Expression of G-Protein $\beta\gamma$ Subunits In Endocrine Pancreatic Cell Lines

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5. årsoppgave i Stadium III - Medisinstudiet ved Universitetet i Tromsø

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University of Tromsø, Institute of Clinical Medicine, September 2004

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RÉSUMÉ

Heterotrimeric guanine nucleotide binding regulatory proteins (G proteins) play an important role in many transmembrane signaling events and are composed of $\alpha\beta\gamma$ subunits. Upon activation, the GTP-bound α subunit interacts directly with various effectors. The β and γ subunits of the G protein form a tightly connected dimer which directly modulate effector molecules such as adenylyl cyclase II and VI, phospholipase A2 and C, cardiac K^+ channels and N type Ca^{++} channels. Several studies have revealed a variation in affinity between the subunits in the dimer depending on the combination of the different β- and several γ-subunits, and that the different combinations do not have the same affinity to the Gα subunits. It has also been shown that the respective $\beta\gamma$ dimers differ in which signaling pathways they are involved in.

A cellular distribution of the different β- and γ subunits in the different endocrine cell types located in islets of Langerhans has not been made. Therefore, in this study, five well-established endocrine pancreatic cell lines, representing insulin (Rin5F and β-TC3)-, glucagon (INR1-G9)-, and somatostatin (Rin14B, Rin1027-B2 and AR42J)- producing cells have been used to study the expression of the different $\beta\gamma$ subunits by using the reverse transcriptase polymerase chain reaction (RT-PCR) technique.

Our results show for the first time that all presently known β subunits and γ3, γ5, γ7, γ10 and γ12 subunits are present in the endocrine pancreas. The β1- and γ12 subunits are present in all pancreatic cell lines tested, whereas γ1, γ2, γ6, γ4 and γ8 subunits were not detected in any of the cell lines tested. A different, cell specific expression was observed for the other subunits. These findings may be of importance regarding the different regulation pattern found for the different effectors in the endocrine pancreas.

1. INTRODUCTION

The family of structurally homologous guanine nucleotide binding regulatory proteins or G proteins plays an important role in transducing extracellular signals from cell-surface receptors to intracellular effectors [1, 2, 3]. G proteins are heterotrimeric protein composed of $\alpha\beta\gamma$ subunits. In most biological systems, the α subunits are generally recognized as the signal carrier that dictates the specificity of signaling pathways. It has been assumed that the β and γ subunits, which form a tightly associated $\beta\gamma$ complex, usually play a more passive role by promoting interactions between the α subunit and the receptor [4]. However, there is now a growing body of evidence demonstrating that $G\beta\gamma$ also participates in a wide range of other G protein functions [for reviews see 5, 6, 7, 8] (see also figure 1.).

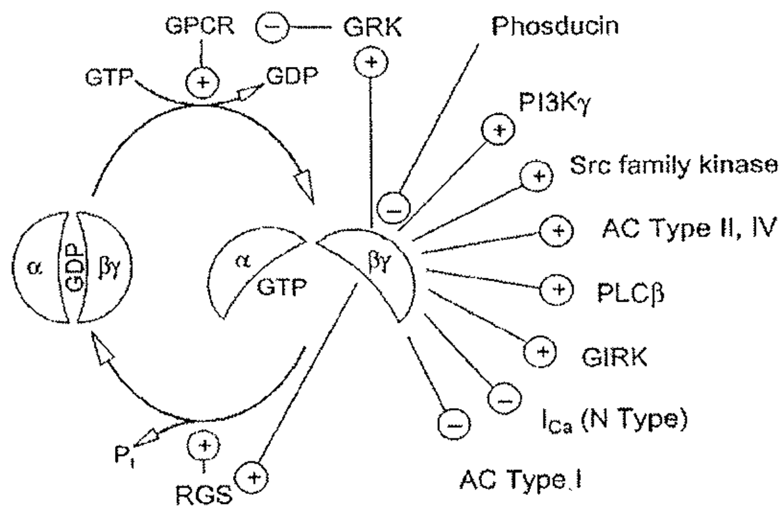

Figure 1: Interactions of $G\beta\gamma$-dimers in the GTPase cycle and signal transduction pathways.

This include the promotion of cholera toxin- and pertussis toxin-catalyzed ADP-ribosylation of the $G\alpha$ subunit [9], interaction with phosducin [10] and receptor kinase [11, 12, 13], and

regulation of the activities of effectors such as adenylyl cyclase types II and VI, phospholipase C, N type Ca^{++} channels and cardiac K^{+} channels [14, 15, 16, 17, 18], activation of mitogen activated protein kinase (MAPK) and phosphotidylinositol-3-kinase (PI3K) [19]. The molecular mechanism by which the Gβγ complex regulates a diversity of signaling processes is still not fully understood.

To date, five forms of Gβ [20] and multiple forms of Gγ subunits have been identified by biochemical, immunological, and molecular cloning studies [21]. At the amino acid levels, the Gβ subunits are highly conserved. In contrast, the Gγ subunits are more divergent. Because of the diversity of the Gγ sequences, it is generally believed that the Gγ subunit determines the functional specificity of the Gβγ complex [22]. *In vitro* assays [23, 24, 25] and yeast two-hybrid analysis [26] have demonstrated that the various βγ combinations interact widely with varying affinities, and indicate that this affinity, in combination with the level of expression of a particular subunit gene, determines which heterotrimeric combinations are active in a given cell.

The purpose of this study was to gain further insight into the molecular distribution and regulatory function of Gβγ subunits in the endocrine pancreas. Investigations regarding Gβγ distribution had not yet been undertaken. Therefore, the knowledge of Gβγ subunit expression will further clarify and possibly specify the G protein-coupled signaling pathways that are involved in endocrine functions of the different hormone-producing cells located in the endocrine pancreas.

2. MATERIALS AND METHODS

2.1. Materials

Cell culture containers were from Nalgene Nunc Int.; RPMI-1640 medium from GIBCO BRL Gaithersburg, MD. USA; Trizol™ (total RNA isolation reagent for liquid samples) and deoxyribonuclease I, amplification grade were from GIBCO BRL Gaithersburg, MD, USA; Mineral oil was from SIGMA; Ready-To-Go™ RT-PCR and PCR beads were from Amersham Biosciences; Ready-To-Go pd$(N)_6$ universal primer was from Pharmacia Biotech; G-protein β-and γ subunit specific primers were synthesized by MedProbe, Norway; Agarose and molecular weight markers were from PROMEGA, Madison, WI, USA. All other chemicals used in this study were of standard laboratory grade.

2.2 Cell-culture

INR1-G9 cells [27], Rin5F cells (ATCC no.: CRL-2058) [28], β-TC3 cells [29], Rin1027-B2 cells [30], Rin14B cells (ATCC no.: CRL-2059) [28], 6A3 cells [31], and AR42J cells (ATCC CRL-1492) [32] were cultured in RPMI-1640 (11mmol glucose) medium supplemented with 5% FBS (fetal bovine serum), 75 μg/ml penicillin and 50 μg/ml streptomycin and incubated in a humidified atmosphere with 95% air and 5% CO_2.

The cells were trypsinised and sub-cloned once or twice weekly. The medium was changed twice weekly and 24 hours prior to an experiment. The cells were always harvested at a logarithmic phase of growth.

2.3 RNA preparation

Total RNA was isolated directly from cell samples with TRIZOL™ and according to the manufacturer's protocol. After removal of the cell culture medium, 750 μL TRIZOL™

reagent was added to each cell container to achieve lysis of the cells. The cells were detached from the container by a cell scraper and homogenized before they were placed in a sterile test tube. The sample suspension was incubated in room temperature for 5 min to permit complete dissociation of the nucleoprotein. To separate the aqueous phase containing RNA, 200μl chloroform was added. The mixture was incubated for 10 min at room temperature before centrifugation for 15 min at 12,000 x *g* at 2 to 8°C. The aqueous phase was transferred to a new sterile test tube, and 500 μl of isopropanol was added to precipitate RNA. After 10 min incubation at room temperature and centrifugation for 10 min at 12,000 x *g* at 2 - 8°C, the supernatant was removed. The RNA pellet was washed once with 1.0 ml 75 % ethanol. After centrifugation at 7,500 x *g*, the RNA was re-dissolved in 50 μl of RNase-free water. The sample was then incubated 10 min at 59°C in a water-bath to dissolve the RNA completely. Quantity and quality of total RNA was judged by spectrophotometry at a wavelength of 260 and 280 nm, respectively.

2.4 Reverse transcriptase polymerase chain reaction (RT-PCR)

For each sample 200 ng DNAse I treated total RNA was used for amplifications. RT-PCR was performed in a total volume of 50 μl using Ready-to Go™ RT-PCR beads and according to the manufacturer's protocol. The respective forward and reverse primers corresponding to the G-protein β-and γ subunits used for amplification are listed in table 1. The samples were processed in a PTC-100 programmable thermal cycler (Hybaid). Cycles were carried out at 94°C for 90 sec, 60°C for 80 sec, and 72°C for 2 min (35 cycles) with a final step at 72°C for 7 min. In some cases the annealing temperature was decreased 2°C or increased by up to 8°C. Primers that correspond to the house keeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) [33] were used as an internal standard. The products

were separated by electrophoresis in TBE buffer on 1.8% - 3% agarose gels depending on the size of amplified fragments, and were finally visualised by staining with ethidium bromide.

3. RESULTS

Total RNA was prepared from seven different pancreatic cell lines and treated with DNase I prior to reverse polymerase chain reaction (RT-PCR) as described more extensively in Materials and Methods. For amplifications, primer pairs corresponding to different β- and γ-subunits were used (see table 1).

The expected molecular sizes (in bp) of each β- and γ-subunit have been theoretically evaluated according to the positions of the primer pairs, as indicated (see also table 1). The obtained PCR products were separated by gelelectrophoresis on 1.5-3.0 % agarose gels and thereafter visualized by staining with ethidium bromide as described in Materials and Methods. The amplification of five β- and nine γ- subunits by RT-PCR is summarized in table 2.

The β1 subunit with a molecular weight of 543 bp of the amplified product was the only β-subunit found in all the cell lines tested. The β2 subunit with an amplified product of 601 bp was found in somatostatin-producing Rin14B cells, Rin1027-B2 cells and glucagon-producing INR1-G9 cells. The β3 subunit PCR product with a molecular weight of 653 bp was detected in insulin-producing Rin5F cells, somatostatin-producing Rin1027-B2 cells, AR42J cells and 6A3 cells. β4- and β5 subunits were both amplified in insulin-producing Rin5F cells, in somatostatin-producing Rin14B and Rin1027-B2 cells and in 6A3 cells with

an estimated molecular weight of 432 and 580 bp, respectively. All the 5 β subunits tested were found in insulin-producing Rin5F and somatostatin-producing Rin1027-B2 cells.

Only a limited number of Gγ subunits were determined by RT-PCR in the endocrine pancreatic cell lines. The γ3 subunit was found in glucagon-producing INR1-G9 cells and somatostatin-producing Rin1027-B2 cells with a molecular weight of 158 bp for the amplified product. The γ5 subunit was found in all the cell lines except in glucagon-producing INR1-G9 cells and somatostatin-producing Rin1027-B2 cells with a PCR product size of 715 bp. The γ7 subunit was found in insulin-producing Rin5F cells and somatostatin-producing Rin14B cells, and the γ10 subunit was found in all cell lines tested except somatostatin-producing Rin14B cells, with molecular weights for their PCR products of 786 and 175 bp, respectively. The γ12 subunit, with a PCR product size of 218 bp, was found in all cell lines tested.

When testing for γ1–, γ2, γ6 –, γ4– and γ8– subunits, no specific PCR products were obtained. Increasing amounts of template and re-amplification attempts did not change these results (data not shown).

Amplification of the house-keeping gene GAPDH was used as internal standard and resulted in a PCR product with a molecular weight of 328 bp for all cell lines tested, respectively (data not shown).

The expected molecular sizes (in bp) of each β- and γ-subunit tested have been theoretically evaluated according to the positions of the primer pairs.

4. DISCUSSION

In this study we investigated for the first time the expression of G-protein β- and γ subunits in isolated cell lines that represent the different endocrine cell types that are located in islets of Langerhans. The results showed a differential expression pattern of βγ subunits that was independent of the endocrine cell type. For example, a different expression pattern for the two insulin-secreting β-cell lines β-TC3 and Rin5F was observed. One possible explanation may be that both tumour cell lines are derived from different species, β-TC3 from mouse and Rin5F from rat [29, 28]. It is hereby also interesting to note that both cell lines represent a different type of transplantable insulinoma, one produced by SV40 transformation (β-TC3) and the other produced by UV irradiation (Rin5F). In addition, cell lines and primers derived from different species have been used for the screening. For some species the β- and γ subunits are not yet determined by molecular cloning making the design of specific primers impossible. This could explain why the G protein γ1, γ2, γ6 or γ4 subunits could not be detected in any cell lines. However, γ8 subunits could not be detected in any of the rat pancreatic cell line cell lines although the primers were derived from published rat sequences.

Islets of Langerhans are a conglomerate of different hormone-producing cell types and many G protein-coupled pathways are present in the pancreas representing autocrine, endocrine and exocrine signal transduction. However, little is known about the involvement of specific βγ dimer combinations in regulating different signaling pathways. Only for some signaling pathways the specificity of βγ dimer combinations have been determined. In the endocrine pancreas insulin secretion is initiated by an increase in cAMP through activation of adenylyl cyclase caused by Ca^{2+} influx, sequestration or electrical activity [34]. Another G protein-coupled effector, phospholipase C (PLC), is involved in glucose-induced insulin

secretion regulation by cholecystokinin and acetylcholine. It is known that both effectors are regulated by βγ subunits. Zhang et al. [35] showed that a specific β subunit determines which effector molecule is regulated. Phospholipase C β2 (PLCβ2) is activated if β1 or β5 complexed with γ2 is present, whereas the mitogen-activated protein kinase (MAPK) or c-jun-N-terminal kinase (JKN) pathways are activated by a βγ2 complex containing β1, but not β5. For AR42J cells it is known that gastrin can activate the expression of CRE-regulated gene ICER involving PKA, and that MAPK and PKC signaling is involved in gastrin induced proliferation [33]. Here, the MAPK pathway might involve β1 subunit but in combination with a γ subunit different then γ2, since this subunit was not found in these cells.

β5 appears to be highly selective with respect to a number of phospholipids-dependent enzymes [36]. For example, β5γ2 will only activate PLCβ1 and PLCβ2, whereas β1γ2 will activate these isoforms as well as PLCβ3, PI3K3, and PI3Kγ. From our findings the β5 subunit is found in Rin5F, Rin14B, Rin1027-B2, β–TC3 and 6A3 cells but not the γ2 subunit. This implies that there are other possible β5γ combinations responsible for activating the above mentioned effectors.

Voltage-dependent Ca^{2+} channels are regulated by muscarinic and somatostatin receptors activation in a G protein-dependent manner; the M4 muscarinic receptor requires Gαi1 to associate with β3γ4, and somatostatin signaling is specific for the Gαo2β1γ3 combination [37, 38]. From our findings, the specific combination β1γ3 is possible in glucagon-producing INR1-G9 cells and somatostatin-producing Rin1027-B2 cells. In INR1-G9 cells, this specific βγ dimer might be involved in the inhibitory actions of somatostatin on glucagon secretion. On the other hand, β1γ3 may trigger inhibition of somatostatin secretion by an autocrine mechanism in Rin1027-B2 cells.

In the present work we have not screened for γ9 or γ11. γ11 has been shown to have structural and functional similarities with γ1 [39], and could therefore be expected to have a similar tissue distribution.

There are several steps in the signaling pathway that might also be significant: The type of G-protein-coupled receptor (GPCR), its location in the membrane and associated cell compartment, the α-subunit involved and effectors present in the cell. There are also several theories regarding the relationship between the α- and the βγ- subunits, for instance there is not jet known in what degree the monomer and dimer are separated during the reactions [40].

From our limited studies, we can conclude that all known G protein β subunits are present in the endocrine pancreas, and so are G protein γ3-, γ5-, γ7-, γ10- and γ12 subunits. We have also shown that the distribution for some of these subunits is specific for some hormone-producing cell lines. Future experiments will be needed to explore the potential specific βγ subunit combinations involved in cellular signaling in the endocrine pancreas.

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6. TABLES

Table 1: **PCR primer sets for amplification of different G-protein βγ subunits**

G-protein subunit	GeneBank Acc #	Nucleotide sequence	Expected size in bp
β1	U34958 rat	FW: 5'-CAT-CTG-CTC-CAG-TTA-CAA-TCT-GAA-3' REW: 5'-AGT-GCA-TCC-CAG-ACA-TTA-CAG-TTG-3'	543
β2	U34959 rat	FW: 5'-TAG-CCA-CAG-CCA-TCC-CAT-CAT-CT-3' REW: 5'-CGA-GAG-GGC-AAT-GTC-AGG-GTC-A-3'	601
β3	L29090 rat	FW: 5'-GTG-GGA-CAC-ACT-GGT-GAC-TGC-ATG-3' REW: 5'-TTG-CCC-TTG-TGA-CCC-TGC-TAC-TC-3'	653
β4	S86124 rmouse	FW: 5'-GCC-TCT-CAC-TGA-ATC-CGT-ACT-TG-3' REW: 5'-TCC-AGG-AVC-CVG-TSG-CCA-CRG-CCA-T-3'	432
β5	L34290 rat	FW: 5'-TGT-GGT-GGT-CTG-GAT-AAT-AAG-TG-3' REW: 5'-CCT-TGA-GAA-CAT-CCT-AGA-CAT-TG-3'	580
γ1	K03255 bovine U38495 mouse	FW: 5'-GAT-GCC-AGT-NAT-YAA-CAT-YGA-RGA-3' REW: 5'-ATA-RTC-TCT-CAC-TTC-KTC-RCA-RCA-3'	130
γ2,6	J05071 bovine	FW: 5'-GGT-MGA-ACA-GCT-SAA-GAT-GGA-3' REW: 5'-GTA-CAG-STG-YBA-BSA-RDG-GRT-C-3'	119
γ3	M58349 bovine U38497 mouse	FW: 5'-GAT-GAA-RGG-TGA-AAC-DCC-TGT-NAA-3' REW: 5'-AGC-GTG-GGC-ATC-ACA-GTA-TGT-CAT-3'	158
γ4	U37527 mouse	FW: 5'-GAA-TGA-AGG-AAG-GAA-TGT-ABA-AYA-A-3' REW: 5'-CTC-CCG-CAC-GTA-GGC-TTC-ACA-GTA-3'	155
γ5	M95780 rat	FW: 5'-GCC-ATA-CAC-ATA-ATC-TTC-TAC-TC-3' REW: 5'-AGC-AAA-GTT-ACA-GGT-CAC-AGA-GG-3'	715
γ7	L23219 rat	FW: 5'-CGA-ACG-CAT-CAA-GGT-TCT-CCA-AG-3' REW: 5'-GTT-ACA-CAC-TCG-GCC-ATA-CCA-G-3'	786
γ8	L35921 rat	FW: 5'-AAC-GCT-AAG-GAT-GAC-CCA-CT-3' REW: 5'-CAG-GCA-CCA-AGT-CAC-CAA-GT-3'	162
γ10	U31383 human	FW: 5'-CGT-GGA-GAG-GAT-CAA-GGT-CT-3' REW: 5'-GGT-ATT-CCT-CAG-CAA-ACT-TC-3'	175
γ12	U37561 human	FW: 5'-AGA-TGT-CGA-GCA-AGA-CAG-CCA-GTA-CC-3' REW: 5'-CTA-TAA-GAT-GGT-GCA-GGT-CTT-CTT-ATC-C-3'	218

Table 2: Expression of G-protein βγ subunits in 7 different endocrine pancreatic cell lines.

G protein subunit	INR1-G9	Rin5F	Rin14B	Rin1027-B2	β-TC3	AR42J	6A3
Beta 1	+	+	+	+	+	+	+
Beta 2	+	+	+	+	-	-	-
Beta 3	-	+	-	+	-	+	+
Beta 4	-	+	+	+	+	-	+
Beta 5	-	+	+	+	+	-	+
Gamma 1	-	-	-	-	-	-	-
Gamma 2,6	-	-	-	-	-	-	-
Gamma 3	+	-	-	+	-	-	-
Gamma 4	-	-	-	-	-	-	-
Gamma 5	-	+	+	-	+	+	+
Gamma 7	-	+	+	+	-	-	-
Gamma 8	-	-	-	-	-	-	-
Gamma 10	+	+	-	+	+	+	+
Gamma 12	+	+	+	+	+	+	+

+ = expression
- = no expression