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Insulin, Glucagon, Pancreatic Polypeptide Hormone and Somatostatin in the Goat Pancreas: Demonstration by Immunocytochemistry

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Abstract

Insulin, glucagon, pancreatic polypeptide hormone (PP) and somatostatin immunoreactive cells were demonstrated in the islet of the goat pancreas by the immunofluorescence procedure. Islet cells showing immunostaining for the hormones appeared to have a characteristic distribution. The demonstration of PP and somatostatin within the pancreas of the goat suggests they may be significant in modulating intra- and extra-islet function in this ruminant species.

Introduction

The mammalian pancreatic islet, apart from the production of insulin and glucagon, also elaborates several peptide hormones including somatostatin, pancreatic polypeptide hormone (PP), gastrin and β-endorphin (Erlandsen *et al.* 1976; Lin 1980; Watkins *et al.* 1980). Several of the islet hormones, such as glucagon, somatostatin and PP, also exist outside the pancreas (e.g. in cells of the gastrointestinal tract [Hokfelt *et al.* 1975; Larsson *et al.* 1976; Leduque *et al.* 1982]) and insulin-like material has recently been demonstrated in the brain (Dorn *et al.* 1981; Baskin *et al.* 1983; Birch *et al.* 1984).

Insulin, glucagon, PP and somatostatin have previously been demonstrated in the pancreatic islet of the human, guinea-pig and rat by immunocytochemistry (Erlandsen *et al.* 1976; Sundler *et al.* 1977; Paulin and Dubois 1978; Baskin *et al.* 1984). These studies have also shown species differences in the cellular distribution of the hormonal cells. More recently Alumets *et al.* (1983) have reported on the ontogeny of several peptide hormones in the pancreas and gut of the fetal pig. There are few such studies in ruminant species. We have recently reported on the immunohistochemical localization of PP and somatostatin in the pancreas of the fetal and adult sheep (Reddy and Elliott 1984; Reddy *et al.* 1984). The cellular localization of insulin, glucagon, PP and somatostatin has not been reported for the goat. Although the goat and sheep are phylogenetically close, the two species show some important differences, for example in the endocrine control of pregnancy (Thorburn and Challis 1979). In the present study, we have examined the pancreas of the goat in order to localize insulin, glucagon, PP and somatostatin by immunocytochemistry and compare the cellular distribution of the hormonal cells with those of other mammalian species reported previously.

Materials and Methods

Pieces of pancreas from four adult goats were removed from the splenic region immediately after decapitation of the animals and fixed in Bouin's fluid at room temperature (22°C) for 24 h. The tissues were dehydrated by increasing concentrations of ethanol, cleared in xylol and embedded in paraffin (56°C). Sections of 5 μm thickness were cut and prepared for histological examination.

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As controls, pancreas from normal Wistar rats and diabetic BB rats were similarly processed for histology.

Guinea-pig antiserum to bovine insulin (Sigma, St. Louis, U.S.A.) was prepared by Dr J. R. Crossley (Auckland, N.Z.) according to the method of Robinson and Wright (1961). Porcine anti-glucagon serum was raised in rabbits by the procedure of Holst and Aasted (1974) and was kindly supplied by Dr J. E. Livesey (Christchurch, N.Z.). Porcine glucagon was supplied by Eli Lilly and Company (Indianapolis, U.S.A.). Rabbit anti-bovine pancreatic polypeptide (anti-BPP) serum and highly purified BPP antigen were generous gifts from Dr R. E. Chance (Eli Lilly Research Laboratories, Indianapolis, U.S.A.). Antibodies to somatostatin (Bachem, California, U.S.A.) were raised in rabbits and were supplied by Dr J. R. Oliver (Adelaide, Australia) and its specificity has been reported previously (Buckerfield *et al.* 1981; Reddy *et al.* 1984).

The indirect immunofluorescent technique of Nairn (1976) was employed. Tissue sections from the goat and normal Wistar rats were incubated for 2 h at 37°C with antisera to insulin, glucagon and somatostatin at a dilution of 1 : 40 and anti-BPP serum at a dilution of 1 : 120 with phosphate-buffered saline (PBS) as the diluent. All sections were washed in excess PBS. Sections which were reacted with anti-glucagon, anti-BPP and anti-somatostatin sera were incubated with sheep anti-rabbit gammaglobulin labelled with fluorescein isothiocyanate (Wellcome Laboratories, U.K.) while sections incubated with anti-insulin serum were reacted with sheep anti-guinea-pig gammaglobulin conjugate (Wellcome Laboratories, U.K.). Both of the labelled conjugates were applied at a dilution of 1 : 10 and incubated for 30 min at 37°C. After the sections were washed in PBS they were mounted in glycerol–saline and the immunofluorescence observed with a Nikon fluorescence microscope and appropriate fields photographed. The specificity of the immunofluorescence staining was tested by replacing the antisera with either normal rabbit or guinea-pig serum, PBS or application of the antisera after absorption with an excess of the corresponding antigens (50 μg/ml of undiluted antisera). After immunofluorescence examination sections were stained with haematoxylin and eosin and viewed with the aid of a microscope.

Tissue sections from diabetic BB rats were immunostained for insulin as described above and examined microscopically.

Results

In the goat immunofluorescent staining was observed in cells of the pancreatic islet which were reacted with antisera to insulin, glucagon, PP and somatostatin. Cells staining for insulin (Fig. 1) were more abundant than those staining for the other three hormones examined in the present study. Insulin cells occupied the central core of the islet. Glucagon-containing cells were usually located in the islet periphery and were fewer in number than those which stained for insulin (Fig. 2). The cellular pattern for PP immunoreactive cells appeared similar to insulin but PP cells usually occupied a dense segment of the islet (Fig. 3). Somatostatin-containing cells were predominantly arranged in a ribbon-like manner within the islet and peripherally, and occasionally as small groups of cells, or singly (Fig. 4).

Pancreatic sections from normal Wistar rats which were incubated with anti-insulin serum gave bright fluorescence of cells occupying the central islet core, while those reacted with anti-glucagon, anti-BPP or anti-somatostatin sera resulted in positive staining of cells located in the periphery of the islet (results not shown). When anti-insulin serum was applied to pancreatic sections from diabetic BB rats in the immunofluorescence procedure, no immunostaining was observed. Immunofluorescent staining was also absent when pancreatic sections were treated with either normal rabbit or guinea-pig serum, PBS or absorbed antisera in the immunohistochemical procedure.

Discussion

This study, to our knowledge, is the first report demonstrating the presence of insulin, glucagon, PP and somatostatin in cells of the pancreatic islet of the goat by immunocytochemistry. The antisera used in this investigation were of sufficient specificity to allow specific localization of the four islet hormones (Reddy and Elliott

1984; Reddy *et al.* 1984). Further, when the same antisera were incubated with Bouin's fixed sections taken from the pancreas of a normal rat the distribution of cells showing

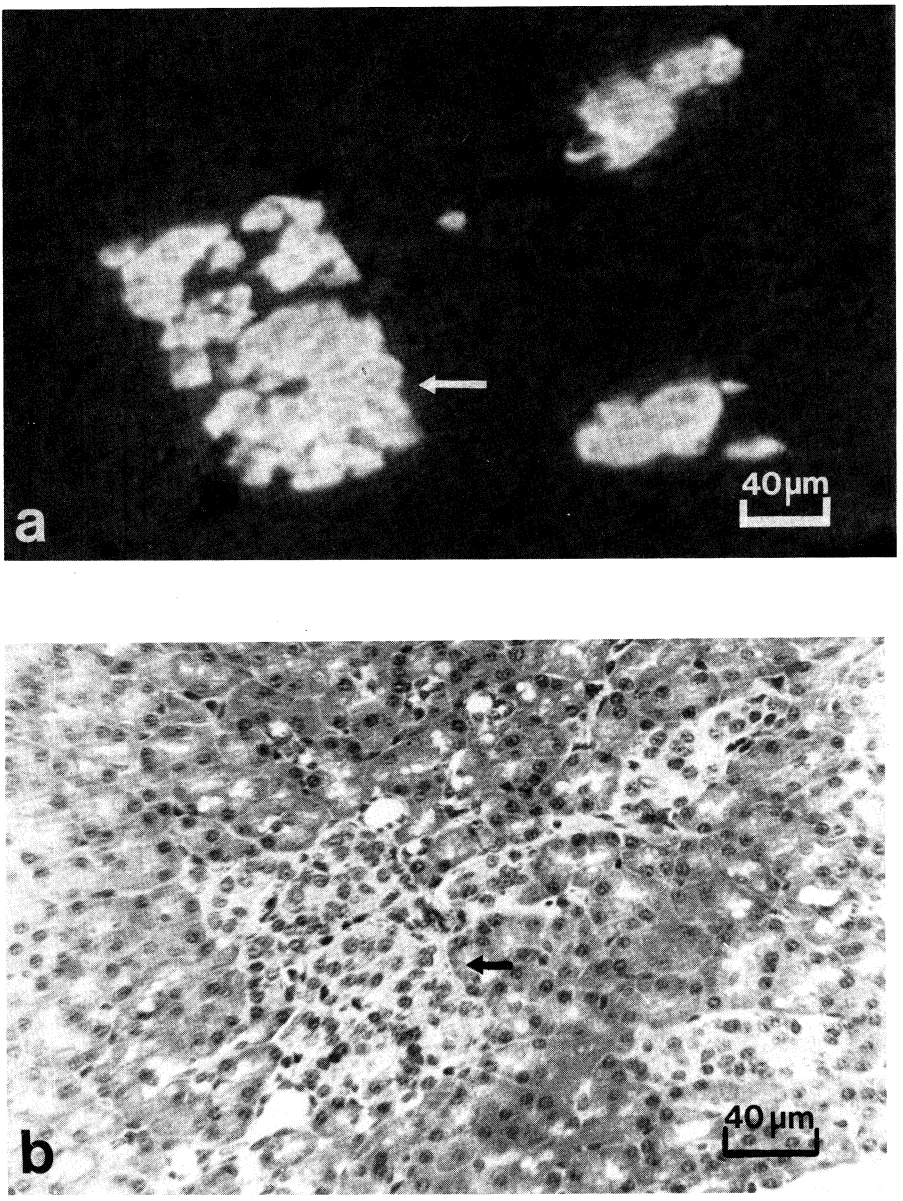

Fig. 1. Immunofluorescence localization of insulin in pancreatic islet of goat. (*a*) Section stained by the immunofluorescence procedure showing insulin cells in an islet. (*b*) Same section subsequently stained by haematoxylin and eosin showing insulin cells in the same islet (arrow).

positive staining for the four hormones was similar to that obtained by others (Hokfelt *et al.* 1975; Erlandsen *et al.* 1976; Orci 1982). In addition, when pancreatic sections

obtained from a diabetic BB rat were treated with anti-insulin serum by the same procedure there was no staining for the hormone, which is consistent with the results

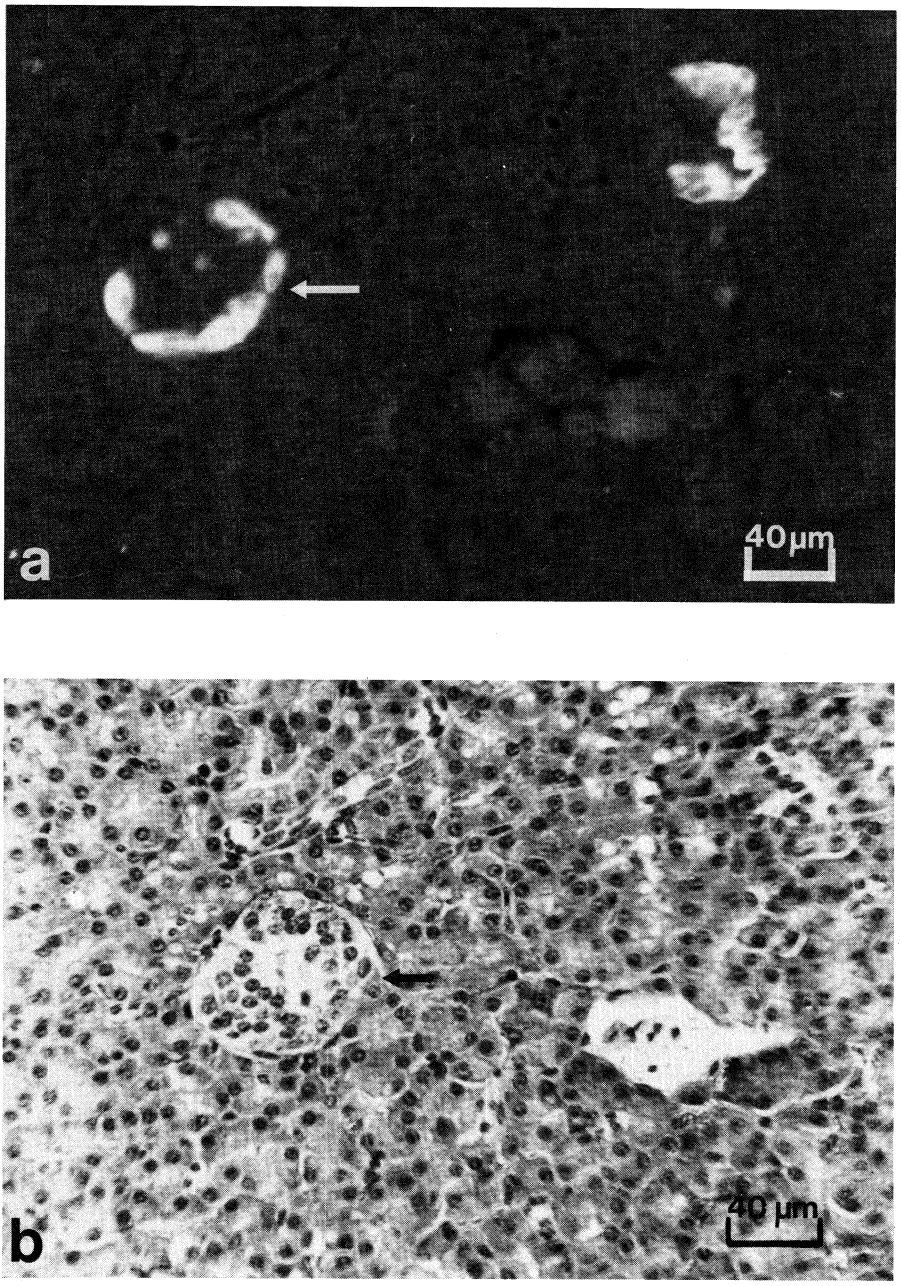

Fig. 2. Immunofluorescence localization of glucagon in pancreatic islet of goat. (*a*) Section stained by the immunofluorescence procedure showing glucagon cells in an islet. (*b*) Same section stained by haematoxylin and eosin showing glucagon cells in same islet (arrow).

of Patel *et al.* (1983) and thus confirms the specificity of the anti-insulin serum used in our study.

Most of the islet cells in the goat pancreas showed bright immunostaining for insulin and appeared to occupy the central region of the islet, whereas glucagon cells were fewer and were distributed peripherally. These results are similar to those reported previously

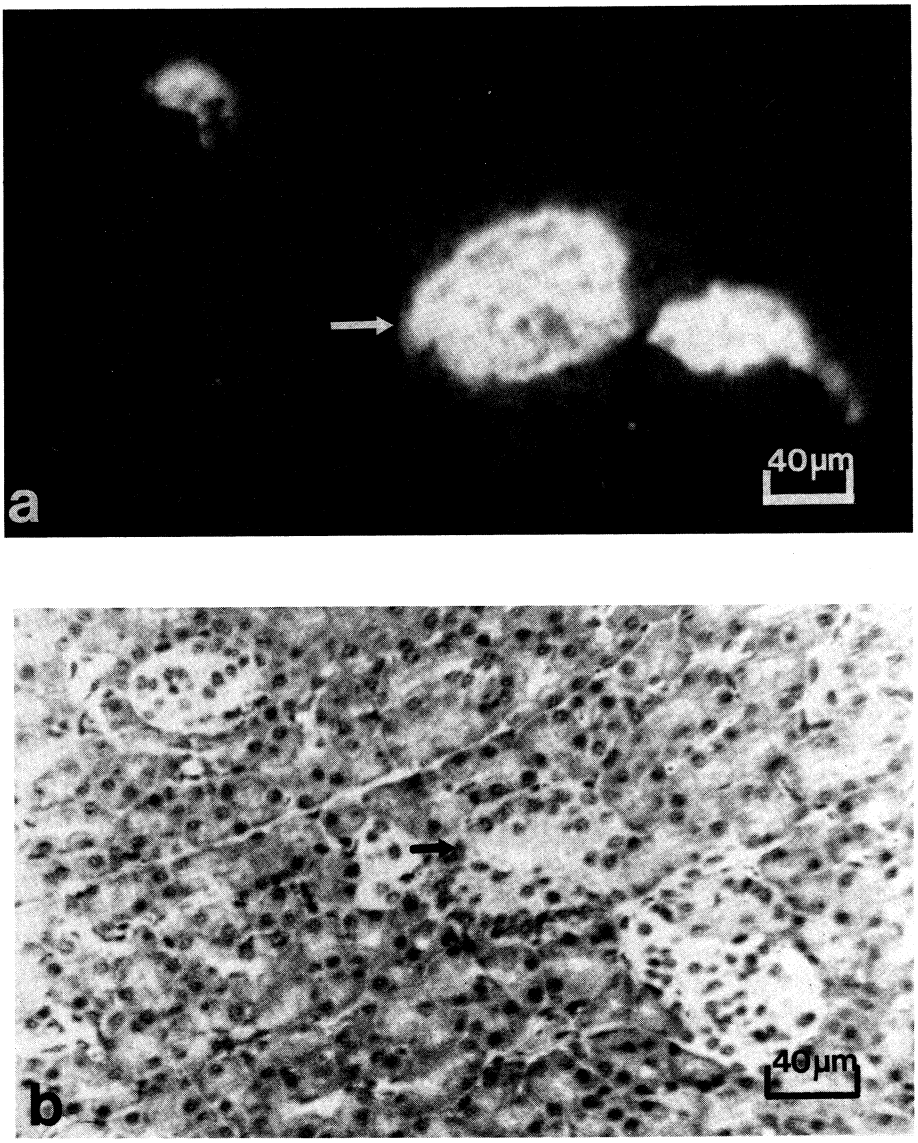

Fig. 3. Immunofluorescence localization of PP in pancreatic islet of goat. (*a*) Section stained by the immunofluorescence procedure showing PP cells in an islet. (*b*) Same section stained by haematoxylin and eosin showing PP cells in same islet (arrow).

for other mammals (Hokfelt *et al.* 1975; Erlandsen *et al.* 1976; Orci *et al.* 1976). The distribution of PP cells in the goat was similar to those for insulin, although the former cells were usually concentrated in one large area of the islet. We have observed a similar

distribution in the sheep (Reddy and Elliott 1984), and our results suggest the presence of PP-rich islets in the two species. The distribution of somatostatin immunoreactive

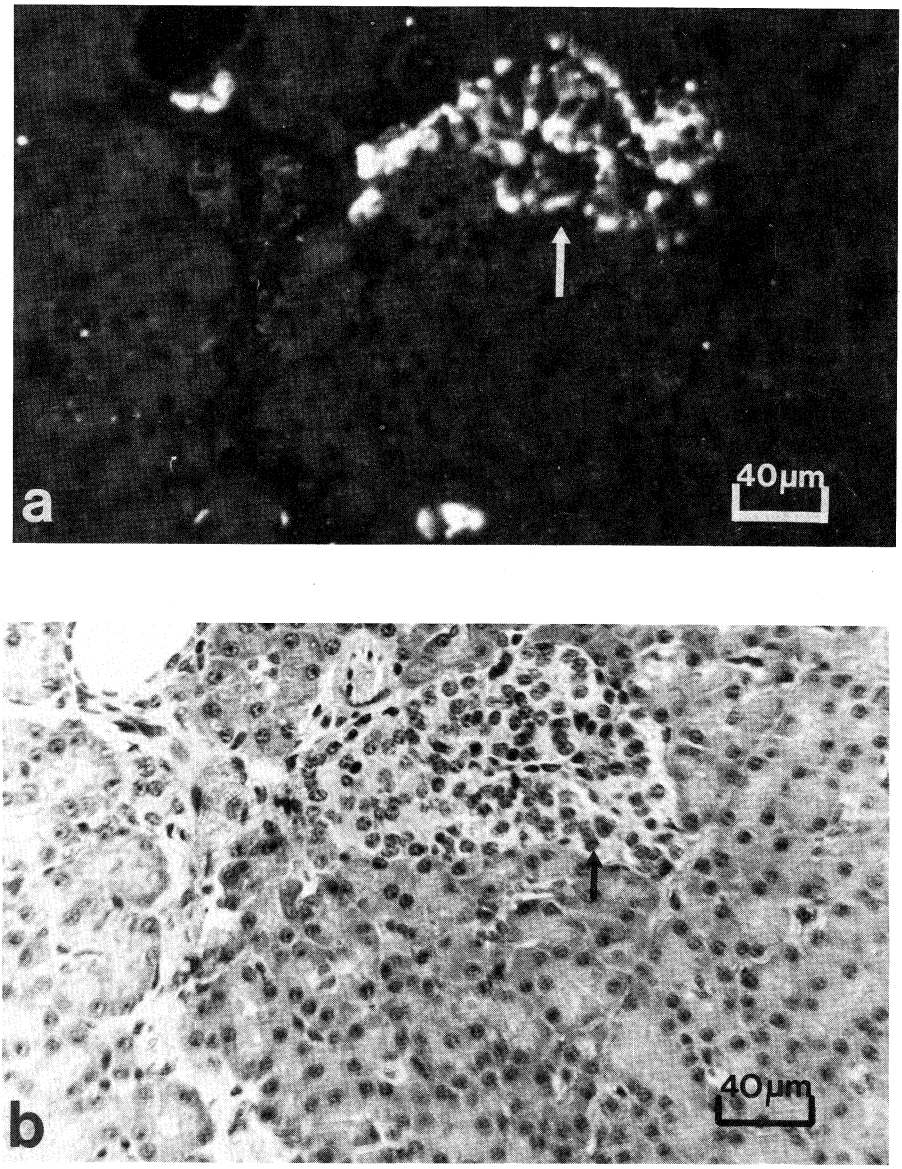

Fig. 4. Immunofluorescence localization of somatostatin in pancreatic islet of goat. (*a*) Section stained by the immunofluorescence procedure showing somatostatin cells in an islet. (*b*) Same section stained by haematoxylin and eosin showing PP cells in same islet (arrow).

cells was also similar to that in the sheep (Reddy *et al.* 1984) but different to those in the rat and human. For example, in the rat such cells are confined to the periphery of the islet (Erlandsen *et al.* 1976; S. Reddy, unpublished observations).

It is now recognized that insulin, glucagon, PP and somatostatin are present in distinct cell types within the islet. In the rat, the inhibitory effect of exogenously administered somatostatin on pancreatic insulin and glucagon release has been demonstrated (Tannenbaum *et al.* 1982) and it is likely that somatostatin from the D cell of the islet could fulfil this role in a paracrine manner. Others have reported that both insulin and PP can exert trophic effects on the acinar tissue (Greenberg *et al.* 1977; Henderson *et al.* 1981). It is therefore likely that these intra- and extra-islet functions of the pancreatic hormones demonstrated in other species are true for the goat also. In the human, rat and guinea-pig, somatostatin cells have also been shown by immunocytochemistry to contain β-endorphin (Watkins *et al.* 1980) but whether these findings are true for the goat and other ruminant species is unknown. Our present immunohistochemical studies suggest that the four hormones share considerable structural homologies with those of other mammals, particularly the sheep, although the rat pancreas produces two electrophoretically distinct insulin molecules (Kakita *et al.* 1982). The presence of several additional biologically active peptides has been demonstrated in the mammalian pancreas, for example, thyrotrophin releasing factor (Koivusalo *et al.* 1981) but it is not known whether the goat also elaborates similar peptides within the islet.

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References

Alumets, J., Håkanson, R., and Sundler, F. (1983). Ontogeny of endocrine cells in porcine gut and pancreas: an immunocytochemical study. *Gastroenterology* **85**, 1359–72.

Baskin, D. G., Gorray, K. C., and Fujimoto, W. Y. (1984). Immunocytochemical identification of cells containing insulin, glucagon, somatostatin and pancreatic polypeptide in the Islets of Langerhans of the guinea-pig pancreas with light and electron microscopy. *Anat. Rec.* **208**, 567–78.

Baskin, D. G., Woods, S. C., West, D. B., van Houten, M., Posner, B. I., Dorsa, D. M., and Porte, D. (1983). Immunocytochemical detection of insulin in rat hypothalamus and its possible uptake from cerebrospinal fluid. *Endocrinology* **113**, 1818–25.

Birch, N. P., Christie, D. L., and Renwick, A. G. C. (1984). Immunoreactive insulin from mouse brain cells in culture and whole rat brain. *Biochem. J.* **218**, 19–27.

Buckerfield, M., Oliver, J., Chub, I. W., and Morgan, I. G. (1981). Somatostatin immunoreactivity in amacrine cells of the chicken retina. *Neuroscience* **6**, 689–95.

Dorn, A., Bernstein, H. G., Hahn, H. J., and Ziegler, M. (1981). Insulin immunohistochemistry of rodent CNS: apparent species differences but good correlation with radioimmunological data. *Histochemistry* **71**, 609–16.

Erlandsen, S. L., Hegre, O. D., Parsons, J. A., McEvoy, R. C., and Elde, R. P. (1976). Pancreatic islet cell hormones. Distribution of cell types in the islet and evidence for the presence of somatostatin and gastrin within the D Cell. *J. Histochem. Cytochem.* **24**, 883–97.

Greenberg, G. R., Mitznegg, P., and Bloom, S. R. (1977). Effect of pancreatic polypeptide on DNA-synthesis in the pancreas. *Experientia* **33**, 1332–3.

Henderson, J. R., Daniel, P. M., and Fraser, P. A. (1981). The pancreas as a single organ: the influence of the endocrine upon the exocrine part of the gland. *Gut* **22**, 158–67.

Hokfelt, T., Effendic, S., Hellerstrom, C., Johansson, U., Luft, R., and Arimura, A. (1975). Cellular localization of somatostatin in endocrine-like cells and neurons of the rat with special references to the A_1-cells of the pancreatic islets and to the hypothalamus. *Acta Endocrinol.* **80** (Suppl. 200), 1–41.

Holst, J. J., and Aasted, B. (1974). Production and evaluation of glucagon antibodies for radioimmunoassay. *Acta Endocrinol.* **77**, 715–26.

Kakita, K., O'Connell, K., and Permutt, A. (1982). Pancreatic content of insulins I and II in laboratory animals: analysis by immunoelectrophoresis. *Diabetes* **31**, 841–5.

Koivusalo, F., Leppaluota, J., Knip, M., and Rayaniemi, H. (1981). Presence of TRF immunoreactivity in marginal islet cells in rat pancreas. *Acta Endocrinol.* **97**, 398–404.

Larsson, L. I., Sundler, F., and Hakanson, R. (1976). Pancreatic polypeptide-A postulated new hormone: identification of its cellular storage by light and electron microscopic immunocytochemistry. *Diabetologia* **12**, 211–26.

Leduque, P., Moody, A. J., and Dubois, P. M. (1982). Ontogeny of immunoreactive glicentin in the human gastrointestinal tract and endocrine pancreas. *Regulatory Peptides* **4**, 261–74.

Lin, T. M. (1980). Pancreatic polypeptide: isolation, chemistry, and biological function. In 'Gastrointestinal Hormones'. (Ed. G. B. J. Glass.) pp. 275–306. (Raven Press: New York.)

Nairn, R. C. (1976). 'Fluorescent Protein Tracing'. (Churchill Livingstone: Edinburgh.)

Orci, L. (1982). Macro- and micro-domains in the endocrine pancreas. *Diabetes* **31**, 538–65.

Orci, L., Baetens, D., Ravazzola, M., Stefan, Y., and Malaisse-Lagae, F. (1976). Pancreatic polypeptide and glucagon: non-random distribution in pancreatic islets. *Life Sci.* **19**, 1811–16.

Patel, Y. C., Ruggere, D., Malaisse-Lagae, F., and Orci, L. (1983). Alterations in somatostatin and other islet cell functions in the spontaneously diabetic BB Wistar rat: biochemical and morphological characterization. *Metabolism* **32**, 18–25.

Paulin, C., and Dubois, P. M. (1978). Immunohistochemical identification and localization of pancreatic polypeptide cells in the pancreas and gastrointestinal tract of the human fetus and adult man. *Cell Tissue Res.* **188**, 251–7.

Reddy, S., and Elliott, R. B. (1984). Immunofluorescence localization of pancreatic polypeptide hormone in the pancreas of fetal and adult sheep. *Aust. J. Exp. Biol. Med. Sci.* **62**, 239–42.

Reddy, S., Oliver, J. R., and Elliott, R. B. (1984). Immunohistochemical demonstration of somatostatin in the pancreas of fetal and adult sheep. *Aust. J. Biol. Sci.* **37**, 25–9.

Robinson, B. H. B., and Wright, P. H. (1961). Guinea-pig anti-insulin serum. *J. Physiol.* **155**, 302–10.

Sundler, F., Håkanson, R., and Larsson, L. I. (1977). Ontogeny of rat pancreatic polypeptide (PP) cells. *Cell Tissue Res.* **178**, 303–6.

Tannenbaum, G. S., Ling, N., and Brazeau, P. (1982). Somatostatin-28 is longer acting and more selective than somatostatin-14 on pituitary and pancreatic hormone release. *Endocrinology* **111**, 101–7.

Thorburn, G. D., and Challis, J. R. G. (1979). Endocrine control of parturition. *Physiol. Rev.* **59**, 863–918.

Watkins, W. B., Bruni, J. F., and Yen, S. S. C. (1980). β-Endorphin and somatostatin in the pancreatic D-cell colocalization by immunocytochemistry. *J. Histochem. Cytochem.* **28**, 1170–4.

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