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# Autonomic Mediation of Glucagon Secretion During Hypoglycemia

## Implications for Impaired $\alpha$ -Cell Responses in Type 1 Diabetes

Gerald J. Taborsky Jr, Bo Ahrén, and Peter J. Havel

This article examines the role of the autonomic nervous system in mediating the increase of glucagon secretion observed during insulin-induced hypoglycemia (IIH). In the first section, we briefly review the importance of the  $\alpha$ -cell response in recovery from hypoglycemia under both physiologic conditions and pathophysiologic conditions, such as type 1 diabetes. We outline three possible mechanisms that may contribute to increased glucagon secretion during hypoglycemia but emphasize autonomic mediation. In the second section, we review the critical experimental data in animals, nonhuman primates, and humans suggesting that, in the absence of diabetes, the majority of the glucagon response to IIH is mediated by redundant autonomic stimulation of the islet  $\alpha$ -cell. Because the glucagon response to hypoglycemia is often impaired in patients with type 1 diabetes, in the third section, we examine the possibility that autonomic impairment contributes to the impairment of the glucagon response in these patients. We review two different types of autonomic impairment. The first is a slow-onset and progressive neuropathy that worsens with duration of diabetes, and the second is a rapid-onset, but reversible, autonomic dysfunction that is acutely induced by antecedent hypoglycemia. We propose that both types of autonomic dysfunction can contribute to the impaired glucagon responses in patients with type 1 diabetes. In the fourth section, we relate restoration of these glucagon responses to restoration of the autonomic responses to hypoglycemia. Finally, in the fifth section, we summarize the concepts underlying the autonomic hypothesis, the evidence for it, and the implications of the autonomic hypothesis for the treatment of type 1 diabetes. *Diabetes* 47:995–1005, 1998

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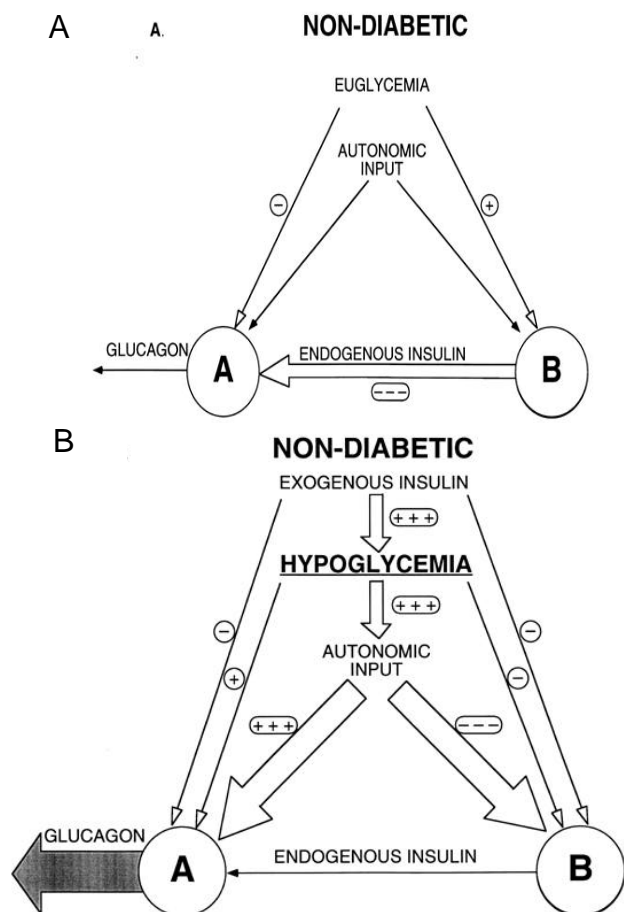
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DAN, diabetic autonomic neuropathy; IIH, insulin-induced hypoglycemia; PP, pancreatic polypeptide; VIP, vasoactive intestinal peptide.

### GLUCAGON SECRETION DURING HYPOGLYCEMIA: CLINICAL IMPORTANCE AND POTENTIAL MECHANISMS

**Clinical importance.** Hypoglycemia is a major acute complication of type 1 diabetes, contributing to both its morbidity and its mortality (1). Attempts to reduce the long-term complications associated with type 1 diabetes, such as retinopathy and nephropathy, by reducing hyperglycemia via intensive insulin therapy have unfortunately exacerbated this acute complication: intensive insulin therapy produces a threefold increase of the incidence of severe hypoglycemia (2,3). For this reason, hypoglycemia is a major limiting factor in improving glycemic control (4) and, therefore, in preventing or reducing the long-term complications associated with type 1 diabetes. Consequently, it is important to understand the physiologic mechanisms that limit the severity and duration of hypoglycemic episodes.

The work of Gerich and Cryer (5,6) established the importance of glucagon and epinephrine in limiting the glucose nadir and promoting rapid recovery of plasma glucose from rapid and transient hypoglycemia in nondiabetic human subjects. Later studies, primarily by Bolli et al., also suggested important roles for glucagon (7) and epinephrine (8) in countering the slow-onset, but progressive, hypoglycemia that is more often encountered clinically (9). Because most patients who have had type 1 diabetes for 1–2 decades have totally lost their glucagon response to IIH and have blunted epinephrine responses, these studies helped to explain the impairment of glucose recovery observed in patients with long-term type 1 diabetes (10–14). In many patients with newly diagnosed type 1 diabetes, both glucagon and epinephrine responses to hypoglycemia are quantitatively normal, as is the rate of glucose recovery from hypoglycemia (15). In contrast, many patients who have had type 1 diabetes for less than a decade have blunted glucagon responses with normal epinephrine responses, but a delayed glucose recovery (15). From these studies, the concept emerged that the glucagon response is critical for normal glucose recovery and that impairment of the glucagon response is a major factor in the susceptibility of patients with type 1 diabetes to episodes of prolonged and severe hypoglycemia (5). Thus, it became important to understand the mechanisms that mediate the normal



**FIG. 1.** Nondiabetic subjects. **A:** During euglycemia, glucagon secretion may be tonically restrained (thin arrow) by 1) a mild inhibitory effect (-) of glucose directly on the  $\alpha$ -cell, 2) the lack of autonomic stimulation of the  $\alpha$ -cell, and 3) a marked inhibitory effect (- - -) of endogenous insulin on the  $\alpha$ -cell. **B:** During hypoglycemia, glucagon secretion is markedly increased (thick arrow), perhaps by 1) a direct stimulatory effect (+) of hypoglycemia on the  $\alpha$ -cell, 2) a marked stimulatory effect (+ + +) of the autonomic nervous system secondary to its activation by central hypoglycemia, and 3) a marked reduction of endogenous insulin (thin arrow), and thus its inhibitory effect on the  $\alpha$ -cell. These stimulatory effects overwhelm any inhibitory effect (-) of exogenous insulin directly on the  $\alpha$ -cell. The marked reduction of endogenous insulin secretion is secondary to 1) an inhibitory effect (-) of hypoglycemia per se on the  $\beta$ -cell, 2) an inhibitory effect (-) of hypoglycemia directly on the  $\beta$ -cell, and 3) a net inhibitory effect (- - -) of the autonomic input to the  $\beta$ -cell.  $\alpha$ - and  $\beta$ -cells are represented as circles labeled A and B, respectively.

glucagon response to hypoglycemia and to determine how these mechanisms are compromised in type 1 diabetes.

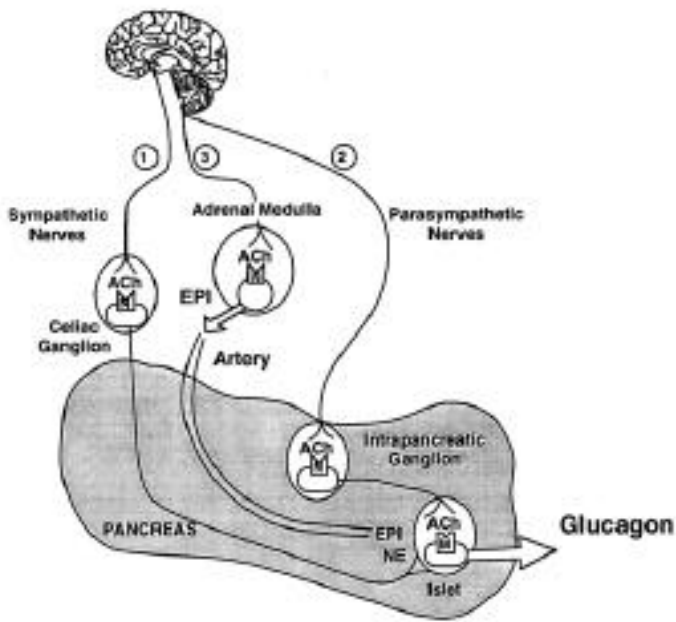
**Potential mechanisms.** Three different mechanisms have been proposed to mediate the  $\alpha$ -cell response to hypoglycemia (Fig. 1). The first is straightforward, namely, that hypoglycemia directly stimulates glucagon secretion from the pancreatic  $\alpha$ -cell. In support of this hypothesis are in vitro experiments in which perfusion of the isolated pancreas with low-glucose media increased glucagon secretion (16). However, this type of experiment may not be as conclusive as it appears, since hypoglycemia also lowers insulin secretion, which can also have effects on the  $\alpha$ -cell. Indeed, lowering the glucose level to which isolated  $\alpha$ -cells are exposed failed to stimulate glucagon secretion (17), suggesting little direct stimulatory effect of hypoglycemia per se on the  $\alpha$ -cell. It remains unclear how much of the

glucagon response in vivo is mediated by glucose recognition directly by the  $\alpha$ -cells.

A second mechanism that could potentially mediate increased glucagon secretion in response to hypoglycemia involves a local inhibitory effect of endogenous insulin on neighboring  $\alpha$ -cells (Fig. 1). Such an action of endogenous insulin was originally proposed by Samols (18), first strongly supported by the extensive data of Weir (19), and later critically reviewed by both (20). This mechanism requires that blood within the islet flows from  $\beta$ -cells to the  $\alpha$ -cells, thereby exposing them to high concentrations of inrailelet insulin, a concept that has both anatomical (21) and physiological (22,23) support. It also requires that insulin or a  $\beta$ -cell product directly inhibit glucagon secretion from the  $\alpha$ -cell. Marked inhibition by insulin has been demonstrated in hamster glucagonoma cells (22), but only modest inhibition has been demonstrated in normal purified rat  $\alpha$ -cells (17), which apparently lack insulin receptors (24). Thus, other  $\beta$ -cell products, such as gamma aminobutyric acid (25), might also mediate the inhibitory effect of the  $\beta$ -cell on the  $\alpha$ -cell. Alternatively, high concentrations of inrailelet insulin could cross-react with the IGF-1 receptors found on  $\alpha$ -cells (26). Whatever the mediator, hypoglycemia would inhibit secretion from the  $\beta$ -cell and thereby remove the tonic local inhibitory restraint on the  $\alpha$ -cell, resulting in increased glucagon secretion (20,27,28). In vivo, exogenous insulin has been shown to impair the glucagon response to arginine in patients with type 1 diabetes who have little endogenous insulin secretion (29). Endogenous insulin also apparently inhibits glucagon secretion, at least in vitro. For example, neutralizing endogenous insulin by perfusion of the isolated pancreas with high titer anti-insulin antibodies increases glucagon secretion in a variety of species (22,30). Also, destruction of islet  $\beta$ -cells prevents low glucose media from stimulating glucagon secretion in vitro (31). Thus, a role for inrailelet insulin or a  $\beta$ -cell product in the glucagon response to hypoglycemia is supported by a variety of in vitro data. Its role in vivo is less clear, however: when endogenous insulin secretion is partially suppressed before hypoglycemia in nondiabetic humans, there is still a quantitatively normal  $\alpha$ -cell response to hypoglycemia (32). Similarly, patients with newly diagnosed type 1 diabetes, who presumably have markedly reduced endogenous insulin secretion, also exhibit a normal increase of glucagon secretion in response to hypoglycemia (32). Thus, either suppression of endogenous insulin is not the mediator of the glucagon response to hypoglycemia in vivo, or complete suppression of endogenous insulin secretion, as seen only during hypoglycemia (32), is necessary to stimulate glucagon secretion (see GLUCAGON RESPONSE for further discussion).

A third potential mediator of the glucagon response to hypoglycemia is the autonomic input to the  $\alpha$ -cell (33) (Fig. 1). There are three major autonomic inputs to the  $\alpha$ -cell: sympathetic nerves, parasympathetic nerves, and the circulating neurohormone epinephrine (Fig. 2). Inclusion of epinephrine as part of the autonomic response to hypoglycemia is consistent with its established role as a key mediator of the metabolic effects of sympathetic activation during other stresses (34).

All three of these autonomic inputs to the  $\alpha$ -cell—sympathetic nerves, parasympathetic nerves, and adrenal medulla—are activated by hypoglycemia (33). Cannon first demon-



**FIG. 2.** Three autonomic inputs to the  $\alpha$ -cell. Preganglionic sympathetic nerves (1) travel in the spinal cord releasing acetylcholine (ACh) from their terminals within the celiac ganglion. The released ACh acts on nicotinic (N) receptors on postganglionic sympathetic neuronal cell bodies, which send fibers that innervate the A-cells. These nerves release norepinephrine (NE) and sympathetic neuropeptides (not shown) at their terminals within the islet. Preganglionic parasympathetic nerves travel in the vagus (2), releasing ACh from their terminals within intrapancreatic parasympathetic ganglia. The released ACh acts on N receptors on postganglionic parasympathetic neuronal cell bodies, which send fibers to the  $\alpha$ -cells. These nerves release ACh at their terminals within the islet, which acts on muscarinic receptors (M) on the  $\alpha$ -cells. They also release parasympathetic neuropeptides (not shown). Preganglionic sympathetic nerves from the spinal cord innervate the adrenal medulla (3), releasing ACh to activate N receptors on chromaffin cells. Activation of these receptors stimulates the release of the sympathetic neurohormone epinephrine (EPI), which reaches the  $\alpha$ -cell via the arterial circulation. Each of these three autonomic pathways is activated by hypoglycemia.

strated over 70 years ago that hypoglycemia stimulates the secretion of epinephrine from the adrenal medulla. In contrast, the activation of pancreatic sympathetic nerves by hypoglycemia has only recently been demonstrated (35,36). The demonstration that parasympathetic nerves to the pancreas are activated during hypoglycemia is indirect: atropine blocks the hypoglycemia-induced secretion of pancreatic polypeptide (PP) from the islet F-cell (33,37).

Each of these three autonomic inputs to the  $\alpha$ -cell is capable of stimulating glucagon secretion (33). Epinephrine stimulates glucagon secretion both *in vitro* (38) and *in vivo* (39). Electrical stimulation of pancreatic sympathetic nerves (40) or local infusion of the classical sympathetic neurotransmitter norepinephrine (41) or a pancreatic sympathetic neuropeptide, such as galanin (42), all increase glucagon secretion. Finally, electrical stimulation of parasympathetic nerves (43,44) or local infusion of the classical parasympathetic neurotransmitter acetylcholine (45) or a pancreatic parasympathetic neuropeptide, such as vasoactive intestinal peptide (VIP), (46,47) all stimulate glucagon secretion. Thus, three different autonomic pathways can potentially stimulate glucagon secretion during hypoglycemia via the actions of

several different transmitters (Fig. 2), suggesting the possibility of redundant autonomic mediation.

Although the three mechanisms above have been discussed separately, there is evidence for autonomic participation both in the effect of glucose on the  $\alpha$ -cell *in vitro* and in the suppression of insulin secretion *in vivo*. For example, *in vitro* perfusion of the pancreas with low glucose media can release norepinephrine from the sympathetic nerve terminals of the pancreas (48), and  $\alpha$ -adrenergic blockade during such perfusions nearly abolishes the glucagon response to glucopenia (49). Thus, surprisingly, there is evidence for autonomic mediation of glucagon response to glucopenia, even *in vitro*. These findings provide an alternative to the interpretation that hypoglycemia directly stimulates the  $\alpha$ -cell.

Evidence *in vivo* suggests that part of the suppression of endogenous insulin secretion seen during IIH is due to activation of the autonomic nervous system. The evidence for the effect of hyperinsulinemia on endogenous insulin secretion includes suppression of C-peptide secretion during hyperinsulinemic euglycemic clamps (32) (Fig. 1). Animal studies had suggested that this inhibition of insulin secretion might be partially neurally mediated (50). This interpretation was confirmed in humans by the observation that patients receiving pancreas transplants (51) showed less suppression of endogenous insulin secretion during hyperinsulinemic clamps. Furthermore, hypoglycemia specifically activates the sympathetic nerves to the pancreas *in vivo* (35), and early experiments with cross-perfused dog pancreas suggest that part of the suppression of insulin secretion during hypoglycemia is sympathetically mediated (52). Thus, it becomes difficult to separate the effects of hypoglycemia that are due to its direct action on the  $\alpha$ -cell or to its suppression of endogenous insulin secretion from those that are mediated via autonomic activation.

#### AUTONOMIC CONTRIBUTION IN NONDIABETIC ANIMALS AND HUMANS

**Animal studies.** In 1989, when we first reviewed the literature on autonomic mediation of glucagon secretion during hypoglycemia (33), the majority of studies in humans were negative. Furthermore, only two studies conducted in animals, one in rats (53) and one in calves (54), suggested a significant autonomic contribution to hypoglycemia-induced glucagon secretion. We hypothesized that redundant stimulation of glucagon secretion by the parasympathetic and sympathoadrenal divisions of the autonomic nervous system (Fig. 2) could explain much of the negative data, and we suggested that new experiments, designed to simultaneously block all three autonomic inputs to the  $\alpha$ -cell, would be required to critically test this hypothesis. One problem with blocking all three autonomic inputs to the  $\alpha$ -cell is the redundancy of postganglionic neurotransmission in pancreatic sympathetic and parasympathetic nerves. Although there are effective antagonists for the actions of the classical neurotransmitters, norepinephrine and acetylcholine, the available antagonists for the peptidergic neuropeptides, galanin and VIP, are not yet reliable *in vivo*. In contrast, ganglionic neurotransmission seems predominately mediated by the release of acetylcholine from preganglionic nerve terminals and its action on the nicotinic receptors of postganglionic cell bodies, including adrenal medullary chromaffin cells (Fig. 2). Therefore, we used nicotinic antagonists to block autonomic activation and demon-

strated a marked reduction in the glucagon response to hypoglycemia in several species (55–58).

Several other studies have provided evidence of central neural mediation. For example, preventing central, but not peripheral, hypoglycemia in dogs with selective carotid and vertebral artery glucose infusions markedly attenuates both parasympathetic (PP) and sympathoadrenal (epinephrine) responses to IIH and eliminates the increase of glucagon seen when the brain and the pancreas are simultaneously exposed to hypoglycemia (59). Further, lesioning of the ventromedial hypothalamus in rats (60) or microdialysis of glucose into this region (61) significantly impairs both autonomic activation and the glucagon responses to IIH. These studies demonstrate the necessity of neural activation for the glucagon response to hypoglycemia in animals.

Other studies in which autonomic inputs were sequentially blocked support the hypothesized redundancy of autonomic stimulation of glucagon secretion during hypoglycemia. For example, the increase of plasma glucagon during IIH in rats was not significantly affected by parasympathetic cholinergic blockade with methylatropine or by  $\alpha$ - and  $\beta$ -adrenergic blockade with tolazoline and propranolol, but the combination of cholinergic and adrenergic blockade markedly impaired the glucagon response (62). There is additional evidence for at least partial redundancy between parasympathetic and sympathoadrenal mechanisms during marked hypoglycemia in mice (56) and calves (54). More recently, in dogs, we found that either the direct sympathetic innervation of the pancreas (63) or adrenal medullary activation (P.J.H., T.O. Mundinger, G.J.T., unpublished observations) can mediate an intact glucagon response to marked hypoglycemia independently of the vagal parasympathetic input. Therefore, a significant autonomic contribution to increased glucagon secretion during hypoglycemia has been documented in nondiabetic animals, and the response appears to be redundantly mediated by the three autonomic inputs to the pancreas (Fig. 2).

However, it was not known whether different mechanisms were operating to regulate glucagon secretion during hypoglycemia in humans and other primates or whether the negative results obtained from the earlier human studies were the result of an unrecognized redundancy similar to that demonstrated in animals. Evidence for the latter interpretation is provided by studies in rhesus monkeys, in which the ganglionic blocking agent trimethaphan was used to impair autonomic activation, while allowing systemic hypoglycemia and hyperinsulinemia. Trimethaphan infusion nearly abolished the autonomic (epinephrine, norepinephrine, and PP) responses and reduced the glucagon response to hypoglycemia of 35 mg/dl (1.9 mmol/l) by ~75% (57), indicating that autonomic activation mediates a substantial portion of the glucagon response in a primate species. In the same study, high doses of atropine combined with  $\alpha$ - and  $\beta$ -adrenergic blockade produced a similar degree of impairment of the glucagon response. Thus, the glucagon response to hypoglycemia in nonhuman primates is mediated via activation of the autonomic nervous system, as it is in other animal species.

**Human studies.** Indirect support for autonomic mediation of the glucagon response in humans has been provided by studies of the acute autonomic dysfunction induced by prior episodes of hypoglycemia (64,65). This phenomenon has been termed hypoglycemia-associated autonomic failure by

Cryer (4). In brief, two prior episodes of hypoglycemia (50 mg/dl) markedly reduce both parasympathetic activation (PP response) and adrenal medullary activation (epinephrine response) to a third episode of hypoglycemia on the following day. The two episodes of prior hypoglycemia also markedly reduce the glucagon response to the third episode of hypoglycemia (64). Similar data are available from studies in rats with much longer periods of recurrent hypoglycemia (66). Because the degree of hypoglycemia in the episodes is closely matched, it is unlikely that the effect of hypoglycemia on the islet, either to directly stimulate the  $\alpha$ -cell or to release the  $\alpha$ -cell from tonic local restraint by high levels of intraislet insulin, was responsible for the impairment of the glucagon response to hypoglycemia. Rather, we suggest that it is related to the impairment of the autonomic activation. Thus, these data indirectly support the hypothesis that the glucagon response to hypoglycemia is autonomically mediated in humans.

The impairment of autonomic activation after recent hypoglycemia appears to be mediated by the increase of circulating cortisol evoked by antecedent episodes of hypoglycemia. Cortisol, in turn, may impair autonomic activation (67) by producing a feedback inhibition of the central corticotropin-releasing hormone (68) thought to be involved in mediating sympathoadrenal activation during hypoglycemia (69). Evidence for the role of cortisol includes induction of autonomic and glucagon impairment by infusion of cortisol at rates that mimic the plasma cortisol response to hypoglycemia (70) and prevention of impaired autonomic and glucagon responses by blocking the cortisol response to antecedent hypoglycemia with metyrapone, an inhibitor of cortisol synthesis (71).

Up-regulation of brain glucose transport may be a separate mechanism that reduces autonomic responses to hypoglycemia. This mechanism is likely to be prominent after long repeated exposure to hypoglycemia, since those are the conditions in which it has been demonstrated both in animals (72,73) and in humans (74). Hypoglycemia-induced cortisol secretion may also be involved in this mechanism of autonomic impairment, since cortisol can induce up-regulation of brain glucose transporters in animals (75). It should be pointed out that up-regulation of brain glucose transport would lessen central glucose deprivation, the stimulus for autonomic activation, whereas the acute effects of cortisol, mediated via suppression of central corticotropin-releasing hormone, would inhibit autonomic outflow even in the presence of central glucopenia.

Additional indirect support for autonomic mediation in humans is provided by studies in patients with chronic autonomic dysfunction resulting from Chagas's disease or Shy-Drager syndrome. These patients have deficient parasympathetic (PP) (76), adrenal medullary (epinephrine) (77), and glucagon responses (76,77) to hypoglycemia. In contrast, there are two studies that found that classical cholinergic and adrenergic receptor antagonists did not reduce the glucagon response to hypoglycemia in nondiabetic human subjects (78,79). However, these antagonists block only the actions of acetylcholine and catecholamines, but not the potential actions of neuropeptides. It is possible that in humans, neuropeptides such as VIP help mediate the glucagon response to hypoglycemia. VIP is found in the parasympathetic nerves (Fig. 2) of the human pancreas (80,81). In animal studies, VIP is released from the pancreas during parasympathetic nerve

stimulation (47) and during IIH (L. Benthem, T.O. Munding, G.J.T., unpublished observations). Finally, local VIP infusion increases glucagon secretion (46,47). Thus, VIP release from pancreatic parasympathetic nerves during IIH could be a mediator of the glucagon response to hypoglycemia in humans. Such peptidergic neurotransmission would explain the failure of classical cholinergic and adrenergic antagonists to block the glucagon response and would reconcile those data with autonomic mediation of the glucagon response.

An alternative explanation is that the lower doses of autonomic blocking agents used in studies in humans (8,79) are not sufficient to totally block the effects of the reflex autonomic activation produced by hypoglycemia. For example, the dose of atropine typically used in human studies (1 mg) does not block a cardiovascular parasympathetic reflex (Valsalva); a fourfold higher dose is needed to suppress this response (82). Thus, studies using different doses of autonomic blockers might resolve these discrepancies between human and animal studies.

Given the failure of adrenergic and muscarinic blockade to reduce the glucagon response to hypoglycemia in humans, other studies specifically designed to address the role of the autonomic nervous system in nondiabetic humans were necessary. In a recent study, the ganglionic blocker trimethaphan was infused to impair autonomic activation in nondiabetic human subjects. Trimethaphan impaired the increases of plasma PP, epinephrine, and norepinephrine during hypoglycemia of 45 mg/dl by 70–80% and reduced the glucagon response by 75% (Fig. 3). This study provides direct confirmation of autonomic mediation in humans (83). Trimethaphan did not affect the glucagon response to arginine, demonstrating that this drug does not inhibit glucagon secretion directly (83). Finally, it should be noted that ganglionic antagonists block the pancreatic release of VIP from postganglionic parasympathetic nerves (47). These data support the hypothesis that activation of the autonomic nervous system has a major role in mediating the glucagon response to hypoglycemia in nondiabetic humans.

#### GLUCAGON RESPONSE TO HYPOGLYCEMIA IN TYPE 1 DIABETES

**Impaired  $\alpha$ -cell responses.** The glucagon response to IIH in patients with type 1 diabetes can be normal, impaired, or totally absent. Before investigators started to examine the effects of intensive insulin therapy, the available data suggested that there was a progressive loss of this response related to the duration of type 1 diabetes. For example, Bolli et al. (15) showed that the glucagon response to IIH in certain groups of newly diagnosed type 1 patients was quantitatively normal, but that the response was clearly blunted in patients who had type 1 diabetes for 1–5 years. Gerich et al. (84) had demonstrated a decade earlier that the glucagon response to IIH was absent in patients who had type 1 diabetes for over a decade. These data suggested that type 1 diabetes per se does not abolish the glucagon response to hypoglycemia, but rather that a time-dependent factor associated with this disease leads first to impairment and then to eventual loss of this  $\alpha$ -cell response. A potential mechanism involving progressive autonomic neuropathy induced by chronic hyperglycemia is discussed below.

After clinical trials of intensive insulin therapy and the associated increased incidence of hypoglycemia (2,3), many

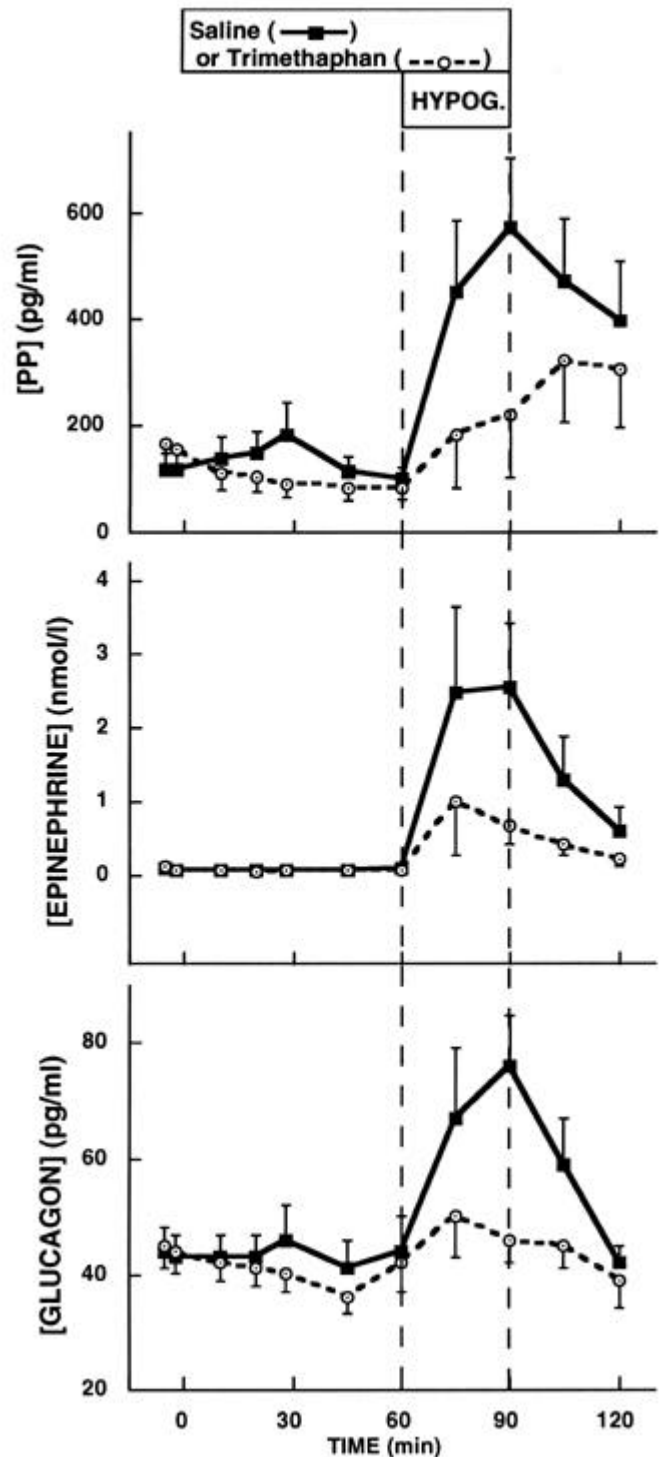


FIG. 3. PP, epinephrine, and glucagon concentrations in the peripheral plasma of seven women before, during, and after a period of 2.5 mmol/l hypoglycemia (HYPOG; vertical dashed lines) during either a saline infusion (—■—) or ganglionic blockade with trimethaphan (---○---). Adapted from Havel and Ahren (83).

reports of impaired glucose counterregulation appeared (10–13), even in patients with diabetes of short duration. This impairment of glucose recovery was related to markedly impaired glucagon (11,13) and epinephrine (12,13) responses to hypoglycemia. There were also reports of a very early loss of the glucagon response to hypoglycemia in newly diagnosed diabetic children (85,86). Reports of severe and pro-

longed nocturnal hypoglycemia in children (87) and of a threefold increase in the incidence of hypoglycemia associated with intensive insulin therapy in adults (2,3) suggested a contribution of prior hypoglycemia to this early loss of  $\alpha$ -cell responses. A potential mechanism involving acute autonomic dysfunction is discussed below.

### Mechanisms

**Hypoglycemia per se.** The mechanism for the slow and progressive loss of the glucagon response to hypoglycemia in type 1 patients has been a subject of controversy. The loss is selective, i.e., patients with long-standing type 1 diabetes have essentially no glucagon response to IHH yet have a normal glucagon response to arginine (84). Thus, the secretory capacity of the  $\alpha$ -cell is normal, yet the  $\alpha$ -cell is either unresponsive to the stimuli specifically associated with IHH (see below) or these stimuli are reduced in type 1 diabetes. As previously discussed, hypoglycemia per se, suppression of endogenous insulin secretion, and activation of the autonomic nervous system have all been proposed as mediators of the stimulation of the glucagon response to hypoglycemia. It is possible that an intrinsic defect in the  $\alpha$ -cell's response to direct hypoglycemic stimulation is responsible for loss of this  $\alpha$ -cell response in type 1 diabetes, as originally proposed by Gerich et al. (84). Restoration of euglycemia in diabetic rats does improve their glucagon responses to hypoglycemia (88) consistent with a partially reversible defect in the  $\alpha$ -cell's recognition of glucose that was induced by the chronic hyperglycemia of diabetes. However, restoration of euglycemia has also been shown to improve both residual  $\beta$ -cell function (89,90) and autonomic responses to hypoglycemia (91). Thus, this observation is consistent with all three potential mediators of the glucagon response to hypoglycemia.

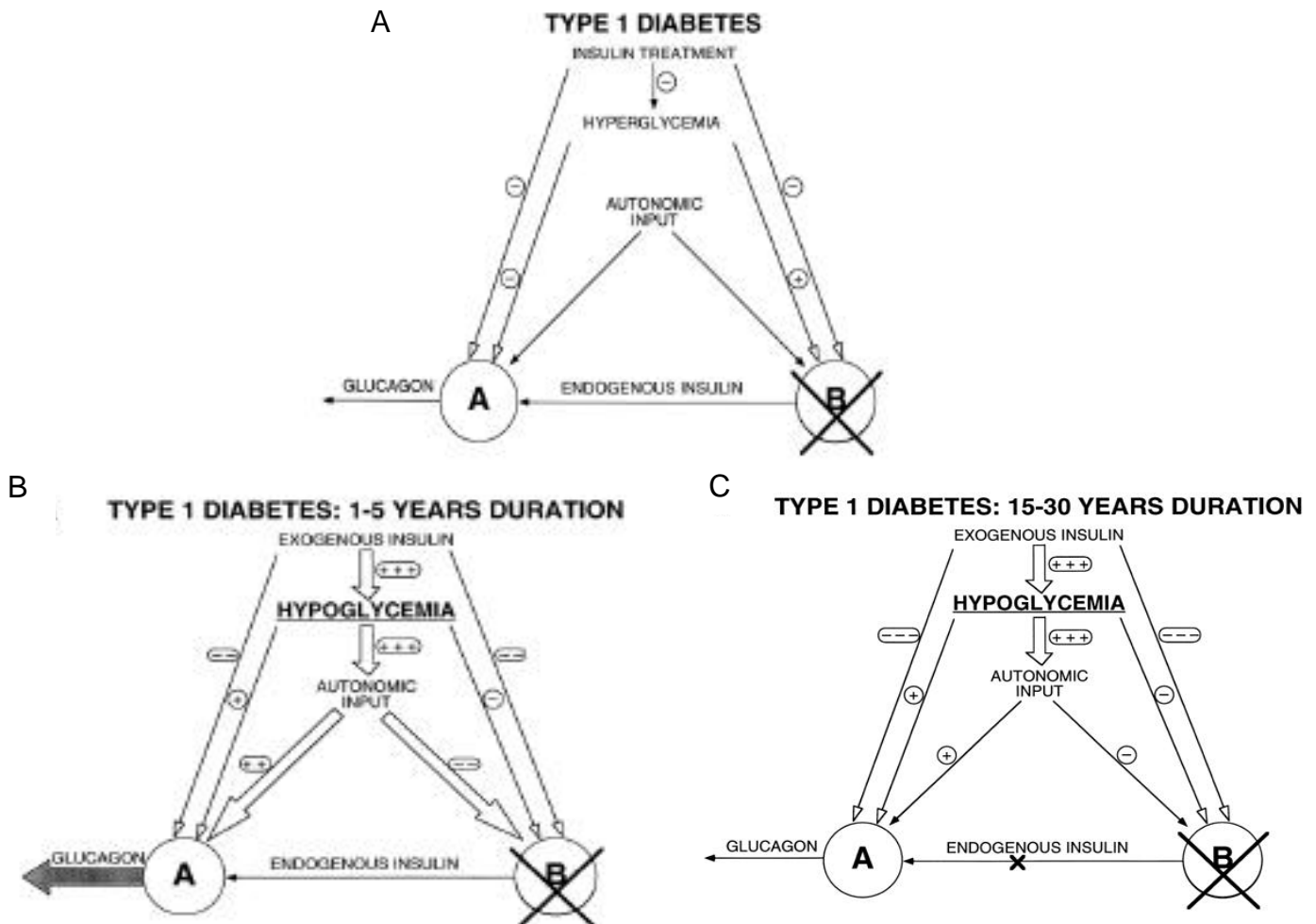
**Loss of  $\beta$ -cell function.** The marked destruction of islet B-cells that occurs at the onset of type 1 diabetes markedly reduces endogenous insulin secretion (89,90), and thereby the magnitude of the fall of endogenous insulin secretion normally produced by hypoglycemia. Thus, that component of the glucagon response hypothesized to occur secondary to suppression of endogenous insulin should be markedly reduced in diabetic islets (27,28) (Fig. 4A), unless the  $\alpha$ -cells become more sensitive to endogenous insulin when the number of  $\beta$ -cells within the islet is dramatically reduced. Indeed, the  $\alpha$ -cell seems to be more sensitive to exogenous insulin, since the suppressive effect on the  $\alpha$ -cell becomes dominant (92) when endogenous insulin is very low, as in the islets of patients with C-peptide-negative diabetes (Fig. 4C). Such net suppression of glucagon secretion by endogenous insulin is illustrated by the effect of hyperinsulinemic euglycemic clamps to inhibit glucagon responses to arginine in patients with type 1 diabetes (29). In contrast, the same level of exogenous hyperinsulinemia potentiates the glucagon response to arginine in nondiabetic subjects (29), presumably via suppression of endogenous insulin secretion (29). Surprisingly, prior marked suppression of endogenous insulin secretion does not potentiate the glucagon response to hypoglycemia (32), suggesting either that this mechanism is not critical in nondiabetic humans or that complete suppression of endogenous insulin, as occurs only during hypoglycemia (32), is required. Nonetheless, the concept persists that in patients with type 1 diabetes, a markedly reduced suppression of endogenous insulin secretion, coupled with the unopposed inhibitory effect of exogenous insulin on the  $\alpha$ -cell, con-

tributes to the impairment of the glucagon response to IHH.

Some have even proposed that the progressive loss of the  $\alpha$ -cell response to hypoglycemia in patients with type 1 diabetes is due to progressive loss of their residual  $\beta$ -cell function (93). This hypothesis implies that many newly diagnosed type 1 diabetic patients retain a small amount of  $\beta$ -cell function, as has been documented both by residual insulin staining in one-third of the islets of autopsied pancreases (94) and by residual C-peptide secretion in vivo (89,90). Some patients may even regain  $\beta$ -cell function upon institution of therapy (95,96). Thus, if only a small percentage of the original  $\beta$ -cells are needed to retain  $\alpha$ -cell responsiveness to IHH, these may still be present in many patients recently diagnosed with type 1 diabetes. Indeed, using a very sensitive C-peptide assay to quantitate remaining  $\beta$ -cell function, Fukuda et al. (93) have demonstrated a correlation between the loss of this residual  $\beta$ -cell function and the loss of glucagon responses to hypoglycemia (93) in type 1 diabetes. These data support the hypothesis that impairment and eventual loss of  $\alpha$ -cell responses to IHH are related to the loss of residual  $\beta$ -cell function in patients with type 1 diabetes.

**Autonomic impairment.** Alternatively, progressive impairment of the autonomic responses to hypoglycemia may contribute to the impairment (Fig. 4B) and eventual loss (Fig. 4C) of the  $\alpha$ -cell response to hypoglycemia in type 1 diabetes. Indeed, in the study noted above, the C-peptide nonresponders also had a delayed epinephrine response to IHH (93), suggesting reduced autonomic input to the  $\alpha$ -cell. Even such small impairments of the autonomic response to hypoglycemia could significantly reduce the glucagon response in patients with type 1 diabetes if the unopposed inhibitory action of exogenous insulin on the  $\alpha$ -cell renders it more dependent on autonomic stimulation. Most investigators have assumed, however, that autonomic dysfunction occurs too late in the natural history of type 1 diabetes to contribute to the relatively early impairment of the glucagon response to IHH. This assumption is based on the common experience that clinical diabetic autonomic neuropathy (DAN) usually occurs after a decade or more of type 1 diabetes. However, clinical DAN is usually recognized late in its course, in its advanced and pervasive form (97), when it is associated with postural hypotension.

More subtle, subclinical forms of neuropathy may be present much earlier in the natural history of type 1 diabetes. These may be detected with more sensitive autonomic tests, such as measurement of heart rate variability (98), scintigraphy of the heart (99), and muscle sympathetic nerve activity (100). Indeed, one-half to three-fourths of patients with type 1 diabetes who did not exhibit the usual signs of clinical DAN had impaired autonomic function, as assessed by these more sensitive tests (99–101). Other studies report the presence of subclinical autonomic neuropathy in one-half of all patients with type 1 diabetes (97). Some of this autonomic dysfunction, however, may be unrelated to the autonomic inputs to the  $\alpha$ -cell. Therefore, it is important to determine if the autonomic responses to IHH per se are impaired in patients with type 1 diabetes before the development of clinical DAN. Results from a recent study have demonstrated just that: the epinephrine and the PP responses to IHH are impaired in patients that have type 1 diabetes without clinical signs of DAN (102). Finally, it is important to recognize that the response of the sympathetic nerves that innervate the  $\alpha$ -cell



**FIG. 4.** Type 1 diabetes. **A:** Basal glucagon levels in insulin-treated type 1 diabetes are either normal (thin arrow) or slightly elevated (not shown) because of the near balance of stimulatory and inhibitory factors. The major stimulatory factor is a marked reduction of endogenous insulin secretion (thin arrow) secondary to a major loss of  $\beta$ -cells (X). The inhibitory factors on the  $\alpha$ -cell include the effects of 1) the exogenous insulin used for treatment, 2) the hyperglycemia usually present despite insulin treatment, and 3) the lack of autonomic stimulation of the  $\alpha$ -cell. **B:** In type 1 diabetes of 1–5 years' duration, the glucagon response to hypoglycemia is usually blunted (medium arrow) compared with that in nondiabetic subjects (Fig. 1), despite the direct stimulatory (+) effect of hypoglycemia on the  $\alpha$ -cell. The smaller glucagon response may be due to a reduction of stimulatory factors, as well as increased sensitivity to inhibitory factors. Thus, there may be less autonomic stimulation (+ +) of the  $\alpha$ -cell than in nondiabetic subjects because of chronic subclinical autonomic neuropathy and/or acute hypoglycemia-associated autonomic dysfunction (see AUTONOMIC IMPAIRMENT). In addition, there is less stimulation of the  $\alpha$ -cell because the disinhibition by endogenous insulin, present in the normal islet, has already been largely removed by the marked destruction of  $\beta$ -cells (X). Finally, this chronic reduction of intraislet insulin levels may render the  $\alpha$ -cell more sensitive to the inhibitory effect (– –) of exogenous insulin. **C:** In type 1 diabetes of 15–30 years' duration, the glucagon response to hypoglycemia is usually absent (thin arrow). The loss of the glucagon response may be due to further reductions of stimulatory factors, as well as to hypersensitivity to inhibitory factors. Thus, there is a further reduction of autonomic stimulation (+) of the  $\alpha$ -cell (compare to Figs. 1 and 4B). There is also less stimulation of the  $\alpha$ -cell by suppression of endogenous insulin (X), since after 15–30 years of type 1 diabetes, almost all  $\beta$ -cells have disappeared. The very low levels of residual islet insulin may render the  $\alpha$ -cell even more sensitive to the inhibitory effects (– – –) of exogenous insulin.  $\alpha$ - and  $\beta$ -cells are represented as circles labeled A and B, respectively.

cannot currently be measured in humans, and thus the impact of its potential impairment would not be ascribed to the autonomic nervous system. Chemically induced or immunologically mediated  $\beta$ -cell destruction in rats is associated with a marked decrease in the number of direct contacts between sympathetic nerves and  $\alpha$ -cells (31), implying a loss of  $\alpha$ -cell responsiveness to this autonomic input. Indeed, this rapidly induced functional sympathetic denervation of the  $\alpha$ -cell is associated with loss of glucagon responses to glucopenia (31). This decrease in the number of direct sympathetic contacts could simply be due to the known shrinkage of the islet that occurs after  $\beta$ -cell destruction. Alternately, recent studies have suggested that  $\beta$ -cells

secrete a neurotrophic factor that maintains sympathetic innervation of the islet (103). Thus, the observation that loss of residual  $\beta$ -cell function is associated with loss of the glucagon response to hypoglycemia may not be the exclusive evidence for a role of endogenous insulin in the  $\alpha$ -cell response to hypoglycemia that it first appeared to be. The alternative, autonomic, interpretation is that loss of residual  $\beta$ -cells somehow widens the synaptic cleft between nerves and  $\alpha$ -cells and, thereby, results in an impairment of the autonomic stimulation of glucagon secretion during hypoglycemia in type 1 diabetes. We conclude that impairment of the autonomic responses to IHH can occur well before the development of clinical DAN and may contribute to the eventual



loss (Fig. 4C), if not the original impairment (Fig. 4B), of the glucagon response to IIH in patients with type 1 diabetes.

The rapid loss of glucagon responses to hypoglycemia in patients intensively treated with insulin and in newly diagnosed children may be due to the "hypoglycemia-associated autonomic failure" described by Cryer (4). In nondiabetic subjects, this rapidly induced and reversible autonomic impairment is associated with a reduction of the glucagon response to subsequent hypoglycemia (64). Further, in diabetic animals with either induced or iatrogenic prior hypoglycemia, there is impairment of both the autonomic and glucagon responses to subsequent hypoglycemia (66,104). It is, therefore, plausible that antecedent hypoglycemia causes an acute reduction of the autonomic stimulation to the  $\alpha$ -cell, which contributes to the impairment of the glucagon response seen either in adult type 1 diabetic patients receiving intensive insulin therapy or in diabetic children who have nocturnal hypoglycemia. To our knowledge, this hypothesis has not been critically tested.

#### RESTORATION OF GLUCAGON RESPONSES TO HYPOGLYCEMIA IN TYPE 1 DIABETES

**Intensive insulin therapy.** The total absence of a glucagon response to IIH in patients who had type 1 diabetes for >15 years (15) led to the assumption that this loss may be irreversible. Indeed, in two groups with type 1 diabetes of 15–18 years duration, 3 months of intensive insulin therapy, with meticulous avoidance of hypoglycemia, failed to improve their epinephrine, PP, or glucagon responses to hypoglycemia (105). However, another study of patients with type 1 diabetes of only 4–7 years duration showed some improvement, but not normalization, of the glucagon response to hypoglycemia after similar therapy (91,106). It is noteworthy that in these latter studies both the epinephrine and PP responses, indices of autonomic inputs to the islet, were also improved. Thus, intensive insulin therapy is associated with partial restoration of autonomic responses to hypoglycemia, and partial restoration of the glucagon responses to hypoglycemia, if the disease is not long standing. The partial restoration of the autonomic responses is consistent with partial reversal of subclinical autonomic neuropathy. Similar conclusions have been reached concerning the influence of duration of type 2 diabetes on the reversibility of defects in autonomic function, such as nerve conduction velocity (107).

**Pancreas transplantation.** Glucagon responses to IIH have also been examined in patients whose long-term type 1 diabetes has been corrected by pancreas transplantation (51,108–110). Although successful pancreas transplantation restores euglycemia (110,111), it is unlikely to restore the responsiveness of the native  $\alpha$ -cells to hypoglycemia, since most of these patients have had diabetes for nearly two decades (108–111). Thus, any improvement seen in the plasma glucagon response to hypoglycemia is assumed to be due to the response of the  $\alpha$ -cells in the transplanted, rather than the native, pancreas (109). Furthermore, the transplanted pancreas should be autonomically denervated because surgical transplantation severs both the parasympathetic and the sympathetic nerves that innervate the pancreas. Despite such pancreatic denervation, the increment of plasma glucagon in response to moderate hypoglycemia is similar to that seen in normal control subjects (109,110). Furthermore, although the secretion of the sympathetic neurohormone epinephrine still

increases during hypoglycemia, and although epinephrine can still reach the  $\alpha$ -cells of the transplanted pancreas, it appears to have little impact, since the glucagon response to hypoglycemia in transplant recipients is unaffected by  $\beta$ -adrenergic blockade (109). The straightforward interpretation of these data is that the glucagon response of the transplanted pancreas is not autonomically mediated.

However, a more detailed analysis of the peripheral plasma glucagon response in transplant recipients suggests that it may be relatively impaired, consistent with denervation of the transplanted pancreas. This interpretation is based on the recognition that most transplantation procedures provide systemic, rather than portal, drainage for the pancreas. Such drainage would eliminate the normal first-pass clearance of glucagon by the liver (112,113) and thus be expected to increase peripheral plasma levels of glucagon. Indeed, both the basal concentrations of plasma glucagon (109,111) and the glucagon response to arginine (109) are exaggerated in transplant recipients. On this basis, one might have expected the glucagon response to hypoglycemia in transplant recipients to be exaggerated as well. Thus, the observation of a quantitatively normal plasma glucagon response to hypoglycemia in the transplant recipients might, in fact, reflect a relative impairment of glucagon secretion. This interpretation is consistent with the impairment of the small glucagon response to mild hypoglycemia seen by other workers in transplant recipients (51,108). Whatever interpretation is correct, the impairment of the glucagon response in transplant recipients is not large, despite the autonomic denervation. Thus, studies in recipients of pancreas transplants suggest that nonautonomic mechanisms contribute substantially to the glucagon response to hypoglycemia.

#### SUMMARY, CONCLUSIONS, AND CLINICAL IMPLICATIONS

In this perspective, we have emphasized the role of the autonomic nervous system in mediating the increase of glucagon secretion observed during IIH. In 1989, when we reviewed the evidence related to this hypothesis (33), we found only a few studies in animals that supported an important role for the autonomic nervous system and a number of studies in humans that argued against this hypothesis. To reconcile these conflicting data, we proposed that the three autonomic inputs to the  $\alpha$ -cell (sympathetic nerves, parasympathetic nerves, and the sympathetic neurohormone epinephrine [Fig. 2]) redundantly mediated the increase of glucagon secretion during IIH in the absence of diabetes. In the subsequent years, we performed a variety of experiments to test that hypothesis using techniques to markedly reduce or abolish the activation of all three of these autonomic inputs during hypoglycemia; we found a marked reduction of the glucagon response in several species (55,56,62,63), including nonhuman primates (57) and, recently, humans (83). Studies by other investigators (59–61) in which central autonomic activation was prevented also demonstrated a markedly reduced glucagon response. We conclude that although peripheral hypoglycemia and suppression of endogenous insulin secretion may be permissive for a normal glucagon response to IIH, the majority of this glucagon response is mediated by redundant autonomic stimulation of the islet  $\alpha$ -cell.

Because it now appears that autonomic mediation is an important determinant of the glucagon response to hypogly-

cemia in humans, we propose here that autonomic impairment, together with  $\beta$ -cell destruction, contributes to the loss of this glucagon response in type 1 diabetes. Specifically, the slow and progressive loss of the glucagon response to hypoglycemia that can occur during the first decade of type 1 diabetes may be due to a progressive impairment of the autonomic responses to hypoglycemia secondary to the development of subclinical DAN. This hypothesis is clinically relevant because early autonomic neuropathy may be more functional than anatomic and may, therefore, be reversible if treated promptly. Indeed, correcting hyperglycemia does partially restore the autonomic and glucagon responses to IHH in patients with type 1 diabetes of short (91,106), but not long (105), duration. A second clinical implication is that agents used to treat or prevent neuropathy should also improve the glucagon response or prevent its impairment, a hypothesis that is currently untested.

An acute form of autonomic dysfunction has recently been recognized: autonomic responses to hypoglycemia can be rapidly impaired by recent episodes of antecedent hypoglycemia (64,65). The autonomic hypothesis implies that this type of autonomic dysfunction should also impair the  $\alpha$ -cell response in nondiabetic individuals, which it does (64). It also implies that acute autonomic dysfunction will further blunt the glucagon response in patients with short-term type 1 diabetes. Although this latter hypothesis has not been tested, if it is true, then intensive insulin therapy, which can produce inadvertent hypoglycemia and thus acute autonomic dysfunction, may actually worsen the  $\alpha$ -cell response in patients with type 1 diabetes. Thus, efforts to reduce diabetic hyperglycemia should be coupled with meticulous avoidance of hypoglycemia (91,105,106). Rapid restoration of autonomic and glucagon responses would then reflect reversal of the acute autonomic dysfunction induced by prior episodes of hypoglycemia, while slower restoration would reflect reversal of those functional defects in peripheral autonomic nerves caused by chronic hyperglycemia. The acute autonomic dysfunction should be fully reversible, while the subclinical autonomic neuropathy may only be partially reversible, particularly if there are structural defects in autonomic nerves induced by either long-term hyperglycemia or total  $\beta$ -cell destruction. Indeed, successful pancreas transplantation, which can restore euglycemia without inducing hypoglycemia, only partially corrects defects in nerve conduction velocity in patients with long-term type 1 diabetes (114). Finally, it is appropriate to reemphasize that the role of autonomic impairment in the deficient glucagon response in patients with type 1 diabetes remains speculative. However, based on the recent evidence for autonomic mediation in nondiabetic humans (83) and given the important implications of this mechanism for restoration of glucagon responses and glucose counterregulation in type 1 diabetes, we feel that expenditure of serious effort to directly test this hypothesis is warranted.

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#### REFERENCES

1. Cryer PE, Gerich JE: Hypoglycemia in insulin dependent diabetes mellitus: interplay of insulin excess and compromised glucose counterregulation. In *Diabetes Mellitus*. Porte D Jr, Sherwin RS, Eds. Stamford, CT, Appleton & Lange, 1997, p. 745-760
2. The Diabetes Control and Complications Trial Research Group: The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *N Engl J Med* 329:977-986, 1993
3. The Diabetes Control and Complications Trial Research Group: Hypoglycemia in the diabetes control and complications trial. *Diabetes* 46:271-286, 1997
4. Cryer PE: Hypoglycemia: the limiting factor in the management of IDDM. *Diabetes* 43:1378-1389, 1994
5. Gerich JE: Lilly lecture 1988: glucose counterregulation and its impact on diabetes mellitus. *Diabetes* 37:1608-1617, 1988
6. Cryer PE, Gerich J: Relevance of glucose counterregulatory systems to patients with diabetes: critical roles of glucagon and epinephrine. *Diabetes Care* 6:95-99, 1983
7. De Feo P, Perriello G, Torlone E, Fanelli C, Ventura MM, Santeusano F, Brunetti P, Gerich JE, Bolli GB: Evidence against important catecholamine compensation for absent glucagon counterregulation. *Am J Physiol* 260:E203-E212, 1991
8. De Feo P, Perriello G, Torlone E, Fanelli C, Ventura MM, Santeusano F, Brunetti P, Gerich JE, Bolli GB: Contribution of adrenergic mechanisms to glucose counterregulation in humans. *Am J Physiol* 261:E725-E736, 1991
9. De Feo P, Perriello G, De Cosmo S, Ventura MW, Campbell PJ, Brunetti P, Gerich JE, Bolli GB: Comparison of glucose counterregulation during short-term and prolonged hypoglycemia in normal humans. *Diabetes* 35:563-569, 1986
10. Arias P, Kerner W, Zier H, Navascues I, Pfeiffer EF: Incidence of hypoglycemic episodes in diabetic patients under continuous subcutaneous insulin infusion and intensified conventional treatment: assessment by means of semi-ambulatory 24-hour continuous blood glucose monitoring. *Diabetes* 8:134-140, 1985
11. Bergenstal RM, Polonsky KS, Pons G, Jaspan JB, Rubenstein AH: Lack of glucagon response to hypoglycemia in type 1 diabetics after long-term optimal therapy with a continuous subcutaneous insulin infusion pump. *Diabetes* 32:398-402, 1983
12. Amiel SA, Tamborlane WV, Simonson DC, Sherwin RS: Defective glucose counterregulation after strict glycemic control of insulin-dependent diabetes mellitus. *N Engl J Med* 316:1376-1383, 1987
13. White NH, Skor DA, Cryer PE, Levandoski LA, Bier DM, Santiago JV: Identification of type 1 diabetic patients at increased risk for hypoglycemia during intensive therapy. *N Engl J Med* 308:485-491, 1983
14. Bolli GB, Dimitriadis GD, Pehling GB, Baker BA, Haymond MW, Cryer PE, Gerich JE: Abnormal glucose counterregulation after subcutaneous insulin in insulin-dependent diabetes mellitus. *New Engl J Med* 310:1706-1711, 1984
15. Bolli G, De Feo P, Compagnucci P, Cartechini MG, Angeletti G, Santeusano F, Brunetti P, Gerich JE: Abnormal glucose counterregulation in insulin-dependent diabetes mellitus: interaction of anti-insulin antibodies and impaired glucagon and epinephrine secretion. *Diabetes* 32:134-141, 1983
16. Gerich J, Charles M, Grodsky G: Characterization of the effects of arginine and glucose on glucagon and insulin release from the perfused rat pancreas. *J Clin Invest* 54:833-841, 1974
17. Pipeleers DG, Schuit FC, Van Schravendijk CF, Van de Winkel M: Interplay of nutrients and hormones in the regulation of glucagon release. *Endocrinology* 117:817-823, 1985
18. Samols E, Tyler J, Marks V: Glucagon-insulin interrelationships. In *Glucagon: Molecular Physiology, Clinical and Therapeutic Implications*. Lefebvre P, Unger RH, Eds. Oxford, U.K., Pergamon, 1972, p. 151-173
19. Weir GC, Knowlton SD, Atkins RF, McKennan KX, Martin DB: Glucagon secretion from the perfused pancreas of streptozotocin-treated rats. *Diabetes* 25:275-282, 1976
20. Samols E, Weir GC, Bonner-Weir S: Intraislet insulin-glucagon-somatostatin relationships. In *Glucagon II*. Lefebvre PJ, Ed. Berlin, Springer, 1983, p. 133-173

21. Bonner-Weir S, Orci L: New perspectives on the microvasculature of the islets of Langerhans in the rat. *Diabetes* 31:883-889, 1982
22. Stagner JI, Samols E: Retrograde perfusion as a method for testing the relative effects of glucose on the A cell. *J Clin Invest* 77:1034-1037, 1986
23. Samols E, Stagner JI: Intra-islet regulation. *Am J Med* 85:31-35, 1988
24. Van Schravendijk CF, Foriers A, Hooghe-Peters EL, Rogiers V, De Meyts P, Sodoyez JC, Pipeleers DG: Pancreatic hormone receptors on islet cells. *Endocrinology* 117:841-848, 1985
25. Rorsman P, Berggren PO, Bokvist K, Ericson H, Mohler H, Ostenson CG, Smith PA: Glucose-inhibition of glucagon secretion involves activation of GABA A-receptor chloride channels. *Nature* 341:233-236, 1989
26. Van Schravendijk CF, Foriers A, Van den Brande JL, Pipeleers DG: Evidence for the presence of type 1 insulin-like growth factor receptors on rat pancreatic A and B cells. *Endocrinology* 121:1784-1788, 1987
27. Unger RH: Insulin-glucagon relationships in the defense against hypoglycemia. *Diabetes* 32:575-583, 1983
28. Samols E: Glucagon and insulin secretion. In *Handbook of Experimental Pharmacology*. Lefebvre PJ, Ed. Berlin, Springer, 1983, p. 485-518
29. Asplin CM, Paquette TL, Palmer JP: In vivo inhibition of glucagon secretion by paracrine beta cell activity in man. *J Clin Invest* 68:314-318, 1981
30. Maruyama H, Hisatomi A, Orci L, Grodsky GM, Unger RH: Insulin within islets is a physiologic glucagon release inhibitor. *J Clin Invest* 74:2296-2299, 1984
31. Tominaga M, Maruyama H, Vasko MR, Baetens D, Orci L, Unger RH: Morphologic and functional changes in sympathetic nerve relationships with pancreatic alpha-cells after destruction of beta-cells in rats. *Diabetes* 36:365-373, 1987
32. Bolli G, De Feo P, Perriello G, De Cosmo S, Compagnucci P, Santeusano F, Brunetti P, Unger R: Mechanisms of glucagon secretion during insulin-induced hypoglycemia in man. *J Clin Invest* 73:917-922, 1984
33. Havel PJ, Taborsky GJ Jr: The contribution of the autonomic nervous system to changes of glucagon and insulin secretion during hypoglycemic stress. *Endocr Rev* 10:332-350, 1989
34. Taborsky GJ Jr., Havel PJ, Porte D Jr: Stress-induced activation of the neuroendocrine system and its effects on carbohydrate metabolism. In *Diabetes Mellitus*. Porte D Jr, Sherwin RS, Eds. Stamford, CT, Appleton & Lange, 1997, p. 141-168
35. Havel PJ, Munding TO, Veith RC, Dunning BE, Taborsky GJ Jr: Corelease of galanin and NE from pancreatic sympathetic nerves during severe hypoglycemia in dogs. *Am J Physiol* 263:E8-E16, 1992
36. Dunning BE, Scott MF, Neal DW, Cherrington AD: Direct quantification of norepinephrine spillover and hormone output from the pancreas of the conscious dog. *Am J Physiol* 272:E746-E755, 1997
37. Schwartz TW: Pancreatic polypeptide: a hormone under vagal control. *Gastroenterology* 85:1411-1425, 1983
38. Samols E, Weir GC: Adrenergic modulation of pancreatic A, B and D cells:  $\alpha$ -Adrenergic suppression and  $\beta$ -adrenergic stimulation of somatostatin secretion,  $\alpha$ -adrenergic stimulation of glucagon secretion in the perfused dog pancreas. *J Clin Invest* 63:230-238, 1979
39. Gerich JE, Karam JH, Forsham PH: Stimulation of glucagon secretion by epinephrine in man. *J Clin Endocrinol Metab* 37:479-481, 1973
40. Marliss EB, Girardier L, Seydoux J, Wollheim CB, Kanazawa Y, Orci L, Renold AE, Porte D Jr: Glucagon release induced by pancreatic nerve stimulation in the dog. *J Clin Invest* 52:1246-1259, 1973
41. Ahren B, Veith RC, Taborsky GJ Jr: Sympathetic nerve stimulation versus pancreatic norepinephrine infusion in the dog: 1. Effects on basal release of insulin and glucagon. *Endocrinology* 121:323-331, 1987
42. Dunning BE, Ahren B, Veith RC, Bottcher G, Sundler F, Taborsky GJ Jr: Galanin: a novel pancreatic neuropeptide. *Am J Physiol* 251:E127-E133, 1986
43. Kaneto A, Miki E, Kosaka K: Effects of vagal stimulation on glucagon and insulin secretion. *Endocrinology* 95:1005-1010, 1974
44. Bloom SR, Edwards AV: Pancreatic endocrine responses to stimulation of the peripheral ends of the vagus nerves in conscious calves. *J Physiol (Lond)* 315:31-41, 1981
45. Kaneto A, Kajinuma H, Kosaka K, Nakao K: Stimulation of insulin secretion by parasympathomimetic agents. *Endocrinology* 83:651-658, 1968
46. Lindkaer S, Jensen J, Fahrenkrug J, Holst JJ, Nielsen OV, Schaffalitzky de Muckadell OB: Secretary effects of VIP on isolated perfused porcine pancreas. *Am J Physiol* 235:E387-E391, 1978
47. Havel PJ, Dunning BE, Verchere CB, Baskin DG, O'Dorisio T, Taborsky GJ Jr: Evidence that vasoactive intestinal polypeptide is a parasympathetic neurotransmitter in the endocrine pancreas in dogs. *Regul Pept* 71:163-170, 1997
48. Christensen NJ, Iversen J: Release of large amounts of noradrenaline from the isolated perfused canine pancreas during glucose deprivation. *Diabetologia* 9:396-399, 1973
49. Hisatomi A, Maruyama H, Orci L, Vasko M, Unger RH: Adrenergically mediated intrapancreatic control of the glucagon response to glucopenia in the isolated rat pancreas. *J Clin Invest* 75:420-426, 1985
50. Stagner J, Samols E, Polonsky K, Pugh W: Lack of direct inhibition of insulin secretion by exogenous insulin in the canine pancreas. *J Clin Invest* 78:1193-1198, 1986
51. Luzi L, Battezzati A, Perseghin G, Bianchi E, Vergani S, Secchi A, La Rocca E, Staudacher C, Spotti D, Ferrari G, Di Carlo V, Pozza G: Lack of feedback inhibition of insulin secretion in denervated human pancreas. *Diabetes* 41:1632-1639, 1992
52. Miller RE, Waid TH, Joyce MP: Direct neural inhibition of insulin secretion in response to systemic hypoglycemia. *Am J Physiol* 230:1090-1094, 1976
53. Patel DG: Role of parasympathetic nervous system in glucagon response to insulin-induced hypoglycemia in normal and diabetic rats. *Metabolism* 33:1123-1127, 1984
54. Bloom SR, Edwards AV, Vaughan NJA: The role of the autonomic innervation in the control of glucagon release during hypoglycemia in the calf. *J Physiol (Lond)* 236:611-623, 1974
55. Havel PJ, Veith RC, Dunning BE, Taborsky GJ Jr: Role for autonomic nervous system to increase pancreatic glucagon secretion during marked insulin-induced hypoglycemia in dogs. *Diabetes* 40:1107-1114, 1991
56. Havel PJ, Akpan JO, Curry DL, Stern JS, Gingerich RL, Ahren B: Autonomic control of pancreatic polypeptide and glucagon secretion during neuroglucopenia and hypoglycemia in mice. *Am J Physiol* 265:R246-R254, 1993
57. Havel PJ, Valverde C: Autonomic mediation of glucagon secretion during insulin-induced hypoglycemia in rhesus monkeys. *Diabetes* 45:960-966, 1996
58. Havel PJ, Taborsky GJ Jr: The contribution of the autonomic nervous system to increased glucagon secretion during hypoglycemic stress: update, 1994. *Endo Rev Monographs* 2:201-204, 1994
59. Biggers DW, Myers SR, Neal D, Stinson R, Cooper NB, Jaspan JB, Williams PE, Cherrington AD, Fizzell RT: Role of brain in counterregulation of insulin-induced hypoglycemia in dogs. *Diabetes* 37:7-16, 1989
60. Borg WP, Doring MJ, Sherwin RS, Borg MA, Brines ML, Shulman GI: Ventromedial hypothalamic lesions in rats suppress counterregulatory responses to hypoglycemia. *J Clin Invest* 93:1677-1682, 1994
61. Borg MA, Sherwin RS, Doring MJ, Borg WP, Shulman GI: Local ventromedial hypothalamus glucose perfusion blocks counterregulation during systemic hypoglycemia in awake rats. *J Clin Invest* 99:361-365, 1997
62. Havel PJ, Parry SJ, Stern JS, Akpan JO, Gingerich RL, Taborsky GJ Jr, Curry DL: Redundant parasympathetic and sympathoadrenal mediation of increased glucagon secretion during insulin-induced hypoglycemia in conscious rats. *Metabolism* 43:860-866, 1994
63. Havel PJ, Munding TO, Taborsky GJ Jr: Pancreatic sympathetic nerves increase glucagon secretion during severe hypoglycemia in dogs. *Am J Physiol* 270:E20-E26, 1996
64. Heller SR, Cryer PE: Reduced neuroendocrine and symptomatic responses to subsequent hypoglycemia after 1 episode of hypoglycemia in nondiabetic humans. *Diabetes* 40:223-226, 1991
65. Dagogo-Jack S, Craft S, Cryer PE: Hypoglycemia-associated autonomic failure in insulin-dependent diabetes mellitus. *J Clin Invest* 91:819-828, 1993
66. Powell AM, Sherwin RS, Shulman GI: Impaired hormonal responses to hypoglycemia in spontaneously diabetic and recurrently hypoglycemic rats. *J Clin Invest* 92:2667-2674, 1993
67. Pacak K, Palkovits M, Kvetnansky Y, Matern P, Hart C, Kopin I, Goldstein DS: Catecholaminergic inhibition by hypercortisolemia in the paraventricular nucleus of conscious rats. *Endocrinology* 136:4814-4819, 1995
68. Luo X, Kiss A, Rabadan-Diehl C, Aguilera G: Regulation of hypothalamic and pituitary corticotropin-releasing hormone receptor message ribonucleic acid by adrenalectomy and glucocorticoids. *Endocrinology* 136:3877-3883, 1995
69. Brown MR, Gray TS, Fisher LA: Corticotropin-releasing factor receptor antagonist: effects on the autonomic nervous system and cardiovascular function. *Regul Pept* 30:321-329, 1986
70. Davis SN, Shavers C, Costa F, Mosqueda-Garcia R: Role of cortisol in the pathogenesis of deficient counterregulation after antecedent hypoglycemia in normal humans. *J Clin Invest* 98:680-691, 1996
71. Pampanelli S, Lalli C, Del Sindaco P, Lepore M, Ciofetta M, Fanelli C, Brunetti P, Bolli GB: Effect of recent, antecedent hypoglycemia and response of cortisol per se, on responses to subsequent hypoglycemia in humans (Abstract). *Diabetologia* 40 (Suppl. 1):A27, 1997
72. McCall AL, Fixman LB, Fleming N, Tornheim K, Chick W, Ruderman NB: Chronic hypoglycemia increases brain glucose transport. *Am J Physiol* 251:E442-E447, 1986
73. Uehara Y, Nipper V, McCall AL: Chronic insulin hypoglycemia induces GLUT-3 protein in rat brain neurons. *Am J Physiol* 272:E716-E719, 1997

74. Boyle PJ, Kempers SF, O'Connor AM, Nagy RJ: Brain glucose uptake and unawareness of hypoglycemia in patients with insulin-dependent diabetes mellitus. *N Engl J Med* 333:1726-1731, 1995
75. Chipkin SR, Van Bueren A, Bercei E, Garrison CR, McCall AL: Effects of dexamethasone in vivo and in vitro on hexose transport in brain microvasculature. *Neurochem Res* 23:649-656, 1998
76. Long RG, Albuquerque RH, Prata A, Barnes AJ, Adrian TE, Christofides ND, Bloom SR: Responses of plasma pancreatic and gastrointestinal hormones and growth hormone to oral and intravenous glucose and insulin hypoglycemia in Chagas's disease. *Gut* 21:772-777, 1980
77. Sasaki K, Matsuhashi A, Murabayashi S, Aoyagi K, Baba T, Matsunaga M, Takebe K: Hormonal response to insulin-induced hypoglycemia in patients with Shy-Drager syndrome. *Metabolism* 32:977-981, 1983
78. Towler DA, Havlin CE, Craft S, Cryer P: Mechanism of awareness of hypoglycemia: perception of neurogenic (predominantly cholinergic) rather than neuroglycopenic symptoms. *Diabetes* 42:1791-1798, 1993
79. Hilsted J, Frandsen H, Holst JJ, Christensen NJ, Nielsen SL: Plasma glucagon and glucose recovery after hypoglycemia: the effect of total autonomic blockade. *Acta Endocrinol* 125:466-469, 1991
80. Bishop AE, Polak JM, Green IC, Bryant MG, Bloom SR: The location of VIP in the pancreas of man and rat. *Diabetologia* 18:73-78, 1980
81. Fahrenkrug J, Pedersen JH, Yamashita Y, Ottesen B, Hokfelt T, Lundberg JM: Occurrence of VIP and peptide HM in human pancreas and their influence on pancreatic endocrine secretion in man. *Regul Pept* 18:51-61, 1987
82. Rothschild AH, Weinberg CR, Halter JB, Porte D Jr, Pfeifer MA: Sensitivity of R-R variation and Valsalva ratio in assessment of diabetic autonomic neuropathy. *Diabetes Care* 42:1549-1554, 1987
83. Havel P, Ahren B: Activation of autonomic nerves and the adrenal medulla contributes to increased glucagon secretion during moderate insulin-induced hypoglycemia in women. *Diabetes* 46:801-807, 1997
84. Gerich J, Langlois M, Noacco C, Karam J, Forsham P: Lack of glucagon response to hypoglycemia in diabetes: evidence for an intrinsic pancreatic alpha cell defect. *Science* 182:171-173, 1973
85. Singer-Granick C, Hoffman RP, Kerensky K, Drash AL, Becker DJ: Glucagon responses to hypoglycemia in children and adolescents with IDDM. *Diabetes Care* 11:643-649, 1988
86. Hoffman RP, Arslanian S, Drash AL, Becker DJ: Impaired counterregulatory hormone responses to hypoglycemia in children and adolescents with new onset IDDM. *J Pediatr Endocrinol* 7:235-244, 1994
87. Matyka K, Crawford C, Wiggs L, Stores G, Dunger DB: High prevalence of nocturnal hypoglycemia in children with type-1 diabetes: analysis of sleep physiology (Abstract). *Diabetologia* 40:A237, 1997
88. Shi ZQ, Rastogi KS, Lekas M, Efendic S, Drucker DJ, Vranic M: Glucagon response to hypoglycemia is improved by insulin-independent restoration of normoglycemia in diabetic rats. *Endocrinology* 137:3193-3199, 1996
89. The Diabetes Control and Complications Trial Research Group: Effects of age, duration and treatment of insulin-dependent diabetes mellitus on residual  $\beta$ -cell function: observations during eligibility testing for the Diabetes Control and Complications Trial (DCCT). *J Clin Endocrinol Metab* 65:30-36, 1986
90. Madsbad S: Prevalence of residual B cell function and its metabolic consequences in type 1 (insulin-dependent) diabetes. *Diabetologia* 24:141-147, 1983
91. Fanelli CG, Epifano L, Rambotti AM, Pampanelli S, Di Vincenzo A, Modarelli F, Lepore M, Annibale B, Ciofetta M, Bottini P, Porcellati F, Scionti L, Santusano F, Brunetti P, Bolli GB: Meticulous prevention of hypoglycemia normalizes the glycemic thresholds and magnitude of most of neuroendocrine responses to, symptoms of, and cognitive function during hypoglycemia in intensively treated patients with short-term IDDM. *Diabetes* 42:1683-1689, 1993
92. Liu DT, Adamson UC, Lins P-ES, Kollind ME, Moberg EA, Andréasson K: Inhibitory effect of circulating insulin on glucagon secretion during hypoglycemia in type 1 diabetic patients. *Diabetes Care* 15:59-65, 1992
93. Fukuda M, Tanaka A, Tahara Y, Ikegami H, Yamamoto Y, Kumahara Y, Shima K: Correlation between minimal secretory capacity of pancreatic  $\beta$ -cells and stability of diabetic control. *Diabetes* 37:81-88, 1988
94. Foulis AK, Liddle CN, Farquharson MA, Richmond JA, Weir RS: The histopathology of the pancreas in type 1 (insulin-dependent) diabetes mellitus: a 25-year review of deaths in patients under 20 years of age in the United Kingdom. *Diabetologia* 29:267-274, 1986
95. Steffes M, Tamborlane W, Becker D, Palmer J, Cleary P: The effect of intensive diabetes treatment on residual  $\beta$ -cell function in the diabetes control and complications trial (DCCT) (Abstract). *Diabetes* 45 (Suppl. 2):18A, 1996
96. Kobayashi T, Nakanishi K, Murase T, Kosaka K: Small doses of subcutaneous insulin as a strategy for preventing slowly progressive  $\beta$ -cell failure in islet cell antibody-positive patients with clinical features of NIDDM. *Diabetes* 45:622-626, 1996
97. Hoeldtke RD, Boden G: Epinephrine secretion, hypoglycemia unawareness, and diabetic autonomic neuropathy. *Ann Intern Med* 120:512-517, 1994
98. Pfeifer MA, Cook D, Brodsky J, Tice D, Reenan A, Swedine S, Halter JB, Porte D Jr: Quantitative evaluation of cardiac parasympathetic activity in normal and diabetic man. *Diabetes* 31:339-345, 1982
99. Schnell O, Kirsch CM, Stemplinger J, Haslbeck M, Standl E: Scintigraphic evidence for cardiac sympathetic dysinnervation in long-term IDDM patients with and without ECG-based autonomic neuropathy. *Diabetologia* 38:1345-1352, 1995
100. Hoffman RP, Sinkey CA, Kienzle MG, Anderson EA: Muscle sympathetic nerve activity is reduced in IDDM before overt autonomic neuropathy. *Diabetes* 42:375-380, 1993
101. Ewing DJ, Neilson JM, Travis P: New method for assessing cardiac parasympathetic activity using 24 h electrocardiograms. *Br Heart J* 52:396-402, 1984
102. Bottini P, Boschetti E, Pampanelli S, Ciofetta M, Sindaco PD, Scionti L, Brunetti P, Bolli GB: Contribution of autonomic neuropathy to reduced plasma adrenaline responses to hypoglycemia in IDDM: evidence for a non-selective defect. *Diabetes* 46:814-823, 1997
103. Myrsten U, Keymeulen B, Pipeleers DG, Sundler F: Beta cells are important for islet innervation: evidence from purified rat islet-cell grafts. *Diabetologia* 39:54-59, 1996
104. Havel PJ, Duesberg C, Elliott DE, Gingerich RL, Feldman EC, Nelson RW: Impaired glucagon responses to insulin-induced hypoglycemia (IIH) are associated with impaired activation of pancreatic parasympathetic nerves in dogs with spontaneous diabetes mellitus (Abstract). *Diabetes* 44 (Suppl. 1):216A, 1995
105. Dagogo-Jack S, Rattarasarn C, Cryer PE: Reversal of hypoglycemia unawareness, but not defective glucose counterregulation, in IDDM. *Diabetes* 43:1426-1434, 1994
106. Fanelli C, Pampanelli S, Epifano L, Rambotti AM, Di Vincenzo A, Modarelli F, Ciofetta M, Lepore M, Annibale B, Torlone E, Perriello G, De Feo P, Santusano F, Brunetti P, Bolli GB: Long-term recovery from unawareness, deficient counterregulation and lack of cognitive dysfunction during hypoglycaemia, following institution of rational, intensive insulin therapy in IDDM. *Diabetologia* 37:1265-1276, 1994
107. Judzewitsch RG, Jaspan JB, Polonsky KS, Weinberg CR, Halter JB, Halar E, Pfeifer MA, Vukadinovic C, Bernstein L, Schneider M, Liang KY, Gabbay KH, Rubenstein AH, Porte D Jr: Aldose reductase inhibition improves nerve conduction velocity in diabetic patients. *N Engl J Med* 308:119-125, 1983
108. Battezzati A, Luzi L, Perseghin G, Bianchi E, Spotti D, Secchi A, Vergani S, Di Carlo V, Pozza G: Persistence of counter-regulatory abnormalities in insulin-dependent diabetes mellitus after pancreas transplantation. *Eur J Clin Invest* 24:751-758, 1994
109. Diem P, Redmon JB, Abid M, Moran A, Sutherland DER, Halter JB, Robertson RP: Glucagon, catecholamine and pancreatic polypeptide secretion in type 1 diabetic recipients of pancreas allografts. *J Clin Invest* 86:2008-2013, 1990
110. Barrou Z, Seaquist ER, Robertson RP: Pancreas transplantation in diabetic humans normalizes hepatic glucose production during hypoglycemia. *Diabetes* 43:661-666, 1994
111. Ostman J, Bolinder J, Gunnarsson R, Brattstrom C, Tyden G, Wahren J, Groth CG: Effects of pancreas transplantation on metabolic and hormonal profiles in IDDM patients. *Diabetes* 38:88-93, 1989
112. Jaspan JB, Ruddick J, Rayfield E: Transhepatic glucagon gradients in man: evidence for glucagon extraction by human liver. *J Clin Endocrinol Metab* 58:287-292, 1984
113. Herold KC, Jaspan JB: Hepatic glucagon clearance during insulin induced hypoglycemia. *Horm Metab Res* 18:431-435, 1986
114. Martinenghi S, Comi G, Galardi G, Di Carlo V, Pozza G, Secchi A: Amelioration of nerve conduction velocity following simultaneous kidney/pancreas transplantation is due to the glycaemic control provided by the pancreas. *Diabetologia* 40:1110-1112, 1997