Galanin—Sympathetic Neurotransmitter in Endocrine Pancreas?

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The effects of sympathetic neural activation on basal pancreatic hormone secretion cannot be explained solely by the actions of the classic sympathetic neurotransmitter norepinephrine. The nonadrenergic component may be mediated by the 29-amino acid peptide galanin in that this neuropeptide meets several of the criteria necessary to be considered a sympathetic neurotransmitter in the endocrine pancreas. 1) Galanin administration inhibits basal insulin and somatostatin secretion and stimulates basal glucagon secretion from the pancreas, qualitatively reproducing the effects of sympathetic nerve stimulation. These sympathomimetic effects appear to be mediated by direct actions of galanin on the islet. 2) Galanin-like immunoreactivity exists in fibers that innervate pancreatic islets. 3) Galanin is released during electrical stimulation of pancreatic nerves. The quantity released is sufficient to reproduce sympathetic nerve stimulation-induced effects on insulin secretion and to contribute to the neural effects on somatostatin and glucagon release. 4) Whether interference with galanin action or release reduces the islet response to sympathetic nerve stimulation remains to be determined. We hypothesize that galanin and norepinephrine act together to mediate the islet response to sympathetic neural activation. If galanin is a sympathetic neurotransmitter in the endocrine pancreas, it may contribute to the inhibition of insulin secretion that occurs during stress and thereby to the hyperglycemic response. Moreover, the local presence of this potent β-cell inhibitor in the islet leads to speculation on galanin’s contribution to the impairment of insulin secretion that occurs in non-insulin-dependent diabetes mellitus and therefore on the potential utility of a galanin antagonist in the treatment of this disease.

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Scores of neuropeptides have been discovered over the past 30 years, and many of them can influence metabolism through actions on the endocrine pancreas under certain experimental conditions. However, knowledge of a peptide’s effects on insulin or glucagon secretion is by itself of limited use in understanding the physiologic or pathophysiologic role of that peptide, because the ability of an exogenous peptide to alter islet hormone secretion does not necessarily reflect a physiologic function in the pancreas. For this reason it may be more useful to begin by inquiring what functions cannot be explained by the actions of previously known regulators and therefore what functions might be subserved by an endogenous neuropeptide. We consider one example.

Systemic stress or direct electrical stimulation of the sympathetic nerves to the pancreas inhibits insulin and stimulates glucagon release. Such changes of pancreatic hormone secretion are thought to serve an important role to defend blood glucose levels under conditions of increased demand or decreased availability of metabolic fuels. Although the classic sympathetic neurotransmitter norepinephrine is usually assumed to mediate sympathetic neural influences on islet function, direct tests of this assumption have produced some surprising results. For example, direct pancreatic arterial infusion of norepinephrine, in a wide range of doses, failed to reproduce the inhibition of basal insulin release seen during sympathetic nerve stimulation in anesthetized dogs (1), although the infusion could inhibit arginine-stimulated insulin release. Furthermore, moderate doses of norepinephrine did stimulate basal glucagon secretion, although to a lesser extent than did nerve stimulation. A second, independent approach revealed that combined adrenergic blockade had little effect on sympathetic nerve stimulation-induced changes of basal pancreatic hormone release (2). These studies suggested that sympathetic neural regulation of basal islet hormone secretion was a function in which neuropeptides may serve an important role. One recently discovered neuropeptide, galanin, stands out

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as a particularly good candidate to mediate nonadrenergic neural influences on the islet.

Galanin is a 29-amino acid peptide with a C-terminal amide characteristic of many biologically active peptides. Galanin was originally isolated from porcine intestinal extracts by virtue of its C-terminal amide and was so named for its N-terminal glycine and C-terminal alanine residues (3). Galanin has little homology with other known peptides, but like other peptides it is synthesized as a large-molecular-weight precursor and then enzymatically cleaved to the 29-amino acid form. A 60-amino acid galanin message-associated peptide (GMAP) neighbors the galanin sequence (4). Because GMAP (as well as galanin) appears to be highly conserved across species, it is thought to have as yet unknown biologic functions (5). The first effects of galanin were reported in 1983; galanin was found to contract smooth muscle preparations from the rat and produce hyperglycemia in conscious dogs (3). The hyperglycemic effect of galanin led to investigation of its influence on the endocrine pancreas, and galanin was found to markedly reduce peripheral insulin levels in response to parenteral glucose in conscious dogs (6). This action of galanin was reminiscent of stress and spurred our interest in this peptide as a possible mediator of nonadrenergic sympathetic neural influences on the endocrine pancreas.

Historically, proof that a substance had a physiologic role as a neurotransmitter required a demonstration that it fulfilled a set of criteria. We have borrowed from the tenets of classic neurobiology and modified the criteria to be specific for a proposed function in the pancreas (Fig. 1). To be considered a sympathetic neurotransmitter in the endocrine pancreas, galanin must 1) have direct actions on islet function that mimic the effect of sympathetic neural activation, 2) be localized in islet sympathetic nerves, and 3) be released by sympathetic neural activation in quantities sufficient to evoke an islet response. In addition, interference with the release or actions of galanin must alter the islet response to neural activation (Fig. 1).

**DOES EXOGENOUS GALANIN IMITIC EFFECTS OF SYMPATHETIC NEURAL ACTIVATION THROUGH DIRECT ACTIONS ON ISLETS?**

Early reports that systemic administration of galanin produced hyperglycemia and prevented the expected increase of insulin levels during endogenous or exogenous hyperglycemia were consistent with an effect of this peptide to inhibit insulin secretion (3,6). Further tests of porcine galanin in anesthetized dogs with the use of sampling of the pancreatic venous effluent demonstrated marked inhibition of basal insulin output, modest stimulation of basal glucagon output, and significant suppression of pancreatic somatostatin output (7), effects qualitatively identical to those produced by electrical activation of the local sympathetic nerves to the pancreas (18). Galanin has since been reported to inhibit basal insulin secretion and the acute insulin response to both glucose and nonglucose secretagogues in vivo and/or in vitro in rats, mice, and dogs (9–15). The potency of galanin, the consistency of the observed effects, and the ability of the porcine form of galanin to inhibit insulin release in all species examined (i.e., the ability to cross species boundaries) set this peptide apart from other possible candidates. For example, the effects of neuropeptide Y (NPY) and calcitonin gene-related peptide (CGRP) on islet function seem to vary with species and experimental system (16–19).

The evidence that galanin's inhibitory influence on insulin secretion is mediated by a direct action of this peptide on the β-cell is substantial. Mediation by an extrapancreatic effect of galanin was ruled out by demonstration that galanin, given directly into the pancreatic circulation in vivo (at a systemically ineffective dose), markedly inhibits insulin output (7) and that galanin inhibits insulin release from the isolated perfused pancreas in vitro (15). Galanin does not appear to inhibit insulin release by influencing local pancreatic neural activity, because galanin administration does not change pancreatic venous norepinephrine levels in vivo (7) and because neither adrenergic nor cholinergic blockade prevents galanin-induced inhibition of insulin secretion in the perfused dog pancreas in vitro (15). Galanin also inhibits insulin release from isolated mouse islets (13) and

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**FIG. 1. Schema of 4 criteria for acceptance of galanin as sympathetic neurotransmitter in endocrine pancreas.**

1. Galanin must mimic the effects of sympathetic neural activation through direct action on the islet.

2. Galanin must be localized in the sympathetic nerves of the islets.

3. Galanin must be released during neural activation in quantities sufficient to evoke an islet response.

4. Interference with galanin action or release must alter the pancreatic response to neural activation.
from monolayer cultures of neonatal rat pancreatic cells (10), strongly supporting a direct effect. Finally, the demonstration of specific receptors for galanin on membranes from a hamster β-cell tumor (20) and the recent report that galanin activates ATP-sensitive K+ channels in an insulin-secreting rat islet cell line (21) provide information regarding the cellular mechanisms by which galanin directly inhibits insulin release. Thus, all available evidence supports the conclusion that the sympathomimetic effect of galanin to inhibit insulin secretion reflects its direct action on the β-cell.

In contrast to galanin’s consistent effects on insulin secretion, observations of this peptide’s influence on glucagon secretion have been more varied. We find that galanin modestly stimulates pancreatic glucagon output when administered either systemically or locally into the pancreatic artery in vivo in dogs (7); a similar moderate increase of both basal and stimulated glucagon levels in response to systemic galanin was found in mice (13). However, galanin has also been reported to have no effect on peripheral glucagon levels in conscious dogs (6) and no effect on glucagon release from the perfused rat pancreas in vitro (11). Finally, although moderate doses of galanin had little effect on glucagon secretion from the isolated perfused dog pancreas, high doses elicited an inhibitory effect (15).

Although it is difficult to reconcile the divergent reports of the effects of galanin on glucagon secretion, clarification may be provided by considering galanin’s effect on pancreatic somatostatin secretion. Galanin markedly inhibits pancreatic somatostatin release from the dog pancreas in vivo (7), as does sympathetic nerve stimulation (8). A similar degree of somatostatin suppression has been suggested to remove a paracrine restraint on the α-cell, resulting in increased glucagon secretion (22). The inhibitory tone of local somatostatin on the α-cell is reflected by pancreatic somatostatin output; it appears high in dogs in vivo and substantially lower in vitro in both the isolated dog and rat pancreas. Thus, it is reasonable to suggest that a net stimulatory effect of galanin occurs when the pancreatic somatostatin tone is initially high and is then markedly reduced by galanin administration. Conversely, in experimental preparations in which pancreatic somatostatin secretion is low, e.g., perfused pancreas and isolated islets, there is little paracrine restraint to remove, and a weak direct inhibitory effect on the α-cell becomes apparent when high doses of galanin are administered. This interpretation is consistent with our findings that galanin stimulates glucagon release in vivo (7) but inhibits glucagon release from monolayer cultures of rat pancreatic cells (unpublished observations). Additionally, galanin-induced inhibition of insulin secretion could also contribute indirectly to the stimulation of glucagon release observed in vivo by reducing putative local endocrine restraint of the α-cell by insulin (23).

Because of galanin’s apparently variable effects on glucagon secretion, its potential contribution to the α-cell response to sympathetic nerve stimulation is difficult to estimate. However, because galanin does increase glucagon output in vivo, it may augment norepinephrine’s direct stimulatory effect on glucagon secretion, possibly via its action to suppress pancreatic somatostatin and insulin secretion.

**IS GALANIN LOCALIZED IN ISLET SYMPATHETIC NERVES?**

Galanin-like immunoreactivity has been detected in extracts of pancreatic tissue from pigs, rats (24), mice (25), humans (S.M. Gabriel, unpublished observations), dogs, and rabbits (unpublished observations). The galanin-like immunoreactivity in the dog pancreas dilutes in parallel with synthetic porcine standards in radioimmunoassay and elutes from Sephadex G-50 in a position very similar to that of synthetic porcine galanin, suggesting there is significant similarity between the canine and porcine forms of the molecule (30). However, the levels of galanin in pancreatic extracts are much lower than those in intestinal or hypothalamic extracts (26). The low pancreatic content of galanin may lead to an erroneous assumption that this peptide cannot serve an important function within the pancreas. However, the islet-specific location of galanin nerves may reconcile the low levels of galanin in pancreatic extracts with an important but highly localized function in that islets represent only 1–2% of total pancreatic mass.

Immunofluorescent staining for galanin in the dog pancreas revealed galanin-like immunoreactivity only in nerve fibers and primarily in those that innervate the islets, as opposed to the fibers innervating acinar tissue and blood vessels (7). This selective islet innervation again sets galanin apart from other peptides present in canine pancreas, e.g., NPY, vasoactive intestinal polypeptide, and CGRP, which do not exhibit such selective localization (16,27; unpublished observations). Galanin-like immunoreactivity was not found in the intrapancreatic ganglia, also in contrast to NPY (16), suggesting that galanin nerves may be sympathetic in character, because pancreatic sympathetic fibers are extrinsic (i.e., their ganglia reside outside the pancreas), whereas parasympathetic fibers are intrinsic (i.e., they arise from intrapancreatic ganglia). An extrapancreatic location of galanin cell bodies is also consistent with the recent failure to detect galanin mRNA in rat pancreatic tissue (5). Thus, galanin appears to be present in sympathetic-like fibers innervating the dog islet.

Similar localization studies of pancreatic galanin-like immunoreactivity in other species are necessary to establish the generality of this observation. Furthermore, it would be of interest to determine if galanin and norepinephrine are colocalized.

It is important to note that all available information regarding both galanin’s presence in the pancreas and its release during neural activation is based on immunologic techniques with the use of antibodies generated against porcine galanin. Because there is species variability in the C-terminal part of the galanin molecule (5), we and others have found that the detection of galanin in nonporcine species is often enabled only by the use of a non-C-terminally directed antibody (28). Therefore, a comparison of pancreatic content or localization of galanin-like immunoreactivity between species must take into account the specificity of the antibody employed.

**IS GALANIN RELEASED DURING Pancreatic NEURAL ACTIVATION IN QUANTITIES SUFFICIENT TO EVOKE AN ISLET RESPONSE?**

Galanin’s proposed role as a local neurotransmitter would dictate that pancreatic neuronal spillover is not likely to make
a significant contribution to circulating galanin levels. For this reason, and because circulating levels of galanin have been reported to be very low (<10 fmol/ml in humans; 29), documentation of galanin release during pancreatic nerve stimulation necessitates direct access to pancreatic venous blood and a sensitive assay for galanin in plasma. Recently, using a non-C-terminally directed antibody to measure galanin in dog plasma, researchers found that electrical stimulation of the pancreatic autonomic nerves markedly increased the concentration of galanin-like immunoreactivity in the pancreatic venous effluent. Calculated pancreatic spillover (arteriovenous concentration difference × plasma flow) increased by >15-fold (30), and the galanin-like immunoreactivity released by pancreatic nerve stimulation coeluted with synthetic porcine galanin. As predicted, this spillover did not influence peripheral levels of galanin. Because pancreatic galanin spillover increases to a similar degree during stimulation of the splanchic nerves (the sympathetic input to the splanchic bed; 31), the galanin appearing in the pancreatic vein during mixed autonomic nerve stimulation probably arises from the sympathetic nerves.

To determine if the amount of galanin released during neural activation is sufficient to evoke an islet response, galanin was infused directly into the pancreatic artery of dogs at a rate that reproduced the increment of pancreatic venous galanin observed during local nerve stimulation. Although it is difficult to estimate the amount of galanin present at its site of action during nerve stimulation, the local islet concentration of galanin must equal or exceed that present in the pancreatic venous effluent. Therefore, reproducing the neurally induced increment of pancreatic venous galanin by infusing exogenous peptide is a conservative approach to evaluation of the potential actions of endogenous galanin. Such local matched infusions of galanin decreased basal insulin output by >50%, equivalent to the inhibition induced by pancreatic nerve stimulation (30). Pancreatic somatostatin output also decreased to a degree similar to that induced by nerve stimulation. Glucagon output increased, but the magnitude of the response was less than that induced by nerve stimulation. Because norepinephrine is also released under these conditions and because exogenous norepinephrine has been found to stimulate glucagon output, it seems plausible that these two neurotransmitters act in concert to mediate the α-cell response to neural activation. Indeed, an interaction of norepinephrine and galanin to influence basal insulin and somatostatin secretion also remains a possibility. In addition, their interaction to impair glucose-stimulated insulin release is likely because each individually inhibits this response. Nonetheless, the amount of galanin released during nerve stimulation appears sufficient to account for the neurally induced inhibition of basal insulin secretion and to contribute to the changes of basal somatostatin and glucagon secretion.

**DOES INTERFERENCE WITH GALANIN ACTION OR RELEASE ALTER PANCREATIC RESPONSE TO NEURAL ACTIVATION?**

This final criterion is the most critical for acceptance of galanin as a sympathetic neurotransmitter in the endocrine pancreas, but it is the most difficult to fulfill because the experimental tools to address this criterion are lacking. We therefore discuss theoretical approaches.

The actions of galanin could be blocked by several types of molecules including 1) a competitive antagonist, 2) an antibody to the galanin receptor, and 3) an antibody to galanin itself. There has been considerable success in the design of competitive antagonists to some peptides (e.g., vasopressin and parathyroid hormone) with the use of various fragments and analogues to define the portion of the amino acid sequence (domain) responsible for receptor binding (necessary but not sufficient to exert bioactivity) and the domain that is actually responsible for the biologic effect. These domains, once defined, may be chemically modified to enhance receptor binding but eliminate intrinsic bioactivity. Although such structure-binding-activity studies are best performed with in vitro screening techniques in view of the large numbers of peptides that are necessary to test, parallel in vivo testing of the most promising candidates is necessary because antagonists that are potent and effective in vitro have sometimes been found to be ineffective or even to exert agonistic properties in vivo. There is no known antagonist of galanin action, and the studies defining the receptor-binding and bioactive domains are just beginning.

Antibodies to the galanin receptor could antagonize galanin action and provide the additional benefit of a long-duration effect. Thus, a galanin-receptor antibody might be useful for longer-term animal studies to elucidate galanin’s physiologic role. However, the galanin receptor has not yet been isolated or purified for use as an antigen to generate galanin-receptor antibodies. Furthermore, certain anti-receptor antibodies can be receptor agonists, not antagonists (32).

A third method for blocking galanin action is immunoneutralization of the neurally released galanin with an antibody to galanin itself. The advantage of this approach is that such antibodies are available. The disadvantage is that immunoglobulins may be too large to easily penetrate the pancreatic vasculature and reach the site of galanin action in high enough concentrations to neutralize the locally released galanin. However, despite the theoretical limitations, some studies have successfully demonstrated a role for other neurotransmitters with this technique (33,34).

Finally, this criterion could be addressed by preventing galanin release—either by prior depletion of galanin from nerve terminals or by actual blockade of release. Pharmaceuticals capable of depleting or blocking the release of the classic neurotransmitters norepinephrine and acetylcholine have been available for some time and have proved to be extremely valuable tools to define the physiologic roles of these neurotransmitters. However, this approach to determining galanin’s role is remote in that the mechanisms controlling its release are not yet understood. Furthermore, this approach requires selective release of galanin, which may not be possible if it is costored with norepinephrine. Evidence for such colocalization in the pancreas is not yet available.

**SUMMARY AND SPECULATION**

We have hypothesized that galanin is a sympathetic neurotransmitter in the endocrine pancreas and acts, in addition to norepinephrine, to mediate the influence of the local sympathetic nerves on the secretion of insulin, glucagon, and
We have discussed the criteria that must be met before this hypothesis can be accepted, the evidence that galanin meets said criteria, and approaches that may be of use in obtaining further evidence.

In summary, 1) exogenous galanin potently inhibits insulin secretion, in various species, by a direct action on the β-cell; galanin also markedly inhibits pancreatic somatostatin secretion and, at least in vivo, modestly stimulates glucagon release. 2) Galanin-like immunoreactivity is located in fibers innervating the dog islet. Further studies are needed to demonstrate similar innervation in other species and to prove its sympathetic character. 3) Preliminary reports suggest that galanin is released during electrical activation of pancreatic nerves in the dog and that the concentrations achieved are high enough to restrain insulin and somatostatin release and augment glucagon release. 4) The critical demonstration that the effects of sympathetic neural activation are reduced by interference with galanin release or action awaits further studies and may necessitate the development of new experimental tools.

We have evaluated a specific hypothesis related to galanin's potential role in the endocrine pancreas. However, galanin, like many other neuropeptides, is widely distributed in the mammalian nervous system. It is present in the hypothalamus and other brain areas; the posterior pituitary; the spinal cord; the nerves of the respiratory, genitourinary, and gastrointestinal tracts; and the adrenal medulla (35). Exogenous galanin can influence pituitary hormone secretion, food intake, neuronal transmission, and smooth muscle tone (35). It is therefore likely that galanin has many local functions in addition to that proposed herein. Work in other areas may not only clarify galanin's diverse roles but may also provide general information about, for example, the mechanism of galanin release and action, which may in turn accelerate the development of the pharmacologic tools needed to further elucidate galanin's function in the pancreas.

However, even rigorous proof that galanin is a pancreatic sympathetic neurotransmitter would represent only the first step toward understanding this peptide's physiologic/pathophysiologic function in the pancreas. The role of the sympathetic nervous system as a whole in the regulation of endocrine pancreatic secretion is not well understood. For example, although it has generally been accepted that the sympathetic nervous system plays an important role in the endocrine pancreatic response to stress, it has only recently been documented that any stress in fact activates pancreatic sympathetic nerves (36). Nonetheless, it is tempting to speculate that pancreatic galanin is released during stress, re-strains insulin secretion, and thereby contributes to the integrated metabolic response.

The potent inhibitory effect of galanin on insulin secretion may also lead to speculation on galanin's role in other states in which β-cell function is impaired, e.g., diabetes. Whereas the impairment of insulin secretion in insulin-dependent diabetes mellitus results from destruction of β-cells, the impairment in non-insulin-dependent diabetes mellitus (NIDDM) is more complicated. Although many theories center on the potential interaction of insulin resistance with a reduction of β-cell mass, the presence of a potent endogenous inhibitor of β-cell secretion in the fibers that innervate the islets suggests that galanin could contribute to or exacerbate the impairment of insulin secretion in NIDDM. If this were so, a galanin antagonist could be useful in the treatment of this disease. Even if elevated levels of endogenous galanin or enhanced islet sensitivity to galanin does not contribute to the development of NIDDM, blocking normal "galaninergic tone" might still be expected to improve islet function.

Clearly, there is much left to learn about the neuropeptide galanin and its role in the endocrine pancreas. From our perspective, however, continued effort seems warranted.

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