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Galanin

Savio G. Barreto

Department of Gastrointestinal Surgery, Gastrointestinal Oncology, and Bariatric Surgery

Medanta Institute of Digestive and Hepatobiliary Sciences, Medanta, The Medicity Gurgaon, India

e-mail: georgebarreto@yahoo.com

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1. General Information

Galanin was isolated in 1983 from porcine intestine by Tatemoto *et al*. (89) using a chemical method designed for the detection of C-terminal amidated peptides. The isolated peptide was named galanin because of its N-terminal glycine and C-terminal alanine residue. Following this, galanin was isolated from other species, including humans (27, 66, 74, 83, 90). It is a neuropeptide that does not belong to any other family of neuropeptides (16). Galanin is involved in the regulation of a multitude of physiological conditions, ranging from central nervous system functions like cognition and memory, sensation of pain, feeding behaviour, and sexual behavior to endocrine functions such as influencing the release of insulin, acetylcholine, norepinephrine, glutamate, dopamine, growth hormone and prolactin, and finally also acting on gastrointestinal motility and secretion (47).

Structure of Galanin

Galanin is a 29 amino acid, C-terminally amidated peptide. The 29 amino acid structure is common to most species, except humans. In humans, galanin exists as a 30 amino acid molecule with no amidation at the C-terminus (27). While there is an absolute conservation of the N-terminal 1-15 amino acids in all species, the inter species variation occurs in the form of amino acid substitutions in the C-terminal portion of the molecule. Interestingly, it is the N terminal portion of galanin that is involved in the receptor-ligand interactions. This mechanism was elucidated based on the finding of extensive cross-recognition of galanin molecules by receptors from several species as well as ligand-binding studies using ^{125}I-monoiodo-Tyr^{26}-porcine galanin (43) or ^{125}I-monoiodo-Tyr^{9}-rat galanin (7) and labelling and autoradiographic studies using porcine, rat or human galanin (30, 43, 49, 63).

Galanin is proteolytically processed from a 123-(porcine, human) or 124-(murine and others) amino acid precursor pro-peptide, “preprogalanin” encoded by a single-copy gene organized into 6 small exons spanning about 6kb of genomic DNA, depending on the species) (48) along with a 59 or 60 amino acid peptide known as galanin message-associated peptide (GMAP) (27, 45, 73, 90). (**Figure 1)**

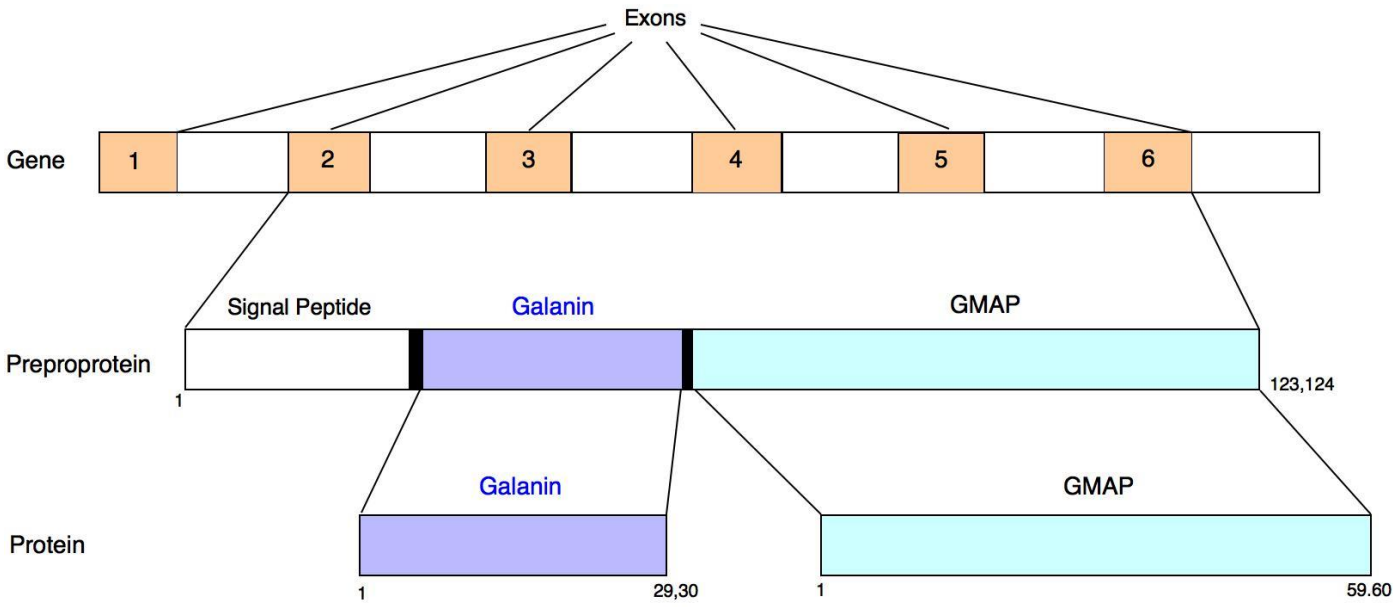

Figure 1. Schematic representation of the mammalian galanin gene and proteins. Galanin from most species is 29 aa long with an amidated C terminal derived from Glycine. Human galaline has a Ser residue in position 30 and is not amidated. Whether there is a physiological role for GMAP or a portion of it is unknown.

Galanin Localization

Galanin is distributed throughout the central and peripheral nervous systems as well as in the gastrointestinal tract where it is present in the myenteric, mucous and submucous plexuses (72). In the spinal cord, galanin has been detected in large amounts in the dorsal horn cells, nerve cell bodies intrinsic to the spinal cord, including neurons of lamina II, intermediate dorsal laminae, neurons around lamina X, and in subpopulations of lower motor neurons (72). In the gastrointestinal tract it influences smooth muscle contraction (75).

Based on tissue-specific expression studies in the rat, highest concentrations of galanin messenger ribonucleic acid (mRNA) were found in the hypothalamus in the central nervous system and in the duodenum in the gastrointestinal tract (45).

Galanin Receptors

To date, there have been 3 major galanin receptors described: galanin receptor 1 (GALR1), galanin receptor 2 (GALR2), and galanin receptor 3 (GALR3), and a single putative receptor (galanin receptor-like or GALRL). The major receptors are members of the G-protein coupled receptor (GPCR) superfamily and they possess substantial differences in their functional coupling and subsequent signalling activities (77).

GALR1, the first functional galanin receptor, was cloned from human Bowes melanoma cells (35). Activation of GALR1 leads to the opening of G-protein-regulated inwardly rectifying K^+ channels (GIRK) (87) and stimulation of mitogen-activated protein kinase (MAPK) activity in a protein kinase C (PKC)-independent fashion (51).

Subsequent work by Howard *et al*. (41), Smith *et al*. (86), and Wang *et al*. (92) led to the discovery of GALR2 and GALR3. The stimulation of GALR2 potentially leads to the downstream activation of a multitude of intracellular pathways. The most common pathway involved is that of phospholipase C (PLC) activation (51) which leads to an increase in inositol phosphate hydrolysis mediating the release of Ca^{2+} into the cytoplasm from intracellular stores (51) and also the opening of Ca^{2+} dependent chloride channels (19, 28, 68, 86, 91). In experiments using Xenopus oocytes, Smith *et al*. (87) demonstrated that cloned GALR3 appears to couple to a $G_{i/o}$-type G-protein to stimulate a PTX-sensitive activation of an inward K^+ current

especially when co-expressed with GIRK1 and GIRK4. GALRL was discovered in 2004 by Ignatov *et al*. (44).

Galanin receptors are distributed all over the body but predominantly in the central and peripheral nervous systems (21, 32, 50, 52, 62, 63, 71, 79, 85), the spinal cord, and the gastrointestinal tract (17, 26, 29, 42, 62, 93). Other sites of galanin receptor location include the pancreas (5, 24, 31), thyroid (34), the iris (88), genitourinary tract (69, 70), and in the autonomic ganglia (4, 54).

2. Galanin and the Pancreas

Galanin Localization in the Pancreas

The location of galanin immunoreactivity in the pancreas has been studied in various animal species including canine (24), murine (56), Australian possum (20), chicken (38), porcine (64), and bovine (65) which show a wide variation in distribution and amount of galanin immunoreactivity between species. In the human pancreas, galanin immunoreactive nerves have been shown to be chiefly localized to the exocrine portion with only irregular innervation of the islets (82), and it is also present in nerve fibres and cell bodies in the intrapancreatic ganglia (2). Li *et al*. (53) also demonstrated the expression of the three galanin receptors in the normal mouse pancreas.

Barreto *et al*. (10) conducted *In situ* hybridization experiments on mouse pancreas using DIG-labeled probes and a protocol based in part on that described by Nuovo *et al*. (67) with an aim to localise galanin receptors. Mouse dorsal root ganglia were used as a positive control for GALR1 and GALR2 which produced positive signal. For the first time, they were able to demonstrate hybridization signals representing GALR1 and GALR2 in the islet cells while that for GALR3 was present in islets and acinar cells. These findings were confirmed on Western Blot analysis.

Galanin's Effects on the Exocrine Pancreas

The earliest work on the effect of galanin on amylase secretion was performed by Ahren *et al*. (1) on isolated Wistar rat pancreatic acini. The effects of galanin on the exocrine pancreas previously reported in literature lacked consistency - a likely result of the complexity of the systems used for such studies, viz. both in vivo and in vitro (isolated acinar cell or lobule preparations or isolated perfused pancreata). The reported effects on basal and stimulated (Carbachol or cholecystokinin (CCK), CCK-8, bethanechol, 2-deoxyglucose, veratridine, bomebsin, secretin and / or potassium chloride) secretion varied from inhibitory (1, 31, 37, 46, 76, 78, 94), in some models, to stimulatory (78) in other. Still other scientists reported no effect (1, 31, 46). These effects of galanin on pancreatic amylase secretion are summarized in **Table 1**. Galanin is also a potent inhibitor of pancreatic bicarbonate secretion (36).

Bhandari *et al*. (18), focussed on the effect of galanin on amylase and protein secretion *in vivo* in the Australian possum and found that while galanin actually increased pancreatic juice secretion rate, it reduced the amylase and protein output.

Based on these initial *in vivo* and *in vitro* effects, it was postulated that galanin acted not by direct action on the acinar cells but via neural elements most likely mediated by the cholinergic system (31, 37).

More recently, the effects of galanin on basal and stimulated exocrine secretion were studied by Barreto *et al*. (8, 13, 15) using a pancreatic fragment / lobule preparation (9). They demonstrated that galanin inhibited caerulein- and glucose-stimulated, but not basal (10) or carbachol-stimulated amylase secretion from isolated mouse pancreatic lobules. The caerulein stimulation of amylase secretion was blocked by atropine and tetrodotoxin implying mediation by cholinergic neurones.

Table 1: Experimental evidence for the action of galanin on pancreatic amylase secretion

Author (year) (Ref #)	System	Stimulator	Effect of galanin
Ahren *et al*. (1988) (1)	Isolated acinar cells	Carbachol	~18% inhibition
		CCK	~36% inhibition
Yagci *et al*. (1991) (94)	*In vivo*	Bethanechol	None
		2-deoxyglucose	Significant inhibition
		CCK	Significant inhibition
Runzi *et al.* (1992) (78)	Isolated perfused pancreas	CCK-8	Significant stimulation
Rossowski *et al.* (1993) (76)	*In vivo*	CCK-8	Significant inhibition
Kashimura *et al.* (1994) (46)	Isolated acinar cells	Carbachol	Significant inhibition
Herzig *et al.* (1993) (37)	*In vivo*	2-deoxyglucose	Almost complete inhibition
	Isolated acinar cells	Carbachol	None
		CCK-8	None
	Isolated lobules	Veratridine	Significant inhibition
Flowe *et al.* (1992) (31)	*In vivo*	2-deoxyglucose	Significant inhibition
		CCK-8	Significant inhibition
		Betanechol	None
	Isolated acinar cells	Betanechol	None
		CCK-8	None
	Isolated lobules	KCl	Significant inhibition
		Veratridine	Significant inhibition

Abreviations used are CCK, cholecystokinin; CCK-8, cholecystokinin octapeptide; KCl, potassium chloride

The caerulein response was also blocked by diazoxide which indicates dependence on insulin secretion. These data demonstrated for the first time that caerulein-stimulated amylase secretion in lobules involves a combined neural-paracrine mechanism. Galanin inhibited the caerulein-stimulated amylase secretion acting on the cholinergic nerves and/or insulin secretory cells. Galanin also inhibited glucose-stimulated amylase secretion. On balance, these data suggested that galanin may modulate caerulein-stimulated amylase secretion by acting on cholinergic nerves and/or islet cells possibly via GALR2 to regulate insulin release (15) (**Figure 2**). Barreto *et al*. (13) went on to study the effect of galanin on supramaximal caerulein-stimulated amylase secretion (in an attempt to understand the role of galanin in the development of acute pancreatitis due to hyperstimulation).

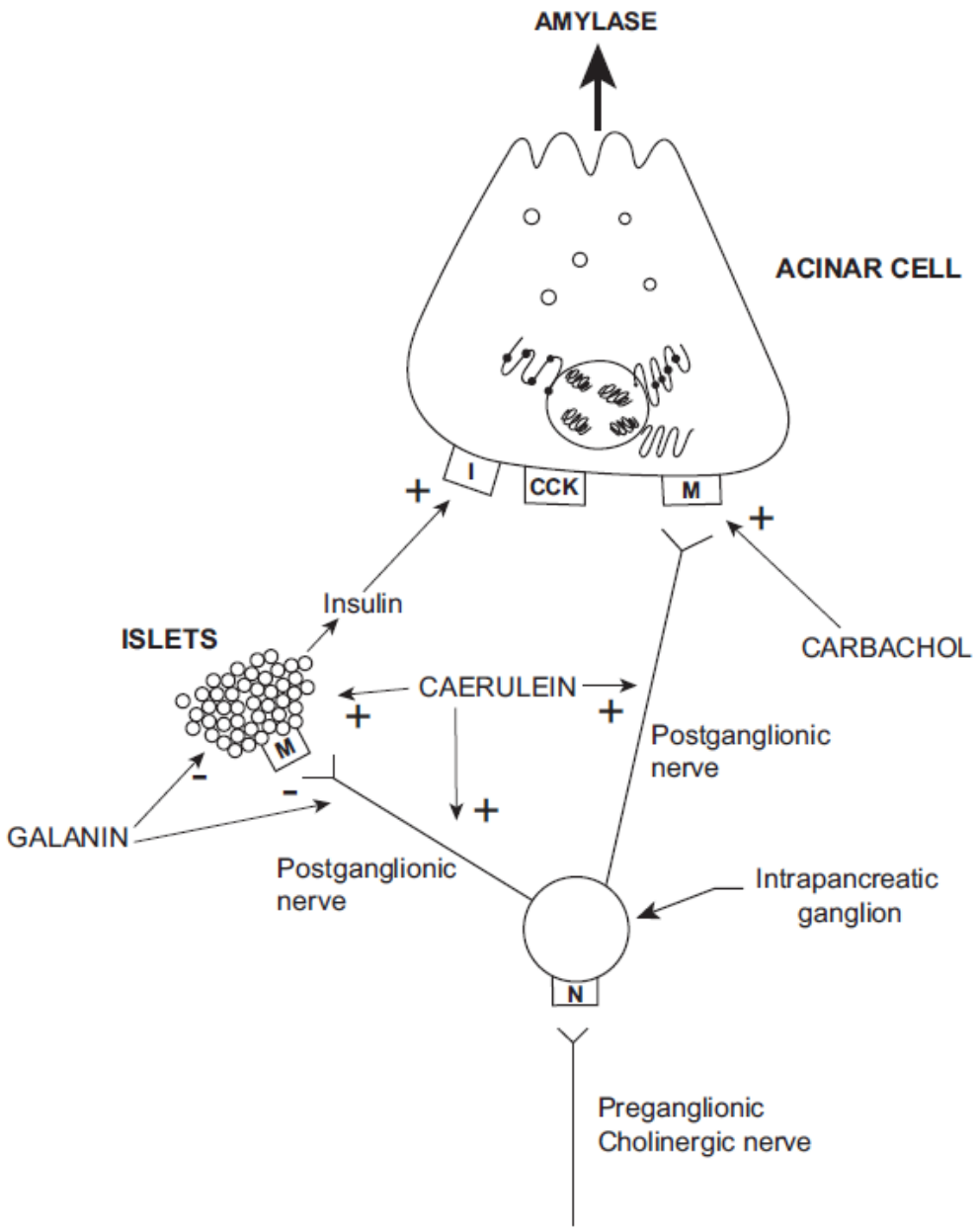

Figure 2: Diagrammatic representation of postulated mechanism of galanin of inhibition of caerulein-stimulated amylase secretion via insulin secretion. From Ref (15). Abreviations: I, insulin receptor; N, nicotinic receptor; M, muscarinic receptor; +, stimulation; -, inhibition. Reproduced from **(15).**

They demonstrated for the first time that exogenous galanin potentiated supramaximal caerulein-stimulated amylase secretion from murine pancreatic lobules. Pre-treatment with atropine and tetrodotoxin caused a partial inhibition of the amylase secretion induced by supramaximal concentration of caerulein, indicating both neural and non-neural components to this effect. The amylase secretion induced by supramaximal caerulein was abolished by pre-treatment with diazoxide implying mediation by insulin. Galanin's potentiating effect was abolished by pre-incubation of lobules with a combination of atropine and diazoxide supporting muscarinic receptor and insulin mediation.

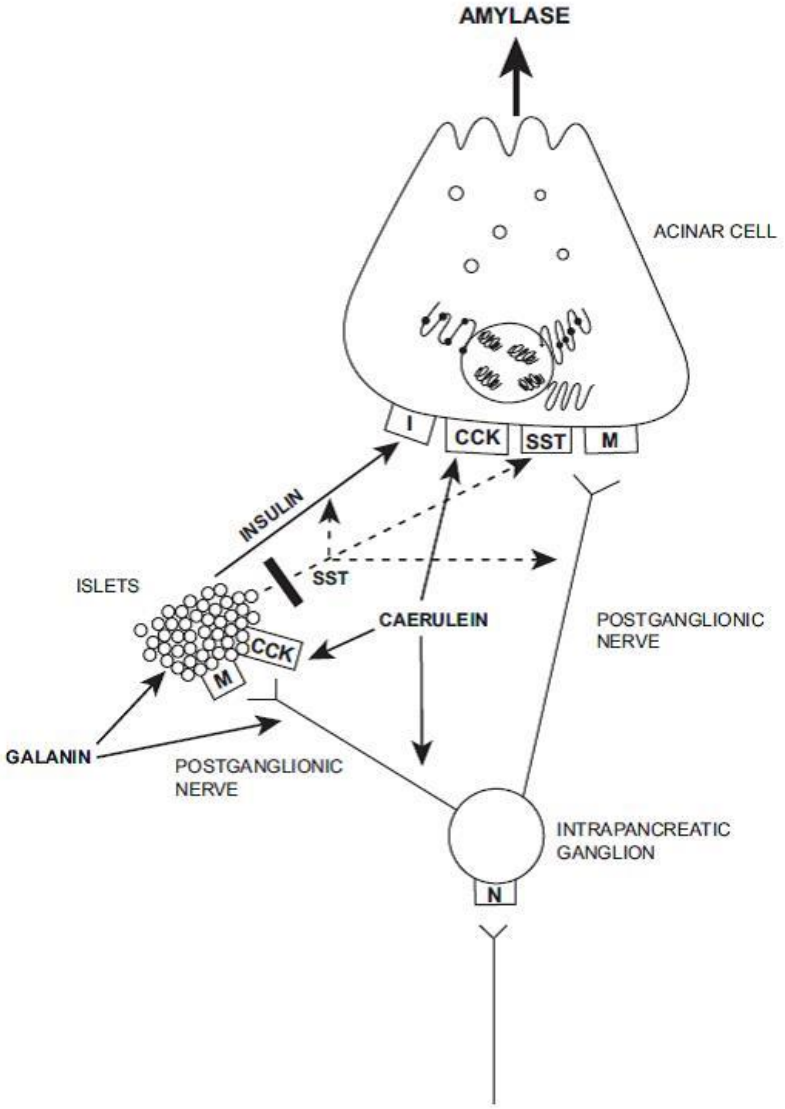

Figure 3: Diagrammatic representation of mechanism by which galanin (GL) potentiates supramaximal Caerulein (Caer)-stimulated amylase secretion. Caer acts directly on cholecystokinin receptors (CCK) on acinar cells and also on postganglionic cholinergic nerves to induce insulin release via activation of muscarinic receptors (M) on the islets. The effect of Caer at this concentration appears to be completely dependent on a potentiating effect of the released insulin acting on insulin receptors (I) located on the acinar cells. Caer also acts on CCK-B receptors on islet (δ) cells to evoke the release of somatostatin (SST), which then exerts an overall inhibitory effect by virtue of its actions on insulin release, SST receptors on acinar cells, and on nerves (broken lines), resulting in a reduction of the amylase secretion evoked by Caer. The black bar indicates blockade of SST secretion. GL acts via GL receptors (not shown) on SST-containing islet cells and/or on postganglionic cholinergic nerves to inhibit the Caer-induced secretion of SST. N, nicotinic receptor (13).

Treatment with Galantide, the non specific receptor antagonist of galanin, resulted in an inhibition of galanin's potentiation indicating the involvement of galanin receptors. Moreover, administration of octreotide also inhibited the galanin effect suggesting a role for somatostatin. In support of this, the galanin potentiation was mimicked by pretreatment with CAHP (cyclo-(7-aminoheptanoyl-Phe-D-Trp-Lys-Thr-[BZL]) - a somatostatin antagonist) and CAHP treatment failed to modify the galanin potentiation, suggesting that galanin probably acts by inhibiting

the release of somatostatin caused by supramaximal caerulein. On balance, these data suggested that at supramaximal caerulein

concentrations, galanin acts via its receptors to further increase caerulein-stimulated amylase secretion by inhibiting the caerulein-induced release of somatostatin (13). The lack of a direct effect on galanin on the acinar cells was confirmed in further experiments (10) **(Figure 3)**.

Galanin's Effects on the Endocrine Pancreas

An important action of galanin on the pancreatic endocrine system is the inhibition of insulin secretion (24). McDonald *et al*. (61) found that intravenous administration of galanin to fasted conscious dogs produced a dose-dependent hyperglycaemia accompanied by decreases in plasma insulin levels, but with no elevation of plasma glucagon levels which they attributed to a galanin-mediated reversible inhibition of insulin secretion. The inhibitory effect of galanin and the resultant hyperglycemia was reported by several other investigators in various animal species (55, 57, 59, 84) though not in human studies (33, 39).

Ahren *et al*. (3) based on *in vitro* study of pancreatic islets from obese hyperglycaemic mice showed that galanin reduced insulin secretion by a direct effect on the beta cells leading to a mild hyperpolarisation with a subsequent decrease in cytoplasmic free Ca^{2+}. Amiranoff *et al*. (6) based on their experiment of galanin on the insulin-secreting β-cell line Rin-m5f with or without overnight incubation with PTX concluded that galanin inhibited insulin release through the inhibition of adenylate cyclase involving a PTX-sensitive inhibitory GTP-binding regulatory protein.

Dunning *et al*. (25) suggested that galanin was the likely noradrenergic neurotransmitter involved in the effects of sympathetic neural activation on basal pancreatic hormone secretion.

The various mechanisms by which galanin inhibits insulin secretion have been summarised by Sharp (80):

1) Galanin decreases β cell electrical activity (23). Galanin increases the activity of the K_{ATP} channel, hyperpolarizes the membrane, and thus inhibits the action of secretagogues which depolarize the β cell membrane (22, 23).
2) Galanin directly inhibits the dihydropyridine-sensitive voltage-dependent L-type channel (40). The inhibition was concentration dependent.
3) Galanin inhibits the activity of adenylyl cyclase and reduces c AMP levels (6, 56, 60)
4) Galanin may also exert its inhibitory effect on insulin secretion at a very late stage in the stimulus secretion coupling by the inhibition of exocytosis (81).

Galanin's Effects on Pancreatic Vascular Perfusion

Based on studies conducted on the anaesthetized Australian possum, it was found that galanin acutely reduced pancreatic vascular perfusion (20). This effect of galanin on pancreatic vascular perfusion was shown to play a role even in the development of acute pancreatitis (18).

Barreto *et al*. carried their findings noted using pancreatic lobules *in vitro* (10, 13, 15) to the *in vivo* caerulein model of acute pancreatitis in mice. In a series of experiments where they combined the non-specific receptor antagonist of galanin (galantide) with the substance P receptor (neurokinin-1 receptor) antagonist L703,606 (12), a salivary tripeptide (feG) (11) and octreotide (14), they found that galanin may be involved in the pathogenesis of acute pancreatitis by more than one mechanism (8), viz. potentiating the damaging effects of CCK on the acinar cell, aggravating microvascular instability by influencing pancreatic vascular perfusion, involvement in neurogenic inflammation, affecting somatostatin secretion and

leucocyte adhesion to capillary endothelium, leucocyte rolling and occlusion. They were also able to show that a GALR3 antagonist (SNAP-37889) was equally effective in ameliorating experimentally-induced acute pancreatitis as the non-specific GALR antagonist (Galantide) (10).

3. Tools to Study Galanin

a. Synthetic Peptide

Galanin (porcine & human) can be obtained from multiple sources including American Peptide (Sunnyvale, CA, USA), Sigma Aldrich and Abbiotec.

b. Antibodies

Antibodies against galanin receptors although commercially available, are not reliable (58) which limits the usefulness of immunohistochemistry as a technique to localise the receptors in the pancreas.

c. *In-situ* hybridisation

(DIG)-labeled nucleic acid probes (GALR1, GALR2, GALR3) from Exiqon (Vedbaek, Denmark)

d. Antagonists

Peptide antagonists against the galanin receptor are available. Galantide (non specific GALR antagonist) from Bachem (Bubendorf, Switzerland), M871 (GALR2 antagonist) from UE Sollenberg, (Stockholm University, Stockholm, Sweden) and SNAP-37889 (GALR3 antagonist) from Prof. LA Blackshaw (University of Adelaide, Adelaide South Australia)

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