, et al. 1990). It can be used to reverse the action of benzodiazepines, as in the treatment of an overdose of diazepam. However, we have reported recently that the receptors turned-on by insulin can be blocked by flumazenil (Jin, et al. 2011).
3.3 Barbiturates and picrotoxin

Barbiturates comprise another class of drugs commonly used therapeutically for anaesthesia and control of epilepsy (Olsen 1981). Phenobarbital and pentobarbital are two of the most commonly used barbiturates. Phenobarbital has been used to treat patients with epilepsy since 1912. Pentobarbital is also an anticonvulsant, but it has sedative side effects. Barbiturates at pharmacological concentrations allosterically increase binding of benzodiazepines and GABA to their respective binding sites (Olsen, et al. 1991). Measurements of mean channel open times and channel conductance show that barbiturates act by increasing the proportion of channels opening to the longest open state (9 msec) while reducing the proportion opening to the shorter open states (1 and 3 msec), resulting in an overall increase in mean channel open time and increased channel conductance (Eghbali et al. 1997; Olsen et al. 1991).

Picrotoxin, a convulsant, is an open-channel blocker of GABA-A receptors. Unlike gabazine and bicuculline, picrotoxin binds inside the channel itself and interacts directly with the channel pore, causing a decrease in mean channel open time (Sedelnikova, et al. 2006).

Experimental convulsants like pentylenetetrazol and the cage convulsant t-butyl bicyclopentaphosphorothionate (TBPS) act in a manner similar to picrotoxin (Rienitz, et al. 1987). The antibiotic penicillin, at mM concentrations, is also an open channel blocker with a net negative charge. It blocks the channel by interacting with the positively charged amino acid residues within the channel pore, consequently occluding Cl⁻ passage through the channel (Macdonald and Olsen 1994).

4. Modification of GABA-A receptors

4.1 Trafficking and membrane insertion

Surface transport and synapse targeting of GABA-A receptors is under tight regulation by intracellular proteins e.g. the GABA-A receptor-associated protein (GABARAP), gephyrin, radixin, phosphatases and kinases (Kittler and Moss 2003). Gephyrin and radixin are involved in plasma membrane clustering of GABA-A receptors (Kittler and Moss 2003). GABARAP has been shown to play an important role in membrane trafficking as well as anchoring the receptor to the cytoskeleton (Kittler and Moss 2003).

Once the receptors arrive at the synapse, additional proteins provide receptor stability within the plasma membrane. The majority of inhibitory synaptic targeting is thought to depend on interactions with the receptors’ subunit and the gephyrin protein (Jacob, et al. 2005; Kneussel and Betz 2000) as deletion of subunit or protein disrupts the number of receptors that reach the cell surface (Essrich, et al. 1998a; Kneussel, et al. 1999). There-
fore, intracellular and plasma membrane mediated GABA-A receptor stability is dependent upon various proteins that help to maintain the efficiency of fast synaptic inhibition.

It has been suggested that receptor insertion primarily occurs at extrasynaptic sites (Bogdanov, et al. 2006; Lüscher and Keller 2004) and GABA-A receptors may utilize lateral mobility to increase synaptic receptor numbers in the post-synaptic zone (Bannai, et al. 2009; Bogdanov et al. 2006). Indeed, unique receptor binding studies showed that when synaptic receptors were blocked, extrasynaptic receptors diffused through the plasma membrane to become functional synaptic receptors (Thomas, et al. 2005). The investigators also found that this lateral mobility only takes approximately two minutes for extrasynaptic receptors to move into synaptic locations (Thomas et al. 2005), suggesting that receptor diffusion may provide a means for receptor number consistency and maintaining homeostasis.

4.2 Phosphorylation

A great number of studies have been devoted to determining the effects and implications of GABA-A receptor phosphorylation (Kittler and Moss 2003; Lüscher and Keller 2004; McDonald, et al. 1998; Moss, et al. 1995). The change by phosphorylation / dephosphorylation, presumably causes a conformational change resulting in changes in protein activity.

Biochemical studies have shown that GABA-A receptor subunits can be phosphorylated by 3'-5'-cyclic adenosine monophosphate (cAMP) dependent kinase (PKA) (Heuschneider and Schwartz 1989), Ca²⁺/phospholipid-dependent protein kinase (PKC) (Sigel and Baur 1988), type II Ca²⁺/calmodulin-dependent protein kinase (Machu, et al. 1993), and protein tyrosine kinase (Moss et al. 1995). PKA phosphorylates serine residues in β1 and β3 GABA-A receptor subunits, while the β2 subunit is not phosphorylated by this kinase (McDonald et al. 1998). PKC targets all GABA-A receptor β subunits in the intracellular domain (McDonald et al. 1998). This latter kinase has also been shown to phosphorylate γ2 GABA-A receptor subunits (Moss, et al. 1992). The mechanisms behind PKA phosphorylation appear to rely on key signaling scaffold molecules because PKA itself is unable to directly bind to the GABA-A receptor subunits (Brandon, et al. 2003). AKAP79/150, a neuronal A-kinase anchoring protein, has been shown to bind to PKA as well as both β1 and β3 subunits, effectively targeting PKA to these GABA-A receptor subunits (Brandon et al. 2003).

Many studies suggest that phosphorylation may alter GABAergic currents through receptor desensitization, changes in single-channel open probability, and ligand-receptor interactions (Kittler and Moss 2003). Experiments in rat hippocampal neurons showed that phosphorylation induces a significant conformational change that directly affects and destabilizes ligand affinity for the receptor (Jones and Westbrook 1997). Further, a study investigating
both β1 and β3 containing receptors expressed in HEK 293 cells showed that PKA phosphorylation regulates desensitization and deactivation parameters by increasing the fast phases of desensitization and prolonging deactivation (Hinkle and Macdonald 2003). A recent study found that PKA phosphorylation increased current through α4β3δ receptors by increasing their single channel mean open frequency (Tang, et al. 2010).

5. GABA and GABA-A receptors in pancreatic islets

5.1 Islet of Langerhans

The islets of Langerhans are encapsulated clusters of endocrine cells located in the exocrine pancreas. These cell clusters were discovered in 1869 by Paul Langerhans. The islets can be thought of as micro-organs that are crucial for glucose homeostasis. An islet typically consists of four types of secretory cells, namely, the insulin-releasing β cells, the glucagon-secreting α cells, the somatostatin-containing δ cells and the pancreatic polypeptide-producing (PP) cells. The proportion of the different cell-types and how they are organized within the islet varies among species. The human islets contain 54% β cells, 34% α cells, and 10% δ cells whereas mouse islets have 75% β cells, 19 % α cells and δ cells were 6% (Brissova, et al. 2005; Cabrera, et al. 2006). In rodent islets, 71% of the β cells are clustered in the center of a generally round islet and are surrounded by a mantle of α and δ cells (Brissova et al. 2005; Cabrera et al. 2006). In contrast, pancreatic islet cells are mixed together in human islets with only 29% of them showing homotypic association (Cabrera et al. 2006).

The endocrine pancreatic islets are at the core of glucose homeostasis. Their rapid response to environmental stimuli and in particular the glucose level is based on their electrical properties. In a β-cell, the glucose metabolism leads to an increase of intracellular ATP/ADP ratio, which in turn closes the ATP-dependent K+ channels (KATP channel). Closure of the KATP de-polarizes the cell membrane that is followed by opening of voltage-gated calcium channels. Calcium ions entering the cell trigger the exocytosis of insulin-containing and GABA-containing vesicles. The secretion of insulin is not a sustained process but is driven in a 3 - 4 min pulsatile fashion by rapid oscillations of intracellular calcium (Bergsten, et al. 1994). Similar cyclic hormone release is observed for glucagon and somatostatin (Hellman, et al. 2009; Salehi, et al. 2007). It is noteworthy, that despite the different cyt架构 of the human and rodent islets, the pattern of hormones release is remarkable similar (Hellman et al. 2009; Salehi et al. 2007).

How the pulsatile release comes about is not understood today, but electrical coupling and auto / paracrine signalling have been suggested to be a part of the process. Electrical coupling was initially suggested, as cytostruc-
atural and electrophysiological studies have shown that gap junctions exist between most rodent β cells (Eddlestone, et al. 1984; Pérez-Armendariz, et al. 1991; Speier, et al. 2007). However, due to lack of evidence showing that the α and δ cells are similarly coupled, it has been hard to explain why glucagon and somatostatin secretions are pulsatile. Importantly, in human islets where most β cells lack electrical-coupling, the pulsatile secretion is still intact (Hellman et al. 2009).

**Figure 3.** A cartoon showing extracellular hormone signalling in pancreatic islets. Briefly, in β cells, insulin has been reported act in an autocrine fashion resulting in both inhibitory (mediated by activation of $K_{ATP}$ channels) (Khan, et al. 2001) and stimulatory effects (via intracellular Ca$^{2+}$ mobilization) (Aspinwall, et al. 1999). Insulin acts on the α cells to inhibit glucagon secretion (Gromada et al. 2007), whereas glucagon enhances both insulin and somatostatin secretion via elevation of cAMP (Gromada et al. 2007). Somatostatin inhibits the release of insulin and glucagon (Singh, et al. 2007). Red arrows indicate inhibitory regulation; green arrows, excitatory regulation.

In addition to electrical coupling, the close, physical proximity of the different pancreatic islet cells provides the structural basis for paracrine regulation of hormonal secretion by extracellular signal molecules released into islet interstitium (Caicedo 2013). Despite intensive research over many years, synchronization of hormone secretion by the pancreatic islet cells is still an enigma. In recent years focus has shifted to the role of extracellular signal molecules such as glucagon like protein-1 (GLP-1), Zn$^{2+}$ and GABA for regulation and coordination of secretion of metabolic hormones (Gromada, et al. 2007).
5.2 Diabetes mellitus

Type 1 diabetes (T1D) accounts for about 5 - 10% of all cases of diabetes world-wide (WHO, 1999). In T1D the pancreatic β cell mass is significantly decreased and this usually leads to absolute insulin deficiency. In most cases, T1D has a strong genetic component and the decreased β cell function is associated with cell-mediated autoimmune attack on the β cells (Devendra, et al. 2004). This includes activation of T-cell-mediated reactions in susceptible individuals leading to an inflammatory response within the islets as well as B-cell response with production of antibodies to β cell antigens including autoantibodies to insulin (IAA), glutamate decarboxylase (GAD), and the protein tyrosine phosphatase IA2 (IA-2AA), all of which are easily detected by sensitive radioimmunoassay (Daneman 2006; Devendra et al. 2004).

Type 2 diabetes (T2D) accounts for 90% of diabetes cases worldwide (WHO, 1999) and the official prevalence rate of T2D in Sweden is 4% (Jansson, et al. 2007). It is characterized by high blood glucose, a progressive decline in β cell function and chronic insulin resistance (DeFronzo 1988). Obesity is a major risk factor for the development of type 2 diabetes (Burke, et al. 1999) and is thought to confer increased risk for type 2 diabetes through the mechanism of associated insulin resistance (Ludvik, et al. 1995).

5.3 GABA and GABA-A receptors in the pancreatic islets

In the pancreatic islets, GAD is highly expressed in insulin secreting β cells. Interestingly, GAD65 is one of the major autoantigens in type 1 diabetes mellitus both in humans and rodents (Yoon, et al. 1999). Human β cells express high levels of GAD65 and somewhat lower of GAD67 (Kim, et al. 1993; Taneera, et al. 2012). Mouse and rat β cells express both GAD isoforms with the GAD67 being predominant in mice. Yet, GAD65 expression is not an absolute requirement for the development of autoimmune diabetes in the NOD mouse (Kash, et al. 1999). The metabolism of GABA in pancreatic islets is thought to be similar to that in the brain. After formation in the cytoplasm of β cells, GABA can remain in the cytoplasm or be stored in vesicles of β cells (Braun, et al. 2007; Reetz, et al. 1991) and has even been reported to be present in islet non-beta-cells (Braun, et al. 2010; Chessler, et al. 2002). In the β cells GABA is loaded into synaptic-like microvesicles (SLMV) by vesicular transporters (Chessler et al. 2002). VIAAT has been detected in rat but not in human islets. In rat islets, the distribution of VIAAT expression parallels that of GAD65, with increased expression in the mantle (Chessler et al. 2002). However, vesicular GABA accounts for only a small fraction of cellular GABA, the remaining GABA is in the cytoplasm. When a β cell is stimulated by glucose, SLMV is released along with
the insulin containing granules (large dense core vesicles) by calcium-dependent exocytosis. Nevertheless, the majority of GABA in cytoplasm is released by a non-vesicular pathway such as reverse transport by transporter (Attwell et al. 1993) or fusion pore (Braun et al. 2007; Smismans, et al. 1997) and the release is not dependent on glucose. Sorenson et. al reported that δ-cells contain and take up GABA, but no GAD was detected (Sorenson, et al. 1991). It has also been reported that in rat islet, considerable amount of GABA was stored in the vesicles containing insulin. When the islet is stimulated by glucose, GABA is released along with insulin (Braun et al. 2007; Karanauskaite, et al. 2009). Our recent microarray data showed that bestrophin 1 & 2 are also expressed in human islets (Taneera et al. 2012). It is thus possible that GABA is released through this anion channels similar to what has been observed in glia. Our data also showed that the GABA transporters are expressed in the islets but at a very low level.

In rodents, the cellular GABA content and release are dose-dependently increased by glutamine but decreased by sustained (24 h) glucose activation (Pizarro-Delgado, et al. 2010). An explanation for this can be the dynamic equilibrium that exists in the GABA anabolic/catabolic pathways. Glutamine dose-dependently increases GAD-mediated formation of GABA, whereas glucose metabolism shunts part of the newly formed GABA to mitochondrial catabolism, involving α-KG-induced activation of GABA-T (Wang, et al. 2006). Furthermore, glucose increases cytoplasmic ATP levels that in turn may suppress GAD activity in β cells and GABA production and release, similar to what has been described for ATP suppression of brain GAD activity (Winnock, et al. 2002).

Another possible source of GABA in the islets is from neurons innervating the islets. However, there is limited evidence for GABAergic nerves innervating the islets and, so far, it has only been reported for rat islets (Sorenson et al. 1991) where the neuronal processes extend into the islet’s mantle (Sorenson et al. 1991).

Several groups have studied expression of GABA-A receptor subunits in pancreatic islets (Bailey, et al. 2007; Braun et al. 2010; Wendt, et al. 2004; Xu, et al. 2006; Yang, et al. 1994). In human and rodent islets α, β plus some other types of GABA-A subunits are present and thus functional GABA-A receptors can be formed. In rodent pancreatic islets, only the α cells express GABA-A receptors (Bailey et al. 2007; Braun, et al. 2004) whereas human α, β and δ pancreatic islet cells all have GABA-A receptors (Braun et al. 2010).

The enzyme GAD that makes GABA is expressed in pancreatic β cells and GAD is a major autoantigen in T1D. The expression of GABA and GAD is dramatically decreased in pancreatic islet cells of T1D patients as compared to controls (Al-Salam, et al. 2009). Based on results from isolated pancreatic islets preparations, it has been suggested that a reduction in cellular and released GABA level is more sensitive than insulin as a marker for
the presence of dead beta-cells in the islets (Wang, et al. 2005). Furthermore, GABA has been found to have β cell regenerative and immune-inhibitory effects (Mendu, et al. 2011; Soltani, et al. 2011). Together, these results indicate a role for GABA in regulating islet cell function and glucose homeostasis, which may find clinical application in the future.

In the rodents, pancreatic islets α cells express GABA-A receptors that hyperpolarize the α cells resulting in inhibition of the glucagon secretion (Rorsman et al. 1989; Xu et al. 2006). Release of GABA, evoked by glucose application, from neighboring β cells contributes substantially to the acute inhibition of glucagon secretion from mouse islets and results in changes in GABA-A receptor expression (Bailey et al. 2007). Insulin promotes activation of GABA-A receptors in the α cells by inducing GABA-A receptor translocation to the cell membrane by activating an Akt kinase-dependent pathway. Activation of the receptors by GABA then leads to hyperpolarization of the α cells and, ultimately, suppression of glucagon secretion (Xu et al. 2006).

The GABA-A receptor antagonist SR95531 has been used to investigate the effects of the endogenous GABA (released from β cells) on rat pancreatic islet hormone secretion. The antagonist increased glucagon secretion two fold at 1 mM glucose and completely abolished the inhibitory action of 20 mM glucose on glucagon release. Basal and glucose-stimulated secretion of insulin and somatostatin were unaffected by the 20 µM SR95531 concentration (Wendt et al. 2004). In an in vitro study, in normal rat pancreatic tissue, GABA did not affect glucagon secretion but inhibited glucagon secretion from diabetic pancreatic tissue fragments (Adeghate, et al. 2000). Since chronically elevated glucose levels result in a reduced GABA discharge from the beta cells, it is conceivable that the subsequent decrease observed in the GABA-mediated suppression of the α cells is responsible for a higher glucagon release, as is observed in diabetes (Smismans et al. 1997). Interestingly, GABA induced a significant increase in insulin secretion from pancreas of normal rats whereas in pancreas from diabetic rats, GABA did not lead to significant increase in insulin secretion (Adeghate and Ponery 2002). Whether this effect involves GABA-A or GABA-B receptors or both is not clear.

In human islets, GABA-A receptors on α cells are inhibitory whereas they are excitatory in β and δ cells due to relatively high intracellular chloride concentration (Fig 4) (Braun et al. 2010). The GABA-A antagonist SR95531 inhibited insulin secretion elicited by 6 mM glucose. Application of GABA depolarized the β cells and stimulated action potential firing in β cells exposed to glucose (Braun et al. 2010). GABA signalling in the human pancreas is clearly more extensive than in rodents as GABA-A receptors are present on α, β and δ cells and GABA is released by β and δ cells. GABA also constitutes an autocrine positive feedback loop on the human β and δ cells (Braun et al. 2010).
Although there is no chemical synapse between the cells in the pancreas, the quantal GABA released from the β cells’ synaptic-like vesicles may still evoke transient currents at least on the beta cells themselves. However, all the electrophysiological studies that have been performed so far have been on cell lines, transfected islets with overexpressed GABA-A receptors or dispersed pancreatic cell where artificial high concentration of GABA has been applied (Braun et al. 2010; Braun et al. 2004; Wendt et al. 2004; Xu et al. 2006), so whether phasic currents are evoked normally in the islets is still not known. The interstitial GABA concentration that may be present in the islets is also unknown. Since GABA is released continuously by a nonvesicular pathway, it is not unreasonable to expect that GABA can also evoke tonic currents in the islet cells that may modulate the hormone release. In this study I will examine if tonic and phasic currents are evoked by GABA in the islets.
Aim of the Study

I Investigate how the metabolic hormone insulin affects the GABA-A receptors in the rat CA1 hippocampal pyramid neurons.

II Investigate the expression & physiological function of GABA-A receptors in intact rat pancreatic islets.

III Exam GABA signalling in human pancreatic islets from normoglycaemic and type 2 diabetic donors.

IV Investigate the subtypes & physiological response of GABA-A receptors in identified cell-types in intact human pancreatic islets.
Methods

1. Preparation of hippocampal slice

Postnatal 16 - 22 days old Wistar rats were used for experiments. Animal was sacrificed by decapitation, and the brain was quickly removed with a spatula and placed into ice-cold artificial cerebrospinal fluid (ACSF). Keeping the brain tissue cold is critical for generating brain slice with high quality. After cutting along the midline with a surgical blade upon filter paper, each hemisphere was glued onto specimen disk with the cutting surface downward. The disk was then immersed into the cutting chamber of the vibratome that is filled with an ice-cold ACSF bubbled with carbogen (95% O2 and 2% CO2). Slice thickness is set to 400 µM. The hippocampal slices are put into a glass petri plate filled with ACSF. The plate is placed on a black background (e.g. black paper) to allow easy visualization of the hippocampi. The hippocampus is isolated from the surrounding tissue using sharp surgical blades. Stretching of slice is avoided to keep it intact. Slices are then incubated in the ACSF solution at 37.0 °C for 1h with carbogen bubbling and then stored at room temperature until experiments are done. During the incubation the tissue recovers from the damage imposed by the cutting process.

2. Isolation of pancreatic islets

The experiments were carried out on isolated intact rat pancreatic islets from 50-52 days old Wistar rats. The rat was sacrificed by 95% CO2, and the pancreatic glands were taken out and kept in Hanks' solution. Following intensively cutting by scissors, the small tissue pieces were distributed to three sterilized glass vials each containing 6 mg collagenase from Clostridium histolyticum (Boehringer Mannheim, Mannheim, West Germany) dissolved in 6 ml Hanks' solution. The vials were shaken rapidly in a 37 °C water bath for 19 min until the tissue had disintegrated, as determined by visual inspection. The digest was sedimented three times in 20 ml Hanks' solution. By using 20 µl pipette, islets were picked by hand. Isolated islets were cultured maximally 4–5 d in RPMI 1640 medium (Sigma, St. Louis, MO) containing 10 mM glucose, supplemented with 10% (vol/vol) fetal calf serum, ben-
zylopicillin (100 U/ml), and streptomycin (0.1 mg/ml). Medium was changed every second day.

3. Patch clamp

The patch clamp technique was first used by Neher and Sakmann in 1976. The principle of the method is to isolate a patch of membrane electrically from the external solution and to record current flowing into the patch. This is achieved by pressing a glass pipette, which has been filled with a suitable electrolyte solution, against the surface of a cell and applying light suction. Providing both glass pipette and cell membrane are clean, a seal whose electrical resistance is more than 1 GΩ is formed. When giga seal is formed, this is called cell-attached mode (Fig 5).

![Figure 5. Schematic of patch clamp configurations](image)

Single-channel recording: when glass pipette is small enough (10-20 mΩ) and fire-polished, a seal with over 10 GΩ electrical resistance can be achieved, which reduces the current noise of the recording, permitting good time resolution of single channel currents, currents whose amplitude is in the order of 1 pA. When the pipette tip is pulled away from cell, the inside-out configuration can be formed. Under this condition, channel is detached from intracellular protein. Thus the channel conductance can be monitored without intracellular regulation (Fig 5).

Whole-cell recording: after forming the giga seal, a gentle suction is applied to break the cell membrane, whose interior then comes into contact
with the solution in the pipette, may be voltage or current-clamped. The cell contents equilibrate over time with the solution within the pipette. Whole-cell mode enables the monitoring of all current going through channels on the whole cell membrane.

4. Single-cell RT-PCR

The intact islet was held by a holding pipette and approached by recording pipette from the other side, as previously described by others (Macdonald and Olsen 1994). The whole-cell currents were recorded randomly from different type of pancreatic cells, in solution containing 20 mM glucose at room temperature. After whole-cell recording, the cytosome was collected for cell type discrimination by using single-cell PCR.

At the end of the patch-clamp recording, the cell cytosome (including the nucleus in some instances) was aspirated into the recording pipette by application of a negative pressure to the pipette (Fig. 6). Harvesting was interrupted before or as soon as the seal was lost. In order to avoid RNase or other contamination, all the instruments were sterilized, and gloves are required. The content of the pipette (5 µl) was then expelled into a test-tube and PCR is later performed in a final volume of 20 µl.

![Figure 6. Schematic of cytosome collection](image)

We tested regular RT-PCR and multiplex PCR in this study, both of which worked correctly. But since eventually the regular RT-PCR showed higher successful rate, we switched entirely to it.

For the regular RT-PCR, in the first step, the reverse transcription was performed with VersoTM cDNA synthesis kit (Thermo Scientific) in a final
volume of 20 µl. The reverse transcription reaction was incubated at 42°C for 30 min and then at 95°C for 2 min. In the second step, 3 µl of the RT product was used as a template. Each 3 µl cDNA was individually amplified using specific primer pairs for insulin glucagon and somatostatin, by a 40 cycles PCR protocol (95 °C, 15 s; 59 °C, 30 s; 72 °C, 60 s). 4 µl of each individual PCR reaction was examined by electrophoresis on a 1.5% agarose gel stained with SYBR Gold.

The two steps of multiplex PCR were performed essentially as described by Ruano D et.al (Göpel, et al. 1999). In the first step, cytosome, RT/Platinum Taq Mix (0.4 µl, invitrogen) and 0.67 pmole of each of the primer pairs (designed to amplify glucagon, insulin and somatostatin mRNA) were added to the buffer (reaction mix) supplied by the manufacturer (final volume 20 µl). The set of primers were verified in jPCR program (Ruano, et al. 1995) to avoid dimmers or possible interactions among different primers. The reverse transcription reaction was conducted at 55°C for 30 min followed directly by 40 PCR cycles (95 °C, 15 s; 59 °C, 30 s; 72 °C, 60 s). In the second step, 0.4 µl of the first PCR product was then used as template. Each cDNA was individually amplified using gene specific primer pair by performing 40 PCR cycles (as described above). 10 µl of each individual PCR reaction was then electrophoresised on a 1.5% agarose with SYBR Green.
Results and Discussion

Paper I

Our results demonstrate that in rat hippocampal CA1 neurons, physiological concentrations (1 nM) of insulin induced tonic currents in the neurons. The tonic current was inhibited by the GABA-A inverse agonist L655,708 (5 µM) that is selective for channels containing the α5 and γ2 subunits in the channel complex. This demonstrates that the insulin-induced current is at least partially carried by α5βγ2 GABA-A channels. We further examined two compounds that are normally inert at α5βγ2 channels: the benzodiazepine flumazenil (1 µM) and zolpidem (100 and 200 nM), a positive modulator at α1, α2 and α3 in αβγ2 channels. Surprisingly, in slices incubated in insulin both flumazenil and zolpidem decreased the holding current revealing the large insulin-induced tonic current. Similar to L655,708, flumazenil (n=8) and zolpidem (n=6) inhibited the tonic current by 76 ± 6% and 81 ± 5%, respectively. The tonic current is generated by novel, high-affinity GABA-A receptors and when in place, regulates the CA1 neurons excitability. The CA1 pyramidal neurons in slices incubated with insulin had, on the average, resting membrane potential of -59.2 ± 0.4 mV (n = 10) whereas in control slices it was -57.6 ± 0.4 mV (n = 6) and accordingly, we found that insulin incubation of the slices significantly decreased the action potential firing rate (23.2 ± 1.2 Hz, n = 10) as compared with ACSF control (27.7 ± 1.6 Hz, n = 6). The GABA-A channels are activated by more than million times lower GABA concentrations than synaptic channels, generate tonic currents and show outward rectification. The single-channel current amplitude is related to the GABA concentration, resulting in a single-channel GABA affinity (EC50) in intact CA1 neurons of 17 pM with the maximal current amplitude reached with 1 nM GABA.

GABA-A channels having the α5 subunit in their channel complex are known to be mostly located extrasynaptically in CA1 neurons but are not or minimally activated by the ambient GABA concentration. How the “new” channels differ from the α5-channels normally in the membrane is not clear but heteromeric subunits in the channel complex, different intracellular modification or associations with intracellular proteins can all give rise to the differences observed (Birnir and Korpi 2007; Ju, et al. 2009). Interestingly, the induced tonic current is inhibited by flumazenil and zolpidem, indicating a distinct pharmacology of these novel GABA-A receptors. In the presence
of zolpidem, there was a significant increase in the action potential firing rate in insulin treated but not in ACSF control neurons. These results are somewhat surprising as zolpidem potentiates the synaptic currents and its effects on the tonic current would at least partially be cancelled by the increased sIPSCs. Since the overall effect of zolpidem in the insulin treated slices was increased excitability of the neurons, it supports the notion (Glykys and Mody 2007; Hamann, et al. 2002; Pavlov, et al. 2009) that tonic rather than synaptic conductance regulate basal neuronal excitability when significant tonic conductance is expressed.

Paper II

Gamma-aminobutyric acid (GABA) is an extracellular signalling molecule in the interstitial space in pancreatic islets. We have examined the expression and function of the GABA-signalling system components in human pancreatic islets from normoglycemic and type 2 diabetic (T2D) individuals. Expression of GABA-signalling system components was studied by microarray, quantitative PCR analysis, immunohistochemistry and patch-clamp experiments on cells in intact islets. Hormone release was measured from intact islets. The results revealed that the GABA-signalling system was compromised in islets from T2D individuals where the α1, α2, β2 and β3 GABA-A receptor subunits were significantly down-regulated. The expression of GABA-A associated proteins, the GABA-B receptor subunits, GABA and Cl- transporters and GAD65 and 67, were all examined. However, there was no significant difference between normoglycemic and type 2 diabetic (T2D) individuals.

GABA originating within the islets evoked tonic GABA-A channel currents in the cells. The currents were enhanced by pentobarbital and inhibited by the GABA-A receptor antagonist, SR95531. The effects of SR95531 on hormone release revealed that activation of the GABA- receptors decreased both insulin and glucagon secretion. High concentrations of SR95531 (100 µM) were needed to increase the insulin secretion in low (1 mM) glucose suggesting a role for high-affinity GABA_A receptors regulating insulin release. The GABA-B receptor antagonist CPG55845 increased insulin release in islets (glucose = 16.7 mM) from normoglycemic and T2D individuals. Our results revealed that the interstitial GABA activates GABA-A receptors, the GABA-B receptor and effectively modulates hormone release in islets from T2D and normoglycemic individuals. GABA appears to be a molecule participating in maintaining the right balance between the insulin and glucagon secretion in the pancreas and, therefore, in the blood. Drugs modulating the GABA-signalling system may have therapeutic potential in T2D.
Paper III

Gamma-aminobutyric acid (GABA) is an extracellular signalling molecule in the interstitial space in the rat pancreatic islets. We have examined the expression and function of the GABA-A receptors in rat pancreatic islets. The expression was studied by quantitative RT-PCR analysis and functional properties were examined in patch-clamp experiments on cells in intact islets. The cell-type was identified by single-cell RT-PCR. The results revealed that 17 different GABA-A receptor subunits are expressed in rat islets. Among all the subunits, α1-4, β3, γ3, δ, θ and ρ1-3 subunits had the highest expression level. We collected cytosomes from 95 cells and of those were able to identify the cell-type of 45 cells resulting in a success rate of 45%. Of the identified cells, 49%, 28% and 7% were α, β and δ cells, respectively. In 16% of the cells, transcript for more than one hormone was detected. GABA originating within the islets evoked tonic GABA-A channel currents in the α cells (n = 7) but not in the β cells (n = 7). The currents were enhanced by pentobarbital (n = 7) and inhibited by the GABA-A receptor antagonist SR95531 (100 µM, n = 6). The whole-cell IV relationship showed outward rectification and the single-channel conductance (holding potential = -70 mV) ranged from about 30 to 65 pS. Pentobarbital increased the open probability and the conductance of the single-channels. Whether the different single-channel conductance is due to different subtypes of GABA-A receptors or different GABA concentrations activating the channels remains to be determined. Our results revealed that the interstitial GABA in rat islets reaches concentration levels that are high enough to active native GABA-A receptors channels that are only present in the glucagon-releasing α cells in rat islets.

Paper IV

Gamma-aminobutyric acid (GABA) is an extracellular signalling molecule in the interstitial space in human pancreatic islets. EXPAND INTRODUCTION From the single-cell PCR, the majority of the human pancreatic cells with GABA-activated currents were β cells expressing insulin (25 cells, 40%). Another 11 cells were α cells (17%) expressing glucagon and 5 cells were δ cells (8%) expressing somatostatin. Another 18 cells (29%) expressed insulin together with either somatostatin (10%) or glucagon (19%) and the remaining 6% of the cells expressed all three hormones.

Our results show that GABA-activates GABA-A receptors in α, β and δ cells in intact human islets, and evokes tonic currents. The α and β cells express high-affinity GABA-A receptors that are activated by interstitial GABA concentrations that may be lower than 10 nM. As the GABA concentration in the islet increases, the channel activity increases until the channels
desensitize often by about $1\ \mu$M GABA concentration. Only in the $\delta$-cells were large, transient, spontaneous currents evoked by GABA with amplitudes up to $\sim200\ \text{pA}$. Whether this suggests that the $\delta$ cell is the only cell-type equipped with synaptic-like GABA-A receptors or if only the $\delta$ cells’ GABA-A receptors are located close enough to a GABA releasing site, where they are exposed to high mM concentrations of GABA, remains to be examined. The tonic currents recorded in all three cell-types may have a role in synchronizing the membrane potential of populations of cells and may affect the action potential frequency whereas the phasic current in the $\delta$-cells suggests an acute regulation of somatostatin secretion via the GABA-A receptors. In human islets, the GABA signaling is clearly more extensive and different from what has been recorded in rodents’ pancreatic islets. We could identify several different subtypes of GABA-A receptors in the cells based on single-channel kinetics, current-voltage relations and pharmacology. We have found SR-95531 insensitive GABA-A receptors in human islet cells. SR-95531 inhibits most GABA-A receptors made from the $\alpha\beta$, $\alpha\beta\gamma$ or $\alpha\beta\delta$ subunit composition whereas e.g. homomeric $\rho$ GABA-A receptors are insensitive to SR-95531 but are blocked by picrotoxin (Chebib and Johnston 1999). In human islets most GABA-A receptor subunits are expressed including $\rho2$ and $\pi$ subunits, which have low expression in the central nervous system (Hedblom and Kirkness 1997; Koulen, et al. 1998). What functional and pharmacological properties are imposed on the GABA-A receptors by inclusion of the $\pi$ subunit is not known today. It is possible that the channels that were inhibited by picrotoxin but not by 100 mM SR-95531, were GABA-A receptors containing $\rho2$ or $\pi$ subunits. The alternative explanation is that at high concentrations, SR-95531 may act as a partial agonist and increase the open probability of some subtypes of GABA-A receptors (Wlodarczyk, et al. 2013). The data provides insight into the mechanisms underlying GABA regulation of different cell-types in intact human islet.
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