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Pancreatic and extrapancreatic galanin release during sympathetic neural activation

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DUNNING, BETH ELAINE, PETER J. HAVEL, RICHARD C. VEITH, AND GERALD J. TABORSKY, JR. *Pancreatic and extrapancreatic galanin release during sympathetic neural activation.* Am. J. Physiol. 258 (Endocrinol. Metab. 21): E436–E444, 1990.—To address the hypothesis that the neutropeptide, galanin, functions as a sympathetic neurotransmitter in the endocrine pancreas, we sought to determine if galanin is released from pancreatic sympathetic nerves during their direct electrical stimulation in halothane-anesthetized dogs. During bilateral thoracic splanchnic nerve stimulation (BTSNS), both peripheral arterial and pancreatic venous levels of galanin-like immunoreactivity (GLIR) increased (Δ at 10 min = +92 ± 31 and +88 ± 25 fmol/ml, respectively). Systemic infusions of synthetic galanin demonstrated that *1*) the increment of arterial GLIR observed during BTSNS was sufficient to modestly restrain basal insulin secretion and *2*) only 25% of any given increment of arterial GLIR appears in the pancreatic vein, suggesting that the pancreas extracts galanin, as it does other neurotransmitters. By use of 75% for pancreatic extraction of circulating galanin, it was calculated that pancreatic galanin spillover (output) increased by 410 ± 110 fmol/min during BTSNS. To reinforce the conclusion that pancreatic sympathetic nerves release galanin, GLIR spillover was next measured during direct local stimulation of the pancreatic sympathetic input produced by electrical stimulation of the mixed autonomic pancreatic nerves (MPNS) in the presence of the ganglionic blocker, hexamethonium. During this local pancreatic sympathetic nerve stimulation, arterial GLIR remained unchanged, but pancreatic venous GLIR increased by 123 ± 34 fmol/ml. Thus pancreatic GLIR spillover increased by 420 ± 110 fmol/min during MPNS in the presence of hexamethonium. We conclude that galanin is released from both pancreatic and extrapancreatic sources during sympathetic neural activation in dogs.

sympathetic nervous system; insulin; glucagon; catecholamines

IT HAS BEEN PROPOSED that the neuropeptide, galanin, acts as a sympathetic neurotransmitter in the enodcrine pancreas (9) and mediates the nonadrenergic inhibition of insulin (IRI) and stimulation of glucagon (IRG) secretion seen during activation of the sympathetic nervous system (3, 8). This hypothesis is based on evidence suggesting that *1*) exogenous galanin qualitatively reproduces the endocrine pancreatic effects of sympathetic activation (7, 17) and *2*) galanin-like immunoreactivity (GLIR) is present in fibers innervating the islets of Langerhans (7). Although the recent demonstration that GLIR spills over into the pancreatic venous effluent (i.e., is released) during electrical stimulation of the local mixed autonomic nerves of the pancreas (MPNS) in dogs (10) is consistent with the hypothesis, acceptance of galanin's putative role as a sympathetic neurotransmitter awaits the further demonstration that it is released during sympathetic nerve stimulation. Because MPNS activates both postganglionic sympathetic and preganglionic parasympathetic fibers, the origin of GLIR released during MPNS remains unclear. We therefore sought to test directly the hypothesis that galanin is a sympathetic neurotransmitter in the pancreas by determining whether pancreatic galanin is released by sympathetic nerves.

In the present study we measured pancreatic spillover and peripheral levels of both GLIR and norepinephrine (NE) in halothane-anesthetized dogs during *1*) generalized sympathetic activation produced by bilateral thoracic splanchnic nerve stimulation (BTSNS) and *2*) selective activation of the local sympathetic input to the pancreas (MPNS in the presence of the ganglionic blocker, hexamethonium). In addition, we quantified pancreatic extraction of infused galanin (reflecting a potential local mechanism to terminate this putative neurotransmitter's action). Finally, we examined whether sufficient galanin is present in the systemic circulation during generalized sympathetic activation (BTSNS) to influence IRI secretion hormonally.

MATERIALS AND METHODS

Animals and surgical procedures. Anesthesia was induced with the ultrashort-acting barbiturate, thiamylal sodium (Surital, Parke Davis, Morris Plains, NJ, 30 mg/kg iv) in adult dogs of mixed breed (19–37 kg) following an overnight fast (~18 h). Anesthesia was subsequently maintained with halothane (0.8%) administered from a calibrated vaporizer (Draeger, FRG) by mechanical ventilation in 100% oxygen. To access the pancreatic venous blood, a laparotomy was performed and an extracorporeal shunt containing a sampling port and an electromagnetic flow probe (Zepeda Instruments, Seattle, WA) was placed between the superior pancreaticoduodenal vein (SPDV) and the portal vein. A femoral artery (FA) and vein were

cannulated for arterial blood sampling and intravenous infusions of saline or galanin.

To stimulate the thoracic splanchnic nerves, bilateral thoracotomies were performed at the seventh intercostal space. The sympathetic trunks were dissected from surrounding tissue along the dorsal rib cage, and bipolar electrodes (Harvard Apparatus, South Natick, MA) were placed on each nerve at the level of approximately T_{10}. The nerve trunks were then severed anterior to the electrodes. In dogs in which the mixed autonomic pancreatic nerves were stimulated, the nerve fibers along the superior pancreaticoduodenal artery were dissected free from the artery along with the adjacent fascia and placed in a bipolar electrode. A 60-min stabilization period followed the surgical procedures before experimentation.

Experimental protocols. To determine whether galanin is released during stimulation of the thoracic splanchnic nerves (BTSNS), the sympathetic trunks were electrically stimulated for 10 min with square wave pulses of 1-ms duration and 10 mA current at a frequency of 8 Hz. The stimulations were performed with a model S-44 stimulator coupled to a PSIU6 stimulus isolation unit (Grass Instruments, Quincy, MA). Stimulation parameters were monitored with an oscilloscope. To allow measurement of pancreatic neurotransmitter spillover and hormone output, paired base-line blood samples were drawn from the FA and the SPDV at 10 and 0 min before, 2.5, 5, and 10 min during, and 5 and 15 min after BTSNS.

To determine the rate of extraction of galanin across the pancreas, synthetic porcine galanin (Bachem, Torrance, CA) was infused for 30 min in 14 dogs via the femoral vein at rates ranging from 1.3 to 26 pmol·kg^{-1}·min^{-1}. GLIR was measured in samples obtained simultaneously from the FA and the SPDV, before (−10 and 0 min), during (5, 10, 20, and 30 min), and after (5 and 15 min) galanin infusions. Pancreatic galanin extraction was defined as $(\Delta[GLIR]_{FA} - \Delta[GLIR]_{SDPV})/\Delta[GLIR]_{FA}$. In six of these dogs receiving the lowest dose (1.3 pmol·kg^{-1}·min^{-1}) samples were also obtained for measurement of pancreatic hormones and glucose to determine the effects of low-dose galanin infusions on islet function (see RESULTS, Fig. 8).

To determine if galanin is released from pancreatic nerves during selective local sympathetic neural activation, MPNS was performed in the presence of the ganglionic blocker, hexamethonium (HEX). Hexamethonium bromide (Sigma Chemical, St. Louis, MO) was injected intravenously in repeated doses of 0.1 mg/kg, until the mean arterial blood pressure dropped by 10–20 mmHg and remained at that lower level. HEX was then infused at a rate of 0.7 μg·kg^{-1}·min^{-1} times the number of doses injected initially. This dosage has previously been shown to eliminate all pancreatic effects of vagal nerve stimulation (2). The mixed autonomic pancreatic nerves running in the arterial sheath (see above) were then stimulated with the same stimulation parameters and sampling times employed for BTSNS.

In all experiments, mean arterial blood pressure and SPDV blood flow were monitored continuously and hematocrit was determined at regular intervals.

Sample handling and chemical determinations. Blood samples for measurement of GLIR were immediately placed in tubes containing a mixture of proteolytic enzyme inhibitors (5). Samples for catecholamine determinations were placed in tubes containing glutathione and ethylene glycol-bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraactic acid, those for insulin (IRI) and glucose measurements were placed in tubes containing EDTA, and those for glucagon (IRG) measurements were placed in tubes containing heparin and benzamidine. All blood samples were kept on ice (<2 h), centrifuged, and stored at −20°C until assay.

GLIR was measured in unextracted plasma by radioimmunoassay (RIA) with a non-COOH-terminally directed antibody raised against synthetic porcine galanin and synthetic porcine galanin standards by a procedure described previously (10). Because the species variable portion of the galanin molecule is apparently limited to the COOH-terminal heptapeptide (14, 19), this assay detects GLIR from all species yet examined. Over 90% of synthetic galanin added to dog plasma is recovered in a single peak upon gel filtration.

Plasma NE and epinephrine (EPI) were measured with a sensitive and specific radioenzymatic assay (18). IRI and IRG were measured by RIA with methods described previously (21) and glucose was measured by a glucose oxidase method.

Chromatography. To determine the molecular forms of GLIR present in arterial plasma in the basal and stimulated states, additional plasma samples were obtained before (*time 0*) and during (7.5 min) BTSNS in three dogs. Plasma samples (1.5 ml) were applied to a 1 × 50-cm column of Sephadex G-50 and eluted with galanin assay buffer. Sixty 1-ml fractions were collected for galanin RIA.

Data analysis. Pancreatic hormone output was calculated according to the following formula: output = ($[\text{hormone}]_{SPDV} - [\text{hormone}]_{FA}$) × (1-hematocrit) × blood flow_{SPDV}. Pancreatic neurotransmitter spillover (synonymous with output) was calculated using the same principle but with equations modified to take into account the extraction of circulating neurotransmitters by the pancreas. Because both NE (1) and galanin (see RESULTS) are extracted by the pancreas, most of the transmitter arriving via the arterial circulation does not appear in the pancreatic vein and therefore simple arteriovenous (AV) concentration difference measurements seriously underestimate pancreatic neurotransmitter release. To correct the AV difference for extraction, neurotransmitter spillover was calculated as follows: spillover = {$[\text{NE or GLIR}]_{SPDV}$ − (arterial contribution to SPDV level)} × (1 − hematocrit) × blood flow_{SPDV}. The arterial contribution to SPDV level is defined to be the amount of neurotransmitter present in the SPDV plasma that arrives via the arterial circulation but escapes pancreatic extraction. Because EPI is extracted at the same rate as NE (1), EPI extraction was used as an index of NE extraction and employed to calculate the arterial contribution to SPDV [NE]. Thus the arterial contribution to SPDV [NE] = $[\text{NE}]_{FA}$ × (1 − pancreatic EPI extraction), where EPI extraction is defined to be

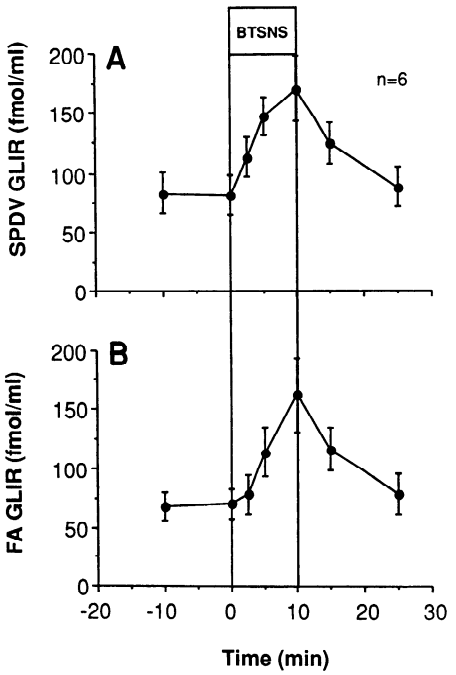

FIG. 1. Pancreatic venous (SPDV, *A*) and femoral arterial (FA, *B*) levels of galanin-like immunoreactivity (GLIR) during 10-min bilateral thoracic splanchnic nerve stimulation (BTSNS) in dogs. Values are means ± SE, $n = 6$.

$([EPI]_{FA} - [EPI]_{SPDV})/[EPI]_{FA}$. In the case of galanin, in the basal state, nearly all plasma GLIR represents a large-molecular-weight cross-reactant in the RIA (10). It is present in equal amounts in FA and SPDV plasma and thus does not appear to be subject to pancreatic extraction (see RESULTS, Fig. 2, and DISCUSSION). Therefore, only the increment over the basal FA level was considered to be true galanin and used in the extraction-spillover calculation. Pancreatic extraction of exogenously infused galanin was measured directly (see above), found to be 75% (see RESULTS), and assumed to be constant and equivalent to the pancreatic extraction of endogenous "true" galanin. Thus, when FA levels of GLIR increased during BTSNS, pancreatic galanin spillover was calculated as follows: spillover = $([GLIR]_{SPDV} - \{[GLIR]_{basal\ FA} + 0.25\ ([GLIR]_{FA} - [GLIR]_{basal\ FA})\}) \times (1 - \text{hematocrit}) \times \text{blood flow}_{SPDV}$ where $[GLIR]_{basal\ FA}$ was defined as the mean of the FA levels at −10 and 0 min (before BTSNS).

During MPNS in the presence of HEX, FA levels of GLIR did not increase, and therefore the complex calculation for GLIR spillover reduces to that used for hormone output. Similarly, because SPDV levels of NE vastly exceeded arterial NE levels during these local nerve stimulations, the calculation for pancreatic NE spillover also reduces to that used for hormone output.

All data were expressed as means ± SE. Statistical comparisons of means within a group at different times were made with a paired *t* test at the time point indicated in the text. Comparisons of means between groups were made with a two-sample *t* test.

RESULTS

Splanchnic nerve stimulations. Figure 1 depicts the levels of GLIR in unextracted plasma obtained from the SPDV and FA during electrical BTSNS in halothane-anesthetized dogs. Pancreatic venous GLIR (Fig. 1*A*) increased rapidly during BTSNS from a base line of 82 ± 18 fmol/ml to 147 ± 16 at 5 min and to 170 ± 27 at 10 min (Δ at 10 min = +88 ± 25 fmol/ml, $n = 6$, $P < 0.01$), then returned to base line (88 ± 17 fmol/ml) 15 min after the nerve stimulation. Arterial levels of GLIR (Fig. 1*B*) also increased during BTSNS from a base line of 69 ± 13 fmol/ml to 114 ± 21 at 5 min and to 162 ± 31 at 10 min (Δ at 10 min = +92 ± 31 fmol/ml, $P < 0.025$), and returned to base line (78 ± 17) 15 min after BTSNS.

Previous studies have demonstrated that the increment of GLIR in SPDV plasma during local autonomic nerve stimulation coeluted with synthetic galanin (10).

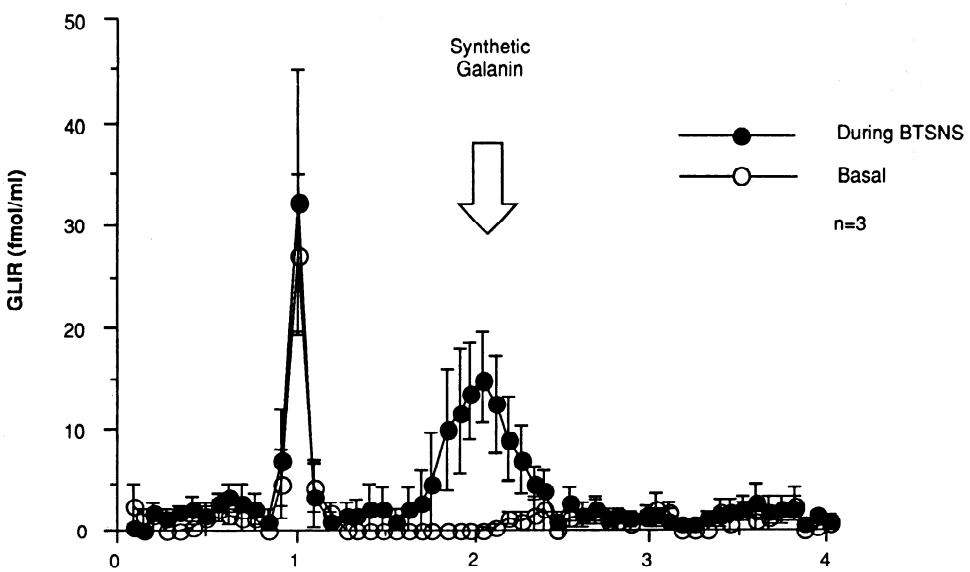

FIG. 2. Elution profile after gel filtration on Sephadex G-50 of galanin-like immunoreactivity (GLIR) in femoral arterial plasma before (basal) and during 10-min bilateral thoracic splanchnic nerve stimulation (BTSNS) in dogs. Ve/Vo, ratio of elution position to void volume. Elution position of synthetic porcine galanin is indicated by arrow. Values are means ± SE of 3 experiments.

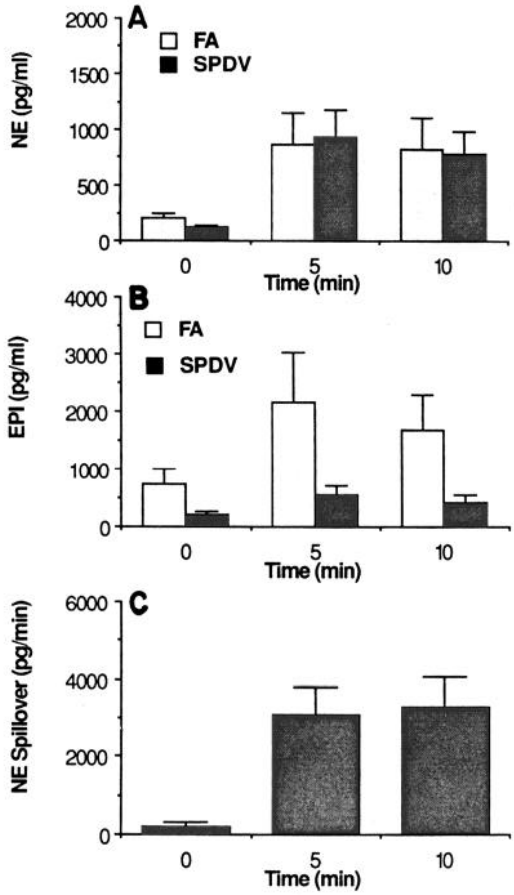

FIG. 3. Plasma concentrations of norepinephrine (NE, *A*) and epinephrine (EPI, *B*) in pancreatic vein (SPDV) and femoral artery (FA) and pancreatic NE spillover (*C*) before (*time 0*) and at 5 and 10 min during bilateral thoracic splanchnic nerve stimulation in dogs. Values are means ± SE, $n = 6$.

To determine whether the increment of GLIR in arterial plasma observed during BTSNS was also caused by an increase of a galanin-sized molecule, gel filtration of arterial plasma was performed. Figure 2 depicts the Sephadex G-50 gel filtration profile of GLIR present in FA plasma before (basal) and during BTSNS. In the basal state, there was one peak of GLIR, eluting in the void volume (V_o). During BTSNS, FA plasma contained two peaks of GLIR, one (V_o) peak nearly identical to that in basal plasma and a second ($V_e/V_o \approx 1.8$–2.3) that coeluted with synthetic porcine galanin. Thus there is little galanin-sized GLIR in basal FA plasma, but the entire increment over basal measured during BTSNS represents a species with an elution position characteristic of galanin.

Figure 3 illustrates the catecholamine levels and NE spillover that occurred before ($t = 0$ min) and during ($t = 5$ and 10 min) BTSNS. As seen in Fig. 3*A*, FA NE levels were similar to SPDV NE levels both before and during BTSNS, analogous to the GLIR data (see Fig. 1). BTSNS increased FA and SPDV levels of NE by 620 ± 280 and 660 ± 210 pg/ml, respectively. As shown in Fig. 3*B*, however, the increases of FA and SPDV EPI levels were not equivalent. BTSNS markedly increased FA EPI (Δ at 10 min = +960 ± 360 pg/ml), presumably because of activation of the adrenal medulla. BTSNS also increased SPDV EPI (Δ at 10 min = +240 ± 100 pg/ml) but to a much smaller extent than it increased FA EPI, reflecting pancreatic extraction of EPI. Because the pancreatic nerves release little EPI and because pancreatic extraction of EPI is known to be very similar to that of NE (1), EPI extraction could be used as an index of NE extraction. By this means (see MATERIALS AND METHODS), pancreatic NE spillover was calculated. As shown in Fig. 3*C*, NE spillover increased during BTSNS from a base line of 180 ± 120 to 3,260 ± 790 pg/min at 10 min (Δ = +3,080 ± 770 pg/min = 18 ± 5 pmol/min, $n = 6$, $P < 0.01$).

Table 1 reports the maximum changes of pancreatic output of insulin (IRI) and glucagon (IRG), of FA glucose levels, mean arterial blood pressure, SPDV blood flow, and hematocrit that occurred during BTSNS, together with the time at which the maximum change occurred. Consistent with sympathetic activation, IRI output was rapdily inhibited and that of IRG was stimulatd. Glucose levels changed little early in the BTSNS (ΔFA glucose at 2.5 min = +8 ± 2 mg/dl, data not shown) but increased markedly (+75 mg/dl) by the 10th min of BTSNS. Therefore, the net stimulation of IRI output that occurred by the end of BTSNS (Δ at 10 min = +250 ± 120%) actually represents a relative inhibition, as apposed to the absolute inhibition that occurred earlier, before hyperglycemia. BTSNS also increased blood pressure, hematocrit, and SPDV blood flow.

Because FA levels of GLIR increased during BTSNS, it was unclear how much of the increase of SPDV GLIR was caused by the increase of circulating GLIR and how much was attributable to local pancreatic neural release of galanin. Thus it was necessary to determine the rate at which the pancreas extracts galanin (analogous to extraction of EPI, Fig. 3*B*) to calculate neuronal spillover of GLIR. Therefore, synthetic galanin was infused systemically at a range of doses, and GLIR was measured in plasma samples obtained simultaneously from the FA and the SPDV. As shown in Fig. 4, there was a linear

TABLE 1. *Effects of bilateral thoracic splanchnic nerve stimulation*

Parameter	Maximum Change	Time, min	$P <$
ΔIRI output, μU/min (%)	−760±180 (−45±9)	2.5	0.005
	+1,450±1,010 (+250±120)	10	NS
ΔIRG output, pg/min (%)	+960±310 (+320±110)	5	0.05
ΔGlucose, mg/dl	+75±21	10	0.02
ΔMAP, mmHg	+30±10	2.5	0.05
ΔPancreatic blood flow, ml/min	+4.2±1.5	2.5	0.05
ΔHematocrit, %	+6.3±2.2	10	0.05

Values are means ± SE; $n = 6$ dogs. Immunoreactive insulin (IRI) and glucagon (IRG); MAP, mean arterial pressure; NS, not significant.

relationship between the increment of arterial GLIR (ΔFA GLIR) resulting from the intravenous infusions and the much smaller increment of pancreatic venous GLIR (ΔSPDV GLIR). As reflected by the slope of this line, for any given increment of FA, GLIR, only 25% of that increment appeared in SPDV plasma. This relationship was constant over the wide range of galanin concentrations, which included six that were in the range of those measured during BTSNS. The slope of the correlation between ΔFA and ΔSPDV GLIR represents one minus the mean extraction of galanin during the 14 infusions; pancreatic extraction of galanin was 75%.

Because only the increment of FA GLIR during BTSNS is likely to be galanin (Fig. 2), the value of 75% extraction was applied only to that increment when calculating (see MATERIALS AND METHODS) GLIR spillover. Figure 5 depicts the calculated net pancreatic spillover of GLIR during BTSNS. GLIR spillover increased during BTSNS from a base line of 70 ± 50 fmol/min to 370 ± 60 at 5 min and to 470 ± 120 fmol/min at 10 min (Δ at 10 min = +410 ± 110 fmol/min, $n = 6$, $P < 0.01$), then returned to base line (100 ± 50 fmol/min) 15 min after the stimulation was teminated.

Pancreatic nerve stimulations during ganglionic blockade. Because of the complex calculations and assumptions involved in determining pancreatic galanin spillover during BTSNS, we sought a second, independent test of the hypothesis that galanin is released from the sympathetic nerves of the pancreas. Therfore, GLIR spillover was also measured during the selective activation of the pancreatic sympathetic nerves produced by local MPNS in the presence of the ganglionic blocker, HEX. Figure 6 depicts SPDV and FA levels of GLIR, SPDV blood flow, and pancreatic spillover of GLIR during these experiments. Pancreatic venous levels of GLIR increased rapidly and markedly during MPNS in the presence of HEX (Fig. 6*A*) from a base line of 84 ± 30 fmol/ml to 208 ± 55 at 5 min and to 187 ± 48 fmol/ml at 10 min (Δ at 5 min = +123 ± 34 fmol/ml, $n = 6$, $P < 0.01$), then returned to base line (78 ± 15 fmol/ml) 15 min after the nerve stimulation. In contrast, FA GLIR did not change significantly from base line (61 ± 11

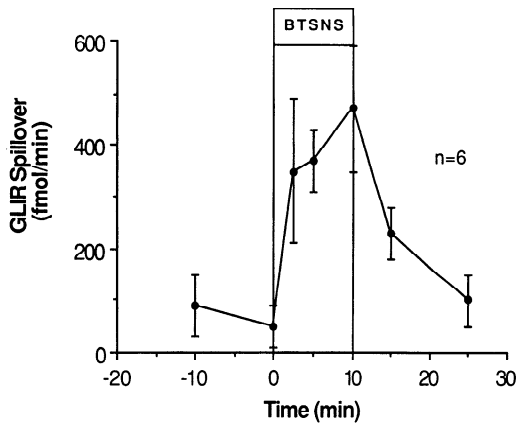

FIG. 5. Calculated pancreatic spillover of galanin-like immunoreactivity (GLIR) during bilateral thoracic splanchnic nerve stimulation (BTSNS) in dogs. Values are means ± SE, $n = 6$.

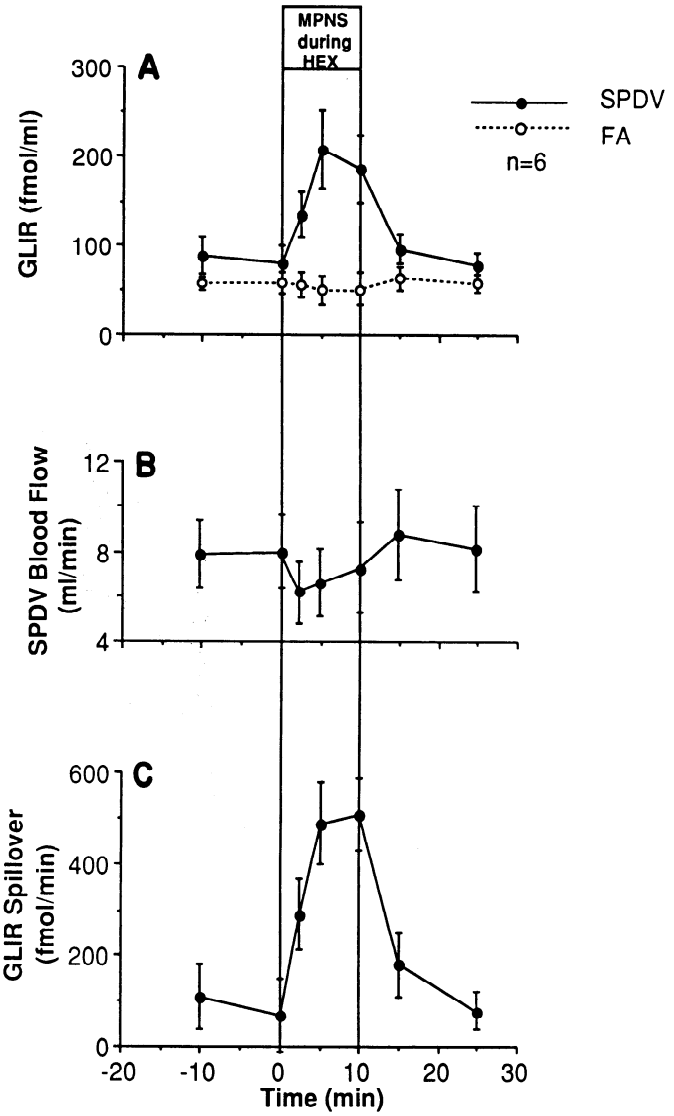

FIG. 6. Pancreatic venous (SPDV) and femoral arterial (FA) levels of galanin-like immunoreactivity (GLIR, *A*), SPDV blood flow (*B*), and calculated pancreatic spillover of GLIR (*C*) during local sympathetic nerve stimulation elicited by mixed autonomic pancreatic nerve stimulation (MPNS) in presence of ganglionic blocker, hexamethonium (HEX), in dogs. Values are means ± SE, $n = 6$.

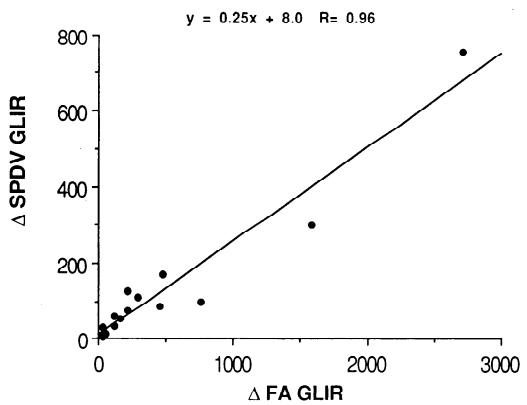

FIG. 4. Change of femoral arterial (FA) vs. pancreatic venous (SPDV) levels (fmol/ml) of galanin-like immunoreactivity (GLIR) during 14 intravenous infusions of synthetic porcine galanin at rates varying from 1.3 to 26 mol·kg^{-1}·min^{-1} in anesthetized dogs. Equation for regression line best fitted to these points is shown. Slope of this line represents 1 mean pancreatic galanin extraction.

fmol/ml) during the local sympathetic nerve stimulation. Pancreatic venous blood flow decreased during MPNS in the presence of HEX, from a base line of 8.0 ± 1.5 ml/min to a nadir at 2.5 min of 6.2 ± 1.4 ml/min (Δ at 2.5 min = −1.8 ± 0.7 ml/min, $P < 0.025$) but returned toward base line by the 10th min of MPNS (7.3 ± 2.0 ml/min). Thus, as shown in Fig. 6*C*, net spillover increased from a base line of 90 ± 80 fmol/min to 490 ± 90 at 5 min and to 510 ± 80 at 10 min during the nerve stimulation (Δ at 10 min = +420 ± 110 fmol/min, $P < 0.01$) and returned to base line (80 ± 40 fmol/min) 15 min after termination of the MPNS.

Figure 7 shows plasma catecholamine levels in the SPDV and FA during the MPNS in the presence of HEX, together with the net pancreatic spillover of NE. As depicted in Fig. 7*B*, SPDV NE increased during MPNS from a base line of 60 ± 10 pg/ml to 2,940 ± 860 at 5 min and to 2,280 ± 630 pg/ml at 10 min (Δ at 10 min = +2,210 ± 630 pg/ml, $n = 6$, $P < 0.01$), whereas FA NE levels did not change significantly from the base line of 80 ± 20 pg/ml. As shown in Fig. 7*B*, SPDV levels of EPI increased modestly during MPNS in the presence of HEX from a base line of 40 ± 10 to 210 ± 50 at 5 min and to 180 ± 40 at 10 min (Δ at 10 min = +140 ± 30 pg/ml, $P < 0.005$), and FA EPI levels did not change significantly from the base line of 70 ± 10 pg/ml. Figure 7*C* depicts net pancreatic spillover of NE that occurred during MPNS in the presence of HEX. NE spillover increased markedly from a base line of 10 ± 100 to 8,920 ± 2,640 at 5 min and to 6,950 ± 2,050 pg/min at 10 min during MPNS (Δ at 10 min = +6,930 ± 2,080 pg/min = +41 ± 12 pmol/min, $n = 6$, $P < 0.025$).

Potential hormonal actions of galanin. To determine if the increase of arterial GLIR that occurred during BTSNS (see Figs. 1 and 2) might influence pancreatic hormone secretion, synthetic galanin was infused systemically at a rate chosen to reproduce the increment of FA GLIR that occurred during BTSNS. Figure 8 illustrates the changes of FA GLIR, IRI output, and FA glucose during systemic infusion of galanin at a rate of 1.3 pmol·kg^{-1}·min^{-1}. As shown in Fig. 8*A*, FA GLIR

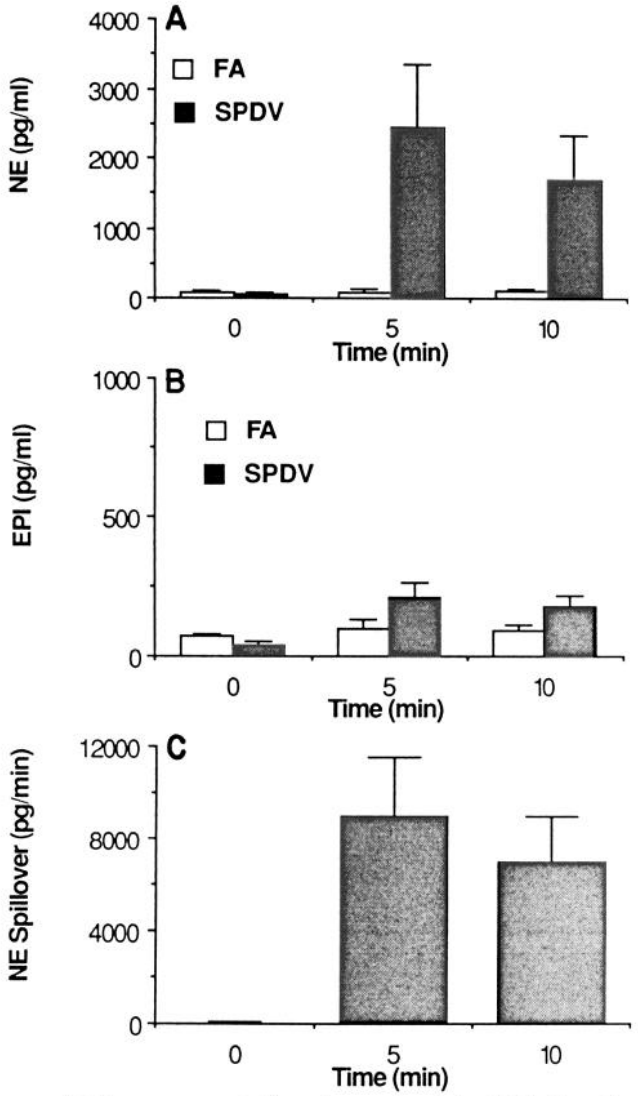

FIG. 7. Plasma concentration of norepinephrine (NE, *A*) and epinephrine (EPI, *B*) in pancreatic vein (SPDV) and femoral artery (FA) and pancreatic spillover of NE (*C*) before (*time 0*) and at 5 and 10 min during mixed pancreatic nerve stimulation in presence of hexamethonium. Values are means ± SE, $n = 6$.

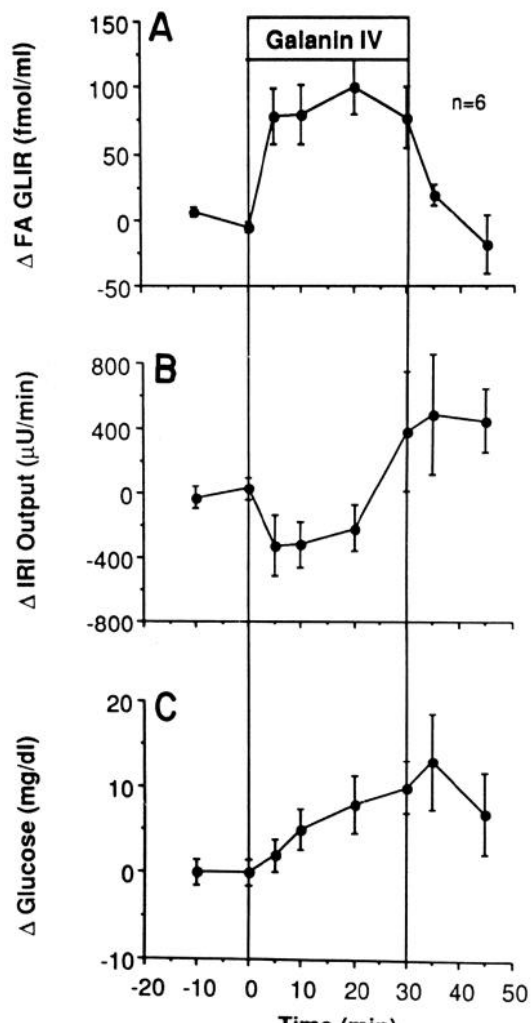

FIG. 8. Change of femoral arterial (FA) galanin-like immunoreactivity (GLIR), insulin (IRI) output, and FA glucose during 30-min intravenous infusions of synthetic porcine galanin at rate of 1.3 pmol·kg^{-1}·min^{-1}. Values are means ± SE, $n = 6$.

increased during systemic galanin infusion from a base line of 65 ± 18 fmol/ml to a mean over the 30-min infusion of 169 ± 14 fmol/ml (Δ = +84 ± 21 fmol/ml, n = 6, $P < 0.01$), then returned nearly to base line (84 ± 21 fmol/ml) 5 min after terminating the infusion. This increment of FA GLIR was very similar to that measured during BTSNS (+92 ± 31 fmol/min, see Fig. 1). As shown in Fig. 8*B*, basal IRI output from the duodenal lobe of the dog pancreas decreased modestly during systemic galanin infusion from a base line of 1,000 ± 230 μU/min to a nadir at 10 min of 685 ± 220 μU/min (Δ at 10 min = −320 ± 140 μU/min = −31 ± 9%, $P < 0.01$). IRI output recovered above base line at 30 min during the infusion (Δ at 30 min = +380 ± 370 μU/min, P = NS). Figure 8*C* depicts the change of glucose levels that occurred during systemic galanin infusion. Glucose levels increased from a base line of 96 ± 9 mg/dl to a peak at 30 min of 106 ± 11 mg/dl (Δ at 30 min = +10 ± 3 mg/dl, $P < 0.025$) and returned toward base line (103 ± 12 mg/dl) 15 min after the infusion. Glucagon output did not change significantly during the systemic galanin infusion (data not shown).

DISCUSSION

The 29-amino acid peptide, galanin, has been shown previously to meet several of the criteria necessary to be considered a sympathetic neurotransmitter in the endocrine pancreas (9). Earlier findings that the effects of sympathetic neural activation on basal pancreatic hormone secretion cannot be explained solely by the actions of NE (3, 8) lent import to the question of whether galanin is released from pancreatic sympathetic nerves. The present study was initiated to address this question by measuring the pancreatic spillover of GLIR and NE during sympathetic neural activation produced by two independent experimental methods.

The first approach was to selectively activate preganglionic sympathetic fibers by electrically stimulating the thoracic splanchnic nerves at the level of T_{10}. During this BTSNS, arterial (FA) and pancreatic venous (SPDV) levels of both GLIR and NE increased rapidly and markedly. Because the magnitude of the increases in the FA and SPDV were very similar (in each case) it might be concluded erroneously that neither NE nor GLIR was released from pancreatic nerves. However, we have previously shown that the pancreas avidly extracts NE (1). Thus the majority of NE in arterial plasma does not contribute to NE concentrations in the pancreatic venous effluent and therefore most of the increment of SPDV NE represents NE of pancreatic origin. Because pancreatic fractional extraction of EPI is very similar to that of NE (1), but the pancreatic nerves usually release little EPI, pancreatic extraction of EPI can be used as an ongoing index of NE extraction when the arterial [EPI] is high. By means of this index of NE extraction, net pancreatic spillover of NE was found to increase during BTSNS, by ~18 pmol/min, despite the lack of an AV [NE] difference across the pancreas. With a more direct measure of pancreatic NE extraction, the AV difference of infused [^{3}H]NE, we have previously reported that BTSNS produces a very similar increase (+16 pmol/min) of calculated pancreatic NE spillover (12).

As was the case for NE, both SPDV and FA levels of GLIR increased during BTSNS. However, we had no ongoing index of galanin extraction to employ for calculation of GLIR spillover in these experiments. Therefore, it was necessary to assess pancreatic galanin extraction in separate experiments by use of exogenous galanin infusions in the absence of changes of endogenous galanin. Doing so, we found that the pancreas extracts GLIR, as it does NE, at a high and constant rate. Measuring simultaneous changes of FA and SPDV GLIR levels during systemic galanin infusion, we found that the pancreas extracts 75% of the circulating GLIR in one pass. This value is very similar to that previously reported for NE (1) and that we found for EPI (74 ± 4%) in the present experiments.

Although it is now accepted that an adrenergically innervated organ extracts catecholamines, presumably by a combination of the well-described neuronal reuptake (*uptake 1*) and tissue uptake (*uptake 2*) (13), initially we found it surprising that the pancreas so avidly extracts a peptide neurotransmitter. However, the mechanism of galanin extraction remains to be determined. It is possible, although unlikely, that there exists a mechanism for peptide recycling by the nerve terminals, analogous to *uptake 1* for NE. It would seem more likely that pancreatic galanin extraction occurs by binding to specific receptors on the target organ (as have been described recently on an insulin-secreting cell line; Ref. 16) and either internalization or degradation into nonimmunoreactive and presumably nonbioactive forms. The finding that galanin is extracted by the pancreas suggests the existence of a mechanism to rapidly inactivate neurally released galanin, keeping its effects local. That there exists such a local mechanism for terminating galanin actions suggests that galanin meets another criterion to be considered a neurotransmitter in the endocrine pancreas. Indeed, this criterion is not often considered in the context of a peptide neurotransmitter, but it is one that has historically been set forth for classical neurotransmitters such as NE and acetylcholine.

Having established that the pancreas extracts synthetic galanin and that the increment of arterial GLIR that occurs during BTSNS coelutes with synthetic galanin, we assumed that the endogenous circulating GLIR was subject to the same pancreatic extraction. Therefore we employed the measured value for extraction (75%) to calculate net pancreatic spillover of GLIR during BTSNS. It was thus determined that there is little net GLIR spillover in the basal state but that pancreatic GLIR spillover increased markedly during the general sympathetic neural activation produced by BTSNS. In light of the pancreatic GLIR release during sympathetic activation, it may be concluded that some and possibly all of the pancreatic "galanergic" nerves are sympathetic.

During BTSNS, IRG secretion was enhanced and IRI output was inhibited; initially there was an absolute decrease below base line, and later insulin secretion was restrained relative to the ongoing hyperglycemia. The known potency of galanin to inhibit basal IRI secretion

(7) and the lack of effect of NE by itself to inhibit basal IRI secretion in vivo in dogs (3) suggest that the neurally released galanin could play a primary role in mediating the early inhibition of basal IRI output that occurred during BTSNS, even though the magnitude of GLIR spillover observed during BTSNS (+410 fmol/min) was only 2.5% of that of NE on a molar basis. It must be allowed, however, that the changes of pancreatic hormone secretion induced by BTSNS are unlikely to be mediated by a single factor. Rather, they probably reflect the net effect of several mediators capable of influencing the endocrine pancreas, including both α- and β-adrenergic effects of the circulating EPI and NE, locally released NE, direct effects of glucose, insulin-inhibiting and glucagon-stimulating effects of locally released galanin, and possibly even modest effects of the circulating galanin (see below).

Although the experiments discussed thus far suggest that galanin is released from pancreatic sympathetic nerves, and may play a role in mediation of the islet response, we sought to reinforce this conclusion with a second approach because the determination of net pancreatic galanin spillover during BTSNS necessitated the use of rather complex calculations and assumptions. Therefore, GLIR spillover was also measured during MPNS in the presence of HEX. This ganglionic blocker was employed in a protocol that has been shown previously to eliminate all endocrine pancreatic effects of direct electrical stimulation of the thoracic vagal nerves (2) and that would therefore be expected to block the intrapancreatic ganglionic transmission of impulses generated by activating preganglionic parasympathetic fibers coursing with the sympathetic fibers in the pancreatic arterial sheath.

Because this local sympathetic nerve stimulation did not raise arterial levels of NE or GLIR, calculation of increases of net spillover did not require estimation of the contribution of arterial NE or galanin to that measured in the SPDV. With this simpler calculation of neurotransmitter output, MPNS in the presence of HEX was found to increase pancreatic spillover of both NE and GLIR. The increment of GLIR spillover observed during MPNS in the presence of HEX was nearly identical to that found to occur during BTSNS. Interestingly, the increment of NE that occurred during MPNS in the presence of HEX was nearly threefold the increment observed during BTSNS. Therefore, the molar ratio of NE to galanin released during MPNS in the presence of HEX was slightly >100:1. This ratio is quite similar to that we found previously to occur during MPNS in the absence of HEX (10).

The finding that MPNS in the presence of HEX releases more NE than does BTSNS, but that the two modes of sympathetic activation release the same amount of GLIR, allows for some limited speculation regarding the nature of the pancreatic sympathetic innervation. Specifically, it suggests that not all pancreatic noradrenergic nerves contain galanin. However, colocalization of galanin and NE in a subpopulation of noradrenergic nerves remains a viable possibility. In addition, it would appear that all preganglionic input to pancreatic galanergic nerves arises above the level of the present BTSNS, and thus is activated by BTSNS, whereas additional NE-containing nerves arise below the level of the BTSNS and are activated only during MPNS. Clearly, it will take both immunocytochemical and further electrophysiological studies to corroborate such speculation.

The present observation, that two different modes of sympathetic neural activation elicited GLIR spillover into the pancreatic venous effluent, suggests that the pancreatic galanergic nerves are extrinsic, postganglionic sympathetic fibers. This is consistent with our earlier studies in which we found galanin-containing fibers innervating dog islets, but no cell bodies in the pancreas containing galanin-like immunoreactivity (7). This classification of galanin nerves is also consistent with a recent description by Kummer (15), of dense galanin staining in cell bodies of paravertebral sympathetic ganglia in cats. Interestingly, in that report, many of the galanin cells also contained neuropeptide Y (NPY). Because dog islets also contain NPY-positive fibers (6), it would be of interest to determine if these two peptides coexist in pancreatic sympathetic nerves. In apparent contrast to the galanin-staining studies in dogs and to the present data demonstrating galanin release during sympathetic neural activation, Su et al. (20) suggest that the few galanergic nerves in rat islets are exclusively intrinsic. These differences remain unresolved but are likely to represent species differences in pancreatic galanergic innervation.

Returning briefly to the generalized sympathetic activation produced by BTSNS, the unexpected increase of circulating GLIR that occurred raised two additional questions. The first issue, the origin of the arterial GLIR, was not addressed directly in this study. However, because an equivalent increase of pancreatic spillover of GLIR occured during BTSNS and local sympathetic stimulation but the local MPNS during HEX did not raise circulating GLIR, it is unlikely that pancreatic GLIR could make a significant contribution to the circulating GLIR measured during BTSNS. Therefore, it appears that generalized sympathetic activation induced by BTSNS released galanin from extrapancreatic as well as pancreatic sources. Because *1*) galanin-immunoreactive nerves are present throughout the gastrointestinal (GI) tract of several mammalian species including the dog (11, 20), *2*) we have found significant amounts of GLIR in extracts of various portions of the canine GI tract (unpublished observations), and *3*) BTSNS would be expected to activate sympathetic nerves of the entire splanchnic bed, it is possible that enough galanin would be released from the GI tract during BTSNS to account for the increase of peripheral levels. It may also be postulated that the circulating galanin derived at least in part from the adrenal. First, in the dog (unpublished observations), as in several other species including humans (4) but excluding rat (14), the adrenals contain high levels of GLIR. Second, BTSNS clearly activates neural input to the adrenal, evidenced by the robust EPI response observed in the present study.

Whether derived from gut nerves or the adrenals or

both, the increase of circulating GLIR seen during BTSNS also raised the question of whether galanin could act as a circulating hormone, contributing to the changes of pancreatic hormone release observed during BTSNS. To address this question, synthetic galanin was infused at a rate that reproduced the BTSNS-induced increase of arterial GLIR. During these matched infusions, the output of IRI was marginally inhibited, glucose levels increased modestly, and IRG output tended to increase (data not shown). Thus, under some circumstances, possibly severe stress, galanin may contribute to humoral regulation of islet function. However, it must be allowed that the halothane-anesthetized laparotomized dogs are inherently stressed, judging by the high "basal" EPI levels observed (730 pg/ml, Fig. 3) that were markedly lower in the presence of HEX (70 pg/ml, Fig. 7). Because we could detect no galanin-sized GLIR in the "basal" state before BTSNS (Fig. 2), an endocrine function for galanin during mild stress seems unlikely, and even during severe stress such as that reproduced by BTSNS, a hormonal role would appear quantitatively less important than galanin's putative role as a local sympathetic neurotransmitter.

In summary, an increasing body of evidence suggests that galanin is a sympathetic neurotransmitter in the endocrine pancreas of the dog. *1*) Galanin is present in islet nerves (7). *2*) Galanin can exert potent, direct, sympathomimetic effects on the endocrine pancreas (7, 17). *3*) There are receptors for galanin on insulin-secreting cell lines (16). *4*) Galanin is released from pancreatic nerves in quantities sufficient to inhibit insulin and somatostatin and to stimulate glucagon release (10). Finally, as suggested by the present studies, *5*) there exists a local mechanism to terminate galanin's actions, consistent with a role as a local neurotransmitter, and *6*) sympathetic nerves of the pancreas release galanin. Further studies are needed to determine the physiological or pathophysiological conditions under which pancreatic galaninergic nerves are activated and to definitively establish galanin's role by blocking the actions of endogenous galanin under those conditions.

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