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# Pancreatic sympathetic nerves contribute to increased glucagon secretion during severe hypoglycemia in dogs

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**Havel, Peter J., Thomas O. Mundinger, and Gerald J. Taborsky, Jr.** Pancreatic sympathetic nerves contribute to increased glucagon secretion during severe hypoglycemia in dogs. *Am. J. Physiol.* 270 (*Endocrinol. Metab.* 33): E20–E26, 1996.—To determine if pancreatic sympathetic nerves can contribute to increased glucagon secretion during hypoglycemia, plasma glucagon and pancreatic glucagon secretion *in situ* were measured before and during insulin-induced hypoglycemia in three groups of halothane-anesthetized dogs. All dogs were bilaterally vagotomized to eliminate the input from pancreatic parasympathetic nerves. One group of dogs received only vagotomy (VAGX). A second group was vagotomized and adrenalectomized (VAGX + ADX). A third group received vagotomy, adrenalectomy, plus surgical denervation of the pancreas (VAGX + ADX + NERVX) to prevent activation of pancreatic sympathetic nerves. In dogs with VAGX only, hypoglycemia increased plasma epinephrine (Epi), pancreatic norepinephrine (NE) output ( $+320 \pm 140$  pg/min,  $P < 0.05$ ), arterial plasma glucagon ( $+28 \pm 12$  pg/ml,  $P < 0.01$ ), and pancreatic glucagon output ( $+1,470 \pm 370$  pg/min,  $P < 0.01$ ). The addition of ADX eliminated the increase of Epi but did not increase pancreatic NE output ( $+370 \pm 190$  pg/min,  $P < 0.025$ ), arterial plasma glucagon ( $+20 \pm 5$  pg/ml,  $P < 0.01$ ), or pancreatic glucagon output ( $+810 \pm 200$  pg/min,  $P < 0.01$ ). In contrast, the addition of pancreatic denervation eliminated the increase of pancreatic NE output ( $-20 \pm 40$  pg/min,  $P < 0.05$  vs. VAGX), the arterial glucagon ( $+1 \pm 2$  pg/ml,  $P < 0.01$  vs. VAGX), and pancreatic glucagon output responses ( $+210 \pm 280$  pg/min,  $P < 0.025$  vs. VAGX) to hypoglycemia. Thus activation of pancreatic sympathetic nerves can contribute to the increased glucagon secretion during severe insulin-induced hypoglycemia in dogs. epinephrine; norepinephrine; vagotomy; adrenalectomy; pancreatic denervation

INCREASED GLUCAGON secretion is a primary counterregulatory factor in the recovery of plasma glucose levels from insulin-induced hypoglycemia (10, 15). However, the question of which mechanisms are responsible for stimulating glucagon secretion during hypoglycemia remains controversial. For example, hypoglycemia *per se* could directly increase glucagon secretion because lowered glucose concentrations increase glucagon secretion from isolated perfused pancreas preparations (41) and from isolated islets (32). However, the pancreas also receives three different autonomic inputs: 1) pancreatic parasympathetic nerves, 2) the circulating neurohormone, epinephrine, and 3) pancreatic sympathetic nerves.

Activation of the adrenal medulla during hypoglycemia was first demonstrated by Cannon more than 70 years ago (8). Adrenal epinephrine release is centrally

mediated via the splanchnic sympathetic nerves. The activation of pancreatic parasympathetic nerves during hypoglycemia has been inferred by increases of plasma pancreatic polypeptide, a hormone secreted by the islet F cells, which is known to be under strong vagal control (21, 38). More recently, we have demonstrated that the sympathetic nerves of the pancreas are activated during central neuroglucopenia (25) and insulin-induced hypoglycemia (19). Although activation of each of these autonomic inputs to the pancreas has been demonstrated to stimulate glucagon secretion in the absence of hypoglycemia (2, 3, 5, 11, 16, 28), it has been difficult to determine the contribution of the autonomic nervous system in mediating increases of plasma glucagon during hypoglycemia. This difficulty may be due, in part, to redundancy between the three autonomic inputs to pancreas in stimulating A cell glucagon secretion during hypoglycemia (for reviews see Refs. 23 and 24).

Results of recent studies support the redundancy hypothesis. In these experiments, pharmacological blockade or ablation of all three autonomic inputs to the pancreas markedly reduced the glucagon response to hypoglycemia (17, 26), whereas in most experiments in which only one or two of the autonomic inputs were blocked or ablated, there was no major effect on the glucagon response (for reviews see Refs. 23 and 33). The hypothesis of redundant autonomic mediation of glucagon secretion is directly supported by the results of a study in which neither methylatropine nor  $\alpha$ - and  $\beta$ -adrenergic blockade alone significantly reduced the increase of glucagon during insulin-induced hypoglycemia in conscious rats, but both atropine and adrenergic blockade together eliminated 75% of the response (22).

The goal of this study was to examine the potential for the direct sympathetic innervation of the pancreas to contribute to increased glucagon secretion during insulin-induced hypoglycemia. Because we hypothesized redundant autonomic mediation of the glucagon response to hypoglycemia, the autonomic inputs to the pancreas were sequentially ablated, concluding with ablation of the sympathetic innervation of the pancreas. Thus the first group of dogs served as a control group and received vagotomy alone (VAGX). The second group of dogs was vagotomized and the adrenal glands were removed bilaterally to prevent the release of adrenal medullary epinephrine (VAGX + ADX). Last, to determine the contribution of the pancreatic sympathetic nerves to the A cell secretory responses to hypoglycemia in the absence of parasympathetic and adrenal medullary activation, a third group of dogs was

vagotomized, adrenalectomized, and the entire pancreas was surgically denervated (VAGX + ADX + NERVX). Arterial plasma glucagon responses and glucagon secretion from the pancreas in situ were measured in the three groups of dogs. Norepinephrine output or spillover from noradrenergic nerves in the in situ pancreas was measured to assess the activation of pancreatic sympathetic nerves during hypoglycemia in the first two groups of dogs and the effectiveness of the pancreatic denervation procedure in the third group of animals.

## RESEARCH DESIGN AND METHODS

**Animals and surgical procedures.** Adult dogs of mixed breed weighing 24–40 kg were used for these studies. After an overnight fast (~18 h), anesthesia was induced with thiamylal sodium (Surital, Parke Davis, Morris Plains, NJ). Anesthesia was subsequently maintained with halothane (0.8%) administered from a calibrated vaporizer (Draeger, Germany) by mechanical ventilation in 100% oxygen. This anesthetic regimen was chosen because it provides full surgical anesthesia but does not suppress parasympathetic or sympathoadrenal activation induced by neuroglucopenic stress, as do some other anesthetics, e.g., pentobarbital (18, 20).

To access pancreatic venous blood, a laparotomy was performed and an extracorporeal Silastic (Dow Corning, Midland, MI) shunt containing a sampling port, an ultrasonic flow probe (Transonic Systems, Ithaca, NY), and a heparin infusion line, was placed between the superior pancreaticoduodenal vein (SPDV) and the portal vein. This procedure allows the measurement of hormone output (26) and neurotransmitter spillover (19, 26) from the right lobe (duodenal region and uncinate process) of the pancreas (~35–50% of the canine pancreas). 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.

In all dogs, the parasympathetic input to the pancreas was surgically ablated by bilateral cervical vagotomy. The right and left cervical vagal trunks were dissected free from their tracts along the carotid arteries, ligated, and cut (26). One group of dogs was vagotomized only. Another group of dogs was vagotomized and in addition the right and left adrenal glands were surgically removed after ligation of the adrenal vasculature and adjacent connective tissue (14). A third group of dogs was vagotomized, adrenalectomized, and in addition the pancreas was surgically denervated by ligating and separating the pancreas and duodenum to the level of the pylorus. All of the mesenteric attachments to the pancreas were ligated and cut and the nerves fibers around the pancreatic blood vessels were dissected free, ligated, and severed (25).

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

**Hypoglycemia protocol.** After the surgical procedures, paired blood samples for glucose, catecholamine, and glucagon determination were drawn from the femoral artery and from the pancreatic venous shunt at -10 and 0 min before insulin injection to establish baseline measurements for the subsequent hypoglycemic period. To produce acute marked hypoglycemia, a bolus of regular porcine insulin (Squibb-Novo,

Bagsvaerd, Denmark) was administered into the femoral vein cannula at a dose of 3.0 U/kg. Paired 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 150 mg/kg followed by an infusion of 15 mg·kg<sup>-1</sup>·min<sup>-1</sup> to equal or exceed baseline arterial glucose levels for 60 min. Paired samples were drawn at 5, 15, 30, 45, and 60 min after the start of the glucose infusion. In all experiments, blood flow in the pancreatic venous shunt was monitored with an ultrasonic transit-time flowmeter (Transonic Systems). Hematocrit was determined at regular intervals throughout the experiments.

**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 HCl. Blood samples for catecholamine determination were placed in tubes containing ethylene glycol-bis(β-aminoethyl ether)-N,N',N'-tetraacetic acid and glutathione. All samples were kept on ice until centrifugation (20 min at 4°C). The plasma was decanted and frozen at -20°C until assayed.

Plasma glucose was assayed by the glucose oxidase method with a Beckman glucose analyzer (Beckman Instruments, Fullerton, CA). Plasma immunoreactive glucagon (IRG) was measured radioimmunologically in unextracted plasma with an antibody that has high specificity for the COOH-terminal portion of the glucagon molecule (39). It has been previously demonstrated that extrapancreatic IRG measured with COOH-terminal specific antisera does not increase during insulin-induced hypoglycemia in dogs (29). Thus measurements of plasma glucagon made with this assay are a reliable index of pancreatic glucagon secretion. Plasma norepinephrine (NE) and epinephrine (Epi) were measured in duplicate with a highly sensitive and specific radioenzymatic assay (13). The intra- and interassay coefficients of variation for the plasma catecholamine assay in this laboratory are 6 and 12%, respectively.

**Calculations and data analysis.** The changes of arterial plasma glucose, arterial plasma Epi, arterial and pancreatic venous plasma NE, pancreatic NE output, arterial and pancreatic venous glucagon, and pancreatic glucagon output were calculated by subtracting the mean of the -10- and 0-min baseline values from the mean of the 45-, 50-, 55-, and 60-min values after the administration of insulin.

Circulating NE is extracted by the pancreas (1). Most of the neurotransmitter arriving via the arterial circulation does not appear in pancreatic venous plasma, and therefore simple measurements of arteriovenous (av) concentration differences will seriously underestimate pancreatic neurotransmitter spillover. To correct the av difference for extraction, pancreatic NE spillover was calculated as follows

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

where square brackets indicate concentration. The arterial contribution to the SPDV level is defined to be the amount of neurotransmitter present in SPDV plasma that arrives via the arterial circulation and escapes pancreatic extraction. Because Epi is extracted at a rate similar to NE (1), Epi extraction has been used to estimate the arterial contribution to SPDV NE levels when arterial Epi levels are high (11). When arterial Epi levels are very low, such as in adrenalectomized animals, Epi extraction cannot be used as an index of NE extraction. Epi extraction during baseline conditions has been measured and found to average  $64 \pm 2\%$ ,  $n = 22$ . We

have found that pancreatic Epi extraction increases to  $78 \pm 1\%$ ,  $n = 35$  during insulin-induced hypoglycemia ( $P < 0.0005$  vs. baseline). Therefore, an extraction rate for NE of 64% was used to calculate NE output at time points during the baseline period, and an extraction rate of 78% was used during hypoglycemia. The arterial contribution to SPDV NE levels during the baseline period is calculated as  $[NE]_{FA} \times (1 - 0.64)$ . The arterial contribution during insulin-induced hypoglycemia is calculated as  $[NE]_{FA} \times (1 - 0.78)$ . Because the NE spillover calculation does not account for the majority of neuronal NE release that is taken back up by the sympathetic nerves, pancreatic NE output is an attenuated index of the activity of pancreatic noradrenergic nerves and is therefore likely to underestimate pancreatic sympathetic activation.

The pancreatic output of IRG was calculated as

$$[IRG]_{SPDV} - [IRG]_{FA} \times SPDV \text{ blood flow} \times (1 - \text{hematocrit})$$

The data in the text, figures, and tables are expressed as means  $\pm$  SE. Statistical comparisons of means within a group were made with a Wilcoxon signed-rank test. Statistical comparisons of means between different groups were made with a Kruskal-Wallis test.

## RESULTS

**Arterial plasma glucose.** Arterial plasma glucose averaged  $107 \pm 3$  mg/dl in VAGX dogs and decreased to  $32 \pm 2$  mg/dl after insulin administration. Baseline glucose levels were lower in both VAGX + ADX and VAGX + ADX + NERVX dogs ( $P < 0.0005$  vs. VAGX) and decreased to a lower nadir ( $15 \pm 2$  and  $13 \pm 2$  mg/dl, respectively) in these dogs than in dogs with VAGX alone ( $P < 0.0002$  vs. VAGX) (Fig. 1).

**Arterial plasma Epi.** Baseline arterial plasma Epi was  $220 \pm 60$  pg/ml in VAGX dogs and increased markedly to  $2,890 \pm 400$  pg/ml during hypoglycemia ( $\Delta = +2,670 \pm 390$  pg/ml,  $P < 0.005$ ). As expected, baseline arterial Epi was lower in both VAGX + ADX and VAGX + ADX + NERVX dogs averaging  $60 \pm 20$  and

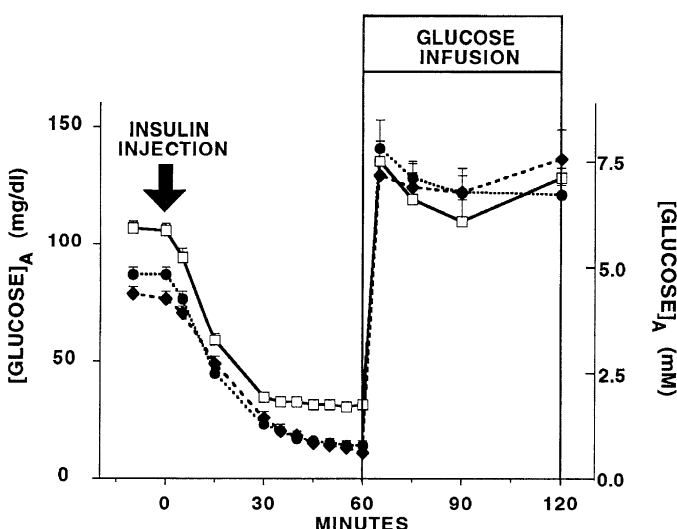


Fig. 1. Arterial (A) plasma glucose before and after insulin injection and during posthypoglycemia glucose infusion in dogs with vagotomy (VAGX;  $n = 9$ ;  $\square$ ) dogs with vagotomy + adrenalectomy (VAGX + ADX;  $n = 10$ ;  $\bullet$ ) and dogs with vagotomy + adrenalectomy + pancreatic denervation (VAGX + ADX + NERVX;  $n = 6$ ;  $\blacklozenge$ ).

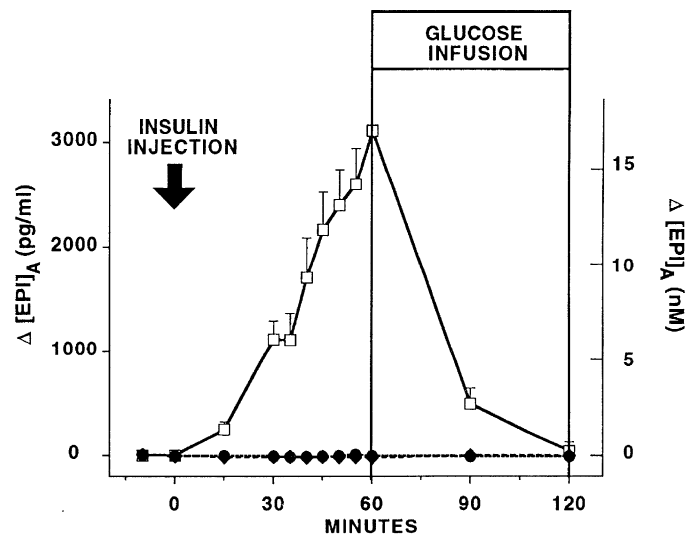


Fig. 2. The change of arterial (A) plasma epinephrine ( $\Delta$ Epi) before and after insulin injection and during posthypoglycemia glucose infusion in dogs with VAGX ( $n = 9$ ;  $\square$ ), dogs with VAGX + ADX ( $n = 10$ ;  $\bullet$ ), and dogs with VAGX + ADX + NERVX ( $n = 6$ ;  $\blacklozenge$ ).

$50 \pm 10$  pg/ml, respectively ( $P < 0.05$  vs. VAGX). Arterial plasma Epi did not increase during hypoglycemia in either VAGX + ADX or VAGX + ADX + NERVX dogs ( $P < 0.0001$  vs. VAGX) (Fig. 2).

**Pancreatic NE output.** Baseline pancreatic NE output was  $420 \pm 80$  pg/min in VAGX dogs. During insulin-induced hypoglycemia pancreatic NE output in these animals increased to  $740 \pm 200$  pg/min ( $\Delta = +320 \pm 140$  pg/min,  $P < 0.05$ ). Although baseline pancreatic NE output was lower in both VAGX + ADX and VAGX + ADX + NERVX dogs ( $P < 0.0025$  vs. VAGX) (Table 1), pancreatic NE output increased to a similar degree during hypoglycemia in VAGX + ADX dogs ( $\Delta = +370 \pm 190$  pg/min,  $P < 0.0125$ ). Pancreatic NE output did not increase during hypoglycemia in VAGX + ADX + NERVX dogs ( $\Delta = -20 \pm 40$  pg/min,  $P < 0.05$  vs. VAGX or VAGX + ADX dogs) (Table 1 and Fig. 3).

**Arterial plasma IRG and pancreatic IRG output.** Baseline arterial plasma IRG was  $25 \pm 3$  pg/ml and increased to  $53 \pm 12$  pg/ml in VAGX dogs ( $\Delta = +28 \pm 12$  pg/ml,  $P < 0.01$ ). Arterial plasma IRG increased by a similar amount in VAGX + ADX dogs ( $\Delta = +20 \pm 6$  pg/ml,  $P < 0.01$ ) but did not increase during hypoglycemia in VAGX + ADX + NERVX dogs ( $\Delta = +1 \pm 2$  pg/ml,  $P < 0.01$  vs. VAGX or VAGX + ADX dogs) (Table 2).

Pancreatic IRG output increased from  $260 \pm 40$  pg/min to  $1,730 \pm 380$  pg/min during hypoglycemia in VAGX dogs ( $\Delta = +1,470 \pm 370$  pg/min,  $P < 0.01$ ). The increase of pancreatic IRG output in VAGX + ADX dogs ( $\Delta = +810 \pm 200$  pg/min,  $P < 0.01$ ) was not significantly reduced from the response in dogs with VAGX only. In contrast, pancreatic IRG output did not increase significantly during hypoglycemia in VAGX + ADX + NERVX dogs ( $\Delta = +210 \pm 280$  pg/min,  $P < 0.025$  vs. VAGX) (Table 2 and Fig. 4).

Table 1. Plasma NE levels and pancreatic NE output before and during hypoglycemia

Treatment	n	pg/ml			NE Output, pg/min
		NE <sub>FA</sub>	NE <sub>SPDV</sub>	BF <sub>SPDV</sub> , ml/min	
VAGX	9				
Baseline		150 ± 30	120 ± 20	10.0 ± 1.1	420 ± 80
During IIH		790 ± 110	290 ± 50	10.1 ± 1.3	740 ± 200
Δ		+640 ± 100*	+170 ± 50*	+0.1 ± 1.0	+320 ± 140*
VAGX + ADX	10				
Baseline		80 ± 20	60 ± 10	10.5 ± 0.8	160 ± 30
During IIIH		320 ± 100	150 ± 30	10.7 ± 1.3	530 ± 180
Δ		+240 ± 90*†	+90 ± 30*	+0.2 ± 1.4	+370 ± 190*
VAGX + ADX + NERVX	6				
Baseline		50 ± 10	40 ± 10	8.0 ± 0.9	90 ± 30
During IIH		140 ± 50	40 ± 10	6.7 ± 0.8	70 ± 20
Δ		+90 ± 40*†	0 ± 10†	-1.3 ± 0.2*	-20 ± 40†

Values during hypoglycemia are means ± SE, 45–60 min postinsulin injection; n, no. of dogs. VAGX, vagotomy; ADX, adrenalectomy; NERVX, denervation; IIH, insulin-induced hypoglycemia; NE, norepinephrine FA, femoral artery; SPDV, superior pancreaticoduodenal vein; BF, blood flow. \*P < 0.05 vs. baseline. †P < 0.05 vs. VAGX.

DISCUSSION

The purpose of this study was to determine if activation of pancreatic sympathetic nerves can contribute to increased glucagon secretion during insulin-induced hypoglycemia. The surgical interventions of vagotomy, adrenalectomy, and pancreatic denervation were employed to successively eliminate the autonomic inputs to the pancreas. The addition of pancreatic denervation to the adrenalectomy and vagotomy significantly reduced the glucagon secretory response, demonstrating that pancreatic sympathetic nerves can contribute to increased glucagon secretion during severe hypoglycemia in anesthetized dogs.

The existence of nerve fibers in the pancreatic islets was first described by Langerhans in 1869 (27). In 1973 Porte et al. (35) demonstrated inhibition of insulin secretion and Marliss et al. (28) demonstrated stimulation of glucagon secretion during electrical stimulation of the sympathetic innervation to the pancreas. These

findings have been confirmed by a number of other investigators (3, 11). Thus it has been hypothesized that activation of the pancreatic sympathetic nerves with its resulting changes of islet hormone secretion could contribute to increased hepatic glucose production during physiological stress (30, 36). Activation of the pancreatic sympathetic nerves during stress was directly demonstrated in 1988 by measuring increased spillover of NE into the pancreatic venous effluent of anesthetized dogs during central neuroglucopenic stress induced by 2-deoxy-D-glucose (25). These nerves are also activated during insulin-induced hypoglycemia and release both NE and the sympathetic neuropeptide galanin in dogs (19). Although one early study suggested that the pancreatic sympathetic nerves can inhibit insulin secretion during hypoglycemia in dogs (31), the present data indicate a role for these nerves to stimulate glucagon secretion during severe hypoglycemia. However, it remains to be demonstrated that pancreatic sympathetic nerves have a role in stimulating glucagon secretion during more moderate hypoglycemia in the absence of anesthesia, vagotomy, and adrenalectomy.

There are several potential reasons why such a role for the pancreatic sympathetic nerves has been difficult to demonstrate. First, hypoglycemia itself might directly stimulate glucagon secretion (32, 41). Second, although activation of the autonomic nervous system as a whole has been demonstrated to mediate the majority of the increase of hypoglycemia-induced glucagon secretion in several species (4, 6, 7, 9, 17, 26), hypoglycemia activates three different inputs to the pancreas (for review see Ref. 23). Third, parasympathetic or sympathoadrenal activation can act redundantly to increase glucagon release during hypoglycemia (22), leading to an underestimation of the autonomic contribution when the pancreatic input from only one of these major subdivisions of the autonomic nervous system is blocked or ablated. The sympathoadrenal input consists of two distinct components: 1) adrenal medullary epinephrine and 2) the direct sympathetic innervation of the pancreas. Investigation of the indi-

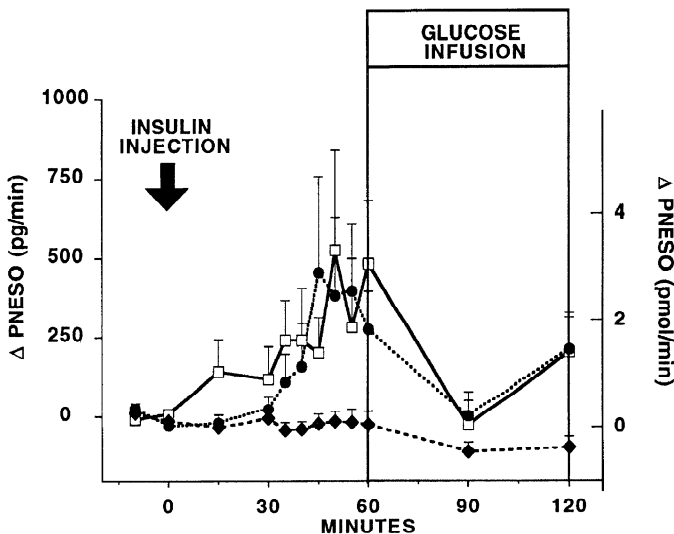


Fig. 3. The change of pancreatic norepinephrine spillover (ΔPNESO) before and after insulin injection and during posthypoglycemia glucose infusion in dogs with VAGX (n = 9; □), dogs with VAGX + ADX (n = 10; ●), and dogs with VAGX + ADX + NERVX (n = 6; ◆).

Table 2. Plasma glucagon and pancreatic glucagon output before and during hypoglycemia

Treatment	n	pg/ml			
		IRG <sub>FA</sub>	IRG <sub>SPDV</sub>	BF <sub>SPDV</sub> , ml/min	IRG Output, pg/min
VAGX	9				
Baseline		25 ± 3	70 ± 8	10.0 ± 1.1	260 ± 40
During IH		53 ± 12	372 ± 83	10.1 ± 1.3	1,730 ± 380
Δ		+28 ± 12*	+302 ± 81*	+0.1 ± 1.0	+1,470 ± 370*
VAGX + ADX	10				
Baseline		36 ± 3	164 ± 51	10.5 ± 0.8	760 ± 270
During IH		56 ± 8	298 ± 58	10.7 ± 1.3	1,570 ± 370
Δ		+20 ± 5*	+134 ± 54*†	+0.2 ± 1.4	+810 ± 200*
VAGX + ADX + NERVX	6				
Baseline		33 ± 4	85 ± 14	8.0 ± 0.9	240 ± 70
During IH		34 ± 4	150 ± 81	6.7 ± 0.8	450 ± 310
Δ		+1 ± 2†	+65 ± 73†	1.3 ± 0.2*	+210 ± 280†

Values during hypoglycemia are means ± SE, 45–60 min postinsulin injection; n, no. of dogs. IRG, immunoreactive glucagon. \**P* < 0.05 vs. baseline; †*P* < 0.025 vs. VAGX.

vidual roles of these two components in mediating changes of pancreatic hormone secretion requires discrete surgical procedures, specifically adrenalectomy or adrenal demedullation and pancreatic denervation. These interventions have not been previously employed to examine the potential contribution of the direct sympathetic innervation, independent from the adrenal medulla, to increased glucagon secretion during hypoglycemia.

In the present study the interventions of adrenalectomy or adrenalectomy plus pancreatic denervation had prominent effects on plasma glucose levels. Baseline plasma glucose was lower in VAGX + ADX and VAGX + ADX + NERVX dogs, suggesting that adrenal Epi and/or glucocorticoids may have been supporting hepatic glucose production in these animals. The plasma glucose levels after insulin administration were also lower in the VAGX + ADX and VAGX + ADX + NERVX animals. This lower nadir occurred despite a significant increase of glucagon secretion observed in the VAGX + ADX dogs. This result by itself suggests a role for the

adrenal medulla to prevent marked hypoglycemia (~30 mg/dl) from progressing to severe hypoglycemia (~15 mg/dl). In a previous study, we found that prevention of the glucagon response to hypoglycemia with a somatostatin infusion also allows insulin to produce glucose nadirs of ~15 mg/dl (19), despite a plasma Epi response equal to that of control animals. This suggests a role for increased pancreatic glucagon secretion to prevent marked hypoglycemia (~30 mg/dl) from progressing to severe hypoglycemia (~15 mg/dl). Taken together, the results of the present study and our previous work (19) indicate that both an intact Epi response and an intact glucagon response to hypoglycemia are required to prevent marked insulin-induced hypoglycemia from progressing to severe and potentially life-threatening levels, and that either response alone is not sufficient to prevent the further decline of plasma glucose levels.

Hypoglycemia in the VAGX dogs produced a large increase of plasma Epi levels which, as expected, was completely abolished by bilateral adrenalectomy in the VAGX + ADX and VAGX + ADX + NERVX dogs. Pancreatic NE output was affected by adrenalectomy, surgical denervation of the pancreas, and perhaps even vagotomy. For example, baseline pancreatic NE output was lower in the VAGX + ADX group than in the dogs with VAGX alone. Such a reduction may be due to the absence of  $\beta_2$ -adrenergic facilitation of neuronal NE release secondary to the loss of circulating Epi (37). Baseline and hypoglycemic pancreatic NE output was also lower in the VAGX + ADX + NERVX dogs, in this case because of the surgical disruption of the postganglionic sympathetic neural pathway. Hypoglycemia increased pancreatic NE spillover in the dogs with vagotomy alone demonstrating significant activation of pancreatic noradrenergic nerves. This contrasts with our previous observations that a similar degree of hypoglycemia (~30 mg/dl) did not produce measurable pancreatic sympathetic activation in nonvatomized animals (19). A possible explanation for the discrepancy is that parasympathetic activity is known to suppress sympathetic activity via a presynaptic action of acetylcholine to inhibit NE release from noradrenergic nerves (34, 40). Such presynaptic cholinergic inhibition of NE

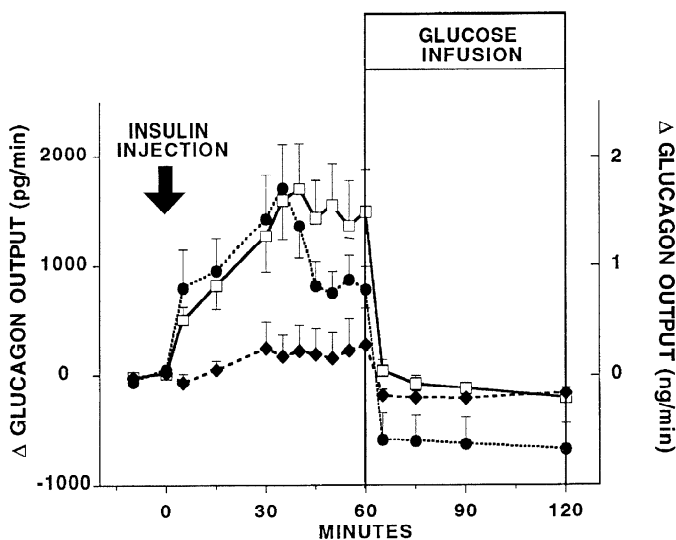


Fig. 4. The change of pancreatic glucagon output before and after insulin injection and during posthypoglycemia glucose infusion in dogs with VAGX (*n* = 9; □), VAGX + ADX (*n* = 10; ●), and dogs with VAGX + ADX + NERVX (*n* = 6; ◆).

release would not be likely to occur in dogs with prior surgical vagotomy, and therefore vagotomy might allow significant activation of pancreatic sympathetic nerves at a less severe degree of hypoglycemia.

Both pancreatic glucagon output and arterial plasma glucagon levels increased markedly during insulin-induced hypoglycemia in VAGX dogs, and these responses were not significantly affected by the addition of adrenalectomy. When activation of pancreatic sympathetic nerves was prevented by surgical denervation of the pancreas, in addition to vagotomy and adrenalectomy, neither arterial plasma glucagon nor pancreatic glucagon output increased significantly during hypoglycemia. Therefore, in the absence of parasympathetic and adrenal medullary activation, pancreatic sympathetic nerves can make a substantial contribution to increased glucagon secretion during severe insulin-induced hypoglycemia in halothane-anesthetized dogs. However, the data also suggest that factors other than activation of pancreatic sympathetic nerves are capable of stimulating glucagon secretion during hypoglycemia. For example, at 15 and 30 min after insulin injection, when the plasma glucose level is still falling, pancreatic glucagon output increases in the VAGX + ADX animals before a detectable increase of pancreatic NE output. This may be due to a lack of sensitivity of the NE output measurements to detect early activation of pancreatic sympathetic nerves (see RESEARCH DESIGN AND METHODS). Alternatively, this early increase of glucagon secretion may reflect an effect of high levels of exogenous insulin to suppress endogenous insulin secretion and release the A cell from paracrine inhibition by intra-islet insulin. However, the expected early glucagon response is absent in the VAGX + ADX + NERVX group, perhaps because either an intact neural input or adrenal medullary Epi secretion is necessary for this indirect effect of exogenous insulin to increase glucagon secretion to occur. Nonetheless, later during the hypoglycemic period there is a clear contribution of the pancreatic sympathetic nerves to the increase of glucagon secretion.

To our knowledge this is the first demonstration of a role for the direct sympathetic innervation of the pancreas to increase glucagon secretion during hypoglycemic stress. It has now been reported that pancreatic NE spillover at euglycemia is larger and that there is a marked increase of pancreatic NE spillover (6-fold) during less severe hypoglycemia ( $36 \pm 2$  mg/dl) in conscious dogs (12) than previously described in anesthetized dogs (19). Thus pancreatic sympathetic nerves are activated during less severe hypoglycemia in the absence of anesthesia-surgery in conscious dogs. However, further experiments in conscious animals are required to determine if pancreatic sympathetic nerves can contribute to increased glucagon secretion during moderate hypoglycemia.

Adrenalectomy without pancreatic denervation did not significantly reduce glucagon responses to hypoglycemia, indicating that, under these conditions, Epi by itself does not mediate a large portion of the glucagon response. Although pancreatic denervation, without

adrenalectomy was not performed in the present experimental series, it is likely that in the absence of pancreatic sympathetic neural activation, Epi could contribute significantly to the glucagon response, as suggested by one previous study (42) and our preliminary data. Thus, in vagotomized animals, adrenal Epi and the pancreatic sympathetic nerves could function in a redundant manner to mediate the glucagon response to hypoglycemia. Accordingly, adrenalectomy and pancreatic denervation together eliminated ~85% of the increase of pancreatic glucagon secretion observed in animals with vagotomy alone. This result supports the findings of a number of other studies that concluded that activation of the autonomic nervous system, and not hypoglycemia per se, mediates the majority of increased glucagon secretion during insulin-induced hypoglycemia (for review see Ref. 24).

In summary, these studies demonstrate that activation of the sympathetic innervation of the pancreas can contribute to increased glucagon secretion during severe hypoglycemic stress. Thus activation of pancreatic sympathetic nerves via increased glucagon secretion may participate in the neuroendocrine counterregulatory response to severe hypoglycemia.

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## REFERENCES

1. Ahren, B., B. E. Dunning, P. J. Havel, R. C. Veith, and G. J. Taborsky, Jr. Extraction of epinephrine and norepinephrine by the dog pancreas in vivo. *Metab. Clin. Exp.* 37: 68-73, 1988.
2. Ahren, B., and G. J. Taborsky, Jr. The mechanism of vagal nerve stimulation of glucagon and insulin secretion in the dog. *Endocrinology* 118: 1551-1557, 1986.
3. Ahren, B., R. C. Veith, and G. J. Taborsky, Jr. Sympathetic nerve stimulation versus pancreatic norepinephrine infusion in the dog. 1) Effects on basal release of insulin and glucagon. *Endocrinology* 121: 323-331, 1987.
4. Biggers, D. W., S. R. Myers, D. Neal, R. Stinson, N. B. Cooper, J. B. Jaspan, P. E. Williams, A. D. Cherrington, and R. T. Frizzell. Role of the brain in counterregulation of insulin-induced hypoglycemia in dogs. *Diabetes* 38: 7-16, 1989.
5. Bloom, S. R., and A. V. Edwards. Pancreatic endocrine responses to stimulation of the peripheral ends of the vagus nerves in conscious calves. *J. Physiol. Lond.* 315: 31-41, 1981.
6. Bloom, S. R., A. V. Edwards, and N. J. A. Vaughan. The role of the autonomic innervation in the control of glucagon release during hypoglycemia in the calf. *J. Physiol. Lond.* 236: 611-623, 1974.
7. Borg, W. P., M. J. During, R. S. Sherwin, M. A. Borg, M. L. Brines, and G. I. Shulman. Ventromedial hypothalamic lesions in rats suppress the counterregulatory responses to hypoglycemia. *J. Clin. Invest.* 93: 1677-1682, 1994.
8. Cannon, W. B., M. A. McIuer, and S. W. Bliss. Studies on the condition of activity in endocrine glands. XIII. A sympathetic and

- adrenal mechanism for mobilizing sugar in hypoglycemia. *Am. J. Physiol.* 69: 46–66, 1924.
9. **Coiro, V., M. Passeri, G. Rossi, L. Camellini, D. Davoli, M. Marchesi, P. Muzzetto, R. Minelli, L. Bianconi, C. Coscelli, and P. Chiodera.** Effect of muscarinic and nicotinic-cholinergic blockade on the glucagon response to insulin-induced hypoglycemia in normal men. *Horm. Metab. Res.* 21: 102–103, 1989.
  10. **Cryer, P.** Glucose counterregulation in man. *Diabetes* 30: 261–264, 1981.
  11. **Dunning, B. E., P. J. Havel, R. C. Veith, and G. J. Taborsky, Jr.** Pancreatic and extrapancreatic galanin release during sympathetic neural activation. *Am. J. Physiol.* 258 (*Endocrinol. Metab.* 21): E436–E444, 1990.
  12. **Dunning, B. E., M. Scott, and D. Neal.** The importance of pancreatic neural activity assessed by quantification of pancreatic polypeptide and norepinephrine release in the conscious dog. *Diabetes* 44, *Suppl.* 1: 133A, 1995.
  13. **Evans, M. I., J. B. Halter, and D. Porte, Jr.** Comparison of double- and single-isotope enzymatic derivative methods for measuring catecholamines in human plasma. *Clin. Chem.* 24: 567–570, 1978.
  14. **Gauthier, C., K. El-Tayeb, M. Vranic, and H. L. A. Lickley.** Glucoregulatory role of cortisol and epinephrine interactions studied in adrenalectomized dogs. *Am. J. Physiol.* 250 (*Endocrinol. Metab.* 13): E393–E410, 1986.
  15. **Gerich, J., J. Davis, M. Lorenzi, R. Rizza, N. Bohannon, J. Karam, S. Lewis, R. Kaplan, T. Schultz, and P. Cryer.** Hormonal mechanisms of recovery from insulin-induced hypoglycemia in man. *Am. J. Physiol.* 236 (*Endocrinol. Metab. Gastrointest. Physiol.* 5): E380–E385, 1979.
  16. **Gerich, J. E., J. H. Karam, and P. H. Forsham.** Stimulation of glucagon secretion by epinephrine in man. *J. Clin. Endocrinol. Metab.* 37: 479–481, 1973.
  17. **Havel, P. J., D. L. Curry, J. O. Akpan, R. L. Gingerich, and B. Ahren.** Autonomic control of pancreatic polypeptide and glucagon secretion during neuroglucopenia and hypoglycemia in mice. *Am. J. Physiol.* 265 (*Regulatory Integrative Comp. Physiol.* 34): R246–R254, 1993.
  18. **Havel, P. J., D. E. Flatness, J. B. Halter, J. D. Best, R. C. Veith, and G. J. Taborsky, Jr.** Halothane anesthesia does not suppress sympathetic activation produced by neuroglucopenia. *Am. J. Physiol.* 252 (*Endocrinol. Metab.* 15): E667–E672, 1987.
  19. **Havel, P. J., T. O. Mundinger, R. C. Veith, B. E. Dunning, and G. J. Taborsky, Jr.** Corelease of galanin and NE from pancreatic sympathetic nerves during severe insulin-induced hypoglycemia in dogs. *Am. J. Physiol.* 263 (*Endocrinol. Metab.* 26): E8–E16, 1992.
  20. **Havel, P. J., T. L. Paquette, and G. J. Taborsky, Jr.** Halothane is less suppressive than pentobarbital on reflex and neural activation of the pancreatic F-cell. *Am. J. Physiol.* 251 (*Endocrinol. Metab.* 14): E111–E116, 1986.
  21. **Havel, P. J., S. J. Parry, D. L. Curry, J. O. Akpan, J. S. Stern, and R. L. Gingerich.** Autonomic nervous system mediation of the pancreatic polypeptide response to insulin-induced hypoglycemia in conscious rats. *Endocrinology* 130: 2225–2229, 1992.
  22. **Havel, P. J., S. Parry, J. S. Stern, J. O. Akpan, R. L. Gingerich, G. J. Taborsky, Jr., and D. L. Curry.** Redundant parasympathetic and sympathoadrenal mediation of the glucagon response to insulin-induced hypoglycemia in conscious rats. *Metab. Clin. Exp.* 43: 860–866, 1994.
  23. **Havel, P. J., and G. J. Taborsky, Jr.** The contribution of the autonomic nervous system to changes of glucagon and insulin secretion during hypoglycemic stress. *Endocr. Rev.* 10: 332–350, 1989.
  24. **Havel, P. J., and G. J. Taborsky, Jr.** The contribution of the autonomic nervous system to increased glucagon secretion during hypoglycemic stress: update 1994. In: *The Endocrine Pancreas, Insulin Action and Diabetes. Endocrine Reviews Monographs*, edited by A. Negro-Vilar and L. E. Underwood. Bethesda, MD: Endocr. Soc., 1994, vol. 2, chapt. 9, p. 201–204.
  25. **Havel, P. J., R. C. Veith, B. E. Dunning, and G. J. Taborsky, Jr.** Pancreatic noradrenergic nerves are activated by neuroglucopenia but not by hypotension or hypoxia in the dog: evidence for stress-specific and regionally selective activation of the sympathetic nervous system. *J. Clin. Invest.* 82: 1538–1545, 1988.
  26. **Havel, P. J., R. C. Veith, B. E. Dunning, and G. J. Taborsky, Jr.** A role for the autonomic nervous system to increase glucagon secretion during marked hypoglycemia in dogs. *Diabetes* 40: 1107–1114, 1991.
  27. **Langerhans, L.** *Contributions to the Microanatomy of the Pancreas.* Thesis, Berlin, 1869. Translated by H. Morrison. Baltimore, MD: The Johns Hopkins Press, 1937.
  28. **Marliss, E. B., L. Girardier, J. Seydoux, C. B. Wollheim, Y. Kanazawa, L. Orci, A. E. Renold, and D. Porte, Jr.** Glucagon release induced by pancreatic nerve stimulation in the dog. *J. Clin. Invest.* 52: 1246–1259, 1973.
  29. **Matsuyama, T., R. Tanaka, K. Shima, K. Nonaka, and S. Tarui.** Lack of a gastrointestinal glucagon response hypoglycemia in depancreatized dogs. *Diabetologia* 15: 471–474, 1978.
  30. **Miller, R. E.** Pancreatic neuroendocrinology: peripheral neural mechanisms in the regulation of the islets of Langerhans. *Endocr. Rev.* 4: 471–494, 1981.
  31. **Miller, R. E., T. H. Waid, and P. Joyce.** Direct neural inhibition of insulin secretion in response to systemic hypoglycemia. *Am. J. Physiol.* 230: 1090–1094, 1976.
  32. **Oliver, J., V. Williams, and P. Wright.** Studies on glucagon secretion using isolated islets of Langerhans of the rat. *Diabetologia* 12: 301–306, 1976.
  33. **Palmer, J. P., and D. Porte, Jr.** Control of glucagon secretion: the central nervous system. In: *Glucagon: Physiology, Pathophysiology, and Morphology of the Pancreatic A-Cells*, edited by R. H. Unger and L. Orci. New York: Elsevier, 1981, p. 135–150.
  34. **Pendry, Y. D., and J. MacLagan.** Evidence for inhibition of sympathetic neurotransmission by endogenously released acetylcholine in the guinea pig trachea. *Br. J. Pharmacol.* 104: 817–822, 1991.
  35. **Porte, D., Jr., L. Girardier, J. Seydoux, Y. Kanazawa, and J. Posternak.** Neural regulation of insulin secretion in the dog. *J. Clin. Invest.* 52: 210–214, 1973.
  36. **Porte, D., Jr., and S. C. Woods.** Neural regulation of islet hormones and its role in energy balance and stress hyperglycemia. In: *Ellenberg and Rifkin's Diabetes Mellitus: Theory and Practice*, edited by H. Rifkin and D. Porte, Jr. New York: Medical Examination Publishing, 1990, p. 175–197.
  37. **Scheurink, A. J. W., A. B. Steffens, H. Bouritius, G. H. Dreteler, R. Bruntink, R. Remie, and J. Zaagsma.** Adrenal and sympathetic catecholamines in exercising rats. *Am. J. Physiol.* 256 (*Regulatory Integrative Comp. Physiol.* 15): R155–R160, 1989.
  38. **Schwartz, T. W., J. J. Holst, J. Fahrenkrug, S. L. Jensen, O. V. Nielsen, J. F. Rehfeld, O. B. Schaffalitzky de Muckadell, and F. Stadil.** Vagal cholinergic regulation of pancreatic polypeptide secretion. *J. Clin. Invest.* 61: 781–789, 1978.
  39. **Tager, A., M. Hohenboken, and J. Markese.** High-titer glucagon antisera. *Endocrinology* 100: 367–372, 1977.
  40. **Vizi, E. S., J. Kiss, and I. J. Elenov.** Presynaptic modulation of cholinergic and noradrenergic neurotransmission: interaction between them. *News Physiol. Sci.* 6: 119–123, 1991.
  41. **Weir, G., S. Knowlton, and D. Martin.** Glucagon secretion from the perfused rat pancreas. *J. Clin. Invest.* 54: 1403–1412, 1974.
  42. **Yamaguchi, N., R. Briand, and R. Gaspo.** Effect of functional adrenalectomy on glucagon secretion and circulating catecholamines during insulin-induced hypoglycemia in the dog. *Can. J. Physiol.* 68: 1183–1188, 1990.