Regulatory Peptides 151 (2008) 19–25

Contents lists available at ScienceDirect

Regulatory Peptides

journal homepage: www.elsevier.com/locate/regpep

The importance of chromogranin A in the development and function of endocrine pancreas

G.M. Portela-Gomes [a,b,*], J.R. Gayen [c], L. Grimelius [a], M. Stridsberg [d], S.K. Mahata [c,e]

[a] Department of Genetics and Pathology, Clinical Chemistry, Uppsala University, Sweden
[b] Department of Gastroenterology, University of Lisbon, Portugal
[c] Department of Medicine, University of California, San Diego, USA
[d] Department of Medical Sciences, Clinical Chemistry, Uppsala University, Sweden
[e] VA San Diego Healthcare System, San Diego, California, USA

ARTICLE INFO

Article history:
Received 14 November 2007
Received in revised form 15 June 2008
Accepted 2 July 2008
Available online 3 August 2008

Keywords:
Chromogranin A knock-out mice
Chromogranins
Endocrine pancreas
Islet hormones
Immunohistochemistry
Immunoassay

ABSTRACT

Background: Chromogranin (Cg) A is expressed in neuroendocrine and neuronal tissues. It is involved in the generation of secretory granules and is cleaved to form biologically active peptides. Targeted ablation of the *Chga* gene resulted in increased plasma catecholamines, high blood pressure, and decreased size and number of adrenal medullary chromaffin granules. The aim of this study was to determine whether *Chga* null mice display changes in the morphology and function of the endocrine pancreas.
Materials and methods: Sections of pancreata from *Chga*−/−, *Chga*+/− and *Chga*+/+ mice, were immunostained with antibodies against synaptophysin, CgA, CgB, secretogranin II and the four major pancreatic islet hormones. Plasma was analysed for glucose, insulin, glucagon, somatostatin and pancreatic polypeptide (PP).
Results: CgA epitopes were undetectable in the islets of *Chga*−/− animals. CgB and secretogranin II epitopes were expressed in the islets of all animal groups albeit with decreased expression in *Chga*−/− islets. The islet number and size were decreased in the *Chga*−/− animals compared with *Chga*+/+. The proportion of insulin cells was decreased but somatostatin and PP cells were increased in *Chga*−/− mice compared to *Chga*+/+ mice. The nuclear size was decreased in insulin cells and increased in somatostatin cells in *Chga*−/− mice. Plasma insulin level was markedly decreased in the *Chga*−/− mice although fasting plasma glucose and glucagon were normal.
Conclusion: Ablation of the *Chga* gene affected the islet volume, the composition, distribution and nuclear size of islet cell types and plasma insulin concentration. Our data indicate decreased insulin cell function and increased glucagon cell function. Our study shows that CgA exerts a significant influence on the endocrine pancreas with importance in maintaining islet volume, cellular composition and function.

1. Introduction

Chromogranin (Cg) A is an acidic glycoprotein of 439 amino acids which is expressed in most neuroendocrine cell types, and has been used as a reliable neuroendocrine immunohistochemical and circulating marker for neuroendocrine tumours. CgA has 8–10 pairs of basic amino acids, which are potential cleavage sites for specific endogenous proteases, giving rise to several peptides [1,2]. Normal neuroendocrine pancreatic cell types express several CgA epitopes to a large extent, in a cell-specific manner [3]. CgA-derived peptides exert a regulatory function on the secretion of different hormones belonging to the neuroendocrine system, *e.g.* inhibition of insulin release [4,5]. CgA has also been reported to regulate granule biogenesis [6,7] and protein trafficking [8,9], where hormone-containing granules could not be formed properly in the absence of chromogranins.

Our knowledge on the function of CgA and CgA-related peptides relies mainly on *in vitro* cell culture studies. More recently, a few *in vivo* studies have been carried out by using genetic approaches to inactivate the gene encoding CgA, namely in one transgenic mouse model with downregulation of CgA expression [7] and in mice with deletion of the *Chga* gene [10,11]. These studies focused on adrenal glands, where targeted ablation of the *Chga* gene resulted in increased plasma catecholamines concentration, high blood pressure, and decreased size and number of chromaffin granules [10]. So far, the endocrine pancreas has not been investigated in these animals.

The aim of the present study was to determine whether the lack of CgA and CgA-derived peptides induces changes in the morphology and function in the endocrine pancreas in *Chga*−/− mice, in order to provide new insights into the *in vivo* functional role of CgA in the endocrine pancreas.

* Corresponding author. Department of Gastroenterology, University of Lisbon, Portugal.
E-mail address: portela_gomes@yahoo.com (G.M. Portela-Gomes).

0167-0115/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.regpep.2008.07.005

2. Materials and methods

2.1. Animals

Adult wild-type (*Chga*+/+), heterozygote (*Chga*+/−) and chromogranin A knock-out (*Chga*−/−) mice with mixed genetic background (129svJ and C57BL/6) were used in this study. All animals were housed on a 12 h light–dark cycle and fed a standard rodent chow. All protocols for animal use and euthanasia were approved by the Animal Care and Use Committee of University of California at San Diego in accordance with National Institute of Health guidelines.

2.2. Immunohistochemical study

The pancreas was dissected out under deep anaesthesia, cut transversally into 3 or 4 pieces which were fixed in 10% buffered formaldehyde for 18 to 20 h at room temperature, dehydrated and embedded in paraffin wax. From each specimen, a series of 15 adjacent sections, 5 µm thick, were cut and attached to positively charged (Superfrost+, Menzel, Braunschweig, Germany) glass slides. Haematoxylin–eosin was used for routine staining.

The sections were immunostained using the indirect two-step dextran-polymer technique (EnVision™ system, DakoCytomation, Glostrup, Denmark), with diaminobenzidine as chromogen, using a Dako Autostainer (DakoCytomation) according to the manufacturer's instructions. The primary antibodies used are characterised in Table 1.

The control stains entailed (i) omission of the primary antibodies, (ii) replacement of the first layer of antibody by non-immune serum diluted 1:10 and by the diluent alone, (iii) preincubation (24 h) of primary antibody with the relevant antigen (10 nmol/ml diluted antibody solution), respectively, before application to the sections.

2.3. Quantification

To obtain the islet volume, the proportions of islet cell tissue in the total pancreatic area were determined in two sections from each paraffin block, 50 µm apart, immunostained with synaptophysin antibodies. The areas of the endocrine and exocrine pancreas were estimated using a Leica DMRE microscope (Leica Microsystems, Wetzlar, Germany) at a magnification of 250×, with an image analysis program Image-Pro-Plus, version 5.1, run under the Windows XP Professional system (Media Cybernetics Inc, Silver Spring, MD, USA).

Differential counts of the four major islet cell types were carried out on adjacent sections, using an Olympus BH-2 microscope with the aid of a square grid in one of the oculars at a magnification of ×400. The whole sections were examined and only cells containing visible nuclear fragments were included in the counts.

The diameters of the nuclear profiles of these cells were estimated using a Nikon Optiphot-2 microscope equipped with a projector V-12A; the microscope images were projected to a table and the nuclei were measured with a ruler at a magnification of ×2180. In nuclei with an ovoid profile the geometrical mean ($\sqrt{a \times b}$, where a is the longest and b is the shortest axis) was taken as the diameter value.

The density of immunoreactive cells for neuroendocrine cell markers was estimated semiquantitatively on a four-tier scale as being either negative or positive in fewer than 10%, between 10 and 50% and more than 50% islet cells, respectively. The immunostaining intensity was also evaluated by using a three-tier scale (weak, moderate, strong).

Table 1
Antibodies used in the study

Antibodies	Code no.	Sequence	Dilution	Source
Synthetic human CgA (m)	LK2H10	250–301	1:10,000	Chemicon International, Temecula, CA, USA
Synthetic human CgA (r)		324–337	1:8000	M. Stridsberg, Dept Med Sc, Uppsala, Sweden
Synthetic human CgA (r)		176–195	1:8000	M. Stridsberg
Synthetic human CgB (r)		16–37	1:8000	M. Stridsberg
Synthetic human CgB (r)		312–331	1:4000	M. Stridsberg
Synthetic human SgII(r)		172–186	1:64,000	M. Stridsberg
Synthetic human glucagon (r)	A0565	5–15	1:1000	DakoCytomation, Glostrup, Denmark
Bovine insulin (g)	Ma37	A chain	1:10,000	P. Westermark, Dept Gen Pathol, Uppsala, Sweden
Human pancreatic polypeptide (r)	A0619	1–36	1:5000	DakoCytomation
Human somatostatin (r)	A0566	1–14	1:16,000	DakoCytomation
Synaptophysin (r)	A0010	–	1:100	DakoCytomation

Antibodies raised in mouse (m), rabbit (r) or guinea-pig (g).
Cg = chromogranin; Sg = secretogranin.

Table 2
Quantitative morphological analyses of the pancreatic islets of *Chga*+/+, *Chga*+/− and *Chga*−/− mice (n=6 in each group)

Parameters	*Chga*+/+	*Chga*+/−	*Chga*−/−
Islet tissue in relation to total pancreatic area (%)	1.8±0.7	5.0±0.7 $p<0.001$[a]	0.2±0.1 $p<0.001$[a] $p<0.001$[b]
Islet no. per mm^2 pancreatic tissue	4.8±0.3	7.4±3.2 NS[a]	3.4±1.0 $p<0.01$[a] $p<0.02$[b]
Area per islet (mm^2)	3.6±2.0	7.8±3.0 $p<0.02$[a]	0.8±0.5 $p<0.01$[a] $p<0.001$[b]

Values are expressed as mean±SEM.
NS, non-significant.
[a] Comparison with *Chga*+/+ mice.
[b] Comparison of *Chga*+/− with *Chga*−/− mice.

2.4. Plasma determinations

Blood glucose was measured in microcuvettes with a Glucose 201 analyzer (HemoCue, Ängelholm, Sweden) using blood from tail tip in conscious mice. Plasma insulin was measured using mouse insulin ELISA kit (Linco Research, St. Charles, Mi, USA), glucagon using mouse glucagon ELISA kit (Wako Chemicals, Richmond, VA, USA), and plasma somatostatin and pancreatic polypeptide using radioimmunoassay (RIA) technique as previously described [12,13]; before blood was drawn, the animals were anaesthetized with rodent anaesthesia cocktail.

2.5. Statistical analysis

Data were expressed as Mean±SEM. Statistical significance was calculated using student's t test and significance was determined at the $p<0.05$ level.

3. Results

3.1. Animals

Ablation of the *Chga* gene resulted in an increased body weight. At 5 months of age the *Chga*−/− mice were about 20% heavier than the *Chga*+/+ mice (*Chga*+/+: 32.2±1.3 gm; *Chga*+/−: 35.5±1.1; *Chga*−/−: 38.0 g±1.4 g; $p<0.001$).

The wet weight of the pancreas was 158.1±8.5 mg in the *Chga*+/+ mice and 154.3±12.5 mg in the *Chga*−/− animals (p=0.81, non-significant).

3.2. Islet morphology

An irregular islet configuration was often seen in *Chga*+/− mice (Figs. 2b, 4b), occasionally in the *Chga*−/− (Figs. 2c, 6c).