Localization of α -endosulphine in pancreatic somatostatin δ cells and expression during rat pancreas development

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Abstract

Aims/hypothesis. α -Endosulphine, a protein that belongs to the cAMP-regulated-phosphoprotein family, has been reported to modulate insulin secretion in vitro through interaction with the pancreatic beta-cell ATP-sensitive potassium (K_{ATP}) channel. In this study, we analysed the tissue distribution of α -endosulphine and determined its pancreatic cellular localization.

Methods. Quantitative tissue distribution of α -endosulphine was studied by RIA on tissue extracts and cellular/subcellular localization was done using immunocytochemistry, morphometry and western blot analysis. α -Endosulphine and somatostatin release from RINT-3 somatostatin-secreting cells was quantified by RIA.

Results. α -Endosulphine, concentrated particularly in the central nervous system, was also detected in a wide variety of tissues including the pancreas. Immunohistochemistry analysis of adult rat pancreatic sections showed that α -endosulphine localized in somatostatin δ cells, where its expression increased during post-natal development. Immunoreactive cells

were detected from foetal age E19, and the number of somatostatin cells co-expressing α -endosulphine increased with developmental age from E19 until adult. α -Endosulphine, highly expressed in the cytoplasm of RINT3 somatostatin-secreting cell line, was recovered in the particulate fraction of RINT3 cell extracts but was not co-secreted with somatostatin.

Conclusion/interpretation. α -Endosulphine is expressed in all tissues tested including pancreas and is also detected in plasma. Pancreatic α -endosulphine is specifically localized in somatostatin δ cells. This cytosolic protein is not co-secreted with somatostatin and could be physically associated with particulate components of the cells. These findings are not in favour of an endocrine/paracrine effect of α -endosulphine on the beta-cell K_{ATP} channel. [Diabetologia (2002) 45:703–710]

Keywords α -Endosulphine, pancreatic islets, δ cells, immunocytochemistry, pancreas development, cellular localization, K_{ATP} channel, ABC transporter, sulphonylurea receptor, cAMP-regulated phosphoproteins.

Sulphonylureas, used in the treatment of Type II (non-insulin-dependent) diabetes mellitus, exert their hypo-

glycaemic effect through closure of the ATP-sensitive potassium channel (K_{ATP}) and thereby stimulate insulin release from pancreatic beta cells [1, 2, 3, 4].

α -Endosulphine was purified from porcine brain as an endogenous equivalent of hypoglycaemic sulphonylurea drugs [5]. It is a 121-amino acid protein with an apparent M_r of 19 kDa and its primary structure has a close similarity with the cyclic AMP-regulated phosphoproteins ARPP-16 and ARPP-19. α -endosulphine is a new member of this family of protein, encoded by a specific gene [6, 7]. ARPP-16 and ARPP-19 are two substrates for protein kinase A (PKA), differing by an additional 16 amino acids extension at the N-terminus

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Abbreviations: K_{ATP} channel, ATP-sensitive potassium channel; ARPP, cyclic AMP-regulated phosphoprotein; PKA, protein kinase A; RIA, radioimmunoassay; IL-1 β , interleukin-1 β ; SUR, sulphonylurea receptor

of ARPP-19 [8]. The ARPP-16 is highly enriched in specific brain areas, mostly in striatal medium size spiny neurones, whereas ARPP-19 is more ubiquitously distributed in all tissues and cell lines [9]. Comparison of amino acid sequences of members of the ARPP family revealed the presence of a highly conserved PKA phosphorylation site. In line with this, α -endosulphine and ARPP-16/19 are phosphorylated in response to activation of PKA in intact cells or striatal slices, and an increased or a decreased phosphorylation of ARPP-16 was noted in response to activation of D₁-type or D₂-type dopamine receptors, respectively, in the striatum [10]. The phosphorylation site is located within a good PKA consensus sequence and is conserved in all vertebrate members of the family that have been identified so far, suggesting that it plays an important regulatory role. However, the physiological function(s) of this family of phosphoproteins has still to be determined.

Human α -endosulphine cDNA was isolated and expressed in a bacterial system [11]. The recombinant protein was tested on beta-cell activity as an endogenous equivalent of sulphonylureas. Human α -endosulphine inhibited sulphonylurea binding to beta-cell membranes, reduced K_{ATP} channel activity and thereby stimulated insulin secretion from MIN6 beta cells at micromolar concentrations [11]. This study aimed to analyse further the biological features of α -endosulphine in view of a possible physiological role of this peptide in the regulation of the K_{ATP} channels.

To address these issues, we analysed the tissue distribution of α -endosulphine and studied its cellular and subcellular localization in the pancreas. In this tissue, the cellular localization and developmental expression of α -endosulphine was determined by immunohistochemistry on rat pancreatic sections, and its subcellular localization was studied in the RINT3 somatostatin-secreting cell line. In addition, the possible secretion of α -endosulphine through the regulated secretory pathway was investigated in RINT3 cells.

Materials and methods

Tissue extraction. Tissues from adult Wistar rats were boiled and extracted in 10 ml 0.5 mol/l acetic acid for each gram of tissue, as previously described [12]. Rat blood samples were collected into glass tubes containing EDTA (15%) and 250 KIU aprotinin (Interchim, Montluçon, France) and centrifuged at 800 g for 10 min at 4°C. Plasma was stored at -30°C before concentration on reversed phase C18 Sep-Pak Plus cartridges (Waters, Milford, Mass., USA) eluted with 50% CH₃CN in TFA-DEA pH 2.5. Eluates were made 10% CH₃CN by evaporation under vacuum. Extracts and concentrated plasma were then run on a reversed phase μ C18 nucleosil HPLC column (Interchim) and the separations were conducted with a linear gradient of 0–50% CH₃CN for 50 min at 1.5 ml/min. One-minute fractions were collected, lyophilized and stored at 4°C before α -endosulphine assay.

Cell culture. MIN6 beta cells were grown in Dulbecco's modified Eagle's medium (DMEM; Life Technologies, Carlsbad, Calif., USA) containing 4.5 g/l glucose and supplemented with 15% foetal calf serum (Life technologies, USA), 100 IU/ml penicillin-100 μ g/ml streptomycin (Life technologies, USA) and 75 nmol/l beta mercaptoethanol (Sigma, St Louis, Miss., USA) [13]. RINT3 and α -TC1-6 cells were grown in DMEM containing 1 g/l glucose and supplemented with 10% foetal calf serum and penicillin-streptomycin [14].

Secretion studies. Experiments were done 6 days after plating. RINT3 cells were washed twice with culture medium containing 1% BSA and 15 mmol/l HEPES and then incubated with the different secretagogues. At the end of each incubation period, cellular supernatant was collected, centrifuged and kept frozen until assayed for α -endosulphine or somatostatin. The cellular α -endosulphine and somatostatin contents were also measured at the end of each incubation period after extraction in PBS containing 0.1% Triton X100. Extracts were frozen until assayed.

α -Endosulphine and somatostatin assay. α -Endosulphine content was determined in HPLC tissue fractions, in culture medium or cell extracts by radioimmunoassay (RIA) after dilution in 0.025 mol/l phosphate buffer containing 0.5% BSA. The antiserum was obtained after rabbit immunization with a synthetic peptide corresponding to the nine amino acid C-terminal fragment specific of α -endosulphine (SKLAGGQVE) conjugated with KLH using a thiol-maleoyl coupling method (Pierce, New York, NY., USA). Recombinant human α -endosulphine was used both as standard and tracer after ¹²⁵I labelling. Free α -endosulphine was separated from bound by adsorption on dextran-coated charcoal. Somatostatin content was determined in culture medium or cell extracts from RINT3 cells as previously described [14].

Immunofluorescence staining of somatostatin-secreting RINT3 cells. RINT3 cells were grown on culture chamber slides and processed for immunocytochemistry as already described [15]. α -endosulphine immunoreactivity was revealed after incubation with rabbit anti- α -endosulphine antibodies (1/250), followed by rhodamine-conjugated goat anti-rabbit IgG (1/125, Sigma). The slides were examined for immunofluorescence using a confocal microscope (Zeiss, Jena, Germany).

Several sets of experiments were carried out with a Triton treatment of living cells just before fixation (30 s in 0.5% Triton-50 mmol/l 2[N-morpholino]ethanesulphonic acid buffer, pH 7.2), so as to eliminate the cytosolic proteins. Cells were immediately fixed and processed for immunostaining as described above.

Immunocytochemistry and morphometry analysis on rat pancreatic sections. Paraffin sections from rat pancreas at various stages (foetal day 17 up to the adult age) were processed for immunohistochemistry. Double labelling was done using simultaneously rabbit anti- α -endosulphine antibodies (1/200) and either mouse anti-insulin (1/2000, Sigma), mouse anti-glucagon (1/2000, Sigma) or mouse anti-somatostatin (1/5000, Novo, London, UK) antibodies. Islet hormone immunostaining was detected using Texas Red-conjugated anti-mouse secondary antibodies (1/200, Jackson Immunoresearch) and α -endosulphine was revealed using FITC-conjugated anti-rabbit secondary antibodies (1/200, Jackson Immunoresearch).

Morphometric analysis was done after a light counterstaining on three sections per animal ($n=3$ animals at each age). In each section, the total pancreatic tissue area was measured using an image analysis software (Leica, Solms, Germany) and

total numbers of cells expressing α -endosulphine or somatostatin were recorded. Results are expressed as number of positive cells/mm² of tissue.

Immunoblotting. Cells and rat adult islets were resuspended in 1 ml ice-cold membrane preparation buffer (10 mmol/l Tris-HCl, 30 mmol/l NaCl, 1 mmol/l dithiothreitol, and 5 μ mol/l phenylmethylsulphonylfluoride, pH 7.5), disrupted using a Dounce homogenizer and centrifuged for 30 min at 20000 g. Pellets and supernatants were considered as the particulate and cytosolic fractions, respectively. For each fraction, 20 μ g of total proteins were separated on SDS-PAGE and blotted on 100% pure nitro-cellulose membranes (Protran, Schleicher & Schuell, Dassel, Germany). Membranes were incubated at room temperature for 1 h with the anti- α -endosulphine antiserum (1/5000), followed by horseradish peroxidase-conjugated donkey anti-rabbit secondary antibodies (1/2000, Amersham Pharmacia Biotech, Lesulis, France). Immunoreactive proteins were detected by chemiluminescence (ECL, Amersham Pharmacia Biotech, France) using X-ray Kodak films BioMax MR1 (Sigma, USA).

Data analysis and statistics. Values are the means $\pm$ SEM of three experiments, performed in triplicate. The differences between experimental values were analysed by the Student's *t* test. Differences were considered statistically significant when *p* was less than 0.05.

Results

The tissue distribution of α -endosulphine was analysed in several rat tissue extracts. Considerable amounts of the protein was recovered in the cortex and the cerebellum areas with concentrations of 262 ± 81 and 32.8 ± 12 pmol/g of tissue, respectively (Fig. 1A). Intermediate concentrations between 5 and 10 pmol/g were found in the pituitary, heart, lung and spleen. A third group comprising the skeletal muscle, pancreas, intestine, stomach, liver, kidney, adipose tissue and blood cells displayed lower concentrations below 5 pmol/g of tissue (Fig. 1A). Rat plasmatic α -endosulphine content was also measured and the circulating concentrations were evaluated as 50 pmol/l. Of note, in the cortex (Fig. 1A, insert) and in all tissues tested, α -endosulphine immunoreactivity eluted from the HPLC column as a single, homogeneous peak with the retention time of the recombinant peptide.

We focussed in this study on the pancreatic cellular and subcellular localization of α -endosulphine. Western blot analysis using the anti- α -endosulphine antibody revealed the presence of a 19-kDa protein in extracts from total rat pancreas (Fig. 1B). The weak signal was in accordance with low pancreatic amounts detected by RIA (Fig. 1A). However, we observed a significant enrichment of α -endosulphine in extracts from pancreatic islets, suggesting a specific localization in the endocrine pancreas. Expression of α -endosulphine were then compared in MIN6 insulin-, α TC1-6 glucagon-, and RINT3 somatostatin-secreting cell lines. The highest expression was found in the somatostatin-secreting cell line RINT3, suggesting a

Fig. 1 A, B. Tissue distribution and pancreatic localization of α -endosulphine. **A** Tissue distribution of α -endosulphine. Tissues from Wistar rat were extracted and fractionated as indicated in Methods and α -endosulphine content was determined in HPLC tissue fractions by radioimmunoassay using an anti- α -endosulphine specific antibody. Insert: α -endosulphine immunoreactivity from rat cortex extract eluted from the HPLC column as a single, homogeneous peak with the retention time of the recombinant peptide. **B** Pancreatic expression of α -endosulphine. Protein samples from total rat pancreas, isolated islets and pancreatic cell lines were separated by SDS-PAGE (20% acrylamide) and transferred to nitro-cellulose. Blots were analysed using the anti- α -endosulphine specific antibody

localization of α -endosulphine in the endocrine δ cells (Fig. 1B).

Immunocytochemistry analysis was then carried out on adult rat pancreatic sections and confirmed the absence of α -endosulphine in the exocrine tissue (Fig. 2). Moreover, double immunostaining using rabbit anti- α -endosulphine antibodies and either mouse anti-insulin (Fig. 2A) or mouse anti-glucagon antibodies (Fig. 2B), shows that α -endosulphine was not detectable in beta cells (which are concentrated in the centre of the islet) nor in the surrounding α cells of the endocrine pancreas. However, double positive cells appear after double staining with an anti-somatostatin antibody (Fig. 2C, D). Single labelled sections

Fig. 2 A–D. Immunocytochemistry analysis on rat pancreatic sections. Paraffin sections from rat adult pancreas were processed for immunohistochemistry. Double immunohistochemistry was performed using simultaneously rabbit anti- α -endosulphine antibodies and either **A** mouse anti-insulin, **B** mouse anti-glucagon or **C** mouse anti-somatostatin antibodies. **D** Magnification of double-labelled cells presented in panel **C**. Pancreatic islet hormones are detected in red and α -endosulphine in green. Double positive cells appear yellow. Consecutive sections of the same islet from a rat adult pancreas are shown (original magnification $\times 500$). Values are the means $\pm$ SEM

incubated with an excess of α -endosulphine peptide (10^{-4} mol/l) or with mismatched secondary antibodies showed no labelling, confirming the specificity of the immunostaining (not shown). Double staining carried out with an anti-pancreatic polypeptide (PP) antibody did not reveal any co localization of α -endosulphine with the pancreatic polypeptide in the PP cells (data not shown).

α -Endosulphine expression was also studied during pancreatic development. Paraffin sections from rat pancreas were analysed at various stages from foetal day 17 until adult age and were processed for immunohistochemistry with anti-somatostatin and anti- α -endosulphine antibodies. Few somatostatin-positive cells were detected from E17 (not shown), while α -endosulphine was not detected until E19. The total number of cells expressing somatostatin, α -endosulphine or both (double-labelled cells) were scored and expressed as number of cells/mm² of pancreatic tissue. Both somatostatin and α -endosulphine expressing cells follow the same developmental pattern, with the highest density of cells for each tissue surface unit observed shortly before weaning (Fig. 3A), as already

described for δ cells [16]. At all stages, α -endosulphine was only found in δ cells, although not all somatostatin δ cells expressed α -endosulphine. The fraction of δ cells expressing α -endosulphine was determined as a function of the development stage. At the foetal stage, 7% of δ cells express α -endosulphine and this ratio increased to 47% shortly before weaning and reached 80% of the δ cells at adult age (Fig. 3B).

The subcellular localization of α -endosulphine was analysed using the RINT3 cells as a model. As shown in Fig. 4A, immunostaining of RINT3 cells followed by microscopic analysis showed a strong and diffuse specific staining in the whole cytoplasm of the cells without any apparent compartmentalization of the protein. However, western blot experiments carried out on particulate and cytosolic fractions, treated at physiological pH, showed that the protein was mainly in the particulate fraction (Fig. 4B). After treatment of the particulate fraction at pH 11, the α -endosulphine signal was recovered mostly in the cytosoluble fraction (Fig. 4B) suggesting that α -endosulphine is not an intrinsic component of biological membranes but could be physically associated with particulate components of the RINT3 cells. The loss of positive staining in living cells after Triton treatment (Fig. 4A) also favours this hypothesis.

We then studied the ability of α -endosulphine to be co-secreted with somatostatin through the regulatory pathway. From RINT3 cells, we observed a rapid release of somatostatin in the presence of 100 μ mol/l tolbutamide or 10 mmol/l KCl (Fig. 5). Under the same stimulatory conditions, no significant release of α -endosulphine from RINT3 cells was detected (Fig. 5). Similar results were obtained after a forskolin treatment of RINT3 cells (data not shown). After a 1-h incubation, α -endosulphine was detected in the incu-

Fig. 3 A, B. Morphometric analysis was performed after a light counterstaining on three sections for each animal ($n=3$ animals for each age). In each section, the total pancreatic tissue area was measured and total numbers of cells expressing α -endosulphine or somatostatin were recorded. **A** Paraffin sections from rat pancreas were analysed at various stages from foetal day 17 until adult age and were processed for immunohistochemistry with anti-somatostatin and anti- α -endosulphine antibodies. The number of cells expressing somatostatin ($\square$) or α -endosulphine ($\blacksquare$) were scored and expressed as number of cells/mm² pancreatic tissue. **B** The fraction of δ cells expressing endosulphine was determined as a function of development. The proportion of somatostatin δ cells expressing α -endosulphine from foetal to adult age was also determined and expressed as percentages. Values are the means $\pm$ SEM

bation medium of RINT3 cells (30 ± 4 fmol/ 10^6 cells/h), representing $6 \pm 1.5\%$ of the total α -endosulphine cellular content, a modest amount compared to the somatostatin (1.2 ± 0.2 pmol/ 10^6 cells/h) representing $80 \pm 6\%$ of the total somatostatin cellular content.

Discussion

α -Endosulphine, a member of the cAMP-dependent protein kinase substrates family, was characterized as a putative regulator of the pancreatic beta-cell K_{ATP} channel activity. Subsequent findings indicated that the α -endosulphine mRNA was expressed in a wide range of human tissues including muscle, brain and endocrine tissues [11]. In this study, a quantitative tissue distribution of the α -endosulphine protein was done on

Fig. 4 A, B. Subcellular localization of α -endosulphine. **A** detection of α -endosulphine by immunofluorescence analysis of RINT3 cells. Living cells (**a**) were immediately fixed or (**b**) were treated before fixation with MES buffer containing triton X100 0.5%. Specific labelling of α -endosulphine was performed using the rabbit anti- α -endosulphine as a primary antibody and the rhodamine-conjugated goat anti-rabbit IgG as a secondary antibody. The slides were examined for immunofluorescence using a confocal microscope. **B** Western blot analysis of α -endosulphine expression in somatostatin-secreting RINT3 cells after pH treatment of the particulate fraction. Crude membranes from RINT3 cells were incubated in 0.1 mol/l bicarbonate buffer at pH 7.4 or pH 11. After treatment, samples were centrifuged as indicated in methods. Particulate (P) and cytosolic (C) fractions obtained were separated by 20% SDS-PAGE and analysed by western blotting with the α -endosulphine specific antibody as described in experimental procedure

a broad variety of tissues, using an antibody recognizing specifically the C-terminal part of the protein. Indeed, α -endosulphine differs from other isoforms of the ARPP family [11] by a four amino acid extension "GQVE" at its C-terminal moiety. The antiserum obtained after rabbit immunization with a synthetic pep-

Fig. 5. Kinetic study of somatostatin and α -endosulphine release from RINT3 cells. Somatostatin (open symbols) and α -endosulphine (filled symbols) secretion was analysed under basal condition ($\square$, $\blacksquare$) and under stimulated conditions in presence of the same medium plus tolbutamide 100 μ mol/l ($\circ$, $\bullet$) or KCl 10 mmol/l ($\triangle$, $\blacktriangle$). Somatostatin and α -endosulphine release were expressed as a percent of the total cellular peptide content (peptide secreted + peptide remaining inside the cell), calculated for each time point. Values are the means $\pm$ SEM of three experiments, performed in triplicate

tide corresponding to the nine amino acid C-terminal fragment of α -endosulphine (SKLAGGQVE), is specific to α -endosulphine and does not recognize ARPP-19 or other members of the ARPP family. The large tissue distribution of the α -endosulphine protein observed in this study, in accordance with that previously reported by Northern blot analysis [11], clearly indicates that α -endosulphine is widely expressed, although most abundantly in the central nervous system, similarly to other ARPP isoforms [10].

α -Endosulphine is able to interact, at the micromolar range, with the beta-cell K_{ATP} channel and thereby stimulates insulin secretion at the same concentrations [11]. Circulating concentrations of α -endosulphine measured by radioimmunoassay in the plasmatic compartment (50 pmol/l) do not argue in favour of an endocrine effect of α -endosulphine on this channel. Accordingly, we analysed more precisely the pancreatic localization of α -endosulphine to check its putative role as an intracellular modulator of insulin secretion. Our results show that pancreatic α -endosulphine is expressed in somatostatin δ cells of the pancreas and suggest that this protein is not an intracellular modulator of the beta-cell K_{ATP} channel. These data raise the question of a putative paracrine modulator of these channels and, using the RINT3 cells as a model, we studied the subcellular localization of α -endosulphine

and its ability to be secreted in the culture medium. α -Endosulphine, is present in the cytosol of RINT3 cells, a result in agreement with a previous subcellular localization of ARPP isoforms in the cytosol of rat neuronal cells [10]. Moreover, α -endosulphine is a cytosoluble protein present in the particulate fraction, suggesting that this protein could interact physically with a component of the particulate fraction, indicating a possible role in protein-protein interaction. A recent study performed on proteins of the ARPP family, focussed on a highly conserved sequence of eight amino acids "KYFDSGDY", likely to play a role in binding to some other cellular constituents [10].

Other biochemical observations performed on proteins of the ARPP family are in favour of an intracellular biological function for these proteins. However, the detection of α -endosulphine in the plasmatic compartment prompted us to study the ability of α -endosulphine to be co-secreted with somatostatin, a paracrine inhibitor of insulin secretion [17, 18]. Somatostatin secretion is generally enhanced by forskolin, a chemical agent which increases the cAMP [13], tolbutamide, a sulphonylurea which closes the δ -cell K_{ATP} channel [19], or KCl which depolarizes pancreatic δ cells. None of these secretagogues were able to induce a clear α -endosulphine release when somatostatin secretion was enhanced, suggesting that α -endosulphine is not co-secreted with somatostatin through the regulated secretion pathway. Preliminary experiments on isolated rat islets and perfused rat pancreas confirm that α -endosulphine and somatostatin are not co-secreted. However, further investigations are needed to explain the presence of α -endosulphine in the plasma compartment and show whether α -endosulphine is able to cross the plasma membrane in the absence of a secretory signal peptide. In this context, it is interesting to note that several peptides and proteins, such as the cytokine interleukin-1 β (IL-1 β), belong to the family of leaderless secretory proteins and are released from the cells by a non-classical secretory route [20]. The lack of a signal peptide, considered as necessary to drive the peptide to the secretory pathway, hampers the targeting to the endoplasmic reticulum and these proteins are thus found in the cytosol from which they gain access to the extracellular medium by intracellular pathways that have not yet been fully characterized. In bacteria and yeast ATP-binding cassette (ABC) transporters are involved in the secretion of leaderless secretory proteins. Interestingly, the mammalian ABC1 transporter could be involved in the secretion of IL-1 β [21]. The interaction of α -endosulphine with the sulphonylurea binding site localized on the sulphonylurea receptor (SUR1) subunit of the K_{ATP} channel, another member of the ABC transporter family [2, 22], is compatible with the hypothesis of an α -endosulphine transport through a similar pathway. We show in this study the presence of α -endosulphine in many tissues including blood cells. Thus, the pres-

ence of α -endosulphine in plasma could be explained by a release from a variety of cell types, possibly through a member of the family of ABC transporters, an hypothesis which needs to be further investigated. However, the relevance of a circulatory effect for a plasmatic protein that is also expressed by many tissues remains to be demonstrated. On the other hand, the high doses necessary to interact and block the K_{ATP} channel make it difficult to speculate on endocrine and paracrine effects of α -endosulphine on the beta-cell K_{ATP} channel to modulate insulin secretion.

α -Endosulphine is a component of a highly conserved family of proteins, with members found in *Drosophila melanogaster*, *Caenorhabditis elegans* or yeast genomes, thereby favouring the hypothesis of conserved functional properties. Hence, the apparent conservation of the phosphorylation site for PKA suggests that regulation by cAMP probably plays an important role in the function of this family of phosphoproteins [10], which could be involved in basic cellular processes. The finding of increasing numbers of δ cells expressing α -endosulphine with development suggests that this protein could play a role in cell differentiation or cell growth during pancreas development. In line with this, a recent study reported a fourfold relative increase in ARPP-16 mRNA in a selected line of pig with increased body and muscle weight, indicating that this protein could play a role in the molecular pathway(s) that regulate postnatal skeletal muscle growth in the pig [23]. Moreover, IL-1 β , a major mediator of inflammation, which exerts pleiotropic effects on many systems [24, 25], induces a differential up-regulation of α -endosulphine gene [26].

In conclusion, we have shown that α -endosulphine cannot be considered to be a classic endocrine and paracrine regulator of the beta-cell K_{ATP} channel activity. These results led us to conclude that α -endosulphine is a physiological molecule which is ubiquitously expressed and which, as a member of a big family of proteins, is probably involved in some basic cellular processes. The finding that developmental age influences α -endosulphine expression suggests this protein is involved in pancreatic δ -cell growth or functional maturation.

References

- Loubatières A (1946) Etudes physiologiques et pharmacodynamiques de certains dérivés sulfamides hypoglycémisants. Arch Inter Physiol 54: 174–177
- Aguilar-Bryan L, Nichols CG, Wechsler SW et al. (1995) Cloning of the β -cell high-affinity sulfonylurea receptor: a regulator of insulin secretion. Science 268: 423–426
- Inagaki N, Gonoi T, Clement JP 4th et al. (1996) A family of sulfonylurea receptors determines the pharmacological properties of ATP-sensitive K^+ channels. Neuron 16: 1011–1017
- Ashcroft SJ, Niki I, Kenna S et al. (1993) The beta-cell sulfonylurea receptor. Adv Exp Med Biol 334: 47–61
- Virsolvy-Vergine A, Leray H, Kuroki S, Lupo B, Bataille D (1992) Endosulphine, an endogenous peptidic ligand for the sulfonylurea receptor: purification and partial characterization from ovine brain. Proc Natl Acad Sci USA 89: 6629–6633
- Virsolvy-Vergine A, Salazar G, Sillard R, Denoroy L, Mutt V, Bataille D (1996) Endosulphine, endogenous ligand for the sulphonylurea receptor: isolation from porcine brain and partial structural determination of the alpha form. Diabetologia 39: 135–141
- Peyrollier K, Héron L, Virsolvy A, Le Cam A, Bataille D (1996) Alpha-endosulphine is a novel molecule, structurally related to a family of phosphoproteins. Biochem Biophys Res Commun 223: 583–586
- Horiuchi A, Williams KR, Kurihara T, Nairn AC, Greengard P (1990) Purification and cDNA cloning of ARPP-16, a cAMP-regulated phosphoprotein enriched in basal ganglia, and of a related phosphoprotein, ARPP-19. J Biol Chem 265: 9476–9484
- Girault JA, Horiuchi A, Gustafson EL, Rosen NL, Grengard P (1990) Differential expression of ARPP-16 and ARPP-19, two highly related cAMP-regulated phosphoproteins, one of which is specifically associated with dopamine-innervated brain regions. J Neurosci 10: 1124–1133
- Dulubova I, Horiuchi A, Snyder GL et al. (2001) ARPP-16/ARPP-19: a highly conserved family of cAMP-regulated phosphoproteins. J Neurochem 77: 229–238
- Héron L, Virsolvy A, Peyrollier K et al. (1998) Human alpha-endosulphine, a possible regulator of sulfonylurea-sensitive KATP channel: molecular cloning, expression and biological properties. Proc Natl Acad Sci USA 95: 8387–8391
- Virsolvy-Vergine A, Brück M, Dufour M, Cauvin A, Lupo B, Bataille D (1988) An endogenous ligand for the central sulfonylurea receptor. FEBS Lett 242: 65–69
- Ishihara H, Asano T, Tsuduka K, et al. (1993) Pancreatic beta cell line MIN6 exhibits characteristics of glucose metabolism and glucose-stimulated insulin secretion similar to those of normal islets. Diabetologia 36: 1139–1145
- Gros L, Demirpence E, Jarrousse A, Kervran A, Bataille D (1992) Characterization of binding sites for oxyntomodulin on a somatostatin-secreting cell line (RIN T3). Endocrinology 130: 1263–1270
- Gros L, Riu E, Montoliu L, Ontiveros M, Lebrigand L, Bosch F (1999) Insulin production by engineered Muscle cells. Hum Gene Ther 10: 1207–1217
- McEvoy RC, Madson KL (1980) Pancreatic insulin-, glucagon-, and somatostatin-positive islet cell populations during the perinatal development of the rat. Biol Neonate 38: 255–259
- Engelking LR (1997) Physiology of the endocrine pancreas. Semin Vet Med Surg (Small Anim) 12: 224–229
- Sjoholm A, Sandberg E, Ostenson CG, Efendic S (2000) Peptidergic regulation of maturation of the stimulus-secretion coupling in fetal islet beta cells. Pancreas 20: 282–289
- Suzuki M, Fujikura K, Kotake K, Inagaki N, Seino S, Takata K (1999) Immuno-localization of sulphonylurea receptor 1 in rat pancreas. Diabetologia 42: 1204–1211
- Hamon Y, Luciani MF, Becq F, Verrier B, Rubartelli A, Chimini G (1997) Interleukin-1 β secretion is impaired by inhibitors of the ATP binding cassette transporter, ABC1. Blood 90: 2911–2915
- Kuchler K, Rubartelli A, Holland B (1997) Unusual secretory pathways: from bacteria to man. Landes Bioscience, Austin

22. Tusnady GE, Bakos E, Varadi A, Sarkadi B (1997) Membrane topology distinguishes a subfamily of the ATP-binding cassette (ABC) transporters. *FEBS Lett* 402: 1–3
23. Janzen MA, Kuhlers DL, Jungst SB, Louis CF (2000) ARPP-16 mRNA is up-regulated in the longissimus muscle of pigs possessing an elevated growth rate. *J Anim Sci* 78: 1475–1484
24. Bendtzen K (1994) Cytokines and natural regulators of cytokines. *Immunol Lett* 43: 111–123
25. Morioka M, Shimodaira K, Kuwano Y, Fukikawa H, Saito H, Yanaihara T (2000) Effect of interleukin-1 beta on aromatase activity and cell proliferation in human osteoblast-like cells (HOS). *Biochem Biophys Res Commun* 268: 60–64
26. Rieneck K, Bovin LF, Josefsen K, Buschard K, Svenson M, Bendtzen K (2000) Massive parallel gene expression profiling of RINm5F pancreatic islet beta-cells stimulated with interleukin-1beta. *APMIS* 108: 855–872