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Expression of TMEM16A and SLC4A4 in Human Pancreatic Islets

Felicia A. Hanzu[1,2], Rosa Gasa[1], Nurdan Bulur[3], Pascale Lybaert[3], Ramon Gomis[1,2], Willy J. Malaisse[3], Renaud Beauwens[4] and Abdullah Sener[3]

[1]Laboratory of Diabetes and Obesity IDIBAPS, CIBERDEM, [2]Department of Endocrinology and Nutrition, Hospital Clinic, Barcelona, [3]Laboratories of Experimental Hormonology, [4]Cell and Molecular Physiology, Université Libre de Bruxelles, Brussels

Key Words
Human • Pancreatic • Islets • TMEM16A • SLC4A4

Abstract
Background/aims: Stimulation of insulin release by D-glucose is accompanied by Cl^- and HCO_3^- efflux from pancreatic islet cells. The efflux of these anions may involve volume-regulated anion channels, including possibly TMEM16A, and the Na^+-HCO_3^--cotransporter SLC4A4. The present study was designed to explore the expression of both TMEM16A and SLC4A4 in human pancreatic islets. Methods: Pancreases were obtained from human cadaveric donors. Immunodetection of TMEM16A and SLC4A4 was performed by immunohistochemistry on sections of fixed pancreas, while real-time PCR for the study of corresponding gene expression was performed on RNA extracted from both total pancreatic pieces and isolated pancreatic islets. Results: RT-PCR yielded lower levels of SLC4A4 in isolated islets than in the total pancreas, whilst a mirror image prevailed for TMEM16A mRNA. Immunohistochemistry of human pancreas, however, indicated comparable immunostaining of SLC4A4 in insulin-producing cells and exocrine pancreatic cells, whilst that of TMEM16A appeared less pronounced in insulin-producing cells than in exocrine cells. Conclusion: The present findings support the view that, in humans like in rodent, the regulation of anion fluxes in insulin-producing cells may involve both SLC4A4 and TMEM16A.

Introduction

The participation of anions in the process of insulin release has not received as much attention as the role played by cations. These are evidences that Cl^- is concentrated above its electrochemical potential inside b-cells [1-3]. Functional and molecular studies suggest that the $Na^+/K^+/2Cl^-$ co-transporter NKCC1 plays an important role in this respect [4-6]. Stimulation of insulin release by D-glucose is accompanied by both chloride and phosphate anion efflux from islet cells possibly at the intervention of volume regulated anion channels (VRAC)

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Felicia A. Hanzu
Hospital Clinic, Villarroel 170
08036 Barcelona (Spain)
Tel. +34 932279884, Fax +34 932275459,
E-Mail fhanzu@clinic.ub.es

[1, 7-9]. The identity of such channels remains unknown. The recently identified TMEM16A channel, first referred to as a calcium-activated chloride channel (CaCC), was recently proposed to be also sensitive to cell swelling in some epithelial cells [10]. Most anion channels are not only permeable to Cl^- but also to HCO_3^-. The participation of HCO_3^- in the process of glucose-stimulated insulin release is supported by the findings that glucose induces alkalinisation of cytosolic pH [11, 12] and that metabolically produced CO_2 generates HCO_3^- within islet cell mitochondria at the intervention of carbonic anhydrase [13, 14]. The precise location of the latter enzyme within the matrix or within the space between the inner and outer mitochondrial membranes is presently unknown. Furthermore, in prior work, the expression of the Na^+-HCO_3^--co-transporter SCL4A4, also known as the electrogenic Na-HCO_3 cotransporter 1 (NBCel), was documented in both rat pancreatic islet cells and tumoral insulin-producing cells of the BRIN-BD11 line [15, 16]. The activation of this transporter in response to β-cell plasma membrane depolarization could also contribute to cytosolic alkalinisation, cell swelling and the apparent increase of Na^+ influx in glucose-stimulated islets [17].

With this information in mind, the present experiments were designed to assess the expression of TMEM16A channel and Na^+-HCO_3^--co-transporter SLC4A4 in human islets, as a first step towards elucidating their possible role in the process of insulin release.

Materials and Methods

Pancreases (n=3) were obtained from human cadaveric donors without any primary or secondary pancreatic quantifiable pathology, from the Transplant Services Foundation of the Hospital Clinic (Barcelona, Spain) and kept by the Biobanc of the Hospital Clínic-Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS; Barcelona, Spain), after informed consent from their families and approval by the hospital's ethics committee. One part of the tissue was fixed in paraformaldehyde, embedded in paraffin and sliced and further processed as required. From the other part islets were isolated, as previously described [18] and a part utilized as total pancreas.

Immunofluorescence studies

Immunohistochemistry assays were performed on formalin-fixed paraffin-embedded sections of human pancreatic tissue. Tissue was fixed overnight in 4% formalin at 4°C, dehydrated, embedded in paraffin and sectioned at 3µm. Sections were then de-paraffinized and rehydrated. Antigen retrieval was performed with citrate buffer (10mM, pH 6.0) using a microwave oven. Sections were blocked in 3% donkey serum/ 0.1% Triton and incubated with primary antibodies overnight at 4°C: rabbit anti-TMEM16A (1:500, Ab72984 from Abcam) [19], rabbit anti- SLC4A4/NBC (1:100; AB3212 from Millipore) [20] and guinea pig anti-insulin (1:1000, Dako). Tissues were washed and incubated for 1 hr at room temperature with secondary antibodies: Cy3-conjugated donkey anti-rabbit and Cy2-conjugated goat anti-guinea pig (1:500; Jackson Immunoresearch). Epifluorescence images were captured with a Leica DM R microscope (Leica Microsystems Gmbh).

RNA isolation

Total RNA was isolated from islets using the RNeasy Mini Kit (Qiagen) and from total pancreas using the RNAgents Isolation System (Promega).

Real time PCR

2 µg of total RNA were reverse-transcribed using random primers and Superscript III (Invitrogen) following the manufacturer's instructions. Real-time PCR was carried in a 480 Roche Light Cycler using GoTaq PCR® system from Promega. Expression levels obtained were normalized to the housekeeping gene TBP (TATA box binding protein). Oligos used were: *TMEM16A* (forward 5'- AAGTACTCGAC GCTCCCGGCC-3'; reverse 5'-ATAAGGAGTTCAGCAGCGTGCCC-3), *SLC4A4* (forward 5'-GCCAGACCCCAGGAGGATGGA-3'; reverse 5'-ACTCTTCGGCAC ATGGACTCCGA-3') and TBP as internal control (forward 5'-ATCCCTCCCCCATGACTCCCATG-3'; reverse 5'-ATGATTACCGCAGCAA ACCGC-3').

Statistical analysis

Results are presented as mean values (±SEM). Geometric means were used for the pancreas/islet ratio of gene expression levels. The statistical significance was determined by Student's *t* test.

Results

In the analyzed human samples (n=3), qRT-PCR yielded lower gene expression levels of SLC4A4 in isolated islets than in the total pancreas, whilst a mirror image prevailed for TMEM16A mRNA (Fig. 1). As a matter of fact, the mean paired pancreas/islet ratio for SLC4A4 (4.51) was one order of magnitude higher (degree of freedom = 4; $p<0.05$) than the mean paired pancreas/islet ratio for TMEM16A (0.50).

At the protein level, immunohistochemistry for TMEM16A and SLC4A4 proteins in human total pancreas and separated islets documented the presence of both SLC4A4 and TMEM16A in both insulin-producing cells and exocrine cells (Fig. 2, 3). In this respect, the immunostaining of SLC4A4 was as intense in insulin-producing cells as in exocrine pancreatic cells, whilst that of TMEM16A appeared less pronounced in insulin-producing cells than in exocrine cells (Fig. 2, 3).

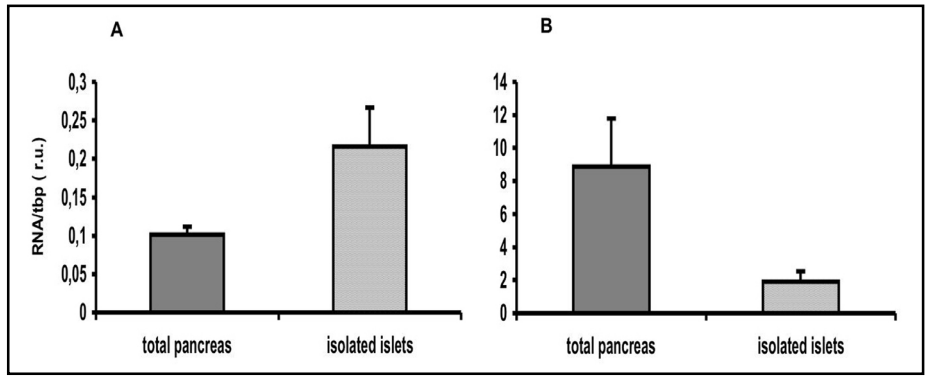

Fig. 1. Gene expression levels of TMEM16A (A) and SLC4A4 (B) as quantified by relative qRT-PCR in human total human pancreas and isolated islets (n=3). TBP: TATA box binding housekeeping protein. Results are expressed as relative units (r.u.) and presented as mean (± SEM).

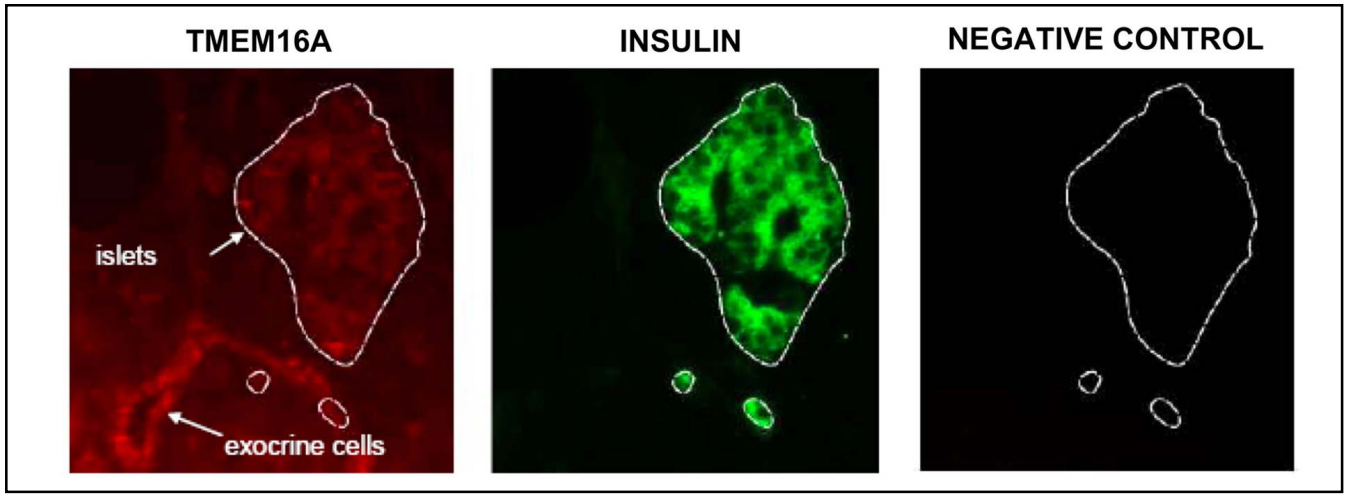

Fig. 2. Immunohistochemistry of TMEM16A in human pancreas. Images (x40 magnification) are representative of n=3 different human pancreases.

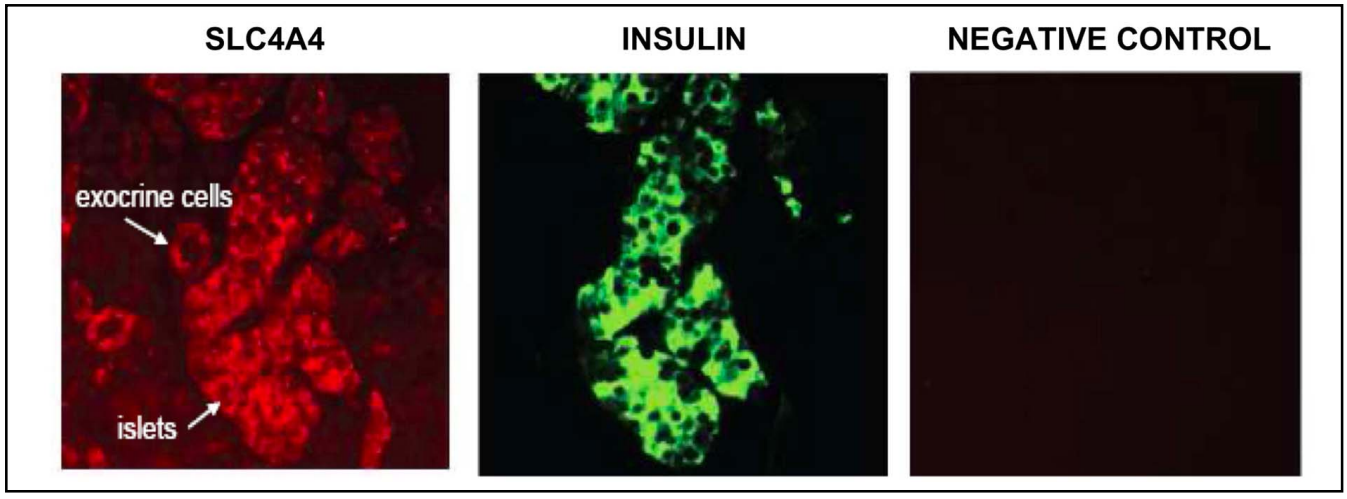

Fig. 3. Immunohistochemistry of SLC4A4 in human pancreas. Images (x40 magnification) are representative of n=3 different human pancreases.

Discussion

To our knowledge, a distinct immunohistochemical positivity for TMEM16A (referred to as DOG1) in the human endocrine pancreas was so far only noticed in a study dealing with the search of novel gastrointestinal stroma tumor biomarker(s) [21]. The present findings reveal the expression of both TMEM16A and SLC4A4

in human insulin-producing pancreatic islet cells. The apparent dissociation between the relative RT-PCR data in isolated islets *versus* total pancreas, on one hand, and intensity of immunostaining in insulin-producing cells *versus* exocrine pancreatic cells, on the other hand, as observed for both TMEM16A and SLC4A4 could suggest cell-specific modulation of post-transcriptional events. In any case, the present work provides relevant information in the perspective of further investigations on the participation of TMEM16A channels and SLC4A4 co-transporter in the regulation of anion fluxes in human insulin-producing cells.

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