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A survey of endocrine cells in the pancreas of the echidna (*Tachyglossus aculeatus*) with special reference to pancreatic motilin cells

JUNZO YAMADA, WILLIAM J. KRAUSE*, NALINI EDWIN†, TOHRU MOCHIZUKI‡ AND NOBORU YANAIHARA‡

*Department of Veterinary Anatomy, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Japan, * Department of Anatomy, School of Medicine, University of Missouri–Columbia, Columbia, Missouri, U.S.A., † Department of Anatomy and Histology, University of Adelaide, Adelaide, Australia and ‡ Laboratory of Bioorganic Chemistry, University of Shizuoka, Shizuoka, Japan*

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INTRODUCTION

The echidna is a unique, primitive, egg-laying protherian mammal. Endocrine cells in the gastrointestinal tract of this species have been studied immunohistochemically and seven types of gut endocrine cells identified (Yamada *et al.* 1985; Matsuzaki *et al.* 1986; Krause & Yamada, 1986). Histological examination of the echidna pancreas has shown that the pancreatic islets are uniformly distributed throughout the head, neck, body and tail regions and that alpha (A) and beta (B) cells also are uniformly distributed in the four regions (Edwin, 1979). Although there are numerous immunohistochemical studies on pancreatic endocrine cells in many species and glucagon-, insulin-, somatostatin- and pancreatic polypeptide-immunoreactive cells are well established as major pancreatic endocrine cells, an immunohistochemical study on pancreatic endocrine cells in the echidna has yet to be done. Furthermore, motilin-immunoreactive cells have been detected recently in the pancreas of *Caiman latirostris* (Reptilia) (Yamada *et al.* 1986). The focus of this study is therefore to examine immunohistochemically not only for well-established pancreatic hormones but also motilin.

MATERIALS AND METHODS

Pancreatic tissues of a suckling pouch young and an adult echidna (*Tachyglossus aculeatus*) were examined in this study. Because of the rare opportunity for obtaining monotremes for this type of scientific study, the observations have been limited to two animals. The suckling pouch-young echidna weighed 67 g, measured 12 cm (snout–rump length) and was established as approximately 30 days post-hatching. Tissue of the pouch-young specimen was fixed in 10% buffered formalin, processed routinely and embedded in paraffin. Pancreatic tissue from the adult echidna was fixed in Bouin's fluid, processed routinely and embedded in paraffin. Specimens from the pouch-young and adult echidnas were a part of collections used in the previous studies of Krause (1972) and Edwin (1987), respectively.

The paraffin-embedded tissues were sectioned serially at 5 μm or 2 μm in thickness and stained immunohistochemically to identify specific endocrine cells using the avidin–biotin–peroxidase complex (ABC) method (Hsu, Raine & Fanges, 1981).

Table 1. *Antisera used*

Antisera[a]	Code or lot	Specificity	Dilution
Insulin[b]	8622014	—	1:1000
Synthetic human cyclic somatostatin[c]	—	—	1:10000
Synthetic porcine glucagon[d]	GL-5	Reacts with pancreatic glucagon	1:10000
Avian pancreatic polypeptide[e]	Lance-10/ 5/81 Bleed	No cross-reaction with glucagon	1:80000
Bovine pancreatic polypeptide[f]	615-R-110-146-16	Cross-reacts with human pancreatic polypeptide	1:100000
Synthetic porcine motilin[d]	R-1104	Reacts against entire molecule	1:8000
Synthetic porcine motilin[d]	R-1106	Reacts against entire molecule but not as wide as R-1104	1:8000
Synthetic canine motilin[d]	R-0220	Reacts with canine motilin	1:10000
Guinea-pig IgG (biotinylated)[g]	40605	—	1:200
Rabbit IgG (biotinylated)[g]	70209	—	1:200
Vectastain ABC kit (Elite)[g]	PK-6100	—	1:2

[a] All antisera were raised in rabbits except those against insulin which were raised in guinea-pigs, and guinea-pig IgG and rabbit IgG which were raised in goats.

[b, g] These antisera were purchased from Immunonuclear Corp., Stillwater; Vector Lab. Inc., Burlingame, respectively.

[c, e, f] These antisera were kindly donated by Dr S. Ito, Niigata; Dr J. R. Kimmel, Kansas; Dr R. E. Chance, Indianapolis, respectively.

[d] Produced by N. Yanaihara.

Details of the antisera used are shown in Table 1. The antisera and control sera were diluted in 0·01 M phosphate-buffered 0·1 M saline (pH 7·3) to prevent non-specific binding of immunoglobulins by ionic interaction. The specificity of each immunohistochemical reaction was determined as recommended by Sternberger (1979); this included replacement of the specific antiserum with the antiserum pre-incubated with the corresponding antigen. Following immunohistochemical staining, the sections were stained lightly with Mayer's haematoxylin, dehydrated, cleared and mounted. The frequency and distribution of immunoreactive cells were determined by light microscopic examination.

RESULTS

The general histology and cytology of the endocrine pancreas of this species is similar to that of higher mammals (eutherian). Cells immunoreactive for porcine insulin, human somatostatin, porcine glucagon, bovine pancreatic polypeptide (BPP), avian pancreatic polypeptide (APP), porcine motilin and canine motilin were found in both the endocrine (islets) and exocrine portions of the pancreas in the pouch-young and adult echidna (Figs. 1, 6). Serotonin (5-hydroxytryptamine)-immunoreactive cells were not observed in the pancreas during this study. The most striking observation is the presence of motilin-immunoreactive cells in the pancreas of this species.

Established immunoreactive endocrine cells

The islets of Langerhans are found scattered among the elements of the exocrine pancreas of the pouch-young and adult echidnas and are limited by a connective tissue

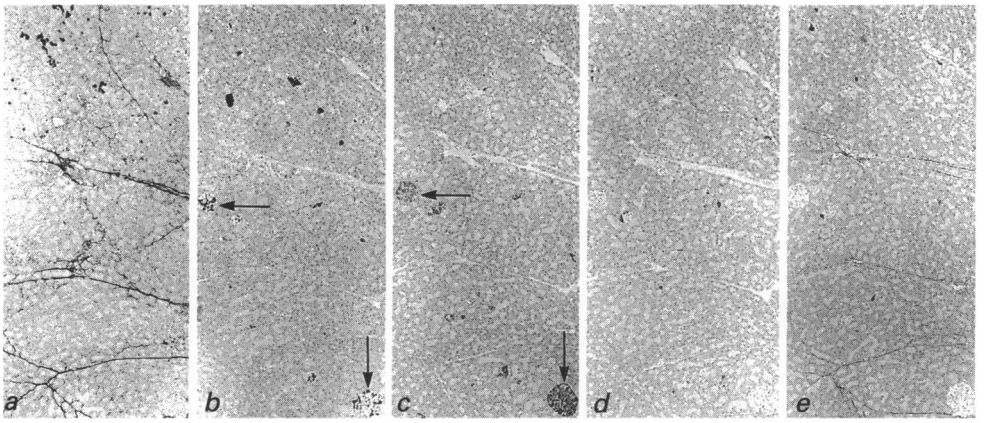

Fig. 1(*a–e*). Five serial sections through the head of an adult echidna's pancreas stained with antisera for glucagon (*a*), BPP (*b*), insulin (*c*), somatostatin (*d*) and motilin (*e*). Five types of immunoreactive endocrine cells are observed in the pancreas. Note that the PP-lobe contains large PP-islets (arrows), comprised primarily of BPP-immunoreactive cells but without glucagon-immunoreactive cells. × 100.

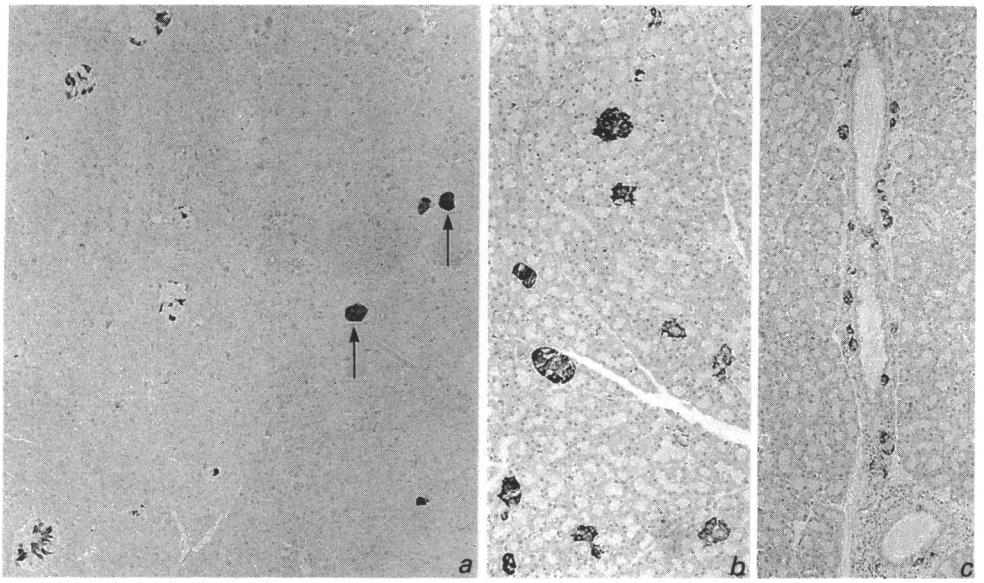

Fig. 2 (*a–c*). (*a*) The left half of the micrograph represents the PP-lobe and the right half the non-PP-lobe. Note that the distribution of insulin-immunoreactive cells and the size of the islets are different between the two lobes. Small insulin (B) islets are found in the non-PP-lobe (arrows). (*b*) The PP-lobe contains numerous BPP-immunoreactive cells and several PP-islets. (*c*) Several BPP-immunoreactive cells are located in and around the interlobular pancreatic ducts. Adult pancreas. × 200.

capsule similar to that seen in eutherian mammals. The islets appear more evenly distributed, smaller in size and more numerous in the pancreas of the pouch-young animal than in the adult. The connective tissue capsule around islets is less well developed when compared to that associated with islets of the adult. The echidna islets have been grouped into two categories based upon their cellular constituents: insulin (B)-islets and pancreatic polypeptide (PP)-islets (Figs. 1*b*, *c*, 2*a*, *b*). The B-islets contain

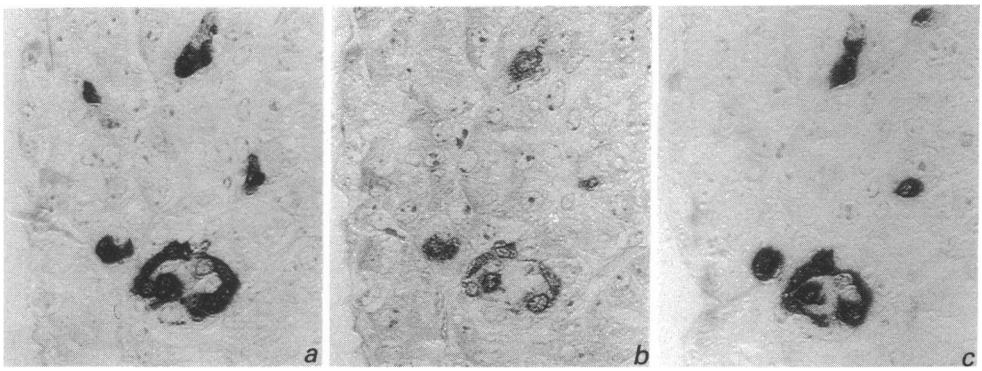

Fig. 3 (*a–c*). Three serial sections through the pouch-young pancreas stained with antisera for BPP (*a*, *c*) and APP (*b*). All APP-immunoreactive cells are immunoreactive for BPP. ×420.

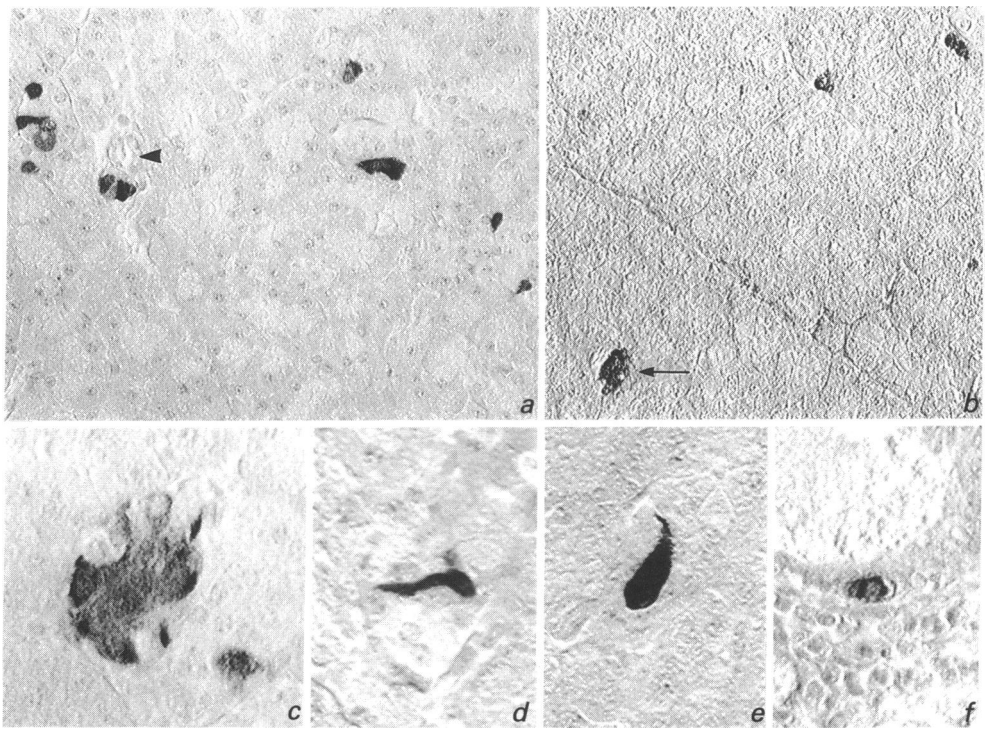

Fig. 4 (*a–f*). A portion of adult pancreas stained with antiserum (R-1104) for porcine motilin. Motilin-immunoreactive cells are found in both endocrine and exocrine portions. Some are located near a small pancreatic duct (arrowhead, *a*). A small cluster consisting of three or four motilin-immunoreactive cells is shown (arrow, *b*). (*c*) Motilin-immunoreactive cells occupy about a half of the islet. (*d*) A motilin-immunoreactive cell in the small islet has a long cytoplasmic process. (*e*) A motilin-immunoreactive cell with a short cytoplasmic process located within an acinus. (*f*) A motilin-immunoreactive cell found in an interlobular pancreatic duct. (*a*, *b*) ×320; (*c–f*) ×430.

all types of pancreatic endocrine cells, with insulin-immunoreactive cells being the most numerous. The PP-islets, on the other hand, consist primarily of PP-immunoreactive cells and a lesser number of insulin-immunoreactive cells. Other types of islet cells are either absent or few in number in this form of pancreatic islets (Fig.

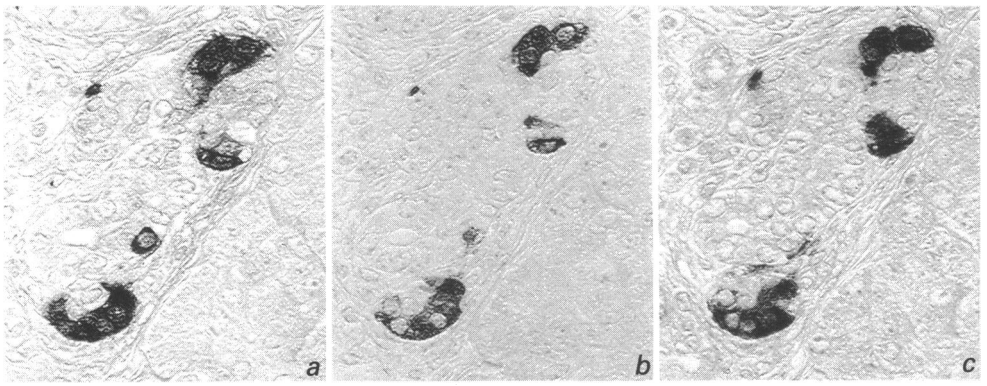

Fig. 5 (*a–c*). Three serial sections of the pouch-young pancreas stained with three types of antisera for motilin. (*a*) Pancreas stained with antiserum R-0220 which was raised against canine motilin. (*b*) Pancreas stained with antiserum R-1104 which recognises the whole molecule of porcine motilin. (*c*) Stained with antiserum R-1106 which recognises the whole molecules of porcine motilin but not so widely as R-1104. All immunoreactive cells stained with the three types of motilin antisera. ×420.

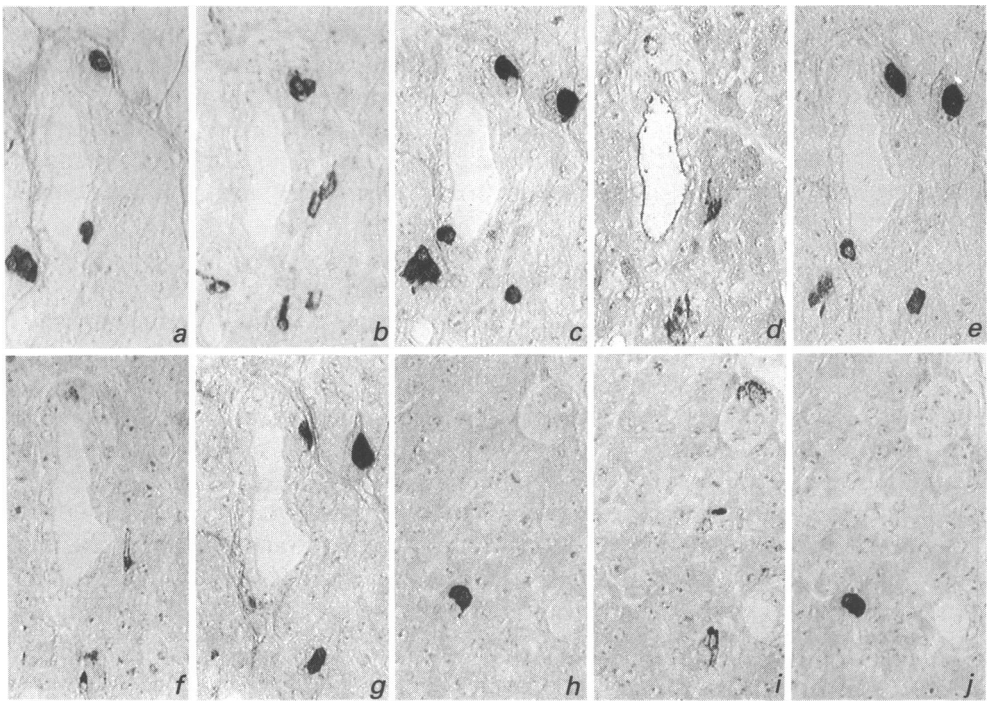

Fig. 6 (*a–j*). Seven serial sections (*a–g*) of the pouch-young pancreas stained with antisera for motilin (R-1104) (*a*, *c*, *e*, *g*), BPP (*b*), insulin (*d*) and glucagon (*f*) and three serial sections (*h–j*) of the pouch-young pancreas stained with antisera for motilin (R-1104) (*h*, *j*) and somatostatin (*i*). Motilin-immunoreactive cells never cross-react with BPP-, insulin-, glucagon- or somatostatin-antisera. ×350.

1). PP- islets are restricted primarily to the PP-lobe of the pancreas, whereas B-islets are scattered throughout the remainder of the pancreas (non-PP-lobe) (Figs. 1, 2). Generally, the PP-islets are larger than B-islets. B-islets are more variable in size, and the proportion of insulin-immunoreactive cells is higher in smaller B-islets than in

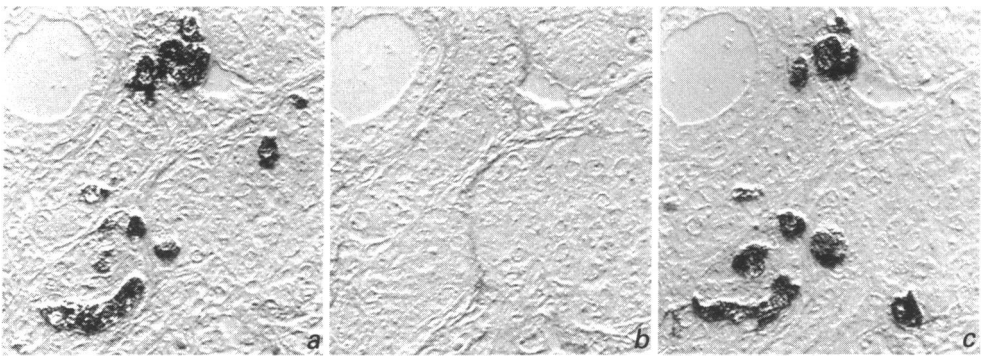

Fig. 7 (*a–c*). Three serial sections of the pouch-young pancreas stained with motilin antiserum R-1104 (*a*, *c*) and R-1104 preabsorbed with porcine motilin (*b*). Immunostaining of pancreatic motilin-immunoreactive cells is abolished by pre-incubation (*b*). × 360.

larger ones. Some small B-islets appear to consist exclusively of insulin-immunoreactive cells.

Insulin- and glucagon-immunoreactive cells are round, columnar or polygonal in shape, while somatostatin- and PP-immunoreactive cells are more polymorphous in nature. On occasion the latter exhibit long cytoplasmic processes. PP-immunoreactive cells can be identified by using either BPP or APP antisera, but the immunoreactivity is more sensitive to the BPP than the APP antiserum (Fig. 3).

All five types of immunoreactive cells are also found in the exocrine portion (Fig. 1), with PP-immunoreactive cells occurring most frequently (Fig. 2*a*, *b*). They appear as solitary cells or as small groups located in and around the intra- and interlobular pancreatic ducts (Fig. 2*c*).

Motilin-immunoreactive cells

Motilin-immunoreactive cells are found in both the islet and the exocrine portion of echidna pancreas, but are more frequently observed in the exocrine portion than in the islets (Figs. 4–7). Isolated motilin-immunoreactive cells, or small groups of them, are randomly scattered among the acinar cells and occasionally located in and around the intra- and interlobular pancreatic ducts (Figs. 4–7). There is no obvious pattern of distribution and they appear to be more numerous in the pancreas of the pouch-young than the adult echidna. On occasion they may occupy nearly half of an islet (Fig. 4*c*). Generally, motilin-immunoreactive cells are round in shape, and sometimes exhibit a long cytoplasmic process (Fig. 4*d*, *e*). The motilin-immunoreactive cells identified are stained with three kinds of antisera for motilin. When thin, serial sections are examined following the use of three types of antisera, all motilin-immunoreactive cells that stain with R-1104 (which recognises the whole molecule of porcine motilin) are stained with R-1106 (which recognises the whole molecule of porcine motilin but not so widely as does R-1104 and R-0220 (which is raised for canine motilin); and the reverse is also true (Fig. 5). The relationship between the motilin-immunoreactive cells and other types of pancreatic endocrine cells is examined using the thin serial section method. Cells immunoreactive for motilin do not cross-react with any of the other pancreatic hormones tested (Fig. 6). All of the controls are negative (Fig. 7).

DISCUSSION

In the present study five types of pancreatic endocrine cells – insulin-, glucagon-, somatostatin-, PP- and motilin-immunoreactive cells – were identified in the pouch-

young and adult echidna. PP-immunoreactive cells in the echidna pancreas were demonstrated by antisera for both avian pancreatic polypeptide (APP) and bovine pancreatic polypeptide (BPP), with PP cells being more sensitive to BPP antiserum than APP antiserum. This observation suggests that the structure of echidna pancreatic polypeptide may more closely resemble that of BPP than APP. PP-immunoreactive cells in the pancreas of the opossum also are immunoreactive to antisera for APP and BPP and, like the echidna, PP-cells stain more intensely with BPP antiserum (Krause, Cutts III, Cutts & Yamada, 1989). Human pancreatic polypeptide (HPP) antiserum also gives a more intense staining reaction than does APP antiserum on the PP-cells in the opossum pancreas (Larsson, Sundler & Håkanson, 1976). PP cells have also been identified in the pancreas of the Australian possum (*Trichosurus vulpecula*) (Reddy, Bibby, Fisher & Elliot, 1986; Edwin, 1987). In crocodiles (Reptilia), pancreatic PP cells of the Brazilian caiman (*Caiman latirostris*) (Yamada *et al.* 1986) and the Nile crocodile (*Crocodilus niloticus*) (Rhoten, 1987) 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 (Buchan, Lance & Polak, 1982), suggesting perhaps a closer relationship between avian and reptilian species.

Distribution of insulin-, glucagon- and somatostatin-immunoreactive cells of the echidna pancreas resembles that of higher mammals. The concept of a specific lobe or pancreatic region rich in PP cells is well established for the eutherian mammals (Orci *et al.* 1976). A specific PP-lobe was identified previously in the head region of the echidna pancreas by Edwin (1987), and PP-immunoreactive cells are the most dominant endocrine cell type in the pancreas of the pouch-young echidna; insulin-immunoreactive cells are the most numerous endocrine cell type in the adult pancreas. The reason for the abundance of PP-immunoreactive cells in the pancreas of the pouch-young echidna might be explained embryologically, as the region observed in this study might have been derived from the ventral primordium which contains numerous PP cells (Larsson *et al.* 1976; Orci *et al.* 1976).

The most significant observation in the present study is the occurrence of motilin-immunoreactive cells in the pancreas of the pouch-young and adult echidna. Motilin, a 22-amino acid peptide first isolated and sequenced from porcine duodenal mucosa (Brown, Mutt & Dryburgh, 1971), stimulates enteric smooth muscle locally (Brown *et al.* 1971; Itoh *et al.* 1976; Christofides, Modlin, Fitzpatrick & Bloom, 1979). Motilin cells have been regarded as one of the major endocrine cell types in the mucosa of the small intestine (Pearce *et al.* 1974; Smith, Davis, Seino & Yanaihara, 1981; Solcia *et al.* 1987). Motilin-like immunoreactivity has also been reported in a variety of tissues distinct from the gastrointestinal tract, most notably the central nervous system (see Nilaver *et al.* 1988) and the pituitary (Jacobowitz, O'Donohue, Chey & Chang, 1981). Motilin-like immunoreactivity has been reported in pancreatic B-cells of the rat, using an antiserum specific to the N-terminus of porcine motilin (Ohashi, Kobayashi & Yanaihara, 1983). However, independent motilin-immunoreactive endocrine cells have been identified in the caiman (*Caiman latirostris*) pancreas (Yamada *et al.* 1986). The present study demonstrates the presence of independent motilin-immunoreactive cells in the pancreas of the echidna, a primitive mammalian species.

Because the pancreas takes its origin from endoderm in that region of the foregut that lines the future duodenum, and because the intestinal epithelium of this particular region of the small intestine contains an exceedingly rich diversity of endocrine cell types including motilin cells in most species, perhaps it is not surprising that a cell type producing motilin is found in the pancreas of some species. Indeed, some pancreatic tumours contain other hormone-producing cells, e.g. gastrinomata and VIPomata

(Heitz, 1984). Such cell types in pancreatic tumours further emphasise the multipotentiality of primordial pancreatic cells at the time of origin.

Intravenous infusion of motilin induces contractions moving from the stomach to the intestine during the interdigestive phase in the dog, and it has been suggested that these contractions are the result of motilin released by the duodenal endocrine cells (Itoh, 1981). Precisely what action exogenous motilin has on pancreatic function is unclear at present, but its administration has been shown to inhibit pancreatic secretion in the dog (Kontrek, Król, Dembiński & Wünsch, 1976). It may be that pancreatic motilin functions in the regulation of pancreatic secretion in a manner similar to that of pancreatic polypeptide. The role motilin plays in the pancreatic islets likewise is unknown.

Because independent motilin-immunoreactive cells have been identified in the pancreas in the caiman (Yamada *et al.* 1986) and echidna it is likely that they exist in other species as well. The current study points out the need for a careful immunohistochemical survey for motilin-immunoreactive cells in the pancreas of several other species, particularly the lower vertebrates.

SUMMARY

Pancreatic endocrine cells were examined in a primitive egg-laying mammal, the echidna, using immunohistochemistry. Immunoreactive endocrine cells were observed using antisera to insulin, glucagon, somatostatin, avian pancreatic polypeptide and bovine pancreatic polypeptide. In addition, motilin-immunoreactive cells were identified in both the endocrine and exocrine pancreas of pouch-young and adult echidnas using three types of motilin antisera. Since the motilin-immunoreactive cells did not cross-react with any other pancreatic hormones tested, they are identified as an independent endocrine cell type.

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