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Immunocytochemical Identification of Islet Cells Containing Calcitonin Gene-Related Peptide-Like Immunoreactivity in the Plains Rat Pancreas (*Pseudomys Australis*)

N Edwin, C M Leigh

ABSTRACT

Background/Aim: Calcitonin gene-related peptide (CGRP) is a product of alternative RNA processing of the calcitonin gene. Its dual occurrence in nerves and endocrine cells makes it likely to be both a neuropeptide and an endocrine (paracrine) messenger. The purpose of this study was to determine whether CGRP-containing cells are present in the pancreas of the plains rat (*Pseudomys australis*).

Methods: Paraffin embedded tissue from four adult plains rats was sectioned and stained using an immunocytochemical avidin-biotin-peroxidase (ABC) method. The sections were incubated with rabbit polyclonal rat CGRP (Peninsula Labs, Belmont, CA) diluted 1:1000, in a solution consisting of 1% bovine serum albumin and 0.05% sodium azide in 0.1 mol/L of PBST overnight in a humid chamber at room temperature. Immunolocalisation was shown in the islets of Langerhans tissue. A negative control using normal serum was used to test the specificity of the individual immunoreaction and on all occasions there was no immunostaining.

Results: For the purpose of colocalisation of CGRP immunoreactive cells, mirror images of stained sections were stained for CGRP and insulin, somatostatin, glucagon and pancreatic polypeptide. The immunostained cells of the same islet were assessed for colocalisation and found to be identical with insulin cells.

Conclusion: The dual localisation of CGRP immunoreactivity in neuronal and endocrine elements provide further evidence for both a neurocrine and hormonal role for CGRP in endocrine and exocrine functions. In the plains rats, we have shown CGRP to be an endocrine peptide.

Keywords: islets, CGRP, immunocytochemistry, endocrine, insulin

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INTRODUCTION

Numerous biologically active peptides, including calcitonin gene-related peptide (CGRP), are known to be expressed in mammalian pancreas and to affect endocrine and exocrine pancreatic functions[1-8]. The thirty-seven amino-acid polypeptide, calcitonin gene-related peptide (CGRP), was originally suggested to exist merely on the basis of analysis of the rat calcitonin gene structure and expression[6]. Subsequently, CGRP was found to occur mainly in neural tissues[6]. Thus, CGRP occurs in neural structures: in the brain[6,9,10], in the spinal cord[6,11] and in nerve fibres in various peripheral organs[6].

The physiological function of CGRP is still unknown; however, its dual occurrence in nerves and endocrine cells makes it likely to be both a neuropeptide and an endocrine (paracrine) messenger[6].

Colocalisation studies showed CGRP-immunoreactive cells in the mouse to be identical with a majority of the insulin cells[6]. In the rat, however, CGRP-immunoreactive cells were identical with somatostatin cells[6].

This investigation, which used Australian plains rat (*Pseudomys australis*), the cellular localisation of CGRP was undertaken, and also colocalisation studies of whether CGRP-immunoreactive cells are identical with a majority of either insulin, somatostatin, glucagon or pancreatic polypeptide cells.

MATERIALS AND METHODS

Four adult male plains rat (*Pseudomys australis*) were anaesthetised by intraperitoneal injection of Nembutal. The pancreas was removed, dehydrated in a series of graded alcohols, cleared and embedded in paraffin. Six thick sections were cut, floated on warm water and mounted on gelatin coated slides. Sections were deparaffinised, rehydrated through a series of alcohols, and then stained for peptide immunoreactivity, in order to identify specific endocrine cells using the avidin-biotin-peroxidase complex (ABC) method.

The sections were incubated with primary rabbit antiserum to polyclonal rat CGRP (Peninsula Laboratories, Belmont, CA), diluted 1:1000, in a solution consisting of 1% bovine serum albumin and 0.05% sodium azide in 0.1 mol/L of PBST overnight in a humid chamber at room temperature. After three

washings in PBS, the sections were incubated in 1 biotinylated goat antirabbit IgG (Vector Laboratories, Burlingame, CA) diluted 1:50 in phosphate buffered serum for 2 hours at room temperature and 2 avidin-biotin-peroxidase complex (Vectastain Elite ABC Reagent, Vector Laboratories) diluted 1:50 in PBST for 1 hour at room temperature. After an additional three washings in PBS, the sections were incubated in peroxidase substrate solution containing diaminobenzidine tetrahydrochloride and nickel (Vector Laboratories) for 3 minutes. Finally, the slides were counterstained with haematoxylin, dehydrated in ethanol, cleared in Histoclear and mounted in pix.

Controls

A negative control using normal serum was used to test the specificity of the individual immunoreaction. There was no immunostaining.

For the purpose of colocalisaton of the CGRP-immunoreactive cells, sections were stained with CGRP and insulin, somatostatin, glucagon and pancreatic polypeptide. The immunostained cells of the same islet were assessed for colocalisation.

RESULTS

The present study demonstrates CGRP-immunoreactive cells in the islet of the plains rat (*Pseudomys australis*) pancreas (Fig 1). Control sections did not show immunostaining, indicating the specificity of the reaction (Fig 2).

Colocalisation studies showed CGRP immunostained cells to be identical with insulin immunostained cells (Fig 3) and not somatostatin (Fig 4), glucagon (Fig 5) or pancreatic polypeptide cells (Fig 6).

DISCUSSION

The present study demonstrates that immunoreactive CGRP has a distribution in the islet's endocrine cells in the plains rat pancreas. This suggests that it may be considered to be an intraislet endocrine or paracrine messenger. CGRP immunoreactive nerve fibres were observed in the rat and mouse, running in the exocrine parenchyma, particularly around blood vessels and they were occasionally seen also within the islets[6]. Immunoreactive CGRP has been found to have a dual distribution in the mouse and rat pancreas, it was found to occur both in nerve fibres and in islets endocrine cells and was considered both an intrapancreatic neuro-peptide and an intraislet endocrine or paracrine messenge[6]. In the rat pancreas, immunoreactive cells with elongated, short and thick processes were mainly distributed at the periphery of the islets of Langerhans and occasionally disseminated among acini as well as in neuronal processes innervating the pancreatic parenchyma and blood vessels in the rat[7]. CGRP has been considered to play both a modulator and endocrine/paracrine role in the control of pancreatic functions[7].

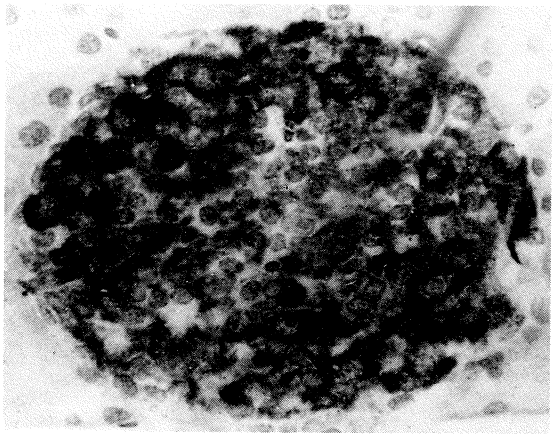

Fig 1 – Endocrine pancreas of plains rat immunostained with an antibody to CGRP ABC technique. Note positive immunostaining (x 540 scale 15 μ).

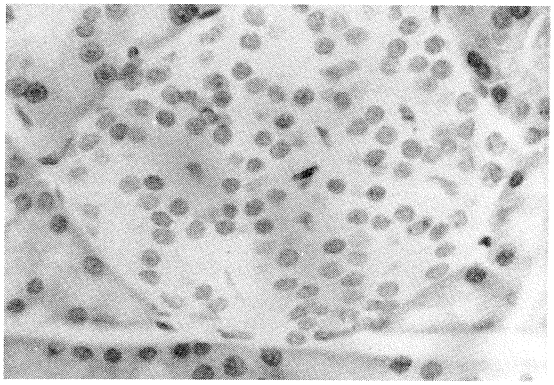

Fig 2 – Control using normal serum to test the antibody; no immunocytochemical staining (x 540 scale 15 μ).

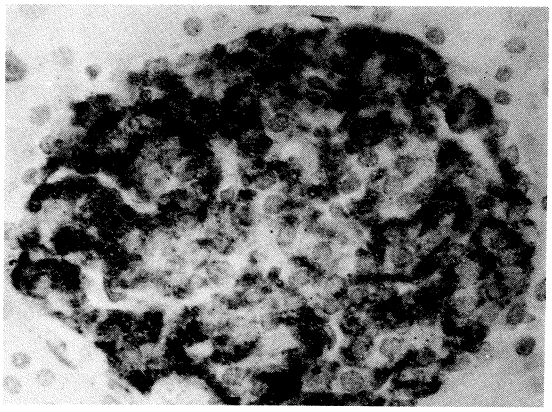

Fig 3 – Endocrine pancreas of plains rat immunostained with an antibody to insulin. Note colocalisation with CARP (x 540 scale 15 μ).

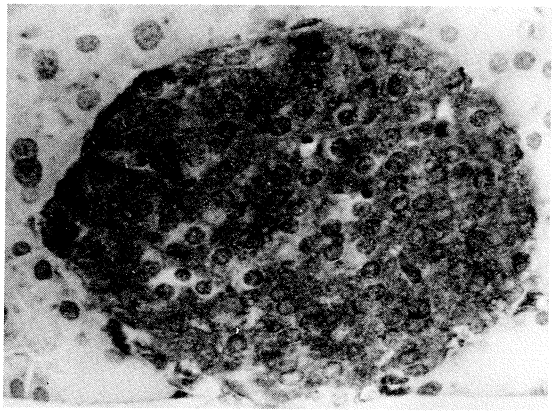

Fig 4 – Endocrine pancreas of plains rat immunostained with an antibody to somatostatic. Note positive immunostaining (x 540 scale 15 μ).

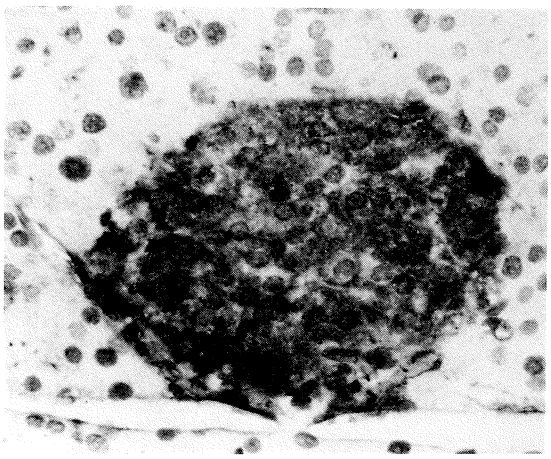

Fig 5 – Endocrine pancreas of plains rat immunostained with an antibody to glucagon. Note positive immunostaining (x 540 scale 15 μ).

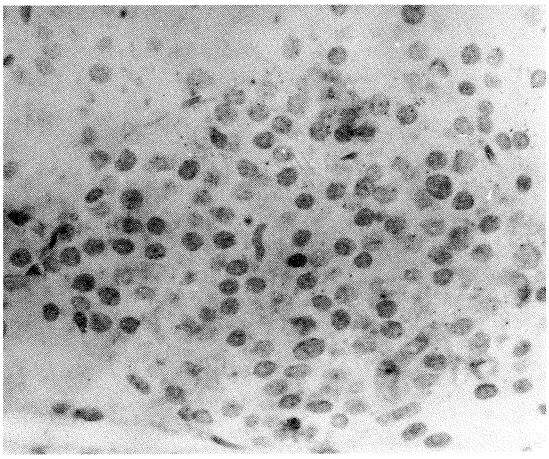

Fig 6 – Endocrine pancreas of plains rat immunostained with an antibody to pancreas polypeptides. Note sparse immunostaining (x 540 scale 15 μ).

In this study, CGRP-immunoreactive cells were found to be identical with insulin cells. This demonstrates a colocalisation of CGRP-immunoreactive cells and insulin cells. Immunocytochemical double staining experiments have previously revealed the CGRP-immunoreactive cells in the mouse to be identical with a majority of the insulin cells[6]. In the rat on the other hand, CGRP-immunoreactive cells were identical with somatostatin cells[6,12]. It was found that CGRP does not stimulate the release of somatostatin from isolated islet cells in the rat[12]. These findings suggest that CGRP may play a regulatory role in the release of insulin[12].

In addition to a particularly powerful vasodilatory effect, CGRP carries out a variety of other biological activities, including selective stimulation of gastrointestinal hormones and inhibition and stimulation of pancreatic secretions[3,13]. The dual localisation of CGRP immunoreactivity in neuronal and endocrine elements, along with the observations that CGRP containing axons and terminals are in close proximity to other neuronal structures – such as dendrites of intrapancreatic ganglia and vasculature provide further evidence for both a neurocrine and a hormonal role for CGRP in endocrine and exocrine functions.

CONCLUSION

Physiologic roles for CGRP remain to be clarified and whether CGRP regulates insulin secretion remains to be proven[2]. CGRP can be detected immunocytochemically in nerve terminals and vasculature associated with the islets of the rat pancreas. They proposed that CGRP passes to the exocrine pancreas, where it acts to stimulate amylase released by an insuloacinar portal system[2]. In the plains rat, CGRP is an endocrine peptide. The results have shown that CGRP immunoreactive cells exist in the pancreatic islets of the plains rat and it may have a paracrine role in the regulation of islet cell function as it is colocalised with insulin-secreting cells.

REFERENCES

1. Larsson LI, Rehfeld JF. Peptidergic and adrenergic innervation of pancreatic ganglia. Scand J Gastroenterol 1979; 14:433-7.
2. Seifert H, Sawchenko P, Chestnut J, Rivier J, Vale W. Receptor for calcitonin gene-related peptide: binding to exocrine pancreas mediates biological actions. Am J Physiol 1985; 249:G147-51.
3. Ahren B, Taborsky GJ Jr, Porte G Jr. Neuropeptidergic versus cholinergic and adrenergic regulation of islet hormone secretion. Diabetol 1986; 29:827-36.
4. Chey WY. Hormonal control of pancreatic exocrine secretion: In: The exocrine pancreas: biology, pathobiology and diseases. Go VLW, Brooks FP, Di Magno EP, Gardner JD, Lebenthal E, Scheele GA, eds. New York: Raven Press 1986:301-33.
5. Holst JJ. Neural regulation of pancreatic exocrine secretion. In: The exocrine pancreas: biology, pathobiology and disease. Go VLW, Brooks FP, DiMagno EP, Gardner JD, Lebenthal E, Scheele GA eds. New York: Raven Press 1986:287-300.
6. Pettersson M, Ahren B, Bottcher G, Sundler F. Calcitonin gene-related peptide: occurrence in pancreatic islets in the mouse and the rat and inhibition of insulin secretion in the mouse. Endocrinology 1986; 11:865-9.
7. Sternini C, Brecha N. Immunocytochemical identification of islet cells and nerve fibres containing calcitonin gene-related peptide-like immunoreactivity in the rat pancreas. Gastroenterol 1986; 90:1155-63.
8. Sternini C, Card PJ. Ultrastructural characterization of calcitonin gene-related X peptide-containing fibres and islet cells in the rat pancreas. Pancreas 1991; 6:375-84.
9. Mason R, Peterfreund RA, Sawchenko PE, Corrigon A, Rivier JE, Vale WW. Release of the predicted calcitonin gene-related peptide from cultured rat trigeminal ganglion cells. Nature 1984; 308:653-5.
10. Rosenfeld MG, Mermod JJ, Amara SG, et al. Production of a novel neuropeptide encoded by the calcitonin gene via tissue-specific RNA processing. Nature 1983; 304:129-35.
11. Gibson SJ, Polak JM, Bloom SR, et al. Calcitonin gene-related peptide immunoactivity in the spinal cord of man and eight other species. J Neurosci 1984; 4:3101-11.
12. Fujimura M, Greeley GH Jr, Hancock MB. Colocalization of calcitonin gene-related peptide and somatostatin in pancreatic islet cells and inhibition of insulin secretion by calcitonin gene-related peptide in the rat. Pancreas 1988; 3:49-52.
13. Goodman EC, Iverson LL. Calcitonin gene-related peptide: novel neuropeptide. Life Sci 1986; 38:2169-78.