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Role for Autonomic Nervous System to Increase Pancreatic Glucagon Secretion During Marked Insulin-Induced Hypoglycemia in Dogs

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To determine the role of the autonomic nervous system (ANS) in mediating the glucagon response to marked insulin-induced hypoglycemia in dogs, we measured arterial and pancreatic venous glucagon responses to insulin-induced hypoglycemia during acute, terminal experiments in halothane-anesthetized dogs in which the ANS was intact (control; $n = 9$), pharmacologically blocked by the nicotinic ganglionic antagonist hexamethonium ($n = 6$), or surgically ablated by cervical vagotomy and cervical spinal cord section ($n = 6$). In control dogs, insulin injection caused plasma glucose to fall by 4.4 ± 0.2 mM to a nadir of 1.7 ± 0.2 mM. Arterial epinephrine (EPI) levels increased by 13,980 ± 1860 pM ($P < 0.005$), confirming marked activation of the ANS. Pancreatic output of glucagon increased from 0.53 ± 0.12 to 2.04 ± 0.38 ng/min during hypoglycemia (change [Δ] +1.51 ± 0.33 ng/min, $P < 0.005$). This increased arterial plasma glucagon from 27 ± 3 to 80 ± 15 ng/L (Δ +52 ± 14 ng/L, $P < 0.025$). Hexamethonium markedly reduced the ANS response to insulin injection (ΔEPI +2130 ± 600 pM, $P < 0.025$ vs. control) despite a similar fall of plasma glucose (Δ −4.1 ± 0.2 mM) and a lower nadir (0.6 ± 0.1 mM). Both the pancreatic glucagon response (Δglucagon output +0.45 ± 0.24 ng/min) and the arterial immunoreactive glucagon response (Δ +5 ± 4 ng/L) were substantially reduced by hexamethonium ($P < 0.025$). Vagotomy plus spinal cord section totally abolished the arterial EPI response to insulin injection despite a larger fall of plasma glucose (Δ −5.4 ± 0.4 mM) and a lower nadir (0.9 ± 0.2 mM). Again, both the pancreatic glucagon response (Δglucagon output +0.27 ± 0.23 ng min) and the arterial glucagon response (Δ +5 ± 2 ng/L) were significantly reduced (both $P < 0.025$ vs. control). We conclude that autonomic activation contributes to the glucagon response to marked insulin-induced hypoglycemia in dogs. *Diabetes* 40:1107–14, 1991

Glucagon secretion increases during insulin-induced hypoglycemia, and this increased secretion is the primary factor responsible for the recovery of plasma glucose levels from acute hypoglycemia (1,2). Hypoglycemia is also known to activate the autonomic nervous system (ANS), increasing the level of circulating epinephrine (3,4) and activating the parasympathetic nerves to the islet as reflected by increased secretion of the vagally sensitive hormone pancreatic polypeptide (5,6). In addition, it has been demonstrated that the direct sympathetic innervation to the pancreas can be activated during glucopenic stress (7). Although each of these autonomic inputs to the pancreas has the potential to increase glucagon secretion (8–10) and therefore to mediate the glucagon response to hypoglycemia, the vast majority of experiments performed to test this question have shown that interfering with one portion of the autonomic activation or blocking its effects with classic receptor antagonists has little or no influence on the glucagon response to hypoglycemia (11–19). These results and the demonstration that lowering the perfusate glucose level can stimulate glucagon secretion from the isolated, perfused pancreas (20) and from isolated islets (21) have led to the dominant view that the increase of glucagon secretion during insulin-induced hypoglycemia is due to a direct effect of low glucose at the level of the islet α-cell.

However, there are suggestions from one animal study (22) that interference with a single element of the ANS delays the glucagon response to hypoglycemia and that a combination of blockade and ablation of the parasympathetic and sympathoadrenal responses significantly reduces the glu-

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cagon response. In reviewing the published experiments examining this question (23), we hypothesized that part of the problem with demonstrating a significant autonomic contribution to the glucagon response to hypoglycemia may be redundant autonomic stimulation of glucagon secretion. Thus, blockade or ablation of the sympathoadrenal input to the islet would leave the parasympathetic input unblocked and vice versa. If this hypothesis is correct, blocking or ablating the activation of all three autonomic inputs to the islet would be needed to determine the true contribution of the whole ANS to the glucagon response.

Therefore, in this study, we either impaired both the parasympathetic and sympathoadrenal inputs to the pancreas pharmacologically with the nicotinic acetylcholine antagonist hexamethonium or eliminated both inputs surgically by cervical vagotomy and cervical spinal cord transection. We then measured arterial and pancreatic venous glucagon responses to insulin-induced hypoglycemia during acute, terminal experiments in halothane-anesthetized laparotomized dogs. Glucagon responses were compared to those in control dogs in the absence of hexamethonium or surgical ablation.

RESEARCH DESIGN AND METHODS

After an overnight fast (~18 h), adult dogs of mixed breed (24–40 kg) were anesthetized with the ultrashort-acting barbiturate thiamylal sodium (Surital, Parke Davis, Morris Plains, NJ). Anesthesia was subsequently maintained with halothane (0.8%) administered from a calibrated vaporizer (North American Draeger, Telford, PA) by mechanical ventilation in 100% O_2. This anesthetic regimen was chosen because it provides full surgical anesthesia but does not suppress parasympathetic or sympathoadrenal activation induced by the neuroglucopenic agent 2-deoxy-D-glucose (24,25), as do some other anesthetics, e.g., pentobarbital sodium (26,27).

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 was placed between the superior pancreaticoduodenal vein (SPDV) and the portal vein (28). This procedure allowed the measurement of hormone output from the right lobe (duodenal region and uncinate process) of the canine pancreas (~35–50% of the 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 experiments.

All surgery and experiments were performed in the presence of full surgical anesthesia. These experiments were acute, terminal procedures. At the conclusion of the experimental protocols, all dogs used for these studies were killed with an overdose of the barbiturate anesthetic thiopental sodium (Pentothal, Abbott, North Chicago, IL) without regaining consciousness.

Pharmacological autonomic blockade. To produce pharmacological autonomic impairment, six dogs received the nicotinic acetylcholine antagonist hexamethonium bromide (0.2–0.5 mg/kg + 1.4–3.5 $\mu g \cdot kg^{-1} \cdot min^{-1}$ i.v.; Sigma, St. Louis, MO) 20–30 min before baseline samples were drawn. The dose was variable because the drug was administered in increments until arterial blood pressure was affected; to ensure significant ganglionic blockade, 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 (9). After the bolus injections, an increment of 0.7 $\mu g \cdot kg^{-1} \cdot min^{-1}$ for each 0.1-mg/kg dose administered initially was infused continuously throughout the experiments. Hexamethonium impairs neurotransmission across parasympathetic and sympathetic ganglia and the adrenal medulla, because postsynaptic signaling in all three involves nicotinic receptors (29).

Surgical autonomic ablation. In six dogs, surgical ablation of the parasympathetic and sympathoadrenal pathways was performed to prevent autonomic activation during hypoglycemia. In these dogs, the right and left cervical vagal trunks were dissected free from the carotid arteries and severed. Then 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 2% lidocaine HCl was injected with a 25-gauge needle directly into the exposed spinal cord in several locations. Five minutes later, 2–3 cm of the cord was excised to insure 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. After the experiments, all dogs were killed with an overdose of anesthetic without regaining consciousness.

Hypoglycemia protocol. One hour after the surgical procedures, paired blood samples for glucose, glucagon, and catecholamine determination were drawn from the femoral artery and 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 pork insulin (Squibb-Novo, 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 <2.2 mM in all control dogs. Hexamethonium-treated dogs or vagotomized, cord-sectioned dogs received less insulin (0.6 U/kg) because lower doses were required to produce decrements of plasma glucose that were equal to or greater than those in the control dogs. Plasma glucose fell to <1.4 mM in all hexamethonium-treated and surgically ablated dogs. 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 in all dogs by administering glucose (50% solution) intravenously as a bolus of 200 mg/kg followed by an infusion of 10–20 mg $\cdot kg^{-1} \cdot min^{-1}$ 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, Seattle, WA). 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

EGTA and glutathione. All samples were kept on ice until centrifugation (20 min at 4°C). The plasma was then decanted and frozen at −20°C until assayed.

Plasma glucose was assayed by the glucose oxidase method with a Technicon autoanalyzer (San Francisco, 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 (30). It has been previously demonstrated that extrapancreatic immunoreactive glucagon measured with COOH-terminal–specific antisera does not increase during insulin-induced hypoglycemia in dogs (31,32). Thus, measurements of arterial glucagon made with this assay are a reliable index of pancreatic glucagon secretion. Plasma norepinephrine and epinephrine were measured in duplicate with a highly sensitive and specific radioenzymatic assay (33). The intra- and interassay coefficients of variation for the plasma catecholamine assay in this laboratory are 6 and 12%, respectively.

The changes of arterial plasma glucose, arterial and pancreatic venous glucagon, pancreatic glucagon output, and arterial norepinephrine and epinephrine 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 injection of insulin. Pancreatic IRG output was calculated as

$$IRG_{SPDV} - IRG_{FA} \times \text{SPDV blood flow} \times (1 - \text{hematocrit})$$

Data are expressed as means ± 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.

RESULTS

Glucose, catecholamine, and glucagon responses in control dogs. In control dogs (n = 9), arterial plasma glucose levels averaged 6.1 ± 0.2 mM before the intravenous administration of insulin (5.0 ± 1.4 U/kg) and decreased by 4.3 ± 0.2 mM to a mean level of 1.9 ± 0.2 mM 45–60 min after insulin injection. The nadir was 1.7 ± 0.1 mM 60 min after insulin injection (Fig. 1).

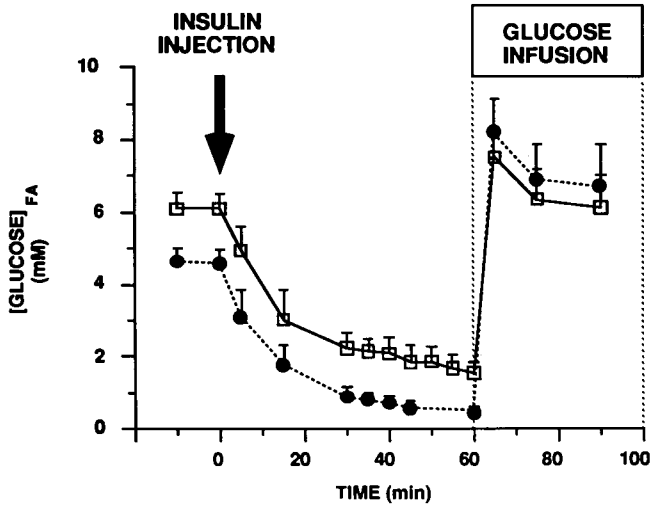

FIG. 1. Comparison of arterial plasma glucose before and after insulin injection and during posthypoglycemia glucose infusion in control (□; n = 9) and hexamethonium-treated (●; n = 6) dogs. FA, femoral artery.

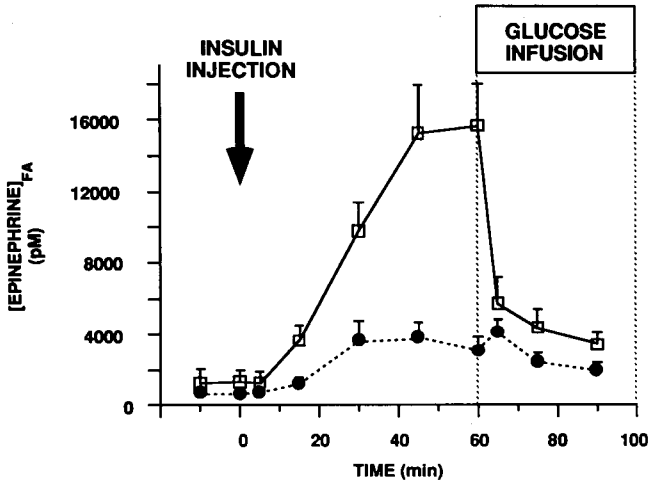

FIG. 2. Comparison of arterial plasma epinephrine before and after insulin injection and during posthypoglycemia glucose infusion in control (□; n = 9) and hexamethonium-treated (●; n = 6) dogs. FA, femoral artery.

Epinephrine levels in arterial plasma were 1640 ± 550 pM before insulin administration and increased to 15,620 ± 2130 pM (45–60 min after insulin injection) during hypoglycemia (change [Δ] +13,980 ± 1860 pM, $P < 0.0005$). Epinephrine rapidly decreased when glucose (200 mg/kg ± 10–20 mg · kg^{-1} · min^{-1}) was infused to reverse the hypoglycemia (Fig. 2). Arterial plasma norepinephrine levels increased from a baseline of 1.02 ± 0.18 to 4.44 ± 0.54 nM (45–60 min after insulin injection; Δ +3.42 ± 0.48 nM, $P < 0.0005$) and also decreased after glucose infusion (Fig. 3).

Arterial plasma glucagon increased from a baseline level of 27 ± 3 ng/L to an average of 80 ± 15 ng/L during hypoglycemia (Δ +52 ± 14 ng/L, $P < 0.005$; Fig. 4). Baseline glucagon levels in SPDV plasma were 107 ± 17 ng/L and increased to 553 ± 97 ng/L during hypoglycemia (Δ +446 ± 91 ng/L, $P < 0.0025$). Pancreatic venous blood flow was 11.1 ± 1.6 ml/min and decreased during hypoglycemia to 8.4 ± 1.3 ml/min (Δ −2.7 ± 0.7 ml/min, $P <$ 0.0025). Hematocrit was 40 ± 1% before and 42 ± 1% after insulin injection (Table 1). Pancreatic glucagon output (see METHODS) increased from a baseline rate of 0.53 ± 120 to 2.04 ± 0.38 ng/min (45–60 min after insulin injection; Δ +1.510 ± 0.33 ng/min, $P < 0.005$; Table 1).

Glucose, catecholamine, and glucagon responses in dogs pretreated with hexamethonium. Plasma glucose before insulin injection was lower in hexamethonium-treated dogs (n = 6) than in control dogs, averaging 4.6 ± 0.2 mM. After insulin injection (0.6 ± 0.1 U/kg), plasma glucose declined by 3.9 ± 0.2 mM, a decrement similar to that observed in control dogs. The nadir of 0.6 ± 0.1 mM (60 min after insulin injection) was significantly lower than in control dogs ($P < 0.0005$; Fig. 1).

Baseline arterial plasma epinephrine levels averaged 660 ± 220 pM, a reduction of ~60% compared with control dogs. During hypoglycemia, epinephrine increased to 2840 ± 660 pM (45–60 min after insulin injection; Δ +2130 ± 600 pM, $P < 0.01$), a significantly smaller response than observed in control dogs ($P < 0.0005$; Fig. 2). Likewise, both baseline and hypoglycemia-stimulated arterial plasma norepinephrine levels were suppressed in hexamethonium-treated dogs, averaging 0.66 ± 0.24 nM before and increasing to 1.86 ± 0.42 nM during hypoglycemia (Δ +1.20 ± 0.36 nM, $P < 0.025$). This response was also significantly smaller than in control dogs ($P < 0.0025$; Fig. 3).

Baseline arterial plasma glucagon was 32 ± 7 ng/L and did not increase significantly, averaging 37 ± 5 ng/L during hypoglycemia (Δ +5 ± 4 ng/L), significantly less than in control dogs ($P < 0.01$; Fig. 4). Pancreatic venous glucagon was 207 ± 98 ng/L and increased marginally to 350 ± 127 ng/L (Δ +143 ± 53 ng/L, $P < 0.025$). Pancreatic venous blood flow was 5.7 ± 0.4 ml/min before and 5.0 ± 0.6 ml/min during hypoglycemia. Hematocrit was 37 ± 2% before insulin injection and did not change during hypoglycemia (Table 1).

Baseline pancreatic glucagon output in hexamethonium-treated dogs averaged 0.55 ± 0.25 ng/min. Glucagon output was 1.00 ± 0.37 ng/min during hypoglycemia. The increase of pancreatic glucagon output during hypoglycemia (Δ +0.45 ± 0.24 ng/min) was not statistically significant and was only 30% of that observed in control dogs ($P < 0.025$ vs. control dogs; Table 1).

Glucose, catecholamine, and glucagon responses in cord-sectioned, vagotomized dogs. Plasma glucose in cord-sectioned, vagotomized dogs ($n = 6$) was 6.2 ± 0.5 mM before insulin injection (0.7 ± 0.1 U/kg) and decreased to 1.0 ± 0.1 mM 45–60 min after insulin ($P < 0.0005$ vs. control dogs) with a nadir of 0.9 ± 0.2 mM at 60 min. The decrease of −5.2 ± 0.4 mM was significantly greater than in control dogs ($P < 0.0025$; Fig. 5).

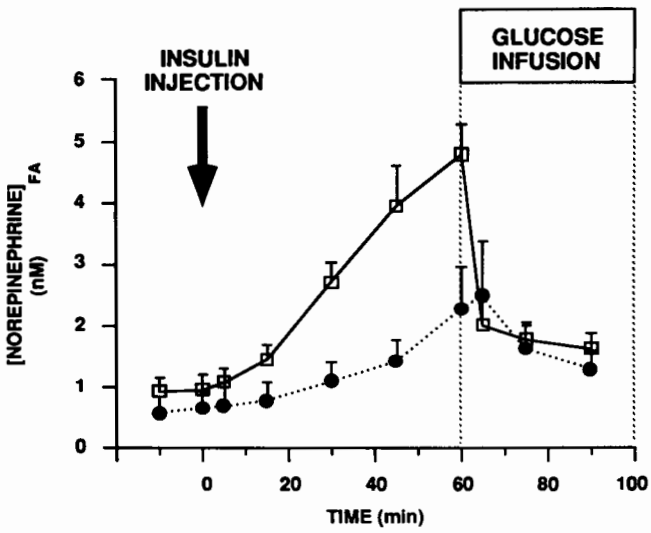

FIG. 3. Comparison of arterial plasma norepinephrine before and after insulin injection and during posthypoglycemia glucose infusion in control (□; $n = 9$) and hexamethonium-treated (●; $n = 6$) dogs. FA, femoral artery.

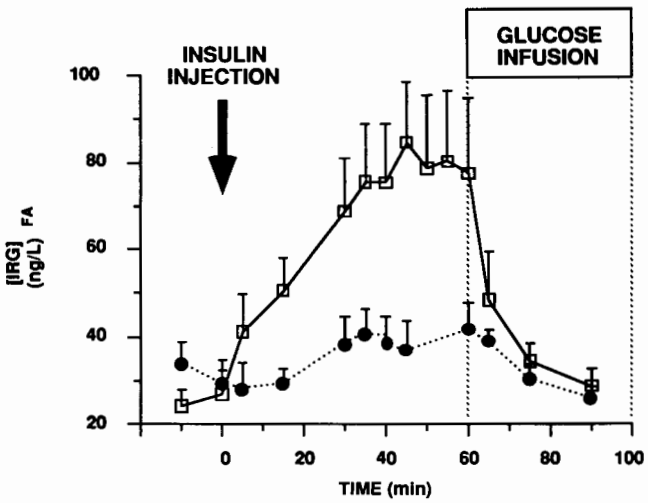

FIG. 4. Comparison of arterial plasma immunoreactive glucagon (IRG) before and after insulin injection and during posthypoglycemia glucose infusion in control (□; $n = 9$) and hexamethonium-treated (●; $n = 6$) dogs. FA, femoral artery.

Arterial plasma epinephrine did not increase significantly in these dogs during hypoglycemia, averaging 220 ± 50 pM before and 490 ± 270 pM 45–60 min after insulin injection (Fig. 6). Similarly, arterial plasma norepinephrine was unchanged during hypoglycemia, with levels of 0.12 ± 0.06 pg/ml and 0.30 ± 0.12 pg/ml before and during hypoglycemia, respectively (Fig. 7).

Baseline arterial plasma glucagon was 32 ± 3 ng/L and did not increase significantly, averaging 37 ± 3 ng/L during hypoglycemia (Δ +5 ± 2 ng/L), which was significantly less than in control dogs ($P < 0.025$; Fig. 8). Pancreatic venous glucagon also did not increase significantly during hypoglycemia in the cord-sectioned, vagotomized dogs, averaging 79 ± 23 ng/L before and 135 ± 54 ng/L after insulin injection (Δ +55 ± 40 ng/L).

Pancreatic venous blood flow was 6.5 ± 0.9 ml/min before insulin administration and 6.3 ± 0.9 ml/min during hypoglycemia. Hematocrit was lower in these dogs (31 ± 2% before and 29 ± 3% during hypoglycemia). The increase of pancreatic glucagon output during the hypoglycemic period (Δ +0.27 ± 0.23 ng/min) was not statistically significant and was <20% of that observed in control dogs ($P < 0.01$; Table 1).

DISCUSSION

Most studies have not found a significant role for the ANS in mediating the glucagon response to insulin-induced hypoglycemia. For example, numerous in vivo studies have examined glucagon responses to hypoglycemia in subjects in which portions of the autonomic activation or its effects were selectively impaired by vagotomy (11), atropine (11,15), adrenalectomy (12,13), quadriplegia (14), or adrenergic antagonists (15–18) and compared them to responses in normal control subjects. These interventions did not attenuate the glucagon response to hypoglycemia. Thus, the prevailing view is that, during hypoglycemia, the α-cell is stimulated directly by low glucose levels. However, hypo-

TABLE 1
Baseline immunoreactive glucagon (IRG) and IRG responses to hypoglycemia

	IRG (ng/L)				
	Femoral artery	SPDV	SPDV blood flow (ml/min)	1 − hematocrit (%)	IRG output (ng/min)
Control (n = 9)					
Baseline	27 ± 3	107 ± 17	11.1 ± 1.6	60 ± 1	0.53 ± 0.12
During hypoglycemia	80 ± 15	553 ± 97	8.4 ± 1.3	58 ± 1	2.04 ± 0.38
ΔPancreatic IRG output					+1.51 ± 0.33
Hexamethonium treated (n = 6)					
Baseline	32 ± 7	207 ± 98	5.7 ± 0.4	63 ± 2	0.55 ± 0.25
During hypoglycemia	37 ± 5	350 ± 127	5.0 ± 0.6	63 ± 2	1.00 ± 0.37
ΔPancreatic IRG output					+0.45 ± 0.24
Vagotomized, spinal cord transection (n = 6)					
Baseline	32 ± 3	79 ± 23	6.5 ± 0.9	69 ± 2	0.17 ± 0.7
During hypoglycemia	37 ± 3	135 ± 54	6.3 ± 0.9	71 ± 3	0.44 ± 0.28
ΔPancreatic IRG output					+0.27 ± 0.23

Values are means ± SE 45–60 min after insulin injection. SPDV, superior pancreaticoduodenal vein; Δ, change.

glycemia can activate three autonomic inputs to the islet during hypoglycemic stress, i.e., adrenal medullary (3,4), parasympathetic (5,6), and sympathetic neural inputs to the pancreas (7), each of which is capable of stimulating glucagon secretion (8–10). Furthermore, there is evidence that both the parasympathetic and sympathetic neural innervation of the pancreas can involve peptidergic rather than classic adrenergic and cholinergic postganglionic neurotransmission (34–37). Thus, the islet effects of these peptide neurotransmitters are probably not blocked by classic postganglionic adrenergic and cholinergic antagonists. Therefore, there is a potential for redundant and peptidergic stimulation of glucagon secretion during insulin-induced hypoglycemia that may have led to an underestimation of autonomic mediation in those studies in which the autonomic activation was only partially impaired or in which classic postganglionic antagonists were used. We have therefore hypothesized that it is necessary to prevent activation of all three autonomic inputs to the islet to reveal the full autonomic contribution to the glucagon response to hypoglycemia (23).

In this study, two approaches for preventing autonomic activation during hypoglycemia were used. One group of dogs received the nicotinic acetylcholine antagonist hexamethonium to block neurotransmission across parasympathetic and sympathetic ganglia and within the adrenal medulla (29). In another group of dogs, the combined surgical procedures of bilateral cervical vagotomy and cervical spinal cord transection were employed. Both approaches would either reduce or eliminate the release of postganglionic neurotransmitters and neurohormones, whether classic or peptidergic. The efficacy of both of these experimental approaches was judged from the reduction of plasma catecholamine responses to the hypoglycemia.

Arterial plasma epinephrine and norepinephrine re-

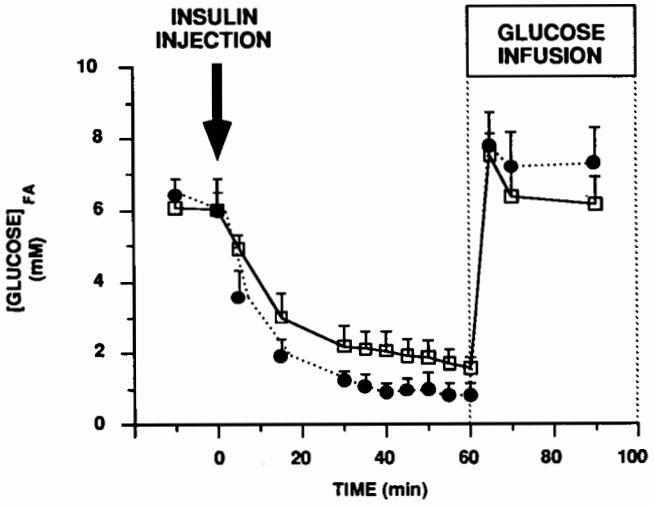

FIG. 5. Comparison of arterial plasma glucose after insulin injection in control (□; n = 9) and vagotomized, cord-sectioned (●; n = 6) dogs. FA, femoral artery.

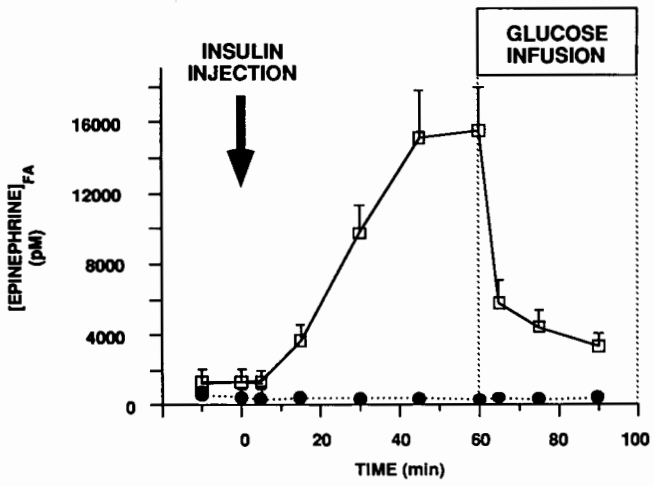

FIG. 6. Comparison of arterial plasma epinephrine before and after insulin injection and during posthypoglycemia glucose infusion in control (□; n = 9) and vagotomized, cord-sectioned (●; n = 6) dogs. FA, femoral artery.

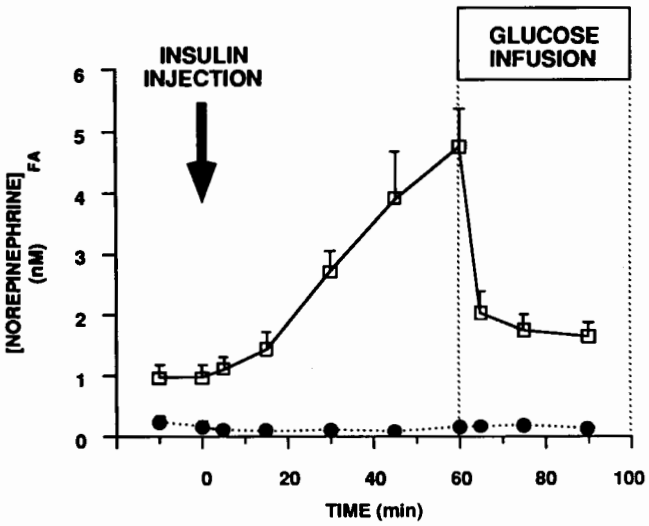

FIG. 7. Comparison of arterial plasma norepinephrine before and after insulin injection and during posthypoglycemia glucose infusion in control (□; *n* = 9) and vagotomized, cord-sectioned (●; *n* = 6) dogs. FA, femoral artery.

sponses in the hexamethonium-treated dogs were reduced by 80%, demonstrating that hexamethonium substantially attentuated sympathodrenal activation despite the presence of a lower plasma glucose nadir, i.e., a stronger stimulus, than in control dogs. In the vagotomized, cord-sectioned dogs, the reduction of sympathoadrenal activation was more effective; arterial plasma epinephrine and norepinephrine responses to hypoglycemia were absent, again despite glucose nadirs that were lower than those in control dogs. In both the hexamethonium-treated and cord-sectioned, vagotomized groups of dogs, arterial glucagon and pancreatic glucagon output responses were markedly attenuated compared with the control dogs. Thus, when the autonomic response to hypoglycemia was pharmacologically impaired or surgically ablated in the dog, the arterial glucagon response to marked hypoglycemia was substantially diminished.

However, the pancreatic glucagon output data show a greater residual response than suggested by the arterial glucagon data (Table 1). For example, in the dogs treated with hexamethonium, nearly 33% of the pancreatic glucagon output response remained. Thus, pancreatic glucagon output may be a more sensitive index of small changes of pancreatic glucagon secretion. Indeed, in previous studies, we observed that stimuli that produce a doubling of pancreatic glucagon output did not produce a significant increase of arterial glucagon levels (38,39). Therefore, although the arterial glucagon responses to insulin-induced hypoglycemia are quite small, there appears to be a greater residual glucagon response revealed by the more sensitive pancreatic glucagon output measurement. Those data suggest that, although the ANS makes a substantial contribution to the glucagon response, it is probably not the only mediator of this response.

The results from this study are in agreement with those of another study designed to eliminate all of the three autonomic inputs to the islet during insulin-induced hypoglycemia. Bloom et al. (22) found that the combination of atropine administration and splanchnic nerve transection impaired the glucagon response to insulin-induced hypoglycemia in conscious calves. Data from a more recent study in dogs are likewise consistent with our study. In a study conducted by Biggers et al. (40), peripheral hypoglycemia, in the absence of autonomic activation, did not increase plasma glucagon levels. The central hypoglycemia normally induced by insulin administration was prevented by infusing glucose into the carotid and vertebral arteries. This experimental manipulation substantially reduced the parasympathetic and sympathoadrenal responses to hypoglycemia and eliminated the glucagon response (40).

In humans, the preponderance of data from studies utilizing partial autonomic impairment demonstrated no reduction of glucagon responses to insulin-induced hypoglycemia (11–17). However, there have been very few human studies in which activation of all three autonomic inputs to the pancreas, whether classic or peptidergic, has been simultaneously blocked. One study, in which the ganglionic blocker trimethaphan camsylate was used, presumably achieving such a combined blockade, showed a reduced glucagon response to insulin-induced hypoglycemia in men (41). Interestingly, in the same study, partial autonomic blockade with atropine did not affect the glucagon response, in agreement with other studies (11,15). The only other evidence for autonomic mediation of glucagon responses to hypoglycemia in humans is much less direct. For example, impaired glucagon secretion during hypoglycemia has been demonstrated in patients with Shy-Drager syndrome (idiopathic autonomic insufficiency; 42) or chronic Chagas' disease (*Trypanosoma cruzi* infection; 43). Both of these pathological states present with combined parasympathetic and sympathetic nervous system dysfunction (44,45). However, because the experimental evidence for redundant autonomic mediation of the α-cell response to hypoglycemia in humans is inconclusive, more complete studies are necessary to definitively establish such mediation of the glucagon response to insulin-induced hypoglycemia in humans.

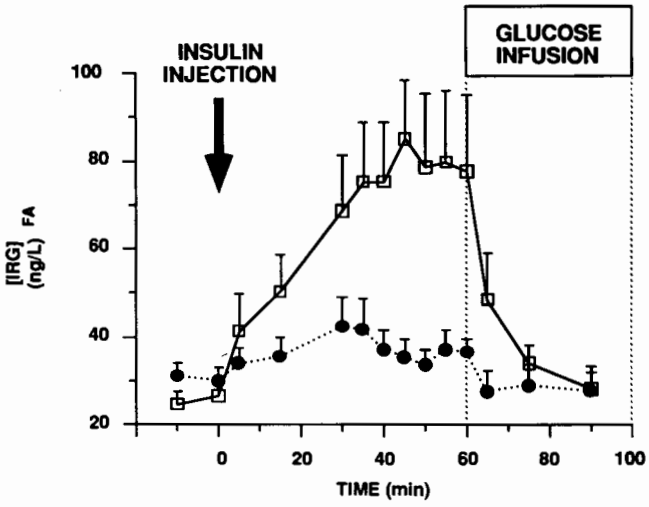

FIG. 8. Comparison of arterial plasma immunoreactive glucagon (IRG) before and after insulin injection and during posthypoglycemia glucose infusion in control (□; *n* = 9) and vagotomized, cord-sectioned (●; *n* = 6) dogs. FA, femoral artery.

The magnitude of the residual glucagon response to extreme hypoglycemia in cord-sectioned, vagotomized animals suggests that the direct effect of low glucose concentrations on the α-cell is not the major factor for increasing glucagon secretion during marked hypoglycemia in anesthetized dogs. These data agree with those of the previously discussed dog study in which glucagon secretion did not increase in response to a substantial decrement of peripheral plasma glucose when central hypoglycemia was prevented (40). In contrast, in vitro studies have suggested a direct effect of low glucose concentration on the α-cell, because lowering the glucose concentration in the media perfusing isolated pancreas preparations (20) or isolated islets (21) increased glucagon secretion.

Likewise, the glucagon response to hypoglycemia in humans and other species may be due to direct stimulation of the α-cell by hypoglycemia, a view consistent with the results from numerous studies employing single, rather than combined, impairment of the autonomic inputs to the pancreas (11–19). Paradoxically, the relative contribution of direct effects of hypoglycemia to the α-cell response may be larger at less-marked degrees of hypoglycemia, such as those employed in many human studies, because the sympathoadrenal activation would be less intense and would therefore provide less autonomic stimulation of the α-cell. Furthermore, because less insulin is required to produce more moderate hypoglycemia, the smaller potentially direct suppressive effects of insulin on the α-cell (46) might allow more modest hypoglycemia to significantly contribute to glucagon secretion. Thus, the results of our experiments do not rule out a direct effect of low glucose concentration on the glucagon response to hypoglycemia under other experimental conditions, particularly in other species, and at differing degrees of hypoglycemia. Rather, these results emphasize the need to reexamine the role of the ANS with approaches that reduce or prevent activation of all of the potentially redundant autonomic inputs to the α-cell.

In summary, in halothane-anesthetized, laparotomized dogs, either surgical or pharmacological interruption of autonomic activation during severe insulin-induced hypoglycemia significantly reduces the glucagon response observed in control dogs. Thus, these experiments demonstrated that autonomic activation can make an important contribution to increased glucagon secretion during marked hypoglycemia in this species. Furthermore, they showed that the direct effect of low plasma glucose concentration at the level of the islet is not the major mediator of the glucagon response when marked hypoglycemia is produced in vivo by the injection of large amounts of insulin. Finally, they suggested the need for similar experiments to determine the contribution of the ANS to glucagon responses in other species and at different degrees of hypoglycemia.

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