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Corelease of galanin and NE from pancreatic sympathetic nerves during severe hypoglycemia in dogs

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Havel, Peter J., Thomas O. Mundinger, Richard C. Veith, Beth E. Dunning, and Gerald J. Taborsky, Jr. Corelease of galanin and NE from pancreatic sympathetic nerves during severe hypoglycemia in dogs. *Am. J. Physiol.* 263 (*Endocrinol. Metab.* 26): E8–E16, 1992.—To determine whether norepinephrine (NE) and galanin are coreleased during reflex activation of the sympathetic nervous system by hypoglycemia, we administered insulin to halothane-anesthetized (0.8%) dogs and measured the spillover of NE and galanin-like immunoreactivity (GLIR) into pancreatic venous plasma. Insulin injection produced hypoglycemia [plasma glucose (PG) = 34 ± 3 mg/dl] but did not activate pancreatic noradrenergic (Δ pancreatic NE output = $+20 \pm 130$ pg/min) or galaninergic nerves (Δ GLIR output = $+40 \pm 50$ fmol/min). To determine whether more severe hypoglycemia would activate these nerves, insulin was administered to dogs infused with somatostatin (SS; 2.5 μ g/min) to block the counterregulatory increase of glucagon secretion. SS reduced the glucagon response to hypoglycemia by >90%, which allowed PG to decrease to 14 ± 1 mg/dl. Pancreatic NE output increased by 470 ± 140 pg/min ($P < 0.005$); however, pancreatic GLIR output did not increase significantly ($\Delta = +70 \pm 50$ fmol/min). When SS was discontinued, pancreatic NE output increased by 490 ± 200 pg/min ($P < 0.025$), and GLIR output increased by an additional $+160 \pm 70$ fmol/min ($P < 0.025$; total Δ from baseline = $+230 \pm 90$ fmol/min, $P < 0.025$), suggesting that SS may restrain pancreatic NE and galanin release. Pancreatic NE and GLIR spillover were also increased during severe hypoglycemia when ganglionic neurotransmission was partially impaired with hexamethonium but not when the neural pathway was interrupted by spinal cord transection. We conclude that NE and galanin are coreleased from pancreatic sympathetic nerves when these nerves are centrally activated during severe hypoglycemia in halothane-anesthetized dogs.

norepinephrine; neuropeptide; somatostatin; glucagon; hexamethonium; halothane anesthesia; spinal cord transection; neuroglucopenia; stress; catecholamines

IT IS WELL ESTABLISHED that sympathetic nerves innervating the pancreatic islets contain and release the classic neurotransmitter norepinephrine (NE) (21, 30). However, recent evidence suggests that the peptide galanin may also be a sympathetic neurotransmitter in the canine pancreas (22). First, galanin-like immunoreactivity (GLIR) is present in sympathetic nerves innervating pancreatic islets (1, 38, 46). Second, the administration of synthetic galanin qualitatively reproduces the changes of pancreatic islet hormone secretion observed during electrical sympathetic nerve stimulation (19, 42, 50, 52), whereas the administration of NE alone is not sufficient to mediate these changes of islet hormone secretion in dogs (5, 20). Finally, the amount of GLIR released during intense electrical stimulation

of sympathetic nerves appears sufficient to mediate the observed impairment of insulin secretion (23). Despite the evidence cited above, there has been no previous demonstration that galanin is in fact coreleased with NE during the presumably less intense reflex activation of pancreatic sympathetic nerves that occurs during systemic stress (30). Such a demonstration would add support to the hypothesis that galanin is a sympathetic coneurotransmitter in the canine pancreas.

Studies of another putative sympathetic neuropeptide that is released by sympathetic nerve stimulation (6, 11, 51), neuropeptide Y (NPY), suggest that neuropeptides may not always be coreleased with classic neurotransmitters, particularly at low neuronal firing rates. Thus low frequencies of electrical stimulation of sympathetic nerves innervating porcine spleen or moderate exercise in humans both produce little NPY release (39, 45), despite significant NE release. Likewise, continuous electrical stimulation of the splanchnic nerves in calves results in much less NPY release than does burst stimulation of the same average frequency (10). There is apparent morphological support for these findings, in that NPY is contained in the large dense granules of sympathetic nerves and not in the small vesicles that also contain NE (7, 18). The implication is that the large granules are recruited for release predominately during intense neural activation. Thus, if galanin is a sympathetic neuropeptide like NPY, it might not be coreleased with NE during the reflex activation of the pancreatic sympathetic nerves that occurs during neuroglucopenic stress (30).

Therefore, to determine whether NE and GLIR are coreleased from the sympathetic nerves of the pancreas during stress, we measured pancreatic NE and GLIR output (spillover) via an extracorporeal pancreatic venous-portal vein shunt during two grades of insulin-induced hypoglycemia in acute terminal experiments performed in halothane-anesthetized laparotomized dogs. In one group of dogs, insulin was administered by itself, resulting in hypoglycemia with a glucose level of ~ 35 mg/dl. In a second experiment, insulin was administered to another group of dogs, in which somatostatin (SS) was infused to inhibit the counterregulatory glucagon response, and thus more severe hypoglycemia was produced (≤ 15 mg/dl).

An additional experiment was performed to confirm that increased pancreatic NE and GLIR responses occur during severe hypoglycemia. In this experiment, severe hypoglycemia was produced by partially blocking hypoglycemia-induced autonomic activation, which, in the

dog, is a major determinant of the glucagon response (31); therefore the glucose nadir after insulin injection is lowered. The autonomic response was reduced by administration of the ganglionic blocking agent hexamethonium. Because the dose employed was submaximal by design, this intervention still allowed activation of pancreatic sympathetic nerves in response to hypoglycemia.

Last, to determine whether increased NE and galanin output resulted from a central reflex that activates pancreatic sympathetic nerves (30) or was possibly due to a local effect of severe hypoglycemia on pancreatic sympathetic nerve terminals (15, 35), we produced equivalent severe hypoglycemia in dogs, in which the sympathetic neural pathway was surgically interrupted at the level of the cervical spinal cord.

METHODS

Animals and pancreatic preparation. After an overnight fast (~18 h), adult dogs of mixed breed (24–40 kg) were anesthetized with the ultrashort-acting barbiturate thiamylal sodium (Surtal; Parke Davis, Morris Plains, NJ). Anesthesia was subsequently maintained with halothane (0.8%) administered from a calibrated vaporizer (Draeger, FRG) by mechanical ventilation in 100% oxygen. This anesthetic regimen was chosen because it has been shown to provide full surgical anesthesia but does not suppress parasympathetic or sympathoadrenal activation induced by the neuroglucopenic agent 2-deoxy-D-glucose (28, 29) as do some other anesthetics, e.g., pentobarbital sodium (53, 54).

To access pancreatic venous blood, a laparotomy was performed, and an extracorporeal Silastic (Dow Corning, Midland, MI) shunt containing a sampling port, an electromagnetic flow probe (In Vivo Metric Systems, Healdsburg, CA), and a heparin infusion line were placed between the superior pancreaticoduodenal vein (SPDV) and the portal vein (5). This procedure allows the measurement of hormone output and neurotransmitter spillover from the right lobe (duodenal lobe and uncinate process) of the canine pancreas (~35–50% of the pancreas). This preparation also receives a portion of the venous drainage from the proximal duodenum. To minimize the duodenal contribution to SPDV blood, the main duodenal input to the SPDV was isolated and ligated between the pancreas and duodenum. The femoral artery (FA) and vein were cannulated for blood sampling and drug infusion, respectively. Saline (0.9%) was infused intravenously at a slow rate throughout the surgery and the experiments.

All surgery and experiments were performed in the presence of full surgical anesthesia. These experiments were acute terminal procedures. At the conclusion of each day's experimental protocol, each animal used in these studies was killed with an overdose of barbiturate anesthetic, thiopental sodium (Pentothal; Abbott Laboratories, North Chicago, IL), without regaining consciousness.

Hypoglycemia protocol. To determine whether hypoglycemia would produce central reflex activation of pancreatic noradrenergic and galaninergic nerves, insulin was administered to four groups of animals as follows: control, SS-infused, hexamethonium-treated, and cord-sectioned dogs. One hour after the surgical procedures, paired arterial and pancreatic venous blood samples for glucose, glucagon, catecholamine, and galanin determination were drawn from the FA and from the pancreatic venous shunt at –10 and 0 min before insulin injection to establish baseline values for the subsequent experiments. To produce acute marked hypoglycemia in control dogs, a bolus of regular porcine insulin (Squibb-Novio, Bagsvaerd, Denmark)

was administered into the femoral vein cannula at a dose of 5.0 ± 1.4 U/kg (range 1.2–12.0 U/kg). Doses were chosen to decrease plasma glucose to <40 mg/dl in all control dogs. The SS-infused dogs received 3.7 ± 1.2 U/kg of insulin (range 1.2–12.0 U/kg). Hexamethonium-treated dogs or cord-sectioned dogs received less insulin (0.6 U/kg) because, in these animals, lower doses were required to produce decrements of plasma glucose that were larger than those in the control dogs. Plasma glucose fell below 25 mg/dl in all SS-infused, hexamethonium-treated, and cord-sectioned dogs. Paired arterial and pancreatic venous blood samples were drawn at 5, 15, 30, 35, 40, 45, 50, 55, and 60 min after insulin injection. After 60 min, the hypoglycemia was reversed by administering glucose (50% solution) intravenously as a bolus of 200 mg/kg followed by an infusion of $10\text{--}20 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ to equal or exceed baseline arterial glucose levels for 30 min. Paired samples were drawn at 5, 15, and 30 min after the start of the glucose infusion. In all experiments, blood flow in the pancreatic venous shunt was monitored with an electromagnetic flowmeter (Zepeda Instruments, Seattle, WA). Hematocrit was determined at regular intervals throughout the experiments.

SS infusion study. To lower the plasma glucose further than in the control dogs, the counterregulatory glucagon response to hypoglycemia was prevented in 11 experiments by infusing synthetic cyclic SS-14 (Bachem, Torrance, CA) intravenously at a rate of 2.5 $\mu\text{g}/\text{min}$.

The hypoglycemia protocol in the SS-infused dogs differed somewhat from the protocol in the other three groups. Two pre-SS samples were drawn at 1 h postsurgery, after which SS was infused for 20 min before the injection of insulin and continued until 60 min after the insulin injection. Samples were drawn at 5, 10, and 20 min into the SS infusion and at 5, 15, 30, 45, 50, 55, and 60 min postinsulin. The SS infusion was then discontinued, and three additional samples were drawn at 65, 70, and 75 min postinsulin, after which glucose was infused for 30 min to reverse the hypoglycemia as in the other experimental protocols.

Hexamethonium experiment. To produce severe hypoglycemia by a second independent method, a submaximal dose of the ganglionic blocking agent hexamethonium was administered before the injection of insulin. Hexamethonium impairs neurotransmission across autonomic ganglia and the adrenal medulla by interfering with postsynaptic signaling via nicotinic receptors (56). Previous data had demonstrated that this dose of hexamethonium substantially diminished hypoglycemia-induced autonomic activation and thereby markedly impaired glucagon responses to hypoglycemia, resulting in a lower glucose nadir after insulin injection (<15 mg/dl) (31). However, this dose of hexamethonium still allows a significant degree of sympathetic nervous system activation during hypoglycemia, as reflected by an increase of arterial plasma NE levels (31). Hexamethonium bromide (Sigma Chemical, St. Louis, MO) was administered intravenously (20–30 min before baseline samples were drawn) in increments until arterial blood pressure was clearly decreased in each animal. Each dog was given repetitive 0.1 mg/kg doses until the blood pressure was decreased by at least 10 mmHg, 90 s after the injection. After the bolus injections, an increment of $0.7 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, for each 0.1 mg/kg administered initially, was infused continuously throughout the experiments (3). Consequently, the dose of hexamethonium was variable and ranged from 0.2 to 0.5 mg/kg plus 1.4 to 3.5 $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$.

Surgical interruption of sympathetic outflow. To determine whether the increased release of pancreatic NE and GLIR was due to a centrally mediated reflex activation of the sympathetic outflow to the pancreas or to a local effect of low plasma glucose levels directly on pancreatic sympathetic nerves, cervical spinal cord transection was performed in six dogs. This procedure

interrupts the sympathetic neural pathway from the brain to the pancreas and adrenal medulla, impairs the counterregulatory increases of glucagon and plasma catecholamines during hypoglycemia, and thereby allows plasma glucose to fall to extremely low levels (31). In these animals, the cervical spinal cord was exposed through a dorsal midline incision by removing part of the spinous process and the arch of the axis. To prevent activation of any nociceptive reflexes, 3 ml of 2% lidocaine hydrochloride were injected with a 25-gauge needle directly into the exposed spinal cord in several locations. Five minutes later, 2–3 cm of the cord were excised to ensure complete interruption of spinal efferents. Bleeding was controlled by tightly packing the vertebral foramen with gauze soaked in mineral oil. These acute terminal procedures were performed in the presence of full surgical anesthesia. At the conclusion of the experiments, each animal was killed with an overdose of anesthetic, without regaining consciousness.

Assays. Blood samples for glucose determination were drawn and placed in tubes containing EDTA. Blood samples for glucagon determination were placed in tubes containing heparin and benzamidine hydrochloride. Blood samples for catecholamine determination were placed in tubes containing ethylene glycol-bis(β -aminoethyl ether)- N,N,N',N' -tetraacetic acid (EGTA) and glutathione. Blood samples for GLIR measurement were drawn and placed in tubes containing a solution of anticoagulants and proteolytic enzyme inhibitors (17). All samples were kept on ice until centrifugation (2,500 revolutions/min for 20 min at 4°C). The plasma was then decanted and frozen at -20°C until assayed.

Plasma glucose was measured by the glucose oxidase method with a Technicon autoanalyzer (Technicon Instrument, San Francisco, CA). Plasma immunoreactive glucagon was measured radioimmunologically in unextracted plasma with an antibody that has high specificity for the COOH-terminal portion of the glucagon molecule (55). It has been previously demonstrated that extrapancreatic immunoreactive glucagon measured with COOH-terminal-specific antisera does not increase during insulin-induced hypoglycemia in dogs (41, 44). Thus measurements of arterial glucagon made with this assay are a reliable index of pancreatic glucagon secretion. Plasma NE was measured in duplicate with a highly sensitive and specific radioenzymatic assay (24). The intra- and interassay coefficients of variation for the plasma catecholamine assay in this laboratory are 6 and 12%, respectively. GLIR was measured in unextracted plasma with a radioimmunoassay with a non-COOH-terminal-directed antibody raised against synthetic porcine galanin and with synthetic porcine galanin standards as previously described in detail (23). Because the species-variable portion of the molecule is apparently limited to the COOH-terminal heptapeptide (36, 48), this assay detects GLIR in all species thus far examined.

Calculations and data analysis. The changes in arterial plasma glucose and glucagon, arterial and pancreatic venous NE and GLIR, pancreatic vein blood flow and hematocrit (Hct), and pancreatic NE and GLIR output were calculated by subtracting the mean of the -10- and 0-min preinsulin baseline values from the mean of the 45-, 50-, 55-, and 60-min values after the injection of insulin, except in the SS-infused dogs, in which the mean of the 50-, 55-, and 60-min samples was used. The changes following the discontinuation of SS infusion were calculated by subtracting the mean baseline values from the mean of the three samples drawn at 5, 10, and 15 min after the SS infusion was discontinued (65, 70, and 75 min postinsulin).

Because both circulating NE (2) and galanin (23) are extracted by the pancreas, most of the transmitter arriving via the arterial circulation does not appear in pancreatic venous plasma, and therefore simple measurements of arteriovenous (a-v) concentration differences will seriously underestimate

pancreatic neurotransmitter release. To correct the a-v difference for extraction, neurotransmitter spillover was calculated as follows

$$\{[\text{NE or GLIR}]_{\text{SPDV}} - (\text{arterial contribution to SPDV level})\} \\ \times \text{SPDV blood flow} \times (1 - \text{Hct})$$

where square brackets indicate concentration. The arterial contribution to the SPDV level is defined as the amount of neurotransmitter present in SPDV plasma that arrives via the arterial circulation and escapes pancreatic extraction. Because epinephrine (Epi) is extracted at a rate similar to NE (2), Epi extraction was used as an index of NE extraction and was employed to calculate the arterial contribution to SPDV NE levels when arterial Epi levels were high. When arterial Epi levels were very low, such as during the baseline period before hypoglycemia in cord-sectioned animals, Epi extraction is not a reliable index of NE extraction. NE extraction during such baseline conditions has been measured and found to average 75% with little variability. Therefore an extraction rate for NE of 75% was used to calculate NE output at time points when Epi levels were low.

Thus the arterial contribution to SPDV NE levels is calculated as

$$[\text{NE}]_{\text{FA}} \times (1 - \text{pancreatic Epi extraction or } 1 - 0.75)$$

pancreatic Epi extraction is calculated as

$$([\text{Epi}]_{\text{FA}} - [\text{Epi}]_{\text{SPDV}})/[\text{Epi}]_{\text{FA}}$$

In the case of galanin, in the basal state, nearly all plasma GLIR represents a large-molecular-weight cross-reactant in the radioimmunoassay (23). It is present in approximately equal amounts in FA and SPDV plasma and thus does not appear to be subject to pancreatic extraction (21). Therefore only the increment over the basal FA level was considered to be true galanin and used in the extraction/spillover calculations. Pancreatic extraction of infused exogenous galanin has been measured and found to average 65% over a wide range of plasma galanin concentrations (23). Thus pancreatic galanin spillover was calculated as follows

$$\text{spillover} = ([\text{GLIR}]_{\text{SPDV}} - [\text{GLIR}]_{\text{basal FA}}) \\ + [0.35([\text{GLIR}]_{\text{FA}} - [\text{GLIR}]_{\text{basal FA}})] \\ \times \text{SPDV blood flow} \times (1 - \text{Hct})$$

The data are expressed as means $\pm$ SE. Statistical comparisons of means within a group were made with a paired *t* test. Statistical comparisons of means of different groups were made with a two-sample *t* test. For multiple comparisons between more than two groups, analysis of variance was performed, and Dunnett's posttest was employed to determine significant differences. The correlation between the mean changes of NE and galanin output was made using linear regression analysis.

RESULTS

Arterial glucose, glucagon, and NE and pancreatic NE and GLIR output responses in control dogs. In control dogs ($n = 9$) the intravenous administration of insulin (5.0 ± 1.4 U/kg) caused plasma glucose to decrease by 77 ± 4 mg/dl to a mean level of 34 ± 3 mg/dl at 45–60 min postinsulin injection (Table 1). The mean nadir was 30 ± 2 mg/dl at 60 min after the insulin injection. Arterial plasma glucagon increased by $+52 \pm 14$ pg/ml ($P < 0.005$, Table 1), and arterial plasma NE levels increased by

Table 1. Baseline arterial glucagon and glucose concentrations and responses during hypoglycemia

Treatment	n	Arterial Glucagon, pg/ml			Arterial Glucose, mg/dl	
		Baseline	$\bar{X}$ 45-60 min	Δ 45-60 min	Baseline	$\bar{X}$ 45-60 min
Control dogs	9	27 $\pm$ 3	80 $\pm$ 15	+52 $\pm$ 14*	110 $\pm$ 3	34 $\pm$ 3
SS infused	11	25 $\pm$ 4	30 $\pm$ 4	+5 $\pm$ 2†	103 $\pm$ 4	14 $\pm$ 1†
Hexamethonium	6	32 $\pm$ 7	37 $\pm$ 5	+5 $\pm$ 4†	83 $\pm$ 4	12 $\pm$ 2†
Spinal cord transection	6	26 $\pm$ 4	31 $\pm$ 8	+5 $\pm$ 7†	90 $\pm$ 7	15 $\pm$ 3†

Values are means $\pm$ SE at baseline and 45-60 min postinsulin injection; n, no. of dogs. SS, somatostatin. Δ , change from baseline. * $P < 0.005$ vs. baseline. † $P < 0.01$ vs. control dogs.

+570 $\pm$ 80 pg/ml ($P < 0.0005$; Table 2). Both arterial glucagon and NE decreased toward baseline during glucose infusion (data not shown).

Pancreatic NE output did not increase during hypoglycemia ($\Delta = +20 \pm 130$ pg/min; Fig. 1A and Table 3). Similarly, there was no significant increase of pancreatic GLIR output ($\Delta = +40 \pm 50$ fmol/min; Fig. 1B and Table 4).

Arterial glucose, glucagon, and NE and pancreatic NE and GLIR output responses after insulin injection in dogs infused with SS. Arterial plasma glucose before SS infusion was 108 $\pm$ 4 mg/dl ($n = 11$). After 10-20 min of SS infusion, before insulin injection, plasma glucose declined slightly to 103 $\pm$ 4 mg/dl ($\Delta = -5 \pm 2$ mg/dl, $P < 0.025$). After insulin injection (3.7 $\pm$ 1.2 U/kg), plasma glucose decreased to 14 $\pm$ 1 mg/dl ($\Delta = -89 \pm 4$ mg/dl), significantly lower than in control dogs, ($P < 0.01$; Table 1). The nadir was 13 $\pm$ 1 mg/dl at 60 min postinsulin injection. Plasma glucose began to increase 10 min after the discontinuation of the SS infusion and reached 29 $\pm$ 6 mg/dl by 15 min ($\Delta = +15 \pm 5$ mg/dl, $P < 0.01$ vs. during SS), after which glucose was infused to equal or exceed baseline levels. Arterial plasma glucagon averaged 32 $\pm$ 5 pg/ml before SS infusion. Plasma glucagon decreased to 25 $\pm$ 4 pg/ml ($\Delta = -7 \pm 3$ pg/ml, $P < 0.0025$) after 10-20 min of SS infusion. SS infusion prevented >90% of the arterial glucagon response to hypoglycemia observed in the control dogs ($\Delta = +5 \pm 2$ vs. +52 $\pm$ 14 pg/ml, $P < 0.01$; Table 1). When the SS infusion was discontinued, plasma glucagon increased rapidly to 71 $\pm$ 13 pg/ml at 15 min ($\Delta = +41 \pm 11$ pg/ml, $P < 0.0025$; data not shown). During the subsequent glucose infusion, arterial plasma glucagon decreased to 29 $\pm$ 5 pg/ml at 30 min. The arterial plasma NE response in SS-infused dogs was similar to that of control dogs; NE increased by +420 $\pm$ 110 pg/ml, $P < 0.0025$ at 50-60 min postinsulin injection

Table 2. Baseline arterial plasma NE concentrations and NE responses during hypoglycemia

Treatment	n	Arterial NE, pg/ml		
		Baseline	$\bar{X}$ 45-60 min	Δ 45-60 min
Control dogs	9	170 $\pm$ 30	740 $\pm$ 90	+570 $\pm$ 80*
SS infused	11	160 $\pm$ 20	560 $\pm$ 110	+420 $\pm$ 110*
Hexamethonium	6	110 $\pm$ 40	310 $\pm$ 70†	+200 $\pm$ 60*†
Spinal cord transection	6	30 $\pm$ 10†	30 $\pm$ 10†	0 $\pm$ 10†

Values are means $\pm$ SE at baseline and 45-60 min postinsulin injection. NE, norepinephrine. * $P < 0.025$ vs. baseline. † $P < 0.05$ vs. control dogs.

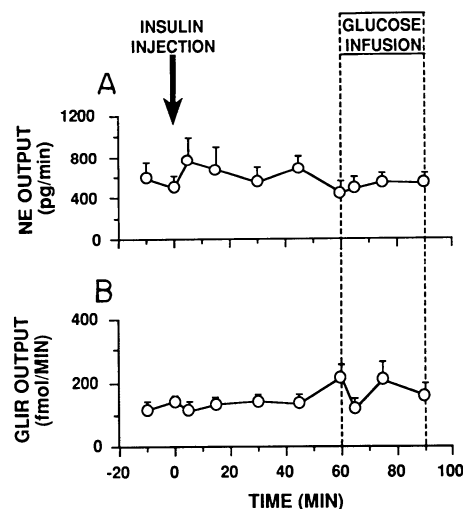


Fig. 1. Pancreatic output of norepinephrine (NE) and galanin-like immunoreactivity (GLIR) before, during, and after insulin-induced hypoglycemia in 9 experiments conducted in control dogs.

(Table 2). Arterial plasma NE decreased after glucose infusion (data not shown).

Pancreatic NE output increased significantly during hypoglycemia in SS-infused dogs, ($\Delta = +470 \pm 150$ pg/min at 50-60 min after insulin injection, $P < 0.005$; Fig. 2A and Table 3). Pancreatic NE output increased by an additional +490 $\pm$ 200 pg/min after the SS infusion was discontinued (total $\Delta = +970 \pm 280$ pg/min). Pancreatic GLIR output did not increase significantly during the SS infusion, despite severe hypoglycemia ($\Delta = +70 \pm 50$ fmol/min, $P < 0.05$); however, pancreatic GLIR output did increase substantially after the SS infusion was discontinued, ($\Delta = +160 \pm 70$ fmol/min, $P < 0.025$; total $\Delta = +230 \pm 90$ pg/min, $P < 0.025$; Fig. 2B and Table 4).

Arterial glucose, glucagon, and NE and pancreatic NE and GLIR responses after insulin injection in dogs pretreated with hexamethonium. After insulin injection (0.6 $\pm$ 0.1 U/kg; $n = 6$), in hexamethonium-treated dogs, plasma glucose declined to 12 $\pm$ 2 mg/dl ($\Delta = -71 \pm 3$ mg/dl), a decrement similar to that observed in control dogs. The nadir of 11 $\pm$ 1 mg/dl (60 min postinsulin injection) was significantly lower than in the control dogs ($P < 0.0005$). Arterial plasma glucagon did not increase significantly during hypoglycemia ($\Delta = +5 \pm 4$ pg/ml); this response was significantly less than in control dogs ($P < 0.01$; Table 1). The arterial plasma NE response to hypoglycemia was suppressed ($P < 0.05$ vs. control dogs) but not eliminated ($\Delta = +200 \pm 60$ pg/ml, $P < 0.025$) in the hexamethonium-treated dogs (Table 2).

Table 3. Plasma NE and pancreatic NE output before and during hypoglycemia

Treatment	n	pg/ml		BF _{SPDV} , ml/min	NE Output, pg/min
		NE _{FA}	NE _{SPDV}		
Control dogs	9				
Baseline		170±30	130±20	11.1±1.6	540±140
During insulin-induced hypoglycemia		740±90	240±20	8.4±1.3	560±100
ΔPancreatic NE output					+20±130
SS infused	11				
Baseline		160±20	110±10	12.2±1.0	420±250
During insulin-induced hypoglycemia		560±110	240±40	11.6±1.1	890±150
ΔPancreatic NE output					+470±150*
Post-SS		770±220	370±90	13.3±1.6	1,380±310
ΔPancreatic NE output					+970±280†
Hexamethonium	6				
Baseline		110±40	110±60	5.7±0.4	310±140
During insulin-induced hypoglycemia		310±70	300±80	5.0±0.6	770±260
ΔPancreatic NE output					+460±170‡
Spinal cord transection	6				
Baseline		30±10	40±10	9.5±1.2	190±70
During insulin-induced hypoglycemia		30±10	30±10	8.2±1.0	130±40
ΔPancreatic NE output					-70±50

Values are means ± SE, at 45–60 min postinsulin injection, except post-SS values that are 65–75 min postinsulin. FA, femoral artery; SPDV, superior pancreaticoduodenal vein; BF, blood flow. * $P < 0.01$. † $P < 0.005$. ‡ $P < 0.025$.

Table 4. Plasma and pancreatic GLIR output before and during hypoglycemia

Treatment	n	pg/ml		BF _{SPDV} , ml/min	GLIR Output, pg/ml
		GLIR _{FA}	GLIR _{SPDV}		
Control dogs	9				
Baseline		57±6	71±11	11.1±1.6	150±40
During insulin-induced hypoglycemia		61±12	91±18	8.4±1.3	190±40
ΔPancreatic GLIR output					+40±50
SS infused	11				
Baseline		84±13	107±18	12.7±0.4	330±80
During insulin-induced hypoglycemia		119±20	133±24	11.6±1.1	400±110
ΔPancreatic GLIR output					+70±50
During insulin-induced hypoglycemia, post-SS		124±23	155±30	13.3±1.6	560±140
ΔPancreatic GLIR output					+230±90*
Hexamethonium	6				
Baseline		46±8	67±18	5.7±0.4	140±50
During insulin-induced hypoglycemia		60±11	99±14	5.0±0.6	250±50
ΔPancreatic GLIR output					+110±30†
Spinal cord transection	6				
Baseline		55±10	89±11	9.5±1.2	290±50
During insulin-induced hypoglycemia		66±13	116±18	8.2±1.0	370±30
ΔPancreatic GLIR output					+80±50

Values are means ± SE at 45–60 min postinsulin injection, except post-SS values that are 65–75 min postinsulin. GLIR, galanin-like immunoreactivity. * $P < 0.025$. † $P < 0.01$.

Pancreatic NE output was increased significantly during hypoglycemia in the hexamethonium-treated dogs ($\Delta = +510 \pm 170$ pg/min, $P < 0.025$; Fig. 3A and Table 3). There was also a significant increase of pancreatic GLIR output during hypoglycemia in these animals ($\Delta = +110 \pm 30$ fmol/min, $P < 0.01$; Fig. 3B and Table 4).

Arterial glucose, glucagon, and NE and pancreatic NE and GLIR output responses after insulin injection in dogs with cervical spinal cord section. Plasma glucose in cord-sectioned dogs ($n = 6$) decreased to 15 ± 3 mg/dl at 45–60 min postinsulin ($P < 0.01$ vs. control dogs; Table 1) with a nadir of 12 ± 2 mg/dl at 60 min. The decrement was -76 ± 8 mg/dl. Arterial plasma glucagon did not increase significantly during hypoglycemia ($\Delta = +5 \pm 7$ pg/ml); this response was significantly less than that in control dogs ($P < 0.01$; Table 1). Arterial plasma NE also did not

change in these animals averaging 30 ± 10 pg/ml before and 30 ± 10 pg/ml 45–60 min after insulin injection ($P < 0.05$ vs. control dogs; Table 2).

Pancreatic NE output did not increase in these animals during the hypoglycemic period ($\Delta = -70 \pm 50$ pg/min; Fig. 4A and Table 3). Similarly, pancreatic GLIR output did not increase significantly during hypoglycemia in cord-sectioned dogs ($\Delta = +80 \pm 50$ fmol/min; Figure 4B, and Table 4).

Correlation of changes of pancreatic NE and GLIR output. The mean changes of NE output in control dogs, in SS-treated dogs (both during and immediately after the SS infusion was discontinued), in hexamethonium-treated dogs, and in cord-sectioned dogs were significantly correlated with the corresponding mean changes of GLIR output ($r = 0.8557$, $P < 0.025$, $n = 5$).

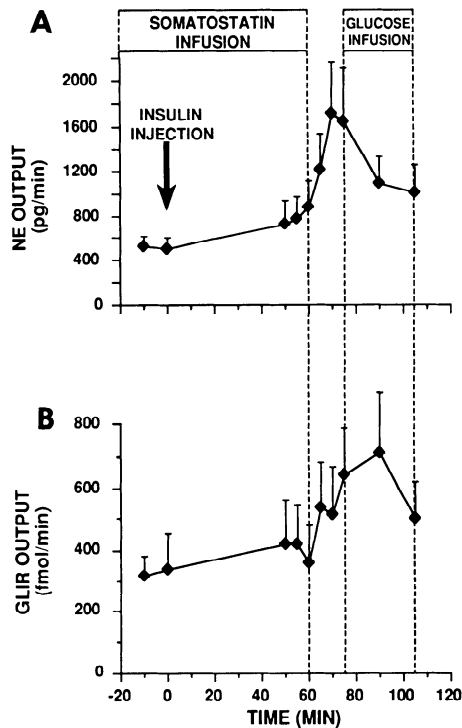


Fig. 2. Pancreatic output of NE and GLIR before, during, and after insulin-induced hypoglycemia in 11 experiments conducted in dogs infused with somatostatin.

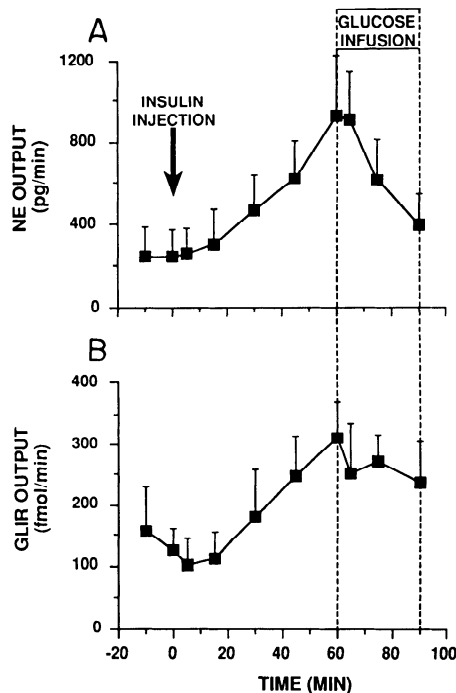


Fig. 3. Pancreatic output of NE and GLIR before, during, and after insulin-induced hypoglycemia in 6 experiments conducted in dogs pretreated with hexamethonium.

DISCUSSION

Recent experimental evidence suggests that the neuropeptide galanin may be a sympathetic neurotransmitter in the endocrine pancreas. Galanin is present in sympathetic nerves innervating the endocrine pancreas (1, 38, 46) and is released with NE during electrical activation of

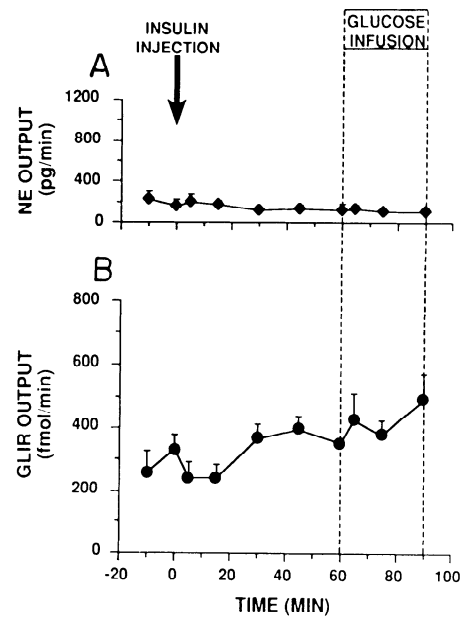


Fig. 4. Pancreatic output of NE and GLIR before, during, and after insulin-induced hypoglycemia in 6 experiments conducted in dogs with spinal cord transection.

these sympathetic nerves (21, 23). Because combined α - and β -adrenergic antagonists do not block all of the effects of electrical sympathetic nerve stimulation on pancreatic hormone secretion (9, 20, 37), the effects of sympathetic nerve activation on pancreatic islet function, which are not adrenergically mediated, could be mediated by galanin (4, 22).

Despite this evidence, there has been no demonstration that galanin is in fact coreleased with NE from the pancreas during endogenous reflex activation of pancreatic sympathetic nerves that occurs during certain types of stress (30). There is evidence to suggest that another neuropeptide, neuropeptide Y, may not be coreleased with NE in significant quantities during low-intensity neural activation (39, 45). Because endogenous nerve impulses that would occur during reflex neural activation are low-frequency potentials, it was important to determine whether galanin is coreleased with NE during the presumably low-intensity reflex activation of the sympathetic nervous system that might occur during stress. In a previous study, we demonstrated that central neuroglucopenic stress induced by administration of 2-deoxy-D-glucose activates the noradrenergic nerves innervating the pancreas (30). Therefore we chose the similar, but reversible, stress of insulin-induced hypoglycemia to activate pancreatic sympathetic nerves in halothane-anesthetized dogs.

In the first experiment (control dogs), insulin injection lowered the plasma glucose to a mean of 34 ± 3 mg/dl, yet neither pancreatic NE nor GLIR spillover increased. Because the administration of a large dose of 2-deoxy-D-glucose clearly increased pancreatic NE spillover in previous experiments (30), we hypothesized that more severe hypoglycemia might be necessary to activate pancreatic sympathetic nerves. However, we had observed that even high doses of insulin did not lower plasma glucose below 30 mg/dl in the control dogs. Therefore we infused SS to

block the counterregulatory glucagon response to hypoglycemia, which allowed the plasma glucose to fall to 15 mg/dl after insulin injection (32). During this severe hypoglycemia, pancreatic NE output was significantly increased 50–60 min after insulin injection. Pancreatic GLIR output did not increase significantly at this time, suggesting either that the change of galanin output was too small to be measured reliably or that pancreatic galanin was not coreleased with NE. Therefore corelease of NE and GLIR was not demonstrated at these time points. However, after the SS infusion was discontinued, pancreatic GLIR output did increase significantly, and NE output increased further, suggesting that NE and GLIR were coreleased.

To confirm NE and galanin corelease during severe hypoglycemia, a second independent approach was used to produce severe hypoglycemia. Data from previous experiments demonstrated that submaximal doses of the ganglionic antagonist hexamethonium were sufficient to reduce the autonomically mediated glucagon response during insulin-induced hypoglycemia, resulting in plasma glucose nadirs equivalent to those in SS-infused and cord-sectioned animals (31). This degree of ganglionic blockade reduced, but did not abolish, the arterial NE response to hypoglycemia, demonstrating only a partial impairment of whole body sympathetic neural activation, suggesting that it might allow activation of pancreatic sympathetic nerves. Indeed, in the hexamethonium-treated dogs, there were significant increases of both pancreatic NE and GLIR output.

To determine whether the increases of pancreatic NE and GLIR output were due to a centrally mediated increase of sympathetic outflow to the pancreas, rather than to an effect of low glucose levels directly on pancreatic sympathetic nerve terminals, as suggested by certain *in vitro* studies (15, 35), severe hypoglycemia was induced in dogs in which the cervical spinal cord was surgically transected. This procedure interrupts the sympathetic neural pathway from the brain to the pancreas and adrenal medulla, impairs counterregulatory increases of glucagon (33) and plasma catecholamines during hypoglycemia, and thereby allows plasma glucose to fall to levels comparable with the SS-infused and hexamethonium-treated dogs (31). No significant increase of either pancreatic NE or GLIR occurred in these animals, despite the presence of severe hypoglycemia, suggesting that increased sympathetic neurotransmitter release from the pancreas *in vivo* is centrally mediated. This conclusion is consistent with the demonstration that the increase of pancreatic NE spillover induced by 2-deoxy-D-glucose administration is markedly impaired by surgical pancreatic denervation (30).

Although both the SS-infused and hexamethonium-treated animals exhibited reflex release of pancreatic galanin with NE, these agents appear to inhibit the neural activation. In the SS-treated animals, there was an increase of both pancreatic NE and GLIR output when the SS infusion was discontinued, suggesting that the SS had been restraining pancreatic NE and GLIR output. SS is well known to directly inhibit the release of numerous hormones, but there are few reports of an

inhibitory effect of SS on neurotransmitter release (27). Alternatively, SS may have restrained pancreatic sympathetic neurotransmitter release via a central action, since central administration of SS analogues can inhibit increases of plasma catecholamines induced by a variety of stresses, including insulin-induced hypoglycemia or 2-deoxy-D-glucose administration (12, 13, 25). Although there was not a significant suppression of the arterial NE response to hypoglycemia in the SS-infused dogs compared with controls, a larger response would have been expected, since the glucose nadir was significantly lower in these animals. Thus SS may have produced a relative inhibition of general noradrenergic outflow during hypoglycemia. In the case of hexamethonium, partial inhibition was expected because this nicotinic antagonist impairs neural transmission in sympathetic ganglia (56). Indeed, the arterial NE response was approximately one-third of that in the control animals, despite more severe hypoglycemia. Despite the limitations of the pharmacological interventions, these experiments were able to provide evidence for the corelease of galanin with NE during reflex activation of the pancreatic sympathetic nerves.

Additional support for corelease of galanin with NE is provided by the correlation of the increments of pancreatic NE output with galanin output across the four studies. This finding is consistent with the demonstration that both NE and GLIR are released during intense electrical stimulation of the sympathetic nerves innervating the dog pancreas (21, 23) and with the results of immunostaining experiments that found that GLIR is colocalized with tyrosine hydroxylase in some, but not all, noradrenergic nerve fibers in the canine pancreas (1).

Although it has long been hypothesized that the sympathetic innervation of the pancreas is involved in the changes of insulin and glucagon secretion observed during systemic stress (8, 34, 43, 47), this hypothesis has been primarily based on indirect evidence. For example, electrical stimulation of the sympathetic nerves to the pancreas inhibits insulin secretion (20, 47) and stimulates glucagon secretion (20, 40). However, it has only recently been established that the sympathetic noradrenergic input to the pancreas is activated during systemic stress (30). If neuronally released galanin does in fact function along with NE as a sympathetic neurotransmitter in the pancreas, then increased pancreatic galanin release during hypoglycemia could inhibit insulin, stimulate glucagon secretion, and thereby increase hepatic glucose production (14).

However, one must be cautious when postulating a physiological role for pancreatic galanin, or pancreatic NE for that matter, in hypoglycemic counterregulation. First, a definitive role for the direct sympathetic innervation of the pancreas in mediating islet hormone responses has not been demonstrated. Second, the NE and GLIR output measured in the present study was from the entire right lobe of the dog pancreas and the proximal duodenum, not solely from pancreatic endocrine tissue. Therefore, these measurements do not necessarily reflect increased noradrenergic and galaninergic input to the islets during hypoglycemia. However, histological studies indicate that the islets receive a preponderance of the

galaninergic innervation of the pancreas (1). Finally, severe hypoglycemia was required to activate the noradrenergic and galaninergic nerves to the pancreas in these anesthetized acutely laparotomized animals. Thus Epi and glucagon, which are secreted well before this severe level of hypoglycemia is reached, may be more important than pancreatic sympathetic nerves for early glucose counterregulation. Therefore, under the conditions of these experiments, NE and galanin could only be involved in hypoglycemic counterregulation during severe hypoglycemia. However, it is possible that, in the absence of anesthesia and surgery, activation of pancreatic noradrenergic and galaninergic nerves may occur during less severe hypoglycemia. Further experiments in chronically catheterized conscious animals would be necessary to address this possibility.

In summary, pancreatic GLIR and NE output increase during severe insulin-induced hypoglycemia in halothane-anesthetized laparotomized dogs, demonstrating that endogenous reflex activation of pancreatic sympathetic nerves can result in corelease of both a peptidergic and classical neurotransmitter. Because these pancreatic neural responses are abolished by spinal cord transection, they appear to be centrally mediated. Although a definitive physiological role for the pancreatic sympathetic nerves remains to be established, the increased release of neuronal NE and galanin could contribute to the counterregulatory changes of islet function for defending plasma glucose during severe hypoglycemia (16, 26).

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