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Bombesin-like immunoreactivity in the pancreas of man and other mammalian species[1]

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Summary. Bombesin-like immunoreactivity has been measured in pancreatic tissues of man (12.4 ± 1.2 pmol/g), pig (15.8 ± 3.2), calf (4.3 ± 0.9), rat (8.5 ± 1.2) and guinea-pig (2.8 ± 0.6) by a specific radioimmunoassay. Gel filtration of the pancreatic extracts revealed 2 major immunoreactive peaks: the earlier peak was eluted in the position of porcine gastrin-releasing peptide, and the later peak was eluted just after the amphibian bombesin standard. Immunocytochemistry demonstrated the presence of bombesin-like immunoreactivity in nerves in the rat pancreas, particularly in the exocrine pancreas, and occasionally in the peri-insular spaces. Isolated rat pancreatic islets were found to contain small quantities of bombesin-like immunoreactivity (0.037 ± 0.003 fmol/islet) suggesting that mammalian bombesin-like peptides may be invovled in the regulation of endocrine as well as exocrine pancreatic secretion.

Mammalian bombesin-like or gastrin-releasing peptide (GRP)-like immunoreactivities have previously been shown by immunocytochemistry to be present in the intrinsic nerves of the gut[3] including the intrapancreatic ganglia of the pig[4], where the peptides with these immunoreactivities may act as neurotransmitters or neuromodulators[3,4]. Infusion studies with amphibian bombesin and GRP, the larger molecular form of bombesin-like immunoreactivity (BLI) isolated from the pig, have shown that these peptides have a wide spectrum of effects on pancreatic endocrine and exocrine functions including stimulation of glucagon, insulin and pancreatic polypeptide release[5,6] and pancreatic enzyme secretion[7,8,9]. In view of the possible role of the bombesin-like neuropeptides in the regulation of pancreatic endocrine and exocrine secretion we decided to quantify these peptides in the pancreas of human and other mammalian species by specific radioimmunoassay and to characterize their molecular forms by gel-permeation chromatography. In addition, isolated rat pancreatic islets were assayed for BLI and the rat pancreas was examined by immunocytochemistry to establish the precise localisation of this immunoreactivity.

Materials and methods. Tissues. Eight histologically normal fresh specimens of human pancreatic tissue were obtained immediately post mortem, or during therapeutic splenectomy or laparatomy in patients suspected of having endocrine tumors. Pancreatic tissues from 4 pedigree Jersey calves (aged 27–38 days) and 4 piglets (aged 6–8 weeks) were removed during anesthesia. Pancreatic tissues from 8 Wistar rats (250–350 g) and 6 Duncan Hartley guinea-pigs (350 g) were removed immediately after death by stunning and decapitation. In addition, pancreatic islets were isolated from 7 groups of 4 rats by a modification of the Lacy-Kostianovsky method[10] and extracted by boiling in 1 ml of 0.5 M acetic acid.

Extraction. Weighed portions of the tissues were immediately minced and extracted in 0.5 M acetic acid (10 ml/g) at 100 °C for 10 min, then frozen and stored at −20 °C until assay.

Radioimmunoassay. The tissue extracts were thawed, centrifuged, and duplicate aliquots of 10 µl and 1 µl of the supernatant were assayed for BLI. Antiserum (BN103) was raised in a rabbit against a (lys[3])-bombesin analogue conjugated to bovine serum albumin with glutaraldehyde[11] and used at a final dilution of 1:640,000. This antiserum crossreacted 96% with synthetic porcine gastrin-releasing peptide (GRP), 0.2% with substance P, and had no crossreaction with other gastrointestinal and pancreatic peptides tested[11]. The radiolabel was ^{125}I-(Tyr[4])-bombesin prepared by the chloramine T method[11] and subsequently purified on a column of Sephadex G-25 (fine) eluted with 0.1 M formic acid containing 1% bovine serum albumin. The assay standards were prepared gravimetrically from synthetic porcine GRP. After 6 days incubation at 4 °C, free label was separated from antibody bound label by adding 2 mg of

Pancreatic content of bombesin-like immunoreactivity in different mammalian species

Species	n	pmol/g wet weight of tissue
Rat	8	8.5 ± 1.2
Guinea-pig	6	2.8 ± 0.6
Calf	4	4.3 ± 0.9
Pig	4	15.8 ± 3.2
Human	8	12.4 ± 1.2
Rat (isolated islet preparations)	7	0.037 ± 0.003 fmol/islet

dextran-coated charcoal per tube. The sensitivity of the assay was 0.4 fmol/tube (95% confidence limits). The intra-assay variation was < 6% and the inter-assay variation was < 10%.

Gel permeation chromatography. In order to study the various molecular forms of BLI present in pancreatic tissues, 4 extracts from different individuals of each species (0.5 ml) were loaded onto a column (0.9 × 60 cm) of Sephadex G-50 superfine, eluted at 4 °C with assay buffer containing 0.2 mol/l sodium chloride at a flow rate of 3 ml/h. The column was re-calibrated between each series of runs with Dextran blue (mol.wt greater than 2×10^6, Kav 0), horse heart cytochrome c (mol.wt 12,384, Kav 0.25) and $Na^{125}I$ (Kav 1) as molecular size markers. Fractions between the void volume and 2 column volumes were assayed at 2 separate dilutions.

Immunocytochemistry. Adult rats were anesthetized with diethyl ether and killed by aortic perfusion with 500 ml of 0.4% p-benzoquinone in phosphate buffered saline over 15 min[12,13]. Specimens were immediately collected from the head and tail of the pancreas and immersed in melting Arcton, pre-cooled in liquid nitrogen. Cryostat sections (6-10 µm) were cut and mounted on glass slides pre-coated with poly-L-lysine[14]. A rabbit antibody was raised to synthetic (Lys^3)-bombesin and used at a dilution of 1:200. An indirect immunofluorescence procedure[15] was carried out using fluorescein-conjugated goat-anti-rabbit gamma globulin (Miles), and sections were viewed with a Leitz-Orthaplan microscope equipped with an UV light source. Antiserum pre-absorbed with bombesin-14 (1 nmol/ml) gave no staining reaction, whereas synthetic substance P pre-absorbed antiserum gave results identical to those with unabsorbed antiserum.

Statistical analysis. Concentrations of BLI were expressed as mammalian bombesin (GRP) equivalents in pmol/g wet weight tissue and quoted as mean ± SEM. The elution coefficient (Kav) for each immunoreactive peak was calculated according to Laurent and Killander[16].

Results. The concentrations of BLI (BLI) in the pancreas of the species examined are shown in the table. The highest concentrations of BLI were found in the porcine and human pancreas, 15.8 ± 3.2 and 12.4 ± 1.2 pmol/g respectively, intermediate concentrations in the rat and calf pancreas, and the lowest concentration in the guinea-pig pancreas (2.8 ± 0.6). BLI was also found in small amounts (0.037 ± 0.003) fmol/islet in isolated rat pancreatic islet preparations. Gel permeation chromatography of pancreatic extracts demonstrated 2 major BLI peaks in each species. The earlier peak (Kav 0.52–0.54) was eluted in the position of GRP, while the second peak (Kav 0.82–0.84) was eluted just after amphibian bombesin (Kav 0.78). These 2 peaks had identical Kav values in the different species, but differed in the relative amounts of immunoreactivity within the peaks (fig. 1). Thus in man and pig the first peak contained approximately 85% of the total recovered immunoreactivity, while corresponding values were approximately 55% for rat and guinea-pig and 30% for the calf. Total recovery of immunoreactivity from the column ranged from 85 to 110%.

BLI was observed by immunocytochemistry in the exocrine rat pancreas in nerve-like structures with a beaded appearance along intra-acinar (fig. 2) and interacinar spaces. No immunoreactive cell bodies were detected in the intraparenchymal ganglia. No bombesin-immunoreactive nerves were found within pancreatic islets in either the cephalic or caudal portions of the gland, but they were occasionally observed in the peri-insular spaces. No BLI was found in the endocrine cells of the islets. Immunoreactive nerve endings were often found in close relationship with blood vessels (fig. 3) and interacinar ducts within the exocrine parenchyma.

Discussion. There is considerable evidence to suggest that the peptidergic nerve fibers of the autonomic nervous system are involved in regulation of gut and pancreatic function[17]. A number of putative peptide neurotransmitters have been located in these fibers by immunocytochemical techniques.

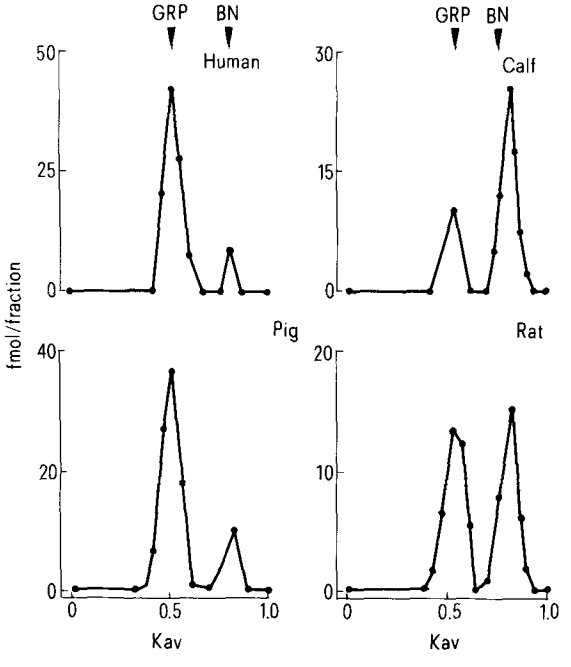

Figure 1. Gel permeation chromatographic profiles of human, porcine, calf and rat pancreatic extracts on a column (0.9 × 60 cm) of Sephadex G-50 superfine. GRP = gastrin-releasing peptide, BN = amphibian bombesin.

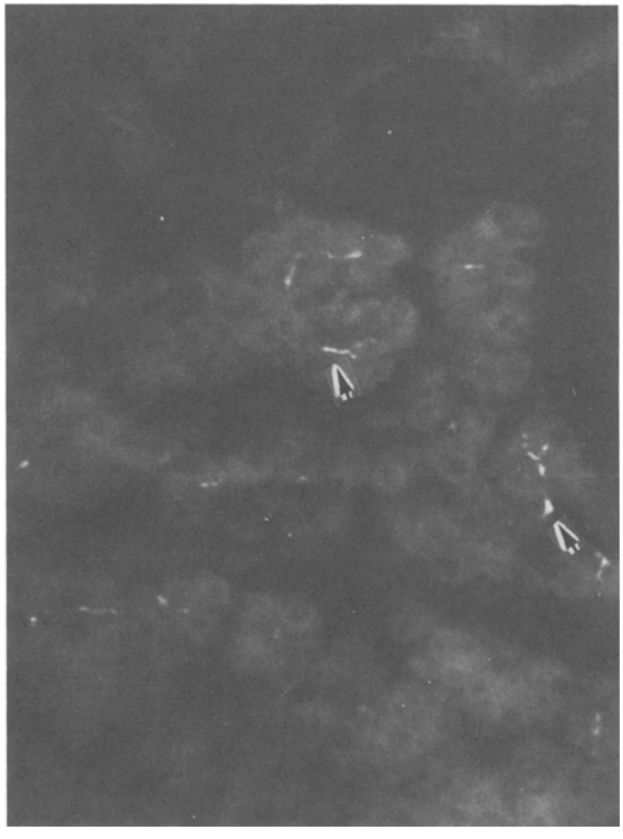

Figure 2. Bombesin-like immunoreactivity in nerves with beaded appearance in intra-acinar spaces (arrow). ×415.

These include somatostatin[18], vasoactive intestinal polypeptide[19], cholecystokinin[20], neurotensin[21] and enkephalins[22]. BLI has been demonstrated in intrinsic nerves throughout the human[3] and mammalian gut[4,23] and its molecular forms may function as stimulatory neuropeptides in a variety of sites[19]. Furthermore, receptors for bombesin have been identified in guinea-pig pancreatic acinar cells[24]. It has been suggested that the post-ganglionic vagal innervation of the endocrine cells in the stomach contains bombesinergic neurons which may regulate the secretion of such peptides as gastrin[25] and so modify gastric acid and endocrine secretion. More recently a 27-amino acid peptide, gastrin-releasing peptide (GRP), has been isolated from porcine upper gastric tissue[26] . This peptide has the same C-terminal decapeptide as bombesin, except for His instead of Gln at position 8 from the C-terminus; the C-terminal nonapeptide of bombesin has been demonstrated to be essential for full biological activity[27].

Quantitative comparison of canine[28] and bovine[29] pancreatic hormone responses to i.v. administration of bombesin and synthetic porcine GRP has demonstrated that the 2 peptides produce a prompt rise in plasma pancreatic glucagon and pancreatic polypeptide concentrations, and are exceptionally potent insulinotropic agents. In the present study we have found significant concentrations of BLI in the pancreas of all species examined. The chromatographic studies on the pancreatic BLI in man and other mammalian species suggest that the larger molecular form predominates in man and pig, whereas both forms are present in approximately equal amounts in rat and guinea-pig, and only 30% of the total immunoreactivity was present as the larger molecular form in the calf. So far the smaller form of mammalian BLI peptide has not been isolated and tested; therefore the significance of different species is, at present, unknown. However, since both bombesin and GRP very potently stimualte pancreatic exocrine and endocrine secretion and the corresponding immunoreactivities are located in nerve fibers in the pancreas, it is likely that these neuropeptides have a role in the regulation of both exocrine and endocrine pancreatic functions. There may be an additional long-term trophic role of these peptides as chronic infusion of bombesin induces hyperplasia and increased DNA synthesis in the rat pancreas[7].

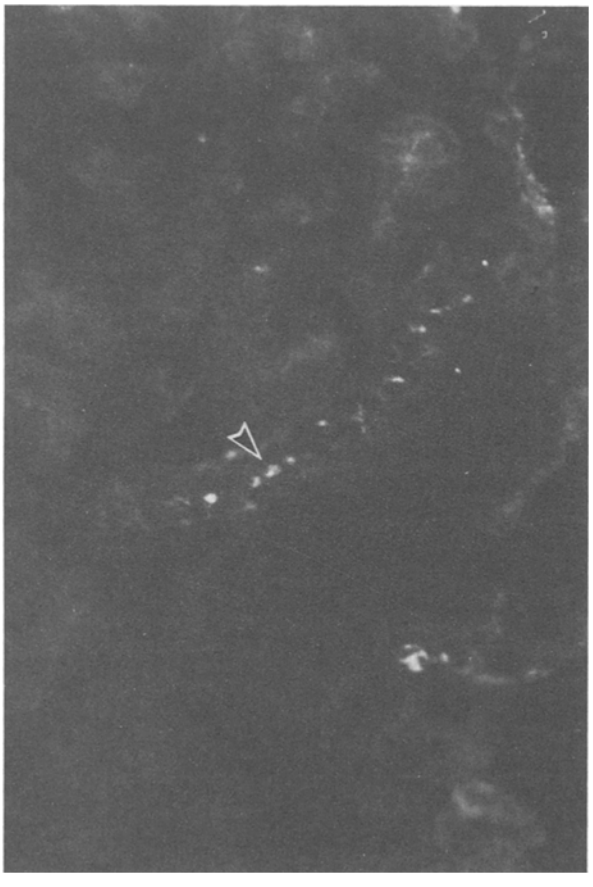

Figure 3. Bombesin-like immunoreactivity in nerves running along blood vessels (arrow). ×720.

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