A LIGHT-MICROSCOPIC IMMUNOCYTOCHEMICAL STUDY OF THE ENDOCRINE PANCREAS IN THE AUSTRALIAN FAT-TAILED DUNNART *(SMINTHOPSIS CRASSICAUDATA)*

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ABSTRACT

The endocrine pancreas of the Australian fat-tailed dunnart (Sminthopsis crassicaudata) was investigated by means of immunocytochemistry using the avidin-biotin-peroxidase technique. This was a light microscopic study using this established technique and has not been previously investigated.

Serial paraffin sections were stained individually with primary antibodies for anti-porcine glucagon, anti-beef pork insulin, anti-human somatostatin, and anti-avian pancreatic polypeptide (APP), anti-bovine pancreatic polypeptide (BPP), anti-serotonin, anti-porcine motilin, showing the same islet. Cells immunoreactive to porcine glucagon, porcine insulin, human somatostatin, APP, BPP were found in endocrine islets, but BPP and APP also appear to be scattered amidst the exocrine portion. Immunoreactive cells were not observed with serotonin and anti-porcine motilin. All controls were negative.

These results in the dunnart pancreas has shown four types of pancreatic endocrine cells. It has also shown that the structure of PP may more closely resemble BPP than APP. This study can be related to studies in echidnas (Tachyglossus aculeatus) and Australian possum (Trichosurus vulpecula). This is a part of an immunocytochemical study investigating the endocrine pancreas in Australian mammals.

Keywords: Pancreatic islets, cell types, light microscopy, Fat-tailed Dunnart (Sminthopsis crassicaudata)

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INTRODUCTION

Immunocytochemical studies of cells containing insulin, glucagon, somatostatin and pancreatic polypeptide (PP) in the endocrine pancreas has been performed on a wide range of vertebrates. In the primitive, egg-laying prototherian mammal, the echidna (*Tachyglossus aculeatus*), immunoreactive endocrine cells were observed using antisera to insulin, glucagon, somatostatin, avian pancreatic polypeptide (APP), and bovine pancreatic polypeptide (BPP), and also motilin[1].

Immunolocalisation of these peptides in marsupials has been performed on possum (*Trichosurus vulpecula*) using immunofluorescence[2], and in the opossum (*Didelphis virginiana*) using PAP method[3]. A light-microscopic and correlated electron-microscopic study on grey kangaroo (*Macropus fuliginosus*), has been done using the PAP method[4]. We have recently shown the localisation of the hormones to specific cell types and the morphology of the secretory granules in an EM study in the marsupial, the fat-tailed dunnart (*Sminthopsis crassicaudata*) using the protein-A gold technique[5]. Although numerous immunohistochemical studies have resulted in the major pancreatic cells being well established, this light microscopic study in the dunnart has yet to be done as also using an established avidin-biotin-peroxidase technique. These results can then be related to a previous study on grey kangaroo using PAP technique[4] and to studies in echidna using avidin-biotin-peroxidase technique[1].

This study is a part of an immunocytochemical study investigating the endocrine pancreas in Australian mammals.

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MATERIALS AND METHODS

Four adult laboratory bred fat-tailed dunnarts (*Sminthopsis crassicaudata*) were obtained from the breeding colony in the Department of Genetics, University of Adelaide. The animals were killed with an overdose of sodium pentobarbitone (Nembutal, Abbot, Ceva Chemicals, Hornsbury, NSW) and perfused initially with a rinsing solution of heparinised saline followed by a phosphate buffered fixative containing 3% formaldehyde and 0.25% glutaraldehyde, ph 7.4 at room temperature. Small pieces of pancreas were excised and immersed in Bouin's fixative, processed routinely and embedded in paraffin. The paraffin embedded tissues were sectioned serially at 1.5, 2, 4 or 5 um in thickness and stained immunohistochemically to identify specific endocrine cells using the avidin-biotin-peroxidase complex (ABC) method[6].

Specific antisera used in the study were anti-porcine glucagon, anti-beef pork insulin, anti-human somatostatin, anti-avian pancreatic polypeptide (APP), anti-bovine pancreatic polypeptide (BPP), anti-serotonin, anti-porcine motilin.

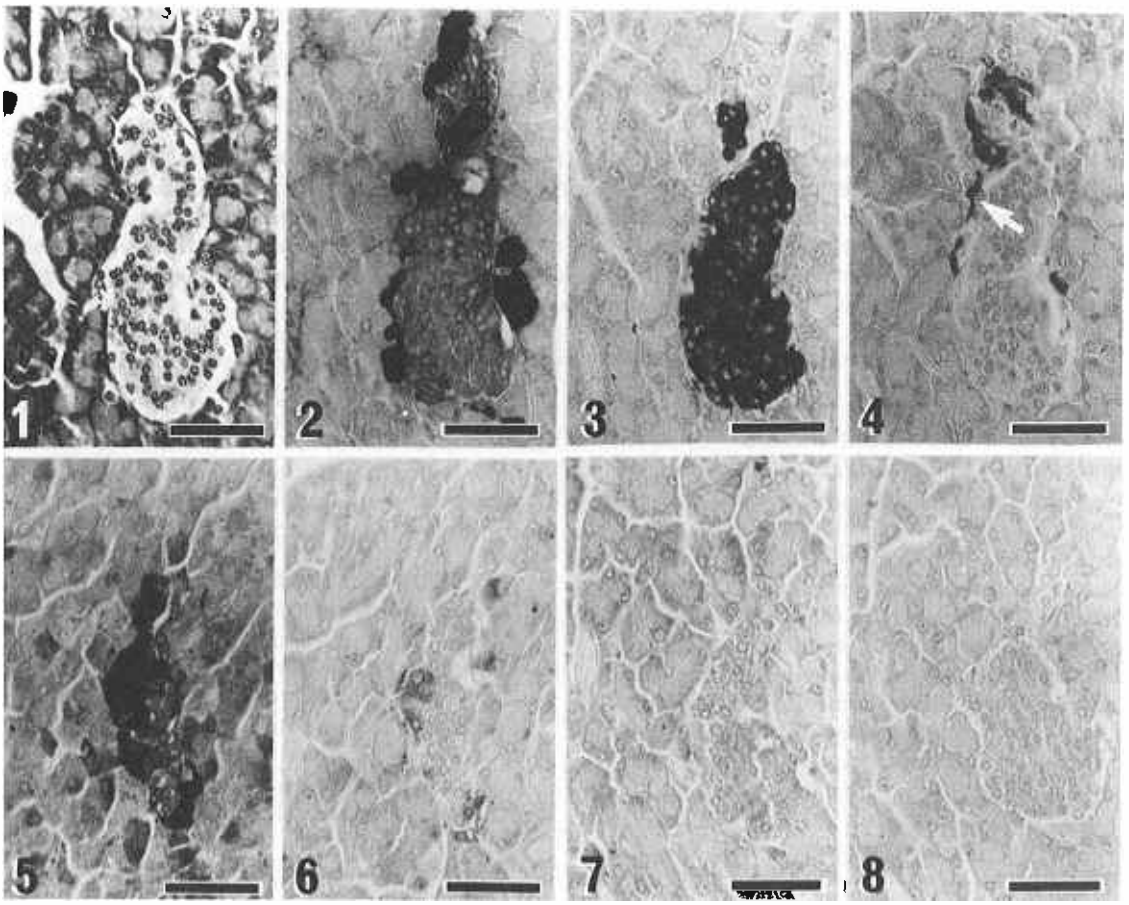
The antisera, their specificities and dilutions used have been reported previously (Yamada)[1,3].

RESULTS

The islets, in the dunnart, were found scattered around elements of exocrine tissue and limited by a connective tissue capsule similar to that seen in eutherian mammals (Fig 1).

Cells immunoreactive for porcine glucagon (Fig 2), porcine insulin (Fig 3), human somatostatin (Fig 4), bovine pancreatic polypeptide (BPP) (Fig 5) and avian pancreatic

Fig 1-8 - Sections of dunnart pancreas stained with haemotoxylin and eosin (Fig 1). Sections of same islet (Fig 2-8) stained with antisera for porcine glucagon (Fig 2), porcine insulin (Fig 3), human somatostatin - arrow showing the elongated process (Fig 4), BPP (Fig 5), APP (Fig 6), showing the distribution of immunoreactive cells. Staining with antisera for serotonin (Fig 7) and motilin (Fig 8) showed no immunoreactive staining. X194. Scale 50 um.



polypeptide (APP) (Fig 6) were found in endocrine islets, but BPP and APP also appear to be scattered amidst the exocrine portion. Serotonin (5-hydroxytryptamine) (Fig 7) and motilin (Fig 8) immunoreactive cells were not observed. All controls were negative.

Insulin and glucagon cells were round, columnar and polygonal in shape, while somatostatin and PP cells were more polymorphous in nature. On occasion, the somatostatin cells were elongated.

The PP immunoreactive cells can be identified by using BPP or APP antisera, but the immunoreactivity is more sensitive to BPP than the APP antiserum (Figs 5, 6), respectively.

DISCUSSION

The dunnart pancreas has shown 4 types of pancreatic endocrine cells–insulin, glucagon, somatostatin and PP cells. PP immunoreactive cells were demonstrated by antisera for both BPP and APP but the immunoreactivity to BPP was greater than to APP antiserum. This suggests that the structure of dunnart pancreatic polypeptide may more closely resemble that of BPP than APP. Similar observations have been made in echidnas - both pouch young and adults[1], and also in opossum[3]. Human pancreatic polypeptide (HPP) antiserum is more sensitive than APP antiserum in opossum pancreas. PP cells have also been demonstrated in the pancreas of the Australian possum (*Trichosurus vulpecula*)[2,8]. In crocodiles (*Reptilia*), PP cells of the Brazilian caiman *(Caiman latirostris)*[9], and the Nile crocodile (*Crocodilus niloticus*)[10], have been identified with mammalian PP and avian PP antisera, whereas PP cells of the Mississippi alligator (*Alligator mississippiensis*) are stained only with APP antiserum[11]. This suggests perhaps a closer relationship between avian and reptilian species.

This study has shown no immunoreactivity to motilin and serotonin (5-hydroxy tryptamine) although motilin-immunoreactive cells have been shown in pouch-young and adult echidnas[1], and in the caiman[9].

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