Cell-specific localization of G protein α-subunits in the islets of Langerhans

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Abstract

G protein α-subunits are involved in the transduction of receptor-mediated regulation of insulin and glucagon secretions. To get further insight into the status of G proteins in α- and β-cells of the Langerhans islets, we have used immunohistochemistry to study the distribution of α-subunits in pancreas sections from the rat.

Our results show that only insulin-immunoreactive β-cells display immunoreactivity for selective antibodies directed against the different members of the Gαs- and Gα12-families (αi, αi2, αi12, αi3 respectively). Immunoreactivities for antibodies directed against members of the Gαq- and Gαi-families showed a more diverse localization: αi and αo2 were only detected in glucagon-immunoreactive α-cells, whereas αi1 was detected in all β-cells but only in a few α-cells. Even though β-cells showed immunoreactivities for αi3-non isoform-selective antibodies, we could not identify the isoform(s) present using selective αi1 and αo2 antibodies. Other members of the Gαq- and Gαg,families (αq3, αq2, αq and αq) were detected in both α- and β-cells.

In conclusion, our findings demonstrate a clear difference in the localization of G protein α-subunits between α- and β-cells, suggesting the involvement of specific receptor transduction pathways for the neuronal/hormonal regulation of α- and β-cell functions.


Introduction

The regulation of insulin and glucagon secretions by hormones and neurotransmitters is mediated through membrane receptors coupled to heterotrimeric GTP-binding proteins (G proteins) (Skoglund & Rosselin 1993, Gillison & Sharp 1994, Seaquist et al. 1994, Marie et al. 1996). These G proteins consist of three subunits: α, β and γ. The α-subunit is well known to regulate specific intracellular effectors. To date, about 22α-subunits have been identified and classified into four subfamilies: Gαs, Gαq, Gαi and Gα12.

Members of the Gαs-family are known to regulate the stimulation of adenyl cyclase (AC) activity by receptor ligands (Cooper et al. 1995, Tausig & Gilman 1995, Antoni 1997). This family contains at least two distinct α-subunits translated from two different genes, Gαs and Gαst (Hepler & Gilman 1992, Neer 1995, Exton 1996), and the presence of both αs and αst subunits has been indicated in β-cells (Walsh et al. 1989, Zigan et al. 1992, 1994). The receptor activation of phospholipase C-β (PLC-β), on the other hand, is known to be mediated through members of the Gαq-family (Cockcroft & Thomas 1992, Bristol & Rhee 1994). To date, five members have been identified in various tissues: αq1, αq11, αq14, αq15 and αq16 (Hepler & Gilman 1992, Neer 1995, Exton 1996). Among these members, αq11 and αq1-subunits are the more widely expressed; their presence in the islets and various β-cell lines have been suggested by the use of non-selective antibodies (Gillison & Sharp 1994, Seaquist et al. 1994, Marie et al. 1996).

The Gαi-family includes the initially separated Gαi1, Gαi2- and Gαi3-families, and the two more recently discovered Gαi4 and Gαqout. A specific effector cannot be attributed to this family since the different subfamilies have been shown to regulate different effectors. Nevertheless, members of the Gαi-family are involved in the transduction of the inhibitory action of hormones and neurotransmitters on insulin secretion (Sharp 1996). Up to seven αi-subunits of this family have been clearly identified in different tissues: three αi1, two αi2, αi2, and αi3-subunits (Hepler & Gilman 1992, Neer 1995, Exton 1996). The exact...
number of \( \alpha \)-isoforms is still unclear due to the generation of multiple \( \alpha \) splice variants and isoforms (Hsu et al. 1990, Shibasaki et al. 1991, Seaquist et al. 1992, Murtagh et al. 1994, Nurnberg et al. 1994, Wilcox et al. 1995).

The last G protein family, the \( G_{\alpha 12} \)-family, contains two members, \( \alpha_{12} \) and \( \alpha_{3} \) (Hepler & Gilman 1992, Neer 1995, Exton 1996), which are thought to be ubiquitously expressed (Spicher et al. 1994).

Although most of the G protein \( \alpha \)-subunits are expressed in a wide variety of tissues, some seem to be more restricted to neuronal tissues (Hepler & Gilman 1992, Neer 1995, Exton 1996). Since \( \beta \)-cells share a number of characteristics with neuronal cells (Steiner et al. 1989, Zigman et al. 1992), it is not surprising that some of the ‘neuronal’ G proteins such as \( \alpha_{s} \), \( \alpha_{o} \), \( \alpha_{z} \) and \( \alpha_{2z} \), are found in \( \beta \)-cells (Hsu et al. 1990, Seaquist et al. 1992, Zigman et al. 1992, 1994). Most of the information concerning the expression of G proteins in \( \beta \)-cells comes from studies using immortalized \( \beta \)-cell lines or whole islet extracts. However, immortalized \( \beta \)-cell lines do not faithfully reproduce pancreatic \( \beta \)-cells (Piolet et al. 1996), and islets of Langerhans contain also three other types of endocrine cells (glucagon-, somatostatin- and pancreatic polypeptide-secreting cells). Therefore, we investigated the expression of G protein \( \alpha \)-subunits in identified \( \alpha \)- and \( \beta \)-cells using double-labeling indirect immunofluorescence on rat pancreas sections.

Our results show for the first time a specific localization of \( G_{\alpha s} \) and \( G_{\alpha 12} \)-families in \( \beta \)-cells and a differential localization of some members of the \( G_{\alpha o} \)- and \( G_{\alpha z} \)-families in \( \alpha \)- or \( \beta \)-cells. This cell-specific distribution of G protein \( \alpha \)-subunits in the islet of Langerhans suggests that different transduction mechanisms may be involved in the receptor regulation of \( \alpha \)- and \( \beta \)-cell functions.

Materials and Methods

Antibodies

The identification of pancreatic endocrine cells was performed using anti-insulin (human, HUI-018) and anti-glucagon (porcine, Glu-001) mouse monoclonal antibodies purchased from Novo (Copenhagen, Denmark). To reveal G protein \( \alpha \)-subunit-immunoreactive cells, we used rabbit polyclonal antibodies. The sources as well as the amino acid sequence against which each antibody was raised are shown in Table 1 and in Begeot et al. (1991) and Rouot et al. (1992).

The two fluorescent antibodies used to detect immunoreactive cells were anti-mouse or anti-rabbit IgG-F(ab')\(_2\) goat fragments which were conjugated either to dichlorotri zinyI amino fluorescein (DTAF) or to Texas Red (Jackson ImmunoResearch, PA, USA).

Pancreas preparation

Pancreata were dissected from three adult male Wistar rats provided by a local source and which had been fed with a standard diet. Each pancreas was cut into approximately 1·0 mm pieces and fixed according to standard techniques. Briefly, the pancreas was fixed (3 h at 4 °C) in freshly prepared 4% v/v paraformaldehyde, 0·5% v/v glutaraldehyde diluted in a phosphate buffer (PB) containing 0·2 mM NaH\(_2\)PO\(_4\) and 0·2 mM Na\(_2\)HPO\(_4\) (pH 7·2). Thereafter, it was rinsed in PB, dehydrated by increasing stepwise ethanol concentrations (70, 90 and 95%), and finally rinsed in 100% 1-butanol before being embedded in paraffin. Fixed tissue was cut into 5 µm-thick sections and collected on gelatin-coated glass slides.

Immunohistochemistry

On the day of experiment, the slides were deparaffinated, permeablized and labeled as described earlier (Marie et al. 1996). Briefly, the slides were deparaffinated by xylene and hydrated by three successive ethanol baths (95, 90 and 70%). Permeabilization of the tissue was performed by a 10 min incubation in Triton X-100 (0·1%) followed by a 30 min incubation in 10% normal goat serum and 0·1% Tween 20 to block non-specific binding sites. The primary antibody was added and incubated overnight at 4 °C and the secondary, fluorescent-coupled antibody (diluted 100 times) was usually added in 0·1% Tween 20 and incubated for 30 min at room temperature, in the dark. After inspection of immunoreactive cells, the second primary and secondary antibodies were added, using identical protocols. The dilutions of the different primary antibodies used are given in Table 1. For all the G protein \( \alpha \)-subunit antibodies used, we performed at least two separate experiments, using both insulin and glucagon antibodies as endocrine cell markers on each of the three isolated pancreata.

Immunoreactive cells were analyzed using an Olympus BH4 light microscope equipped for fluorescent microscopy with selective excitation and absorption filters for DTAF and Texas Red. Images of immunoreactive cells were grabbed using a highly sensitive black and white tube-camera (Hiashuva, Tokyo, Japan) and were stored and analyzed using the image program Clu2 (Galai, Rehovot, Israel) run on a Compaq computer. Photomicrographs were obtained by photographing the stored images from the video screen after artificial re-coloring (Hasselblad camera and Polaroid film). Thus, immunoreactive cells for G protein antibodies will appear in red and those immunoreactive for either insulin or glucagon antibodies in green. These stored red and green video images of the same pancreas section were superimposed on the same film. Hence, in the superimposed photomicrographs, yellow labeled cells indicate cells which show immunoreactivity for both the G protein \( \alpha \)-subunit antibody and either the insulin or the glucagon antibody, since green and red give yellow. The filter combination for Texas Red and DTAF was specific for detecting green or red fluorescence as shown in our superimposed
A striking finding was that immunoreactivity for an antibody directed against the identical C-terminal sequence of α₁ and α₁ff and thus recognizing these two subunits, was only detected in insulin-immunoreactive cells (see Table 1). To clarify the expression of these subunits in β-cells and further elucidate their selective localization, we used two other antibodies directed against specific sequences of α₁ and α₁ff respectively (see Table 1).

Immunoreactivity for the α₁-selective antibody was only detected in insulin-immunoreactive β-cells. This is shown in the superimposed Fig. 1A3 by the presence of yellow labeled cells only in the core of the islet, where β-cells are exclusively localized. Using the specific α₁ff antibody, we could confirm a recent study (Zigman et al. 1992) indicating the presence of α₁ff in the islet, and further demonstrate its selective localization in β-cells. As illustrated in the superimposed Fig. 1B3, α₁ff was not present in glucagon-immunoreactive cells since both green and red labeled cells could still be distinguished. The round cells appearing in yellow in the center of the islet in the superimposed Fig. 1B3 do not reflect immunoreactivity but auto-fluorescence, probably originating from blood cells within the vascular bed of the islet.

Thus, the results obtained using these three antibodies demonstrate that both α₁ and α₁ff are present in glucagon-immunoreactive cells. This agrees with a previous study reporting glucagon-immunoreactive cells in β-cells using an antibody recognizing α₁ff (Lau et al. 1991).

Results

Localization of the G₁α₁-family, α₁ and α₁ff

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-cells, whereas none of them could be detected in glucagon-immunoreactive -cells.

Localization of two members of the family, 

We used two antibodies specifically directed against or to study their respective distribution in the islets. Our results show that immunoreactivity for but not was restricted to the periphery of the islet.

This selective expression of in glucagon-immunoreactive -cells is demonstrated by the detection of yellow labeled cells only when glucagon was used as the endocrine cell marker (superimposed Fig. 1C3). In contrast, immunoreactivity for the selective antibody was detected in both insulin- and glucagon-immunoreactive cells (refer to Table 1).

Localization of the family, 

Table 1 depicts the results obtained using different antibodies directed against different members of the family. As shown in Table 1 and Fig. 2A1–3, immunoreactivity for the selective antibody was present in the core of the islet but also in some glucagon-immunoreactive -cells in the periphery of the islet. This is clearly shown in the superimposed Fig. 2A3, where cells labeled in red, green and yellow can be seen. Furthermore, immunoreactivity for two antibodies directed against were found in both insulin- and glucagon-immunoreactive cells (see Table 1).

Unexpectedly in our study, -cells did not show any immunoreactivity for the or -selective antibodies (see Table 1 and Fig. 2B3). In -cells, -immunoreactivity was evidenced by the yellow labeled cells in Fig. 2B3. The generation of multiple -splice variants and isoforms has been recently reported (Hsu et al. 1990, Shibasaki et al. 1991, Seaquist et al. 1992, Murtagh et al. 1994, Nurnberg et al. 1994, Wilcox et al. 1995). Unfortunately, selective antibodies for these isoforms are not yet available. Thus, to test for the presence of other -isoforms in -cells, we used two antibodies which are likely to react with all the isoforms. These antibodies were raised against specific sequences which are shared by the

Figure 1: Immunohistochemical localization of G-protein -subunit in pancreata from adult Wistar rats. The photomicrographs in columns 1 and 2 show, after artificial re-coloring, immunoreactivity for antibodies directed against insulin (A1) and glucagon (B1 and C1) in green, and immunoreactivities for antibodies against G protein -subunits, (A2), (B2) and (C2) in red. The photomicrographs in column 3 show the superimposed photomicrographs from column 1 and 2. Stock antibody solutions were diluted 5 × (55), 50 × (50) and 75 × (55), and 100 × for insulin or glucagon. In all three experiments, auto-fluorescence from blood cells can be seen within the vascular bed of the islet.

different αi-isofoms identified. Both α- and β-cells showed immunoreactivity for these two antibodies (see Table 1), confirming the presence of αi-isofom(s) in β-cells.

Two more α-subunits, α2 or α3 of this family were recently detected in rat islets, though with no indication of their cellular distribution in the islet (Zigman et al. 1994). Using selective antibodies, we found that α2 and α3 were present in both α- and β-cells, ruling out therefore a cell-selective expression of these subunits in the islet structure (see Table 1).

Localization of the two members of the Gα12-family, α12 and α13

The two members of the Gα12-family, α12 and α13, are thought to be ubiquitously expressed (Spicher et al. 1994). However, as shown by the superimposed Fig. 3A3, immunoreactivity for the α12-selective antibody was only detected in the core of the islet, in insulin-immunoreactive β-cells. Similarly, a selective localization to β-cells was also found for α13. Indeed, immunoreactivity for the α13 antibody was only detected in the core of the islet, in β-cells, and not in the peripheral glucagon-immunoreactive cells as shown in the superimposed Fig. 3B3.

Discussion

The use of double-labeling indirect immunofluorescence allowed us to study the distribution of different G proteins α-subunits in α- and β-cells of the islet of Langerhans. Our results confirm that members of the Gαs-family, αi and αolf, are expressed in the islet (Seaquist et al. 1992, Zigman et al. 1992, Wilcox et al. 1995). The most striking finding was that both subunits were exclusively localized in β-cells. One may ask whether this selective localization could be due to non-specific binding of our antibodies to β-cells. This seems unlikely since we used three antibodies directed against different but specific sequences of the two α-subunits. A more plausible interpretation of our results is that receptor-mediated regulation of AC is different in α- and β-cells. This idea is supported by recent studies demonstrating that the regulation of the different AC isoforms identified is not only mediated through α-subunits, but also involves βγ-complexes, Ca2+-calmodulin and protein kinase C (Cooper et al. 1995, Taussig & Gilman 1995, Antoni 1997). Moreover, the interplay between these different pathways has been shown to be specific for each of the AC isoforms identified. Thus, it is possible that α- and β-cells contain different AC isoforms.

Interestingly, we found that the two α-subunits, αq and α11, of the Gαq-family displayed a different localization in α- and β-cells. Our results suggest that the hormonal/neuronal stimulation of insulin secretion through the PLC pathway may be mediated through only one α-subunit, αq, whereas both αq and α11 would be involved in α-cells. The presence of only αq in β-cells is rather puzzling; however, a possible explanation could be that the receptor regulation of PLC in β-cells also involves the βγ-complexes (Walseth et al. 1989, Bristol & Rhee 1994). In
various tissues, this regulation has been shown to be mediated through the βγ-complexes released from the pertussis toxin (PTX)-sensitive Gαi-family (Walseth et al. 1989, Bristol & Rhee 1994). However, the involvement of PTX-sensitive G proteins in the hormonal/neuronal-mediated activation of PLC in β-cells is still under debate (Wilcox et al. 1995, Verspohl & Herrman 1996).

Members of the Gαi-family are implicated in mediating the receptor-coupled inhibition of insulin secretion (Skoglund & Rosselin 1993, Gillison & Sharp 1994, Seaquist et al. 1994, Marie et al. 1996, Sharp 1996). In agreement with an earlier study (Zigman et al. 1994), we confirmed the presence of Gαi and Gα2 in β-cells. We also demonstrated the localization of these two subunits in α-cells.

Our observation that both Gαi and Gα3 were present in β-cells is supported by previous studies using different β-cell lines (Skoglund & Rosselin 1993, Gillison & Sharp 1994, Seaquist et al. 1994, Marie et al. 1996). However, in two other studies using Western blot techniques on rat islet homogenates, Gαi was not detected (Berrow et al. 1992, Komatsu et al. 1995). This discrepancy could be due to the different methods and antibodies used or to cross-reactivity of our Gαi antibody with the Gα3-subunit, since the sequence of Gαi (amino acids 93–112) used to generate the antibody differs by only one amino acid from that of Gα3. This seems unlikely since Gαi was only found in a few α-cells, whereas Gα3 was always present in both α- and β-cells. Thus, the labeling obtained by our Gαi antibody might indeed indicate that this Gαi-subunit is preferentially localized in the β-cells.

Neither Gαo1 nor Gαo2 was detected in β-cells, and only Gαo2 was found in α-cells. This is surprising, since the antibodies used were generated from specific sequences obtained from the two Gαo splice variants first identified in HIT-T15, a β-cell line (Hsu et al. 1990, Rouot et al. 1992). It is unlikely that these antibodies would not recognize native Gαo in rat β-cells as they were successfully used in an immunohistological study of neuronal differentiation in mouse brain (Rouot et al. 1992). Furthermore, both these selective antibodies were raised against the same domain of Gαo1- and Gαo2-subunits (amino acids 291–302), and the Gαo-selective antibody showed immunoreactivity in α-cells. Therefore, it can be postulated that β-cells express a novel Gαo-isofrom. This hypothesis is supported by a previous study in HIT cells indicating the presence of at least three Gαo-isofroms in β-cells (Seaquist et al. 1992). Nevertheless, the number and the nature of Gαo-isofrom(s) present in pancreatic β-cells remain to be clarified.

In line with a recent study using different cell lines including the β-cell line RINm5F (Spicher et al. 1994) we found that both Gα12 and Gα13 of the Gα12-family were localized in the β-cells of the islet. However, our observation of a selective localization in β-cells is in contradiction to the reported ubiquitous expression (Spicher et al. 1994, Fields & Casey 1997). An interesting speculation behind this selective localization comes from a study showing that Gα13 mediates β2-adrenergic regulation of Na/H-exchanger-1 (Voyno-Yasenetskaya et al. 1994). This β2-adrenergic receptor is also coupled to AC through Gα (Voyno-Yasenetskaya et al. 1994). Thus, the selective

Figure 3 Immunohistochemical localization of G-protein α-subunit in pancreata from adult Wistar rats. The photomicrographs in columns 1 and 2 show, after artificial re-coloring, immunoreactivity for antibodies directed against insulin (A1) and glucagon (B1) in green, and immunoreactivities for antibodies against G protein α-subunits, α12 (A2), α13 (B2) in red. The photomicrographs in column 3 show the superimposed photomicrographs from column 1 and 2. Stock antibody solutions were diluted 50 × (α12) and 50 × (α13), and 100 × for insulin or glucagon.

localization of $G_{\alpha}$ and $G_{\alpha13}$ could indicate a similar receptor coupling in $\beta$-cells.

Another important aspect, which cannot be resolved by light microscopy techniques, is the intracellular localization of $\alpha$-subunits in the $\beta$-cells (Seaquist et al. 1992, 1994). This question is currently being addressed in the laboratory using immunocytochemistry and electron microscopy.

In conclusion, our results reveal a clear difference in the localization of G protein $\alpha$-subunits between $\alpha$- and $\beta$-cells, suggesting the involvement of specific receptor transduction pathways for the neuronal/hormonal regulation of $\alpha$- and $\beta$-cell functions.

References


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