Galanin-like immunoreactive endocrine cells in bovine pancreas

EMMANUEL T. BALTAZAR[1], NOBUO KITAMURA[1], EIICHI HONDO[1], ERLINDA C. NARRETO[2] AND JUNZO YAMADA[1]

[1] *Department of Veterinary Anatomy, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Japan, and* [2] *Department of Veterinary Anatomy, Physiology and Pharmacology, College of Veterinary Medicine, Central Mindanao University, Bukidnon, Philippines*

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ABSTRACT

Pancreata of fetal, neonatal and adult cattle were studied immunohistochemically for galanin. The results revealed galanin-like immunoreactivity both in the endocrine cells and in the neural elements. The galanin-like immunoreactive endocrine cells (Gal-LIEC) were confined to the large islets, and were not observed in the islets of Langerhans and exocrine pancreas. They were first detected at the third prenatal month. Their developmental profile showed an increase from fetal to early neonatal stage with a subsequent decrease towards adulthood. The considerable number of Gal-LIEC from late prepartum to early postpartum stage may imply functional significance of galanin during the perinatal development of cattle. Coexistence of galanin and insulin was also observed which may suggest autocrine interaction between the 2 hormones.

Key words: Immunohistochemistry; insulin; large islets; cattle.

INTRODUCTION

Galanin, a 29-amino acid peptide, was originally isolated from porcine upper intestine (Tatemoto et al. 1983). Subsequent biological studies on this peptide have validated its importance in various mammalian species. It exhibits a broad spectrum of physiological activities, e.g. control of smooth muscle contractility and gastrointestinal motility (Tatemoto et al. 1983; Bauer et al. 1989), and modulation of the release of several neurotransmitters and hormones (Bauer et al. 1989; Harling et al. 1990). Outstanding among the latter biological effects is the ability of galanin to regulate insulin secretion in pigs (Messell et al. 1990), dogs (McDonald et al. 1985; Dunning et al. 1986), rats (Lindskog & Ahrén, 1989; Rünzi et al. 1992), mice (Lindskog & Ahrén, 1987) and humans (Ahrén et al. 1991).

Immunohistochemical demonstration of galanin in the neural elements of the pancreas of several species i.e. pigs (Messell et al. 1990; McDonald et al. 1992; Adeghate et al. 1996), dogs (Dunning et al. 1986; Ahrén et al. 1990; McDonald et al. 1992), cats (Furuzawa et al. 1996), rats, mice (Lindskog et al. 1991), humans (Ahrén et al. 1991; McDonald et al. 1992; Shimosegawa et al. 1992) and chickens (Hiramatsu & Ohshima, 1995), has afforded a morphological basis for its peptidergic neurotransmitter/neuromodulator role in the pancreas. Conversely, the potential role of galanin as a pancreatic hormone, and/or paracrine and/or autocrine substance/s of the pancreatic endocrine cells remains obscure hitherto as no report has so far been published on its occurrence in the endocrine cells of the pancreas.

Our preliminary immunohistochemical screening of neuropeptides in bovine pancreas, however, revealed galanin not only in the neural elements but also in the endocrine cells of the large islets. The large islet is one of the 2 distinct types of pancreatic islets in bovine (Bonner-Weir & Like, 1980). Compared with the small islets, the classic mammalian islets of Langerhans, which are 25–200 μm in diameter, embedded in exocrine tissue, and present in fetus, calf and adult, the large islets, which consist primarily of insulin

Correspondence to Dr Emmanuel T. Baltazar, Department of Veterinary Anatomy, Obihiro University of Agriculture and Veterinary Medicine, Obihiro 080-8555, Japan. Tel.: +81 155 495115, ext. 5353; fax: +81 155 495354; e-mail: baltazar@obihiro.ac.jp

endocrine cells, are 100–1600 μm in diameter, enmeshed in interlobular connective tissue, and prominent in fetus and neonate but negligible in adults (Bonner-Weir & Like, 1980). This islet type, which has also been observed in buffalo (Lucini et al. 1998), sheep (Titlbach et al. 1985) and goat (Baltazar et al. 1999, unpublished) may be a characteristic feature of ruminants.

The present study was conducted to characterise the pancreatic endocrine cells immunoreactive for galanin at various stages of bovine development. Possible colocalisation of galanin and insulin was also investigated.

MATERIALS AND METHODS

Tissue sample collection and preparation

Pancreata of Holstein cattle were collected from 8 fetuses, 14 calves and 3 adults. The fetal pancreata were obtained from a slaughterhouse, accidentally aborted fetuses, and normal fetuses of dissected cattle. The fetal ages were estimated based on crown-rump length measurement (Gomes, 1978). For calves and adults, pancreatic tissues were sampled after the animals were anaesthetised with xylazine and killed by exsanguination from the left common carotid artery.

The tissue samples were fixed in 10% formalin in sodium phosphate buffer, pH 7.4. Tissue blocks were taken from the body of the pancreas, where the large islets are predominantly located (Bonner-Weir & Like, 1980). To remove fixative, the blocks were washed for several hours in water continuously displaced from the container at a very low flow rate. They were immersed and allowed to settle in 10% and then 30 % sucrose solution in 0.1 M sodium phosphate-buffered saline (PBS), pH 7.4. After embedding in Tissue-Tek O.C.T. compound (Miles, Elkhart, USA), they were frozen in liquid nitrogen and sectioned at 20 μm thickness using a cryostat (1720 Digital Kryostat, Leitz, Germany).

Immunohistochemistry

The cryostat sections were mounted on glass slides coated with poly-L-lysine and processed for immunohistochemistry employing the avidin-biotin-peroxidase complex (ABC) method (Hsu et al. 1981). The incubation periods were 2 h with normal goat serum (1:50 dilution), overnight with rabbit antiporcine galanin (1:3000 dilution, IHC-7153, Lot Nos. 950503-3 and 950503-2, Peninsula Laboratories, Belmont, USA), 30 min with biotinylated anti-rabbit IgG in goat (1:200 dilution, BA-1000, Vector Laboratories, Burlingame, USA), and 30 min with ABC reagent (1:2 dilution, PK-6100, Vector Laboratories). The immunoreactive site was revealed with 0.05 M Tris-HCl buffer, pH 7.4, containing 0.05% 3,3′-diaminobenzidine and 0.01% H_2O_2. The sections were then dehydrated through graded concentrations of ethanol (70% to 100%), cleared in xylene and mounted.

Negative controls were carried out by substituting the primary antiserum with PBS and galanin antiserum pre-absorbed with excess amount of its corresponding antigen. In these cases, no immunoreactivity was observed. Furthermore, the manufacturer of the antiserum has claimed negative cross-reactivity of galanin antibody with porcine secretin and neuropeptide Y, human peptide histidine methionine-27, galanin message-associated peptide 1-41 and 44-59, and vasoactive intestinal peptide. Since possible cross-reaction with other still unknown peptides cannot be excluded, the immunoreactivity demonstrated in the present study was referred to as 'galanin-like immunoreactivity'.

Quantitation of galanin-like immunoreactive endocrine cells

Stereological technique was employed quantitatively to characterise the galanin-like immunoreactive endocrine cells (Gal-LIEC) in the large islets (Weibel, 1979). Volume density was adopted as structure parameter and area as test reference system.

Large islets were examined at ×40 objective magnification using a mechanical stage. Photomicrographs of the fields containing areas of the large islets were taken using instant film. The Gal-LIEC in the photomicrographs were shaded darkly while referring back, as a countercheck, to the microscopic fields under examination. The shaded photomicrographs were placed under a scanner (GT-8500, Seiko-Epson, Japan) and scanned using Adobe Photoshop 3.0J software (Adobe Systems, USA). The images were stored in a Macintosh computer and then viewed on the monitor screen. The areas of the Gal-LIEC and large islets were quantified using the NIH Image program (Version 1.61) developed at the US National Institutes of Health and available on the Internet at http://rsb.info.nih.gov/nih-image. With the wide variation in size, irregularity in shape and distribution of the large islets in the pancreas, the volume density was estimated directly for the animal. It was then calculated from the ratio of the sum of the Gal-LIEC

areas to that of the large islet areas on all the photomicrographs taken from at least 3 sections per animal. The volume density was multiplied by a factor (100) and the results were presented as mean percent volume with their respective standard deviation (S.D.). S.D. was computed using the statistical approach described for ratio estimate (Cochran, 1977).

Colocalisation of galanin and insulin

Another set of tissue sections was immunostained for insulin using immunofluorescence. Guinea pig anti-bovine insulin (1:1000 dilution, Catalog No. 20056, Lot No. 8622014, Immuno Nuclear Corp., Stillwater, USA) was used as primary antiserum and rhodamine-labelled anti-guinea pig IgG in goat (1:100 dilution, Catalog No. 55389, Lot No. 00071, ICN/Cappel, Ohio, USA) as secondary antibody. After photomicrography, sections were eluted (Tramu et al. 1978), reimmunostained for galanin by ABC method as described above, and examined for coexistence of insulin- and galanin-like immunoreactivities. Photomicrographs were taken on the same portions of the section initially photographed.

RESULTS

Distribution of galanin-like immunoreactivity

Galanin-like immunoreactivity was observed in the endocrine cells of the large islets, nerve cell bodies of the intrapancreatic ganglia, and nerve fibres of the exocrine and endocrine pancreas. The neural elements showed constant intense immunoreaction, while that of the endocrine cells ranged from weak to strong.

Morphology and distribution of galanin-like immunoreactive endocrine cells

The Gal-LIEC were round to oval in shape and some had short cytoplasmic processes. They were restricted to the large islets, and not detected in the islets of Langerhans and exocrine pancreas. In the large islets, they were irregularly distributed.

Developmental profile of galanin-like immunoreactive endocrine cells

Galanin-like immunoreactivity was observed at the second prenatal month, the earliest stage studied, when it was present only in the nerve fibres of the pancreas. Gal-LIEC were first detected at the third month (Fig. 2*a*).

Table. *Percentage volume of galanin-like immunoreactive endocrine cells (Gal-LIEC) relative to the large islet of the pancreas at various stages of bovine development*

Age	Mean percent relative volume of Gal-LIEC ± S.D.
Fetus	
2.1 mo (CRL 7 cm)	ND
3.2 mo (CRL 17 cm)	Negligible
4.1 mo (CRL 29 cm)	0.80 ± 0.14 (n = 13)
5.3 mo (CRL 44 cm)	
Animal 1	1.91 ± 0.22 (n = 15)
Animal 2	1.87 ± 0.59 (n = 15)
7.3 mo (CRL 70 cm)	3.10 ± 0.43 (n = 11)
8.0 mo (CRL 81 cm)	3.58 ± 0.64 (n = 19)
8.4 mo (CRL 85 cm)	4.10 ± 0.57 (n = 16)
Calf	
1 d	5.43 ± 0.90 (n = 15)
5 d	
Animal 1	8.88 ± 0.98 (n = 18)
Animal 2	9.43 ± 0.47 (n = 18)
7 d	
Animal 1	6.26 ± 0.60 (n = 15)
Animal 2	7.22 ± 0.59 (n = 15)
10 d	4.60 ± 0.57 (n = 11)
13 d	3.88 ± 0.42 (n = 18)
21 d	
Animal 1	3.01 ± 0.53 (n = 15)
Animal 2	2.75 ± 0.57 (n = 15)
1 mo	0.97 ± 0.47 (n = 6)
3 mo	0.13 ± 0.05 (n = 7)
6 mo	Negligible
11 mo	Negligible
1 y	Negligible
Adult	
1.5 y	NA
3 y	NA
8 y	NA

ND, not detected NA, not applicable since the density of the large islets in the pancreas was negligible; n, number of areas evaluated.

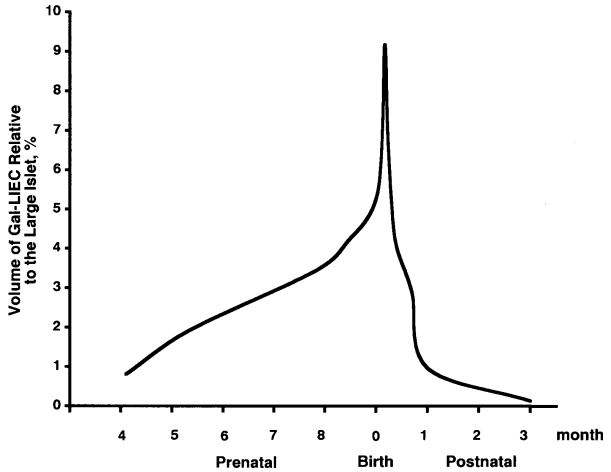

Fig. 1. Developmental profile of galanin-like immunoreactive endocrine cells (Gal-LIEC) in the large islets of the bovine pancreas.

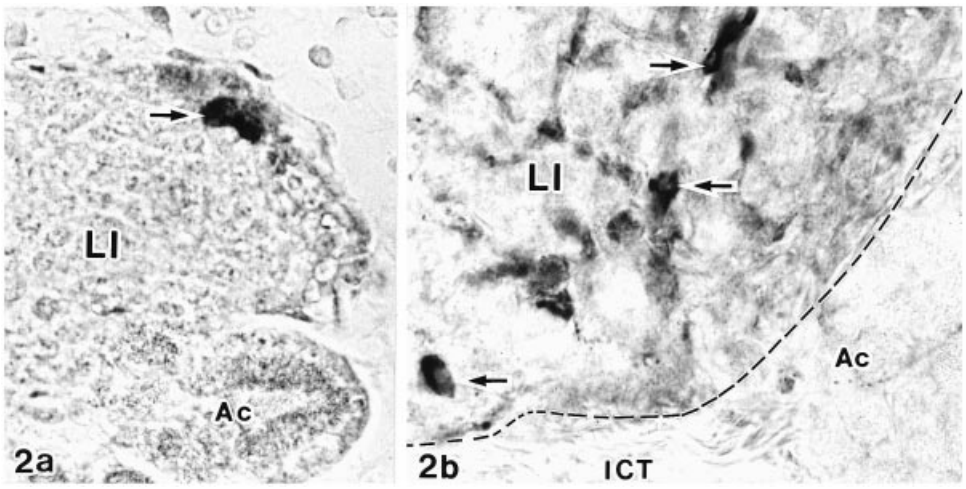

Fig. 2. Galanin-like immunoreactive endocrine cells (arrows) in the large islet (LI) of the pancreas of bovine fetus. (*a*) CRL 17 cm. First detection in a 3-mo-old fetus. ×490. (*b*) CRL 80 cm, 8-mo-old fetus. ×380. Ac, acini; ICT, interlobular connective tissue. Broken line outlines the boundary of the large islet.

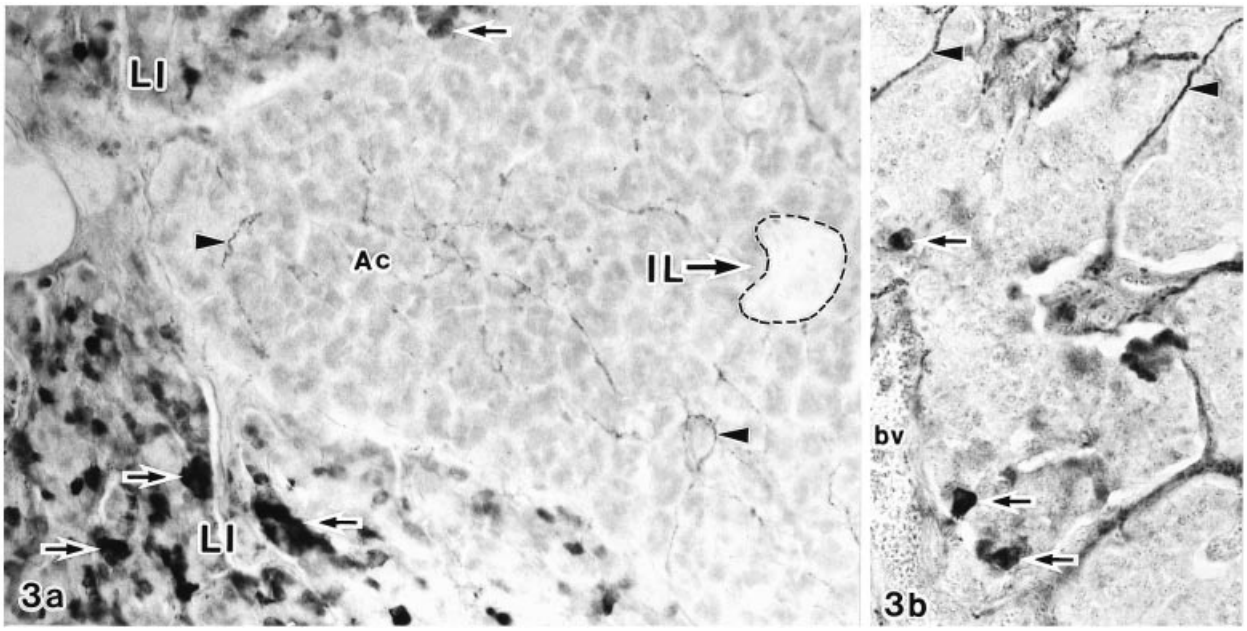

Fig. 3. Galanin-like immunoreactive endocrine cells, (Gal-LIEC) (arrows) and nerve fibres (arrowheads) in the calf pancreas. (*a*) 5-d-old neonate. The Gal-LIEC are present in the large islet (LI) but not in the islet of Langerhans (IL). ×75. (*b*) 1-mo-old calf. An area of a large islet containing Gal-LIEC. ×235. Ac, acini; bv, blood vessel.

The volume of Gal-LIEC relative to the large islets at various stages of bovine development is shown in the Table. The developmental profile of Gal-LIEC in the large islets (Fig. 1) shows that their relative volume increased from ~1% to ~4% as fetal age advances from 4 mo to 2 wk before term. Shortly before birth, the Gal-LIEC level increased considerably until a peak, ~9%, was reached at the fifth neonatal day (Figs 1, 2*b*, 3*a*). A subsequent decline followed down to ~1% at 1 mo postnatal (Figs 1, 3*b*). The Gal-LIEC level continued to decrease until it was negligible during calf maturation, and undetectable in the adult.

Colocalisation of galanin and insulin in the large islet

The large islets contained predominantly insulin-like immunoreactive endocrine cells. Some of these cells also demonstrated galanin-like immunoreactivity

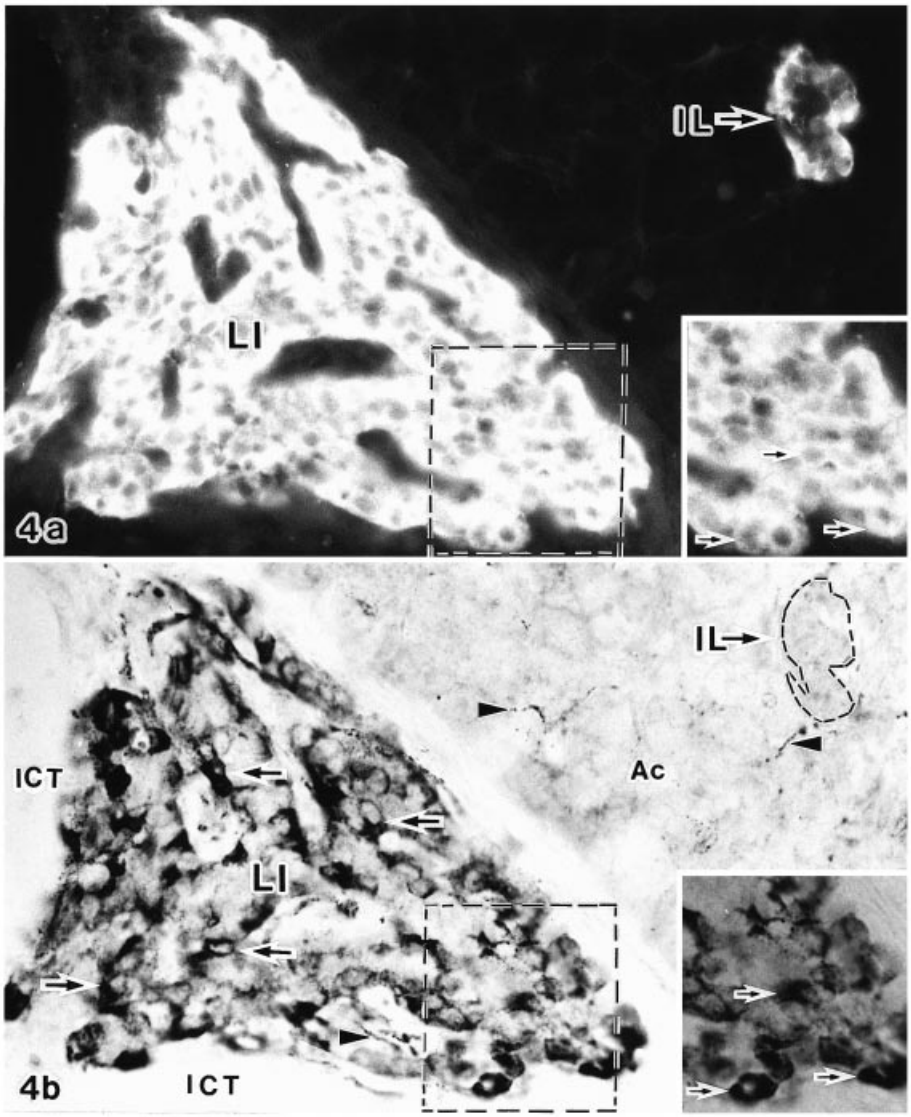

Fig. 4. Colocalisation of insulin- and galanin-like immunoreactivities. (*a*) A section stained for insulin using immunofluorescence, shows insulin in both the large islets (LI) and islets of Langerhans (IL). (*b*) The same section immunostained for galanin using ABC method after elution of the insulin antibody shows Gal-LIEC (large arrows) in the large islet (LI) but not in the islet of Langerhans (IL). Galanin-like immunoreactive nerve fibres (arrowheads) are also shown. (*a*, *b*) ×315. Ac, acini; ICT, interlobular connective tissue. Insets show higher magnification of the area enclosed in boxes by broken lines indicating some endocrine cells (small arrows) immunoreactive for both insulin and galanin. ×380.

(Fig. 4*a*, *b*). All the Gal-LIEC, however, were found to be immunoreactive for insulin.

DISCUSSION

The present study describes galanin-like immunoreactivity in the bovine pancreas. Galanin was demonstrated immunohistochemically using porcine galanin antiserum, the same type of antibody employed in the aforementioned other species. The high homology between the amino acid sequences of porcine and bovine galanin indicates conservation of structure and possibly function (Vrontakis et al. 1991). They only differ in the 16th, 18th, 23rd and 26th amino acid components (Rökaeus, 1987; Rökaeus & Carlquist, 1988).

The immunolocalisation of galanin in the endocrine cells, apart from their association with the neural elements, in the pancreas of bovine fetuses and neonates is a striking difference from the results reported in the adult pig (Messell et al. 1990), dog (Dunning et al. 1986), and human (Shimosegawa et al. 1992), in which an absence of galanin immunoreactivity in the endocrine cells has been reported. Whether this discrepancy is attributed to developmental stage-related variation or species difference remains to be clarified.

The most remarkable finding is the exclusive localisation of Gal-LIEC in the large islets, and their nonappearance in the small islets, which are the classical islets of Langerhans. In the large islets, the Gal-LIEC were first detected at the third prenatal month, 1 mo after the reported first appearance of the large islets in the pancreas (Bonner-Weir & Like, 1980). The developmental profile of Gal-LIEC indicates that their relative volume in the large islets increases gradually as the fetal age advances to about 2 wk before birth. Shortly before parturition, the Gal-LIEC level increases remarkably until a peak is reached at fifth neonatal day and then eventually drops. The sharp increase in the Gal-LIEC level may be associated with the metabolic alterations attributed to the adaptation from intra to extrauterine life, whereas the decline may be related to the changes in metabolism during the digestive system shift from monogastric to functional rumen. With the developmental trend of Gal-LIEC, it may be speculated that galanin in the large islets exerts its most dramatic effect during the perinatal development of cattle.

Previous physiological studies have shown the regulatory role of galanin on insulin release in several mammalian species (McDonald et al. 1985; Dunning et al. 1986; Lindskog & Ahrén, 1987; Lindskog & Ahrén, 1989; Messell et al. 1990; Ahrén et al. 1991; Rünzi et al. 1992). The possible role of pancreatic Gal-LIEC may, therefore, be related to the insulin cells in the large islets of the bovine pancreas. To shed more light on this, it may be worth considering the other conspicuous morphological changes taking place in the pancreas particularly during the perinatal period. A marked degranulation of B-cells in the small islets, the islets of Langerhans (Bonner-Weir & Like, 1980), has been observed precisely at the time when Gal-LIEC were at the highest level. The B-cells in the large islet, on the other hand, remain well-granulated (Bonner-Weir & Like, 1980). The reason for the disparity in the degree of granularity of the B-cells between the 2 types of islet population is unknown. However, galanin could have a role in maintaining the granularity of the B-cells in the large islets.

Galanin as an endocrine constituent in the large islets may regulate insulin secretion via hormonal and/or paracrine and/or autocrine mechanisms. The colocalisation of galanin and insulin provides a morphological basis for autocrine interaction between the 2 hormones. Likewise, the presence of cytoplasmic elongations of Gal-LIEC and their close contiguity with insulin cells may suggest a paracrine mode of action of galanin on insulin release in the large islets.

In conclusion, galanin appears to be a peptide that characterises the large islets of the pancreas during the perinatal development of cattle.

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