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The Canine Sympathetic Neuropeptide Galanin: A Neurotransmitter in Pancreas, A Neuromodulator in Liver

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Our laboratory has investigated the role of the neuropeptide galanin in the sympathetic neural control of both the canine endocrine pancreas and liver. Galanin mRNA and peptide were found in the neuronal cell bodies of the celiac ganglion, which projects fibers to both organs. Galanin fibers formed dense networks around the islets. Galanin was released from these nerves and the amount released appeared sufficient to markedly inhibit basal insulin secretion. We therefore propose that galanin is a sympathetic neurotransmitter in canine endocrine pancreas. Galanin was also found in hepatic nerves usually co-localized with tyrosine hydroxylase, a sympathetic marker. Further, intraportal administration of the sympathetic neurotoxin, 6-hydroxydopamine, abolished galanin staining in the hepatic parenchyma. We evaluated the role of galanin in mediating the actions of sympathetic nerves to increase hepatic glucose production and decrease hepatic arterial conductance. Local infusion of synthetic galanin had little effect by itself, but it did potentiate the action of norepinephrine to stimulate hepatic glucose production, demonstrating a neuromodulatory action. In contrast, galanin had no effect on hepatic arterial blood flow. We therefore propose that in the liver galanin functions as a neuromodulator of norepinephrine's metabolic action.

Key words: Insulin Secretion – Hepatic Glucose Production – Norepinephrine

Introduction

This brief review summarizes the work of our laboratory on the role of the neuropeptide, galanin, in the sympathetic neural control of the canine endocrine pancreas [1] and liver [2]. Over the last fifteen years, we have produced data which, taken together, suggest that in the canine pancreas galanin functions as a sympathetic neurotransmitter, independent of norepinephrine (NE), mediating the inhibition of basal insulin secretion seen during activation of the pancreatic sympathetic nerves. In contrast, in the canine liver, our combined data suggest that galanin functions as a neuromodulator, potentiating NE's direct stimulation of hepatic glucose production, while exerting little stimulatory effect of its own. Below, we have outlined the evidence obtained in our laboratory that supports these two conclusions.

Galanin and Sympathetic Neural Control of the Endocrine Pancreas

Neural localization of pancreatic galanin

The traditional view has been that the sympathetic nerves innervating the pancreas release the classical neurotransmitter, norepinephrine (NE), which in turn inhibits insulin secretion [3] by activating $\alpha 2$ adrenergic receptors [4] on the islet β-cell. However, catecholamines can also activate $\beta 2$ adrenergic receptors on the β-cell [5], which stimulate insulin secretion, particularly *in vivo* [6–7]. Thus, NE is more appropriately viewed as a mixed agonist having opposing effects, particularly on basal insulin secretion [8]. This mixed agonism makes it difficult to predict the net effect of NE on insulin secretion. We therefore infused varying concentrations of NE directly into the pancreatic artery of anesthetized dogs and measured its effects on both basal insulin secretion and pancreatic blood flow [9], the latter as an index of NE's known effect on vasoconstriction. We found that increasing doses of NE produced the expected progressive vasoconstriction. However, none of these local NE infusions could mimic the marked inhibition of insulin secretion observed during the electrical stimulation of the sympathetic nerves of the pancreas. We therefore began to search for co-transmitters that might mediate the inhibitory effects of sympathetic neural activation on basal insulin secretion.

One approach was to examine the post-ganglionic sympathetic neurons that supply the pancreas for the presence of neuropeptides that inhibit the β-cell. The majority of these neuronal cell bodies are located in the celiac ganglia. We therefore used *in situ* hybridization to detect the mRNA of one such neuropeptide, galanin [10], and found galanin mRNA in every cell body in the dog celiac ganglion [11] (see Fig. 1). To determine if this galanin message was translated, we re-examined these neuronal cell bodies for galanin peptide using immunohistochemistry. We found galanin-positive staining in over 90% of the neu-

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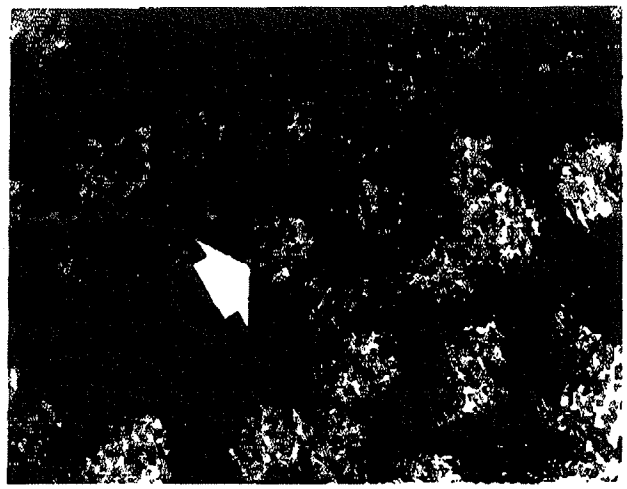

Fig. 1 A darkfield photomicrograph showing *in situ* hybridization of a radiolabeled oligonucleotide probe complementary to galanin mRNA (white dots) in the neuronal cell bodies of dog celiac ganglion. The large filled arrow identifies a single neuronal cell body ([11], reproduced with permission).

ronal cell bodies of the dog celiac ganglion [12]. Although the celiac ganglion projects to many abdominal organs besides the pancreas, the high percentage of neuronal cell bodies that contained galanin mRNA and peptide suggested that pancreatic sympathetic nerves would also contain galanin. This expectation was confirmed by immunohistochemical localization of galanin-like immunoreactivity in the nerve fibers within the pancreas and galanin co-localization with tyrosine hydroxylase [12], an enzyme marker of sympathetic nerves. However, innervation of the pancreas does not necessarily imply innervation of the islet, since the vast majority of pancreatic tissue is exocrine, rather than endocrine. We therefore determined whether galanin fiber nerves preferentially innervated the islet and found dense networks of galanin fibers throughout the islet (Fig. 2), with sparse innervation of the surrounding pancreatic acinar tissue. Based on these data, we concluded that galanin-containing sympathetic nerves densely innervate dog pancreatic islets. However, the above data were still insufficient to prove that galanin played an important role in the sympathetic control of insulin secretion.

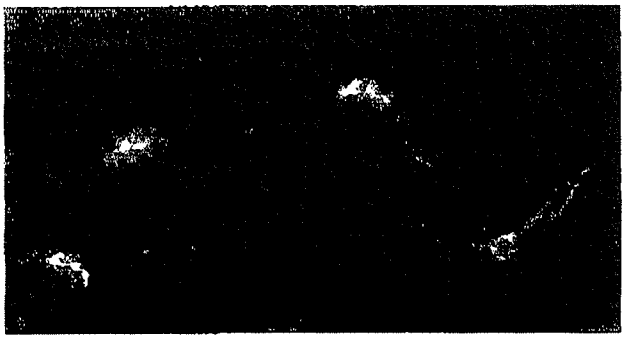

Fig. 2 A lightfield photomicrograph (15×) of a section of dog pancreas showing immunofluorescent staining of a network of galanin fibers surrounding three individual islets and one large blood vessel (lower right) ([24], reproduced with permission).

Release and action of pancreatic galanin

To demonstrate that galanin was released from pancreatic sympathetic nerves, we electrically stimulated either the thoracic sympathetic chain above the pancreas [13] or the local sympathetic nerves surrounding the pancreatic artery [14]. Either stimulation produced a marked increase in the spillover of galanin into the major vein draining the dog pancreas (Fig. 3), demonstrating that galanin was released from pancreatic sympathetic nerves, at least during their electrical stimulation. To determine if the amount of galanin released was sufficient to influence islet function, we next infused synthetic galanin into the pancreatic artery at a rate that reproduced the increment of pancreatic venous galanin observed during local nerve stimulation [14]. This rate of infusion produced an inhibition of basal insulin output that was similar in magnitude but more sustained than that produced by stimulation of the sympathetic nerves of the pancreas. This inhibitory effect of galanin on insulin secretion may be mediated by any of the three currently identified galanin (GAL) receptors (R), since the mRNA for GALR1 [15] and GALR2 [16] has been detected in insulinoma cell lines, and since an antagonist impairing the ability of galanin to inhibit insulin secretion [17] binds with high affinity to GALR3 [18]. Thus, local galanin infusion reproduced the insulin inhibitory response to sympathetic nerve stimulation [14], which local NE infusion could not [9]. These data suggest that galanin acts independently of NE to mediate the inhibition of basal insulin secretion seen during sympathetic nerve stimulation. Thus, it is proposed that the neuropeptide galanin be classified as a sympathetic neurotransmitter in the canine endocrine pancreas [1].

Galanin and Sympathetic Neural Control of the Liver

Neural localization of hepatic galanin

As previously demonstrated, galanin mRNA and peptide were found in nearly all of the neuronal cell bodies of the dog celiac ganglion. This ganglion supplies sympathetic innervation to the liver as well as to the pancreas. Therefore, we hypothesized that galanin would also be found in the sympathetic nerves of

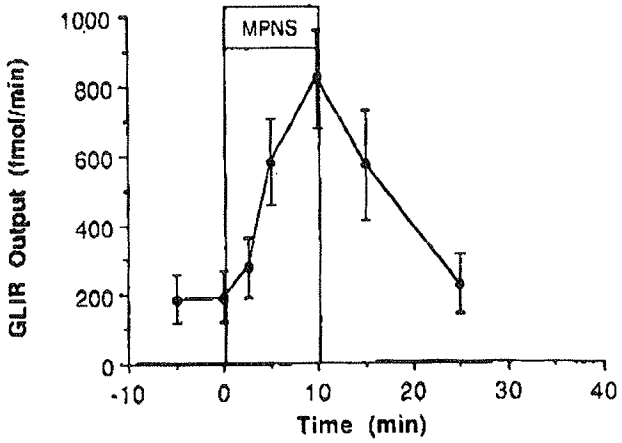

Fig. 3 Increased pancreatic output of galanin-like immunoreactivity (GLIR) during 10 minutes of mixed pancreatic nerve stimulation (MPNS) in halothane-anesthetized dogs ([14], reproduced with permission).

the liver. We tested this hypothesis in two ways. First, we examined the co-localization of galanin in hepatic nerves with the sympathetic enzyme marker, tyrosine hydroxylase [19]. Second, we determined if the sympathetic neurotoxin, 6-hydroxydopamine (6-OHDA), would reduce galanin staining in liver nerves and reduce the concentration of galanin immunoreactivity in extracts of dog liver [19]. Hepatic nerves that were positive for galanin by immunohistochemistry were typically positive for tyrosine hydroxylase. More convincingly, we found that portal venous infusions of 6-OHDA, which depleted hepatic norepinephrine content by over 90%, depleted hepatic galanin content by a similar amount. In addition, the staining of galanin fibers in hepatic parenchyma was nearly abolished (see Fig. 4), although some galanin staining was preserved in the nerve fibers that surrounded hepatic blood vessels. It was therefore interesting to note that the hepatic glucose production response to hepatic nerve stimulation in 6-OHDA pretreated dogs was markedly diminished compared to controls, in contrast to the vasoconstrictor response which was barely reduced [19]. These findings led us to determine whether galanin was released from these nerves, and if galanin had a role in mediating these changes of hepatic glucose production and hepatic vasoconstriction.

Release and action of hepatic galanin

To determine if the galanin contained in hepatic sympathetic nerves was actually released, we produced both a general activation of sympathetic nerves by electrically stimulating the thoracic sympathetic chain above the liver and a local activation by electrically stimulating the nerves surrounding the hepatic artery [20]. The electrical stimulation of either the thoracic sympathetic chain or the local hepatic nerves markedly increase the spillover of galanin from the liver (Fig. 5). To determine if there was a substantial release of galanin from hepatic nerves that were not sympathetic, we also electrically stimulated the hepatic nerves in dogs that were pretreated with intraportal infusions of the sympathetic neurotoxin, 6-OHDA [19]. 6-OHDA pretreatment nearly abolished the stimulation-induced spillover of galanin from the liver (Fig. 5), suggesting that galanin was released almost exclusively from the sympathetic nerves of the dog liver. The activation of hepatic sympathetic nerves was accompanied by an increase of glucose production from the liver and a decrease of hepatic arterial conductance (increased vasoconstriction). However, it was still unclear if galanin contributed to either of these sympathetic actions.

To determine if galanin contributed to these responses, we infused synthetic galanin alone or in combination with NE directly into the hepatic artery of halothane-anesthetized dogs and measured both hepatic glucose production and hepatic arterial conductance [21]. On its own, galanin had little effect on either hepatic glucose production or hepatic arterial conductance, while NE by itself had dramatic effects on both. However, the combination of galanin and NE produced a larger increase of hepatic glucose production than either galanin or NE alone, demonstrating that the sympathetic neuropeptide, galanin, potentiates this component of the liver's response to the classical sympathetic neurotransmitter, NE. The receptor mediating this potentiating action of galanin could be GALR2, since it is the only one of the three currently identified galanin receptors whose mRNA has been detected in liver [18, 22–23]. In contrast, galanin had no such effect on the ability of NE to decrease hepatic arterial conductance, demonstrating that ga-

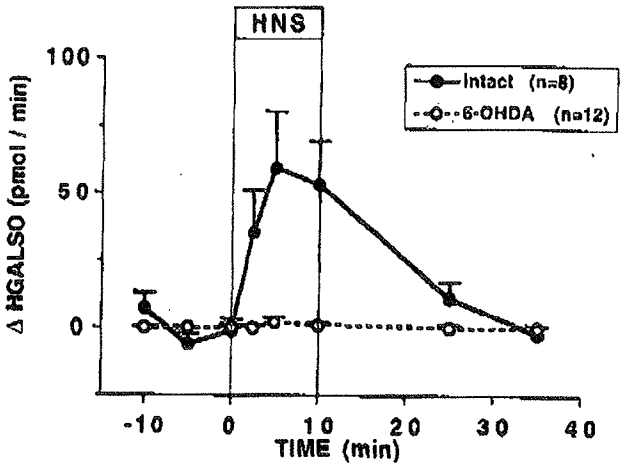

Fig. 5 Hepatic galanin spillover (HGALSO) during hepatic nerve stimulation (HNS) in intact halothane-anesthetized dogs (closed circles, solid line) and in dogs pre-treated with intraportal infusions with 6-OHDA (open circles, dashed lines) ([19], reproduced with permission).

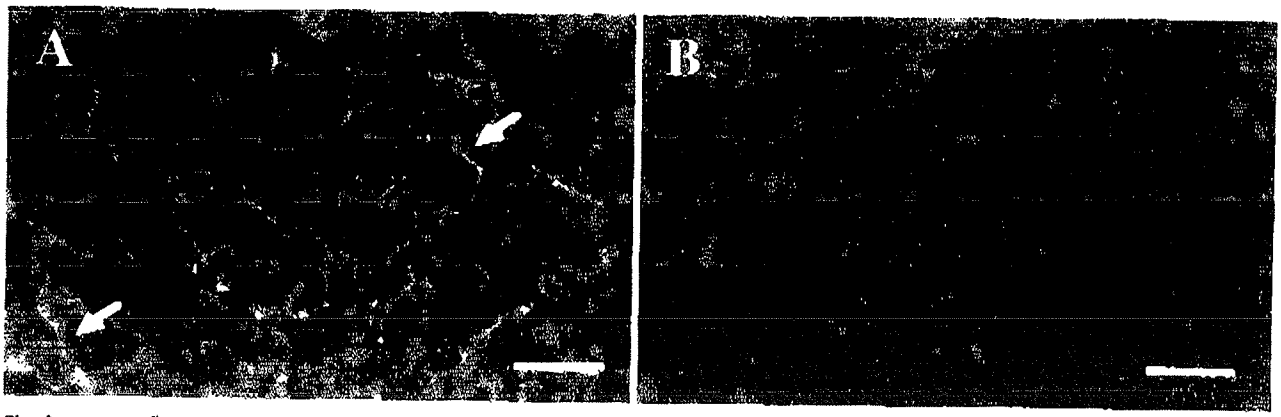

Fig. 4 Immunofluorescent staining of galanin in a section of liver parenchyma from a control dog (A) and from a dog pretreated with an intraportal infusion of 6-OHDA (B). Filled arrows identify individual nerve fibers in a control dog. ([19], reproduced with permission).

lanin did not potentiate the vascular action of NE on the liver, only its metabolic effect.

Conclusion

In summary, galanin is a sympathetic neuropeptide present in, and released from the sympathetic nerves innervating the canine pancreas and liver. In the canine pancreas, it appears to function as a neurotransmitter, independent of the classical sympathetic neurotransmitter NE, and may therefore mediate the inhibition of basal insulin secretion produced by activation of pancreatic sympathetic nerves. In the canine liver, galanin has no significant neurotransmitter action, independent of NE, but rather functions as a neuromodulation of NE's metabolic, but not vascular, action. Galanin may thereby contribute to the stimulation of hepatic glucose production seen during activation of hepatic sympathetic nerves.

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References

1. Dunning BE, Taborsky GJ Jr. Galanin-sympathetic neurotransmitter in endocrine pancreas? Diabetes 1988; 37: 1157–62
2. Taborsky GJ Jr, Boyle MR, Mundinger TO, Baskin DG, Porte D Jr, Kowalyk S. Sympathetic nerves of the liver: their activation during stress and their use of peptidergic neurotransmitters. In: Shimazu, T (ed). Liver Innervation. London: John Libbey and Company 1996: 115–22
3. Porte D Jr, Williams RH. Inhibition of insulin release by norepinephrine in man. Science 1966; 152: 1248–50
4. Schuit FC, Pipeleers DG. Differences in adrenergic recognition by pancreatic A and B cells. Science 1986; 232: 875–7
5. Ahren B, Taborsky GJ Jr, Porte D Jr. Neuropeptidergic versus cholinergic and adrenergic regulation of islet hormone secretion. Diabetologia 1986; 29: 827–36
6. Porte D Jr. Beta adrenergic stimulation of insulin release in man. Diabetes 1967; 16: 150–5
7. Ahren B, Lundquist: Effect of selective and non-selective beta-adrenergic agents on insulin secretion *in vivo*. Eur J Pharmacol 1981; 71: 93–104
8. Robertson RP, Porte D Jr. Adrenergic modulation of basal insulin secretion in man. Diabetes 1973; 22: 1–8
9. Ahren B, Veith RC, Taborsky GJ Jr. Sympathetic nerve stimulation versus pancreatic norepinephrine infusion in the dog: 1) Effects on basal release of insulin and glucagon. Endocrinology 1987; 121: 323–31
10. McDonald TJ, Dupre J, Tatemoto K, Greenberg GR, Radziuk J, Mutt V. Galanin inhibits insulin secretion and induces hyperglycemia in dogs. Diabetes 1985; 34: 192–6
11. Verchere CB, Kowalyk S, Shen GH, Brown MR, Schwartz MW, Baskin DG, Taborsky GJ Jr. Major species variation in the expression of galanin messenger ribonucleic acid in mammalian celiac ganglion. Endocrinology 1994; 135: 1052–9
12. Ahren B, Bottcher G, Kowalyk S, Dunning BE, Sundler F, Taborsky GJ Jr. Galanin is co-localized with noradrenaline and neuropeptide Y in dog pancreas and celiac ganglion. Cell Tiss Res 1990; 261: 49–58
13. Dunning BE, Havel PJ, Veith RC, Taborsky GJ Jr. Pancreatic and extrapancreatic galanin release during sympathetic neural activation. Am J Physiol 1990; 258: E436–44
14. Dunning BE, Taborsky GJ Jr. Galanin release during pancreatic nerve stimulation is sufficient to influence islet function. Am J Physiol 1989; 256: E191–8
15. Parker EM, Izzarelli DG, Nowak HP, Mahle CD, Iben LG, Wang J, Goldstein ME. Cloning and characterization of the rat GALR1 galanin receptor from Rin14B insulinoma cells. Brain Res Mol Brain Res 1995; 34: 179–89
16. Howard AD, Tan C, Shiao L, Palyha OC, McKee KK, Weinberg DH, Feighner SD, Cascieri MA, Smith RG, Van Der Ploeg L, Sullivan KA. Molecular cloning and characterization of a new receptor for galanin. FEBS Letters 1997; 405: 285–90
17. Kakuyama H, Mochizuki T, Iguchi K, Yamabe K, Hosoe H, Hoshino M, Yanaihara N. [Ala^6, D-Trp^8] Galanin (*1–15*)ol is a potent galanin antagonist on insulin release. Biomed Res 1997; 18: 49–56
18. Wang S, He C, Hashemi T, Bayne M. Cloning and expressional characterization of a novel galanin receptor. J Biol Chem 1997; 272: 31949–52
19. Mundinger TO, Verchere CB, Baskin DG, Boyle MR, Kowalyk S, Taborsky GJ Jr. Galanin is localized in sympathetic neurons of the dog liver. Am J Physiol 1997; 273: E1194–202
20. Kowalyk S, Veith R, Boyle M, Taborsky GJ Jr. Liver releases galanin during sympathetic nerve stimulation. Am J Physiol 1992; 262: E671–8
21. Taborsky GJ Jr, Mundinger TO. Differential actions of hepatic sympathetic neuropeptides. Diabetes 1997; 46: 237A
22. Fathi Z, Cunningham AM, Iben LG, Battaglio PB, Ward SA, Nichol KA, Pine KA, Wang J, Goldstein ME, Iismaa TP, Zimany IA. Cloning, pharmacologic characterization and distribution of a novel galanin receptor. Brain Res Mol Brain Res 1997; 51: 49–59
23. Habert-Ortoli E, Amiranoff B, Loquet I, Laburthe M, Mayaux JF. Molecular cloning of a functional human galanin receptor. Proc Natl Acad Sci USA 1994; 91: 9780–3
24. Taborsky GJ Jr. Insulin and glucagon secretion *in vivo* and its neural control. In: Jefferson LS, Cherrington AD (eds). Handbook of Physiology. New York: Oxford University Press 1999: Chapter 1, A, 5 (in press)

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