

The Contribution of the Autonomic Nervous System to Changes of Glucagon and Insulin Secretion during Hypoglycemic Stress*

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I. Introduction

A. Goal of the review

DURING hypoglycemic stress, glucagon secretion increases and insulin secretion decreases. The traditional view is that these changes are due to the effect of low circulating glucose levels acting directly on the pancreatic islet. In contrast, a number of studies suggest that these responses are due to activation of autonomic nervous system that accompanies hypoglycemia *in vivo*, a view that we favor. Although central nervous system and autonomic neural control of the endocrine pancreas has been reviewed before (1-4), this review will focus specifically on the autonomic activation produced by glucopenic stress and its potential contribution to the accompanying changes of glucagon and insulin secretion. The goal of this review is not to negate the traditional view that hypoglycemia has direct actions on the islet, but rather to suggest that the autonomic contribution may have been underestimated in a whole class of experiments because of unrecognized redundancy in the autonomic input to the islet.

The first section will review the supporting evidence that each of three autonomic inputs to the pancreas is activated during this stress. The three inputs are: 1) pancreatic parasympathetic nerves, 2) adrenal medullary epinephrine, and 3) pancreatic sympathetic nerves. The various techniques for measuring these autonomic inputs to the pancreas will be discussed, and the factors which influence the magnitude of the autonomic activation will be outlined.

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The second section reviews the published experiments designed to determine the autonomic contribution to changes of glucagon and insulin secretion during glucopenic stress. These studies are examined in the light of the hypothesis that the autonomic input is redundant during moderate glucopenia. The experimental approaches needed to critically test this hypothesis are outlined.

B. Autonomic input to the pancreas

Early anatomic studies demonstrated that parasympathetic nerves innervate the pancreatic islets. Preganglionic parasympathetic, cholinergic nerves travel in the vagus nerves and terminate at ganglia within the exocrine pancreas and within the islet (4) (Fig. 1). The islets have been shown to contain 10-fold higher levels of choline acetyltransferase than adjacent exocrine tissue (5), suggesting extensive cholinergic innervation. The postganglionic parasympathetic nerves are predominantly cholinergic (Fig. 1); however, recent studies have demonstrated the existence of peptidergic fibers as well (6). Studies in a number of animal species have demon-

strated that parasympathetic nerves can influence islet function because electrical activation of the vagus stimulates the secretion of both insulin (7-9) (Fig. 2) and glucagon (7-9) (Fig. 3).

Activation of the sympathoadrenal system results in the discharge of preganglionic sympathetic nerves, which arrive at the adrenal medulla via the splanchnic nerves and synapse on the postganglionic epinephrine-secreting adrenal chromaffin cells (10) (Fig. 1). Epinephrine released from the adrenal reaches the pancreas via the systemic circulation (Fig. 1). Epinephrine can inhibit insulin secretion (11) (Fig. 2) and stimulates glucagon secretion (12) (Fig. 3).

The classical description of the sympathetic innervation of the pancreas is that all preganglionic sympathetic nerve fibers travel in the splanchnic nerves and synapse in the coeliac ganglia (13, 14). From there, postganglionic sympathetic fibers travel with the arterial blood vessels and enter the pancreas as part of the mixed autonomic nerve (Fig. 1). However, recent studies suggest that other preganglionic sympathetic fibers enter the pancreas di-

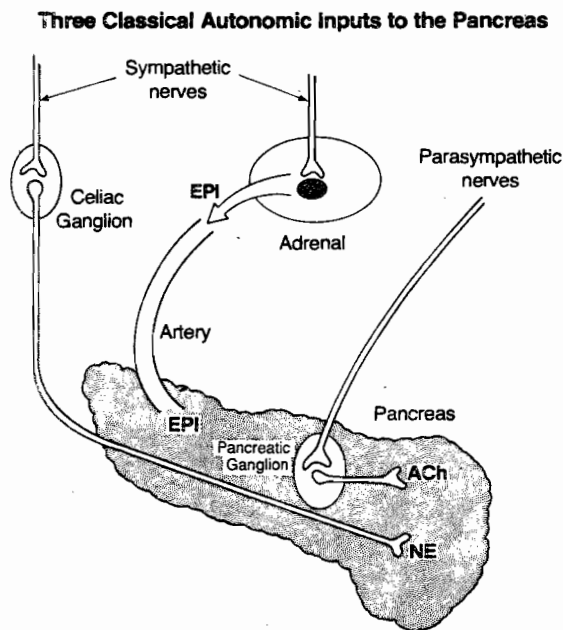


FIG. 1. Three classical autonomic inputs to the pancreas: 1) Preganglionic parasympathetic nerves terminate in intrapancreatic ganglia from whence postganglionic cholinergic nerves arise. 2) Certain preganglionic sympathetic nerves terminate on the neuroendocrine cells of the adrenal medulla and stimulate the release of epinephrine, which reaches the pancreas via the systemic circulation. 3) Other preganglionic sympathetic nerves terminate in the coeliac ganglion from whence postganglionic sympathetic nerves innervate the pancreas and release norepinephrine. Because all three autonomic inputs involve nicotinic transmission in autonomic ganglia, the activation of all three can be simultaneously eliminated with ganglionic blockade, e.g. hexamethonium.

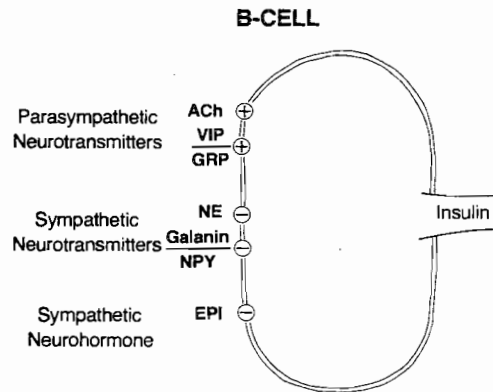


FIG. 2. Effects of three autonomic inputs on insulin secretion. +, Stimulation of insulin release; -, inhibition of insulin release; ACh, acetylcholine; VIP, vasoactive intestinal polypeptide; GRP, gastrin-releasing peptide; NPY, neuropeptide y; EPI, epinephrine.

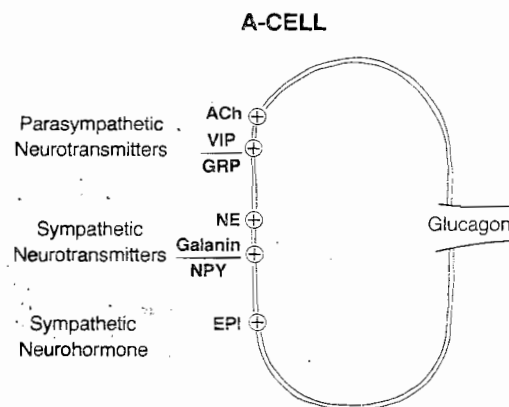


FIG. 3. Effects of three autonomic inputs on glucagon secretion. +, Stimulation of glucagon release; ACh, acetylcholine; NE, norepinephrine; EPI, epinephrine.

rectly and innervate intrapancreatic sympathetic ganglia (15, 16). Sympathetic nerve terminals have been found in close proximity to individual islet cells (4, 14). Although the predominant neurotransmitter of sympathetic nerves is norepinephrine, recently several neuropeptides have been identified in certain sympathetic nerves including those innervating the pancreas (6). Sympathetic nerve activation inhibits insulin (17-19) (Fig. 2) and stimulates glucagon release (18-20) (Fig. 3). Thus, the pancreas receives three different types of autonomic input: parasympathetic nerves, circulating adrenal medullary epinephrine, and sympathetic nerves.

C. Methods of producing hypoglycemia and glucopenia experimentally

Glucopenic stress can be produced either by administering agents that lower plasma glucose levels or by administering agents that impair central glucose utilization (Fig. 4). The iv injection of insulin rapidly results in dose-dependent decrements of the plasma glucose level (21, 22) (Fig. 4). Because the uptake of glucose by the brain is solely dependent on plasma glucose levels (23, 24), central neuroglucopenia results. An advantage of insulin-induced hypoglycemia is that measurement of arterial plasma glucose levels provides an accurate index of the degree of central glucopenia produced. This method also has disadvantages. High circulating insulin levels can have direct inhibitory effects on glucagon and insulin secretion (25, 26) or secondary effects via a direct action of insulin on the central nervous system to produce autonomic activation (27). Further, after the injection of exogenous insulin, it is difficult to assess endogenous insulin secretion. This problem can be circumvented by measurement of plasma C-peptide, which is released on an equimolar basis with insulin (28). In addition, insulin may suppress glucagon indirectly by lowering the level of circulating amino acids. Lastly, the low glucose level itself may have local islet effects on glucagon (29, 30) and insulin secretion, complicating attempts to evaluate the importance of the concomitant autonomic activation on islet hormone secretion.

Phlorizin, like insulin, can be used to produce central glucopenia by lowering the plasma glucose level. Phlorizin inhibits glucose transport in the renal tubule, preventing the reabsorption of glucose from the glomerular ultrafiltrate. Blockade of reabsorption causes glucose to be lost into the urine (Fig. 4), subsequently lowering plasma glucose levels. However, the change of plasma glucose produced by phlorizin over a short period of time is relatively small (31, 32) making it difficult to produce acute hypoglycemic stress.

Intravenous or intracerebroventricular injection of some analogs of glucose that compete with glucose for

Methods for Producing Central Glucopenia

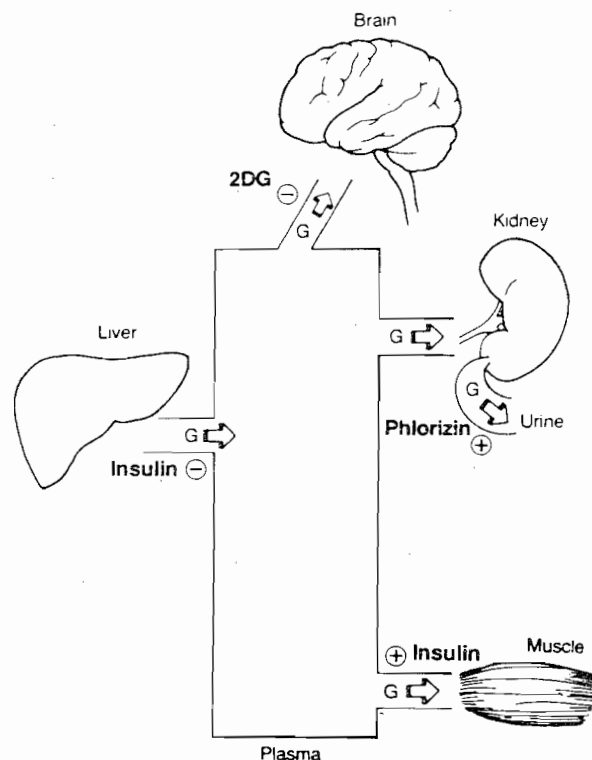


FIG. 4. Three methods for producing central glucopenia: 1) Insulin (I) injection lowers the plasma glucose level and thereby decreases glucose transport into the central nervous system, a tissue whose plasma glucose uptake is solely dependent on glucose and not insulin levels. Insulin lowers the plasma glucose by inhibiting hepatic glucose production and by stimulating glucose uptake into muscle and adipose tissue. 2) Phlorizin produces a more modest and slower fall of plasma glucose level and thereby produces modest central glucopenia. Phlorizin lowers plasma glucose by inhibiting the reabsorption of filtered glucose in the renal tubule so that the renal threshold for glucose drops dramatically, and significant glucose is lost to the urine even at euglycemia. 3) 2-DG is an analog of glucose that competes with glucose for transcellular transport and intracellular phosphorylation. While it has these actions in muscle and adipose tissue, as well as brain, the near total dependence of the central nervous system on glucose metabolism leads to severe central fuel deprivation despite the presence of normal or even elevated levels of glucose in plasma.

membrane transport and intracellular phosphorylation create an intracellular glucopenia (33) (Fig. 4). Because, under normal conditions, the brain is almost entirely dependent on glucose for fuel, administration of these agents results in central neuroglucopenia. The glucopenia-producing analogs include 2-deoxy-D-glucose (2-DG), 5-thioglucoase, and 3-O-methylglucose. In contrast to insulin injection, the administration of glucose analogs often result in an elevation of plasma glucose levels (34-36). Part of this measured hyperglycemia may be the result of counterregulatory responses. However, because these analogs can cross-react in many plasma glucose assays, a glucose-specific assay technique (36) is neces-

sary to ensure that measured increases of plasma glucose are not the result of such cross-reactivity. Thus, during 2-DG-induced neuroglucopenia, there is no easily measured index of the degree of glucopenia produced as is provided by plasma glucose decrements during insulin-induced hypoglycemia. The dose of 2-DG administered or the magnitude of the plasma epinephrine response are the alternate indices that are used to quantify glucopenic stress with this method. Caution should be exercised when comparing results of experiments using insulin with those using 2-DG, as these agents differ markedly in the manner in which they produce glucopenia.

I. Activation of Pancreatic Parasympathetic Nerves

1. Cholinergic activation

Indices of cholinergic activation. Increased gastric acid secretion (37) and increased sudomotor activity (38) during hypoglycemia suggest that this stress activates parasympathetic nerves. However, these measures cannot be used as an index of the activation of the cholinergic fibers that innervate the pancreas. The ideal index of such activation would be the measurement of the spillover of the cholinergic neurotransmitter, acetylcholine, into the pancreatic venous effluent. However, this approach is currently impractical because acetylcholinesterase rapidly degrades acetylcholine making its measurement in plasma extremely difficult. One alternative approach is recording the firing rates of efferent pancreatic parasympathetic nerves. Using this method, Nilima (39) has demonstrated increased activity in the pancreatic branch of the vagus during 2-DG-induced glucopenia in rats. There is, however, another more practical and less technically demanding alternative: the measurement of plasma levels of pancreatic polypeptide (PP).

PP is synthesized by the F-cells of the pancreatic islets (40) and is released either during insulin-induced hypoglycemia (41-43) or during 2-DG-induced glucopenia (44, 45). The release of PP during insulin-induced hypoglycemia is abolished vagotomy (46) and is nearly eliminated by administration of atropine before either glucopenic stimulus (36, 44, 46) (Fig. 5). Thus, the PP response requires activation of parasympathetic nerves and is predominantly mediated by acetylcholine acting on muscarinic receptors (Fig. 5). When the level of parasympathetic input to the pancreas is directly controlled by electrically stimulating the vagus nerves, increasing the stimulation frequency produces graded increases of plasma PP levels (46). Thus, plasma levels of this peptide may be useful as a quantitative, not just qualitative, index of cholinergic input to the pancreatic islet.

Although PP secretion is very responsive to cholinergic

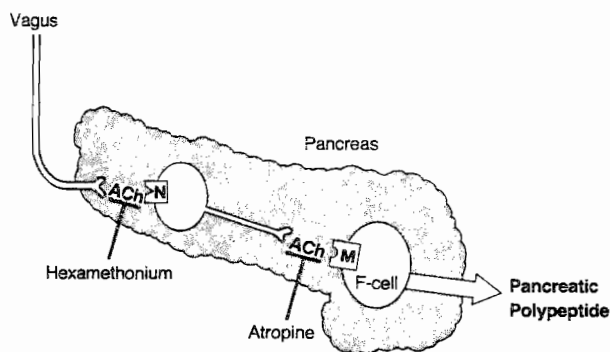


FIG. 5. Control of PP secretion. Vagal nerve fibers entering the pancreas release acetylcholine (ACh) and stimulate nicotinic receptors (N) in intrapancreatic ganglia. The fibers arising from these ganglia release acetylcholine, which stimulates muscarinic receptors (M) on the islet F cells to release PP.

activation, α -adrenergic agonism can inhibit, and β -adrenergic agonism can stimulate PP release (47). However, the dominant catecholamines in plasma, epinephrine and norepinephrine, have little net effect on either basal (47) or insulin hypoglycemia-induced PP release (48), perhaps because of the opposing α - and β -effects of these mixed agonists. Thus, cholinergic stimulation appears to be the dominant controller of PP secretion during hypoglycemia; therefore PP is a useful marker of cholinergic input to the islet during this stress.

2. Pathways involved in and factors that influence parasympathetic activation during glucopenia. The central pathways involved in glucopenia-induced activation of pancreatic parasympathetic nerves have not been well defined. However, the lateral hypothalamus is involved in the gastric acid response to glucopenia (49), and electrical stimulation of the same area stimulates both insulin (50) and glucagon release (51), indirectly suggesting that the lateral hypothalamus is involved in the central pathway that controls the parasympathetic outflow to the endocrine pancreas. In addition, because the plasma PP response to centrally administered cholecystokinin is blocked by central atropine pretreatment, central cholinergic neurons acting on muscarinic receptors may be involved in this pathway (52).

The activity of pancreatic parasympathetic nerves during glucopenic stress is influenced by factors that affect either the initial stimulus or the central pathways. With regard to the stimulus, one might expect the magnitude of the glucopenia to be an important factor in determining the magnitude of the parasympathetic response as it is in determining the magnitude of the plasma epinephrine response to glucopenia (22, 34) (Fig. 6A, see next section). While this expectation is at least partially correct, there is an important difference between the plasma PP and epinephrine responses to glucopenia. First, the plasma PP response to 2-DG-induced glucopenia does

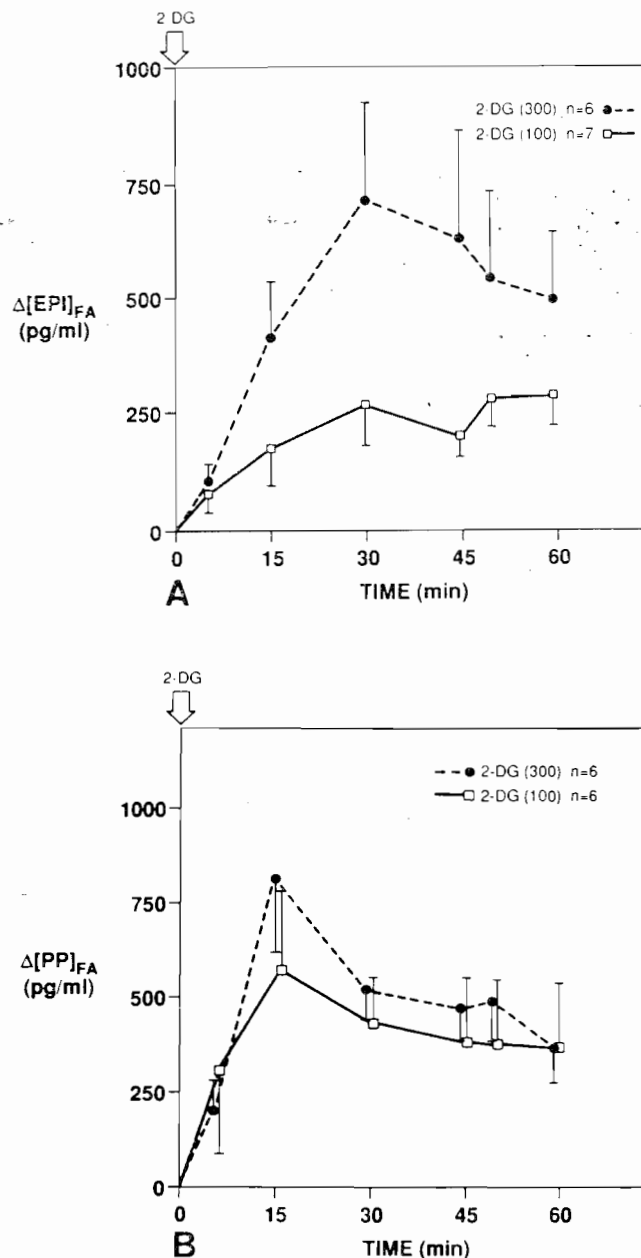


FIG. 6. A, Increased epinephrine response to graded 2-DG-induced central glucopenia. The plasma epinephrine (EPI) increment above baseline (Δ) in femoral arterial plasma in response to either modest glucopenia (2-DG, 100 mg/kg iv bolus; \square — \square) or moderate glucopenia (2-DG, 300 mg/kg iv bolus; \bullet — \bullet) in halothane-anesthetized dogs. [Reproduced with permission from: Havel *et al.*: *Am J Physiol* 252:E667, 1987 (34).]

B, Similar PP responses to graded 2-DG-induced central glucopenia. The plasma PP increment above baseline (Δ) in arterial plasma (FA) in response to either modest glucopenia (2-DG, 100 mg/kg iv bolus; \square — \square) or moderate glucopenia (2-DG, 300 mg/kg iv bolus; \bullet — \bullet) in halothane-anesthetized dogs. [Reproduced with permission from: Havel *et al.*: *Am. J. Physiol.* 251:E111, 1986 (53).]

not increase significantly when the dose of 2-DG is increased from one that produces mild glucopenia to one that produces moderate glucopenia (53) (Fig. 6B), suggesting that the PP response is maximal before marked glucopenic stress occurs. Second, during very mild hypoglycemia, there is a clear plasma PP response (54) when the plasma catecholamine responses are relatively small (22, 38). Thus, pancreatic parasympathetic nerves are activated, and may achieve maximal activation, at a lesser degree of glucopenia than required for maximal activation of the sympathoadrenal system. Ideally, these observations should be confirmed in experiments measuring PP and catecholamine responses to a range of glucose levels during hypoglycemia induced by insulin.

Pentobarbital anesthesia abolishes the PP response to mild or moderate glucopenic stress (36), either by inhibiting by central pathways involved in the response or peripheral parasympathetic neurotransmission. Another general anesthetic, halothane, does not inhibit the PP response to mild glucopenia (53). Thus, although both anesthetics produce surgical anesthesia, they appear to differ dramatically in their effects on the pathways controlling the activation of pancreatic parasympathetic nerves. These studies suggest that the type of anesthesia used in experiments may determine the contribution of parasympathetic activation to changes of islet hormone secretion observed during glucopenic stress.

Another important factor in the PP response to glucopenia is the integrity of the peripheral neural pathway. It is not surprising that vagotomy abolishes the PP response to glucopenic stress (46) (Fig. 5). Because pyloric transection also eliminates the PP response (55), it appears that the preganglionic vagal fibers required for the response may traverse the stomach before they enter the pancreas (56). Less severe disruptions of the parasympathetic neural pathway, such as the autonomic neuropathy of diabetes, can reduce the PP response to hypoglycemia (57). Even diabetics without clinical signs of autonomic neuropathy have a reduced PP response (58, 59). Drugs that block parasympathetic ganglionic transmission, such as hexamethonium, also eliminate the PP response to glucopenia (60) (Fig. 5). In addition, the analgesic, morphine, reduces the PP response to insulin-induced hypoglycemia in man (61), possibly by reducing acetylcholine release from postganglionic parasympathetic nerves (62) or from autonomic ganglia (63) as demonstrated for the related endogenous compounds, the enkephalins. As previously discussed, the muscarinic acetylcholine antagonist, atropine, markedly reduces the PP response to glucopenic stress (36, 44, 46) (Fig. 5). Thus, surgical, pathophysiological, or pharmacological disruption of the vagal pathway can be expected to impair the PP response and the cholinergic contribution to changes

of glucagon and insulin secretion during glucopenic stress.

B. Peptidergic activation

A number of peptides have been localized in nerves of the pancreas with immunohistochemical techniques (6). Of these, vasoactive intestinal polypeptide (VIP) (64, 65) and gastrin releasing peptide (GRP) (66) have been found in intrapancreatic ganglia and islets suggesting that these peptides are located in the cell bodies of intrinsic postganglionic parasympathetic nerves.

Administration of exogenous VIP (67-69) or GRP (70, 71) mimics the effects of parasympathetic nerve stimulation in that both stimulate insulin (Fig. 2) and glucagon (Fig. 3) release. Moreover, both VIP (69, 72, 73) and GRP (74) are released from the pancreas during electrical stimulation of vagal nerves. However, there has, as yet, been no demonstration of either VIP or GRP release from the pancreas during glucopenic stress. Nonetheless, it is important to recognize that if neuropeptides do function as parasympathetic neurotransmitters in the pancreas during glucopenic stress, their effects on islet function may not be blocked by the cholinergic, muscarinic antagonist, atropine.

C. Summary

1) Plasma PP levels serve as a useful index of parasympathetic input to the islet. 2) With this index, glucopenia-induced activation of parasympathetic nerves to the pancreatic islet has been demonstrated. 3) This activation is evident during relatively mild glucopenia and may be maximal during moderate glucopenic stress. Thus, the effect of parasympathetic activation on glucagon and insulin secretion may depend on the severity of the glucopenic stimulus. 4) In addition to acetylcholine, peptides (VIP and GRP) may serve as postganglionic parasympathetic neurotransmitters in the endocrine pancreas. The actions of these candidate neuropeptides on islet hormone secretion may not be blocked by atropine.

III. Activation of the Adrenal Medulla

A. Catecholamine measurement

Over 50 yr ago, Cannon *et al.* (75) demonstrated that insulin-induced hypoglycemia releases epinephrine from the adrenal medulla. Despite this early demonstration, the accurate quantification of the amount of epinephrine released was limited by the catecholamine assay techniques then available. The early fluorometric and bioassay methods were insensitive and therefore required sampling directly from the adrenal venous effluent, a technique impractical for many experiments. Some alternative indices of adrenal medullary activation have

not proven useful either. For example, urinary catecholamine measurement provides more of a chronic than an acute measurement of adrenal medullary activity. These technical limitations were overcome with the development of specific and sensitive radioenzymatic assays for catecholamines in peripheral plasma (76, 77). More recently, high pressure liquid chromatographic separation of catecholamines combined with electrochemical detection has been used successfully to measure peripheral plasma catecholamine levels (78, 79).

With these newer more sensitive methods, most studies have shown that glucopenic stress increases plasma epinephrine levels markedly and plasma norepinephrine levels modestly (80, 81). There are species differences in the relative amounts of the two catecholamines contained in and secreted from the adrenal medulla (82). In the rat, adrenalectomy eliminates the increased urinary excretion of norepinephrine, as well as epinephrine, in response to 2-DG-induced glucopenia (83). In the past, this finding has been interpreted to suggest that the norepinephrine was of adrenal medullary origin. However, recent studies in the rat demonstrate that circulating epinephrine tonically facilitates norepinephrine release from sympathetic nerves (84), suggesting that this norepinephrine may not have been derived from the adrenal. In the dog, adrenal deprivation does not reduce the arterial plasma norepinephrine response to 2-DG-induced glucopenia (85), indicating that the increase of norepinephrine was the result of spillover from peripheral noradrenergic nerves.

The site of blood sampling influences the measured epinephrine response. In venous plasma, the increment of epinephrine will be lower than that in arterial plasma because most tissues extract epinephrine (86). The arterial measurement of plasma epinephrine is preferred because it accurately reflects the concentration of the adrenal medullary hormone to which target tissues are exposed (87).

B. Pathways involved in the adrenal medullary response

A number of peptides and neurotransmitters can produce adrenal medullary activation when administered centrally (88-90), and electrical stimulation of several brain areas can increase plasma epinephrine levels (91-93). However, the central pathways and the neurotransmitters involved in glucopenia-induced activation of the adrenal medulla have not been well defined. Experiments in conscious dogs have demonstrated that carotid artery glucose replacement does not reduce the small epinephrine response to mild insulin-induced hypoglycemia (94). Another dog study showed that vertebral artery glucose replacement alone does not reduce the larger increase of epinephrine during more severe hypoglycemia, while ca-

rotid plus vertebral artery glucose replacement does abolish this response (95). Together these studies suggest that neither brain areas supplied by the vertebral circulation, presumably the upper spinal cord and brain stem, nor the carotid circulation, the cortex, thalamus, and hypothalamus, are by themselves critical for the central perception of glucopenia in conscious animals, but that both regions may be required for an intact epinephrine response. However, one study has indirectly suggested that hindbrain regions are important in glucopenia-induced adrenal activation because blocking the flow of cerebrospinal fluid into the fourth ventricle prevents the hyperglycemic response that follows the administration of 2-DG into the lateral ventricle (96). This suggestion is supported by experiments demonstrating that epinephrine release in response to hypoglycemia was not reduced in sheep in which the cerebrum, thalamus, and hypothalamus were removed (97). Thus, it appears that there are multiple centers in the central nervous system capable of eliciting sympathoadrenal responses during glucopenia. However, it seems likely that forebrain glucoreceptors are the dominant controllers in normal, conscious animals and that when they are compromised by surgery or by anesthesia, then the epinephrine responses to more severe glucopenia may be mediated by glucoreceptors in the lower brain areas.

The adrenal medullary response to glucopenia is neurally mediated, however, because interruption of the peripheral neural pathway, at any point, eliminates the epinephrine response to glucopenic stress. Thus, spinal cord transection (98, 99), adrenal denervation (100), or ganglionic blockade with the nicotinic antagonist, hexamethonium (60, 99, 101), prevent or markedly reduce the epinephrine response to glucopenia (Fig. 1). These methods can be used to experimentally assess the contribution of adrenal activation to islet responses to glucopenic stress.

C. Factors that influence the adrenal medullary response

A number of stimulus-related factors can influence the magnitude of adrenal medullary activation during glucopenia. First, the severity of the glucopenic stimulus determines the magnitude of the plasma epinephrine response. Indeed, unlike parasympathetic activation, which appears to be maximal during relatively mild glucopenia (53) (Fig. 6B), the adrenal medullary response increases progressively as the magnitude of the glucopenic stimulus is increased (22, 34, 38) (Fig. 6A), perhaps because of progressive recruitment of lower brain glucoreceptive centers. Thus, the relative contribution of adrenal medullary activation to the islet hormone responses to glucopenia will increase with the severity of the glucopenic stimulus. When insulin-hypoglycemia is used to

induce glucopenic stress, the glucose nadir achieved, rather than either the absolute decrement or the rate of fall of the glucose level (102, 103), is the major determinant of the magnitude of the epinephrine response.

Certain anesthetics, such as pentobarbital, prevent the epinephrine response to modest glucopenia and significantly reduce the response to more severe glucopenia (104), presumably by impairing central neurotransmission (105). In contrast, low levels of halothane anesthesia have been demonstrated to not suppress glucopenia-induced adrenal medullary activation (34). The analgesic, morphine, does not inhibit the epinephrine response to 2-DG, although morphine does reduce the plasma catecholamine response to surgery, a stress with a nociceptive component (106).

Patients with diabetic autonomic neuropathy have reduced epinephrine responses to insulin-induced hypoglycemia (107, 108). This reduction is correlated with the duration of diabetes (109), suggesting that the degree of autonomic neuropathy, or other effects of diabetes, determines the degree of impairment.

Adrenergic drugs can also influence the plasma epinephrine response to neural activation of the adrenal medulla. For example, the α_2 -adrenergic agonist, clonidine, reduces basal epinephrine levels (110) and the epinephrine response to insulin-induced hypoglycemia (111), possibly by suppressing central sympathetic outflow (112, 113).

However, clonidine may also activate peripheral inhibitory α_2 -adrenergic receptors on the chromaffin cell analogous to those on noradrenergic nerve terminals (114, 115). Finally, the plasma levels of epinephrine can be increased not only by potentiating the release of epinephrine, but also by impairing the removal of the secreted epinephrine from plasma. For example, β -adrenergic blockade with propranolol increases not only the plasma epinephrine response to hypoglycemia (116), but also that to exogenous epinephrine infusion (117), suggesting that β -adrenergic receptors have a role in the clearance of epinephrine from plasma.

D. Summary

- 1) Glucopenic stress activates the sympathetic outflow to the adrenal medulla resulting in the release of adrenal catecholamines, primarily epinephrine.
- 2) Quantification of adrenal medullary activation requires sensitive and specific assay techniques for plasma catecholamines and arterial sampling of plasma epinephrine.
- 3) The magnitude of the adrenal medullary response and therefore its contribution to the islet hormone responses to glucopenia is increased with the severity of glucopenic stress.
- 4) A number of factors, including the integrity of the neural pathway, general anesthetics, and α - and β -adrenergi

drugs, influence the plasma epinephrine response to glucopenic stress.

IV. Activation of Pancreatic Sympathetic Nerves

1. Noradrenergic activation

Although stress-induced activation of pancreatic noradrenergic nerves has often been postulated (118, 119), the ability of central glucopenia to activate these noradrenergic nerves, *in vivo*, has only recently been demonstrated (85). Such experiments have been hindered by the lack of techniques for assessing local noradrenergic activity. The development of specific and sensitive assays for norepinephrine in plasma has partially solved this problem (76-79). However, because the pancreas makes a negligible contribution to systemic norepinephrine levels (85), peripheral norepinephrine measurements are not an index of pancreatic noradrenergic activity. Therefore, it is necessary to measure the concentration of norepinephrine in the pancreatic venous effluent and to calculate pancreatic norepinephrine spillover, in order to accurately assess pancreatic noradrenergic activity (85). Although the concentration of norepinephrine in the pancreatic venous effluent is probably proportional to that in the synaptic cleft, it is certainly much lower because a large portion of the norepinephrine released from noradrenergic nerves is taken up by these nerves or by the adjacent tissues before it spills over into the venous effluent (120, 121) (Fig. 7). Another important

consideration when measuring pancreatic venous norepinephrine levels is that circulating norepinephrine of nonpancreatic origin, part of which escapes extraction by the pancreas, contributes to pancreatic venous norepinephrine levels (Fig. 7). In dogs, this contribution usually averages 25%; 75% of the arterial norepinephrine entering the pancreas is extracted before reaching the venous effluent (122). Because arterial norepinephrine levels increase during glucopenic stress (80, 81, 85), accurate assessment of pancreatic norepinephrine spillover requires that the arterial contribution to pancreatic venous norepinephrine levels first be subtracted when calculating pancreatic norepinephrine spillover (Fig. 7). With this approach, activation of pancreatic noradrenergic nerves has now been demonstrated in the dog during 2-DG-induced neuroglucopenia (85). Further, these experiments demonstrated that the increase of pancreatic noradrenergic activity is stress specific, because pancreatic norepinephrine spillover did not increase during two other stresses, hypoxia and hemorrhagic hypotension, both of which produced arterial plasma epinephrine and norepinephrine responses similar to those observed during the glucopenic stress (85).

Other methods have also been used to assess organ-specific noradrenergic activity. For example, recording of the firing rate of sympathetic nerves has demonstrated increased activity of the nerves innervating the liver (39) and peripheral muscle (123) during glucopenia. However, this technique has not been applied to the measurement of pancreatic sympathetic activity during glucopenia. The measurement of the organ turnover of norepinephrine after preloading of the nerves with radiolabeled norepinephrine is another method used to assess noradrenergic activity in individual organs (124, 125). This method has been used to suggest increased activity of pancreatic noradrenergic nerves during the chronic stress of cold exposure (126) but has not been used during acute glucopenic stress.

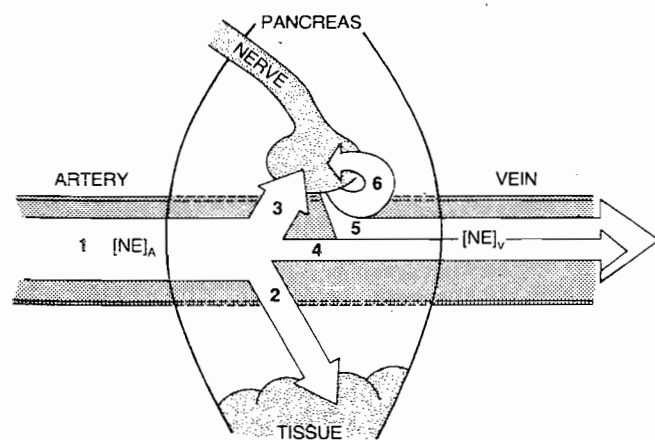


FIG. 7. Factors involved in estimating pancreatic noradrenergic activity. Circulating norepinephrine (NE) (1) enters the pancreas via the pancreatic artery. Approximately 75% of this incoming NE is extracted during transit through the pancreas, either by uptake into tissues (2), or nerves (3). Only 25% of arterial NE escapes pancreatic extraction (4) and contributes to the pancreatic venous NE level (4 + 5). The nerves within the pancreas release NE (5 + 6), but the vast majority is taken back up by the nerves (6), leaving the minority (5) to spill over into pancreatic venous plasma. This spillover is therefore an attenuated index of neuronal NE release. Pancreatic venous NE spillover (5) is calculated as the venous NE level (4 + 5) minus the arterial contribution (4) multiplied by the pancreatic venous plasma flow.

B. Pathways involved in the activation of noradrenergic nerves

There is little information on the pathways by which glucopenic stress activates the noradrenergic nerves of the pancreas; there is, however, some analogous information available regarding the generalized noradrenergic activation produced by glucopenic stress. For example, the systemic plasma norepinephrine response to glucopenia is centrally mediated because intracerebroventricular administration of glucopenia-producing glucose analogs increases peripheral plasma levels of nonadrenal norepinephrine (85). Higher brain areas appear to be involved in the stimulation of general noradrenergic outflow because the plasma norepinephrine response to

insulin-induced hypoglycemia in dogs is abolished by carotid plus vertebral artery glucose replacement, but not by vertebral artery glucose replacement alone (95).

The activation of pancreatic nerves by local glucopenia has been indirectly suggested based on *in vitro* experiments demonstrating that lowering the glucose concentration in the media perfusing isolated pancreata evokes a glucagon response that can be blocked by α -adrenergic antagonists (127, 128). More direct is the demonstration that extremely low glucose concentrations result in the release of norepinephrine into the venous effluent of isolated canine pancreata (129). Despite these *in vitro* data which suggest that pancreatic noradrenergic nerves can be activated directly by glucopenia, *in vivo* studies suggest that the majority of the activation is centrally mediated. In dogs, intracerebroventricular 2-DG increases pancreatic norepinephrine spillover, acute surgical denervation of the pancreas nearly abolishes the pancreatic norepinephrine response to systemic 2-DG, and the local intrapancreatic arterial infusion of 2-DG fails to reproduce the pancreatic norepinephrine response (85).

C. Factors that influence the activation of noradrenergic nerves

Factors that influence the magnitude of the systemic norepinephrine response to glucopenic stress can do so by affecting: 1) the stimulus; 2) the central neural pathways; 3) the peripheral pathways; 4) or the noradrenergic nerve terminals. Increasing the severity of the glucopenic stimulus increases the magnitude of the arterial norepinephrine response (34), as it does the magnitude of the arterial epinephrine response (see above). Inhibition of central neurotransmission by pentobarbital anesthesia markedly reduces the rate of appearance of norepinephrine in systemic plasma (130) and impairs the norepinephrine response to glucopenic stress (104). Low-dose halothane anesthesia does not suppress either the increase of systemic norepinephrine levels or the increase of the plasma norepinephrine appearance rate during glucopenic stress (34), despite the presence of full surgical anesthesia.

Noradrenergic activation involves ganglionic transmission because the nicotinic antagonist, hexamethonium, inhibits the plasma norepinephrine response to glucopenia (60). Barbiturates may also impair peripheral neurotransmission by their inhibitory action on sympathetic ganglia (131). The autonomic neuropathy of diabetes reduces the plasma norepinephrine response to glucopenia (108), presumably by impairing the function of peripheral sympathetic nerves.

α -Adrenergic drugs can modulate the amount of norepinephrine released during hypoglycemia by interacting

with an inhibitory α_2 -adrenergic receptor on peripheral adrenergic nerve terminals (114, 115). For example, the norepinephrine response to insulin-induced hypoglycemia is enhanced during α -adrenergic blockade with phentolamine (116); conversely the α_2 -adrenergic agonist, clonidine, decreases the norepinephrine response to insulin-induced hypoglycemia (111). Although these effects are consistent with an action on nerve terminals, similar effects could result from central actions of these drugs to modulate noradrenergic outflow (112, 113).

It should be mentioned that the preceding discussion concerns factors that modify whole body noradrenergic activity; the data to suggest that these same factors modulate the activity of pancreatic noradrenergic nerves in a similar fashion are not yet available. However, during severe stress, levels of systemic norepinephrine that could exert significant effects on insulin secretion and carbohydrate metabolism may be achieved (132).

D. Peptidergic activation

Both neuropeptide Y (NPY) and galanin (6, 133) are candidate sympathetic neurotransmitters in the pancreas. NPY has been localized in certain nerves containing norepinephrine in a variety of tissues (134), including the pancreas (135). Synthetic NPY has been demonstrated to inhibit glucose-induced insulin secretion in rats (136) and mice (135) (Fig. 2). However, in the dog, NPY is relatively impotent in influencing islet hormone secretion (137). NPY release from the pancreas (138) and from other tissues (139) has been measured during electrical stimulation of sympathetic nerves. However, the release of NPY during glucopenic stress has yet to be demonstrated.

Galanin has also been localized in pancreatic nerves which innervate the islets (140). Synthetic galanin potentially inhibits insulin secretion in dogs (140) and rats (141, 142) (Fig. 2) and also stimulates glucagon secretion in dogs (140) (Fig. 3). Recent studies have demonstrated that galanin is released from the pancreas during electrical stimulation of pancreatic nerves in quantities sufficient to influence islet function (143); however, pancreatic galanin release during glucopenic stress has not yet been demonstrated. It is important to recognize that if galanin, NPY, or other peptides function as sympathetic neurotransmitters in the endocrine pancreas, then their actions may not be blocked by the classical adrenergic antagonists; thus, the importance of sympathetic nerves in the control of insulin and glucagon release might be underestimated in such experiments.

E. Summary

1) Accurate assessment of pancreatic noradrenergic activity requires access to the pancreatic venous effluent,

measurement of arterial and pancreatic venous norepinephrine levels, and measurement of pancreatic norepinephrine extraction. 2) Glucopenic stress activates the noradrenergic nerves innervating the pancreas. This activation may influence the glucagon and insulin responses to glucopenia. 3) Pentobarbital anesthesia reduces and α -adrenergic blockade increases peripheral norepinephrine responses to glucopenic stress. 4) In addition to norepinephrine, peptides may function as sympathetic neurotransmitters in the pancreas, and the effects of these neuropeptides on islet hormone secretion may not be blocked by adrenergic antagonists.

V. Islet Effects of Autonomic Activation Induced by Glucopenic Stress

A. Introduction

Increased glucagon secretion is the primary factor responsible for the recovery of plasma glucose levels from hypoglycemia (144, 145). However, the question of what mechanisms increase glucagon secretion during hypoglycemia remains controversial. As detailed in the previous sections, glucopenic stress activates the three autonomic inputs to the islet; parasympathetic nerves, circulating adrenal medullary epinephrine, and sympathetic nerves. The major problem in assuming that these autonomic inputs actually mediate the increase of glucagon secretion observed during hypoglycemic stress, as has been demonstrated for several other stresses (146-148), is the traditional view that low glucose levels may stimulate glucagon release directly. For example, an isolated buffer-perfused pancreas (149) and isolated islets (150) increase their glucagon secretion in response to lowered glucose concentrations. Further, low glucose levels could reduce somatostatin or endogenous insulin secretion from adjacent D cells (151) or B cells, releasing the A cell from tonic paracrine inhibition (152, 153), and thus contribute indirectly to the glucagon response.

At one extreme, one could postulate that the direct islet effects of low glucose (for review see Ref. 154) are dominant and that autonomic activation makes no additional contribution. At the other extreme, it may be that the direct effect of hypoglycemia is minor and that the effect of the autonomic nervous system (for review see Ref. 155) is dominant. These opposite extremes are, in fact, reflected in the results and conclusions from numerous studies performed to determine the relative contributions of hypoglycemia *per se* vs. the activation of the autonomic nervous system in mediating increases of glucagon secretion observed during hypoglycemic stress. This section of the review will reexamine the results of these experiments in light of the hypothesis that moderate glucopenic stress activates redundant autonomic inputs to the islet, which, if unrecognized,

bias the experiment against finding a significant contribution of the autonomic nervous system to the islet response. Finally, the experimental approaches necessary to critically test this hypothesis are outlined and discussed.

B. Effects of glucopenia-induced autonomic activation on glucagon secretion

1. *Parasympathetic activation.* In 1974, Bloom and associates (156) reported that pretreatment with the cholinergic muscarinic antagonist, atropine, delayed the rise of plasma glucagon in response to insulin-induced hypoglycemia in calves, suggesting that cholinergic neural activation mediates the early glucagon response to glucopenia. Atropine also has been shown to reduce the plasma glucagon response to insulin hypoglycemia in rats (157). However, studies in man (158, 159) and in baboons (160) have failed to demonstrate any reduction of the glucagon response to insulin hypoglycemia by atropine.

The studies performed with 2-DG appear to give opposite results. Atropine did not affect the plasma glucagon response to 2-DG in either rats (101) or calves (44); atropine did, however, reduce the glucagon response to a low dose of 2-DG in man (45). In addition, the glucagon response to 2-DG-induced glucopenia in mice was markedly reduced by atropine pretreatment (161).

There are several potential explanations for these apparently conflicting results. First, the degree of glucopenia produced in the experiments probably varied, which may have resulted in differences in the relative contribution of cholinergic activation to the glucagon response, and thus a variation in the effect of atropine to attenuate the response. If only mild glucopenia were produced, cholinergic nerves might be activated selectively because, as previously discussed, the parasympathetic input to the pancreas appears to be activated by less severe degrees of glucopenia than the sympathoadrenal system. When moderate glucopenia is produced, substantial activation of the sympathetic nervous system occurs as well. This dual activation may result in a redundant stimulation of glucagon secretion, which effectively masks the cholinergic component of the response (Fig. 3). Second, if parasympathetic neuropeptides, *e.g.* VIP or GRP, both of which stimulate glucagon secretion (see previous section), are released during the glucopenic stress, their contribution to the glucagon response may not be blocked by atropine. Finally, there may be species differences in the cholinergic vs. peptidergic mediation of the glucagon response to glucopenia. This last explanation appears less likely because atropine reduces the glucagon response to 2-DG (45), but not that to insulin hypoglycemia (158, 159), in the same species, man. Conversely, in the rat, the glucagon response to

insulin hypoglycemia is reduced by atropine (157), but the response to 2-DG is not reduced (101). Therefore, it seems more likely that a factor related to the stimulus, (e.g. the degree of glucopenia produced), determines whether or not cholinergic mediation of the glucagon response to glucopenia is dominant and, therefore, whether it will be revealed by muscarinic blockade with atropine.

In contrast, the release of both acetylcholine and parasympathetic neuropeptides would be blocked by surgical vagotomy. Studies in man have both demonstrated (162) and failed to demonstrate (158) a significant reduction of the glucagon response to insulin hypoglycemia by truncal vagotomy. In these two studies, the plasma glucose nadirs produced by insulin injection, and thus the degree of glucopenia produced, were similar. It is therefore difficult to explain the apparent contradictory findings of these experiments.

2. Sympathoadrenal activation. Several methods have been used to determine the influence of the sympathoadrenal system on the glucagon response to glucopenic stress. Adrenergic antagonists have been administered to both man and animals to prevent the α -adrenergic and/or the β -adrenergic effects of both neuronally released norepinephrine and adrenal epinephrine on glucagon release. Both Walter *et al.* (163) and Lilavivat *et al.* (116) have shown that neither β -blockade with propranolol nor α -blockade with phentolamine alone reduced the plasma glucagon response to insulin hypoglycemia in man. Combined adrenergic blockade was not tested in these experiments. In the latter study, measurement of the plasma catecholamine levels revealed that β -blockade enhanced the plasma epinephrine response and that phentolamine increased norepinephrine release during insulin hypoglycemia in man. Thus, increased release of either of the two catecholamines during blockade of a single receptor type could have resulted in hyperstimulation of the other unblocked adrenergic receptor. However, other studies showed that combined α - and β -blockade did not reduce the glucagon response to insulin hypoglycemia in man (164) or in rats (165). It is possible, in these experiments, that either redundant parasympathetic neural activation, the potential influence of sympathetic neuropeptidergic activation, e.g. galanin or NPY, or both were responsible for the glucagon response that persisted during the combined blockade (Fig. 3).

Other studies, however, have found a significant adrenergic component in the glucagon response to glucopenic stress. In mice, phentolamine pretreatment modestly reduced the glucagon response to 2-DG-induced glucopenic stress, but propranolol had no effect on this response (161). Palmer and co-workers (166) demon-

strated that the glucagon response to a large dose of 2-DG in baboons was reduced by propranolol alone and that there was a further reduction with phentolamine alone or with the combination of the two blockers. Because the stress produced marked sympathoadrenal activation (20-fold increase of plasma epinephrine), these studies demonstrate a major adrenergic contribution to the glucagon response when the glucopenic stress is severe. However, the increases of glucagon were not abolished altogether, suggesting redundancy in the response (Fig. 3).

The influence of adrenal epinephrine alone on the plasma glucagon response to insulin hypoglycemia has been studied in adrenalectomized man; adrenalectomy did not impair the glucagon response (167, 168).

In experimental animals, the release of norepinephrine from sympathetic nerves innervating the pancreatic islets and that of epinephrine from the adrenal medulla can be prevented by sectioning the splanchnic nerves. In man, the condition of trauma-induced tetraplegia presumably results in a similar state of functional sympathoadrenalectomy. Splanchnic nerve section in calves delays the glucagon response to either insulin hypoglycemia (156) or 2-DG-induced glucopenia (44), suggesting a sympathoadrenal contribution to the early glucagon response. In tetraplegic man, the glucagon response to insulin hypoglycemia is not impaired when compared with normal control subjects (169). Sacca *et al.* (170) showed that the combination of adrenal demedullation and chemical sympathectomy with reserpine did not reduce the glucagon response to insulin-induced hypoglycemia in rats.

In summary, although there is conflicting evidence as to whether the sympathoadrenal system participates in the glucagon response to moderate glucopenic stress, several points should be considered when data from these types of experiments are evaluated. First, the degree of glucopenia induced in the experiments may be critical, because severe glucopenia results in a glucagon response that is impaired by adrenergic antagonists. In addition, adrenergic antagonists may not prevent glucagon release mediated by sympathetic neuropeptides, e.g. galanin or NPY, both of which could stimulate glucagon release (see previous section). Further, parasympathetic activation may compensate for an experimentally-induced reduction of sympathoadrenal input to the A cell, and thus mask the sympathetic contribution to the glucagon response during moderate glucopenia (Fig. 3).

3. Evidence that combined autonomic activation participates in the glucagon response to glucopenic stress. Although many studies have been designed to determine the contribution of autonomic activation to the glucagon response to glucopenic stress, the above review empha-

sizes the difficulty of interpreting negative data from blocking or ablation experiments when multiple or redundant stimulation of the A cell is likely (Fig. 3). Two alternative approaches have been used to demonstrate that the glucagon response to glucopenic stress is indeed mediated by the autonomic nervous system as opposed to the direct effect of a low blood glucose level on the islet A cells. The first is to produce central but not peripheral glucopenia by administering glucopenic agents into the brain only (Fig. 4). Frohman and Nagai (171) induced central neuroglucopenia by administering 2-DG into the lateral cerebral ventricles of conscious dogs and demonstrated a large increase of plasma glucagon levels, despite the presence of significant hyperglycemia, which would tend to restrain the glucagon response (172, 173). This increase may have been mediated by autonomic neural activation. However, the neurohypophysial hormones, oxytocin and vasopressin, are known to stimulate glucagon release (174-176) and are also released during hypoglycemia (177-179). Dunning *et al.* (180) have demonstrated a role for neurohypophysial hormones in mediating the glucagon response to another stress, hemorrhagic hypotension.

The second approach is the converse of the first, *i.e.* to prevent only the central but not the peripheral glucopenia. In experiments employing this approach, glucose was infused into the carotid and vertebral arteries during insulin-induced hypoglycemia. This intervention prevents the plasma epinephrine and norepinephrine responses to the stress and eliminates the glucagon response (181), suggesting that the glucagon response was neurally mediated.

In addition to the evidence above that the autonomic nervous system helps mediate the glucagon response to glucopenic stress, evidence that demonstrates that parasympathetic and sympathoadrenal activation function redundantly to mediate the glucagon response to moderate glucopenic stress is needed to resolve the conflicting views in the current literature. Such studies would involve near complete blockade or ablation of the autonomic input to the pancreas to demonstrate the autonomic contribution, followed by selective blockade or ablation of each input to demonstrate redundancy. Unfortunately few studies have been performed in which both parasympathetic and sympathoadrenal input to the islet are simultaneously impaired. Because both parasympathetic and sympathoadrenal input to the pancreas involve transmission across nicotinic ganglia (Fig. 1), administration of a ganglionic blocking agent, such as hexamethonium, is one intervention that could markedly reduce or abolish the input of both autonomic branches to the A cell (Fig. 3).

One such study showed that an atropine-resistant glucagon response to 2-DG in rats was markedly reduced by

hexamethonium, suggesting a possible sympathetic or peptidergic redundancy in the atropine-treated animals (101). This observation has been confirmed in other species. Hexamethonium pretreatment results in a 90% reduction of the glucagon response to 2-DG-induced glucopenia in the mouse (161) and to insulin-induced hypoglycemia in the dog (99). These studies unequivocally demonstrate that autonomic activation is essential for an intact glucagon response to glucopenic stress in dogs and mice. Further support for these observations is provided by a study that showed that the glucagon response invoked by insulin-induced hypoglycemia in normal man is absent in patients with Shy-Drager syndrome (182), a degenerative neurological condition accompanied by both parasympathetic and sympathetic nervous system dysfunction (183).

Bloom and Edwards examined the effects of pharmacological blockade of cholinergic stimulation with atropine, or prevention of sympathoadrenal activation by surgical section of the splanchnic nerves, or both treatments together, on the plasma glucagon response to glucopenic stress in two studies in conscious calves. In one study glucopenia was induced with iv 2-DG (44); in the other by insulin-induced hypoglycemia (156). In these studies they found that atropine alone delayed the plasma glucagon response to insulin-induced hypoglycemia but not to 2-DG, that splanchnic nerve section alone delayed the glucagon response to either 2-DG or insulin-induced hypoglycemia, and that the two treatments together significantly impaired the glucagon responses to both glucopenic stimuli. In the studies with insulin-induced hypoglycemia, the administration of atropine to splanchnicotomized animals induced a greater degree of hypoglycemia than in intact animals, accompanied by convulsions, demonstrating that the glucagon response mediated by the autonomic nervous system is a critical component of the counterregulatory response for defending plasma glucose levels. These studies provide evidence that the glucagon response to glucopenia in calves is redundantly mediated by cholinergic and sympathoadrenal activation (Fig. 3). They further suggest that the low glucose level during insulin-induced hypoglycemia is relatively impotent in increasing glucagon secretion.

The combination of atropine and tetraplegia did not impair the glucagon response to insulin hypoglycemia in man (184). However, a large component of vagally mediated glucagon secretion has been demonstrated to be atropine resistant (9), and thus peptidergic activation may have contributed to the glucagon response in this study (Fig. 3).

An alternative method by which both the parasympathetic and sympathoadrenal input to the islet can be eliminated in experimental animals is the combined surgical interventions of vagotomy and spinal cord section.

In addition to preventing cholinergic and adrenergic activation in response to glucopenic stress, this technique ensures that any potential peptidergic activation will be prevented as well. In one study in which this procedure was utilized, plasma glucagon levels were not measured. The investigators assessed the recovery of plasma glucose levels after insulin injection in anesthetized dogs. They found that glucose recovery was unaffected by either cervical vagotomy or by spinal cord transection alone; however, the combined procedures markedly impaired the restoration of plasma glucose levels (185). These experiments suggest a redundancy in the parasympathetic and sympathetic contributions to recovery of plasma glucose levels from hypoglycemia; they do not, however, conclusively demonstrate that autonomic input to the pancreatic islet is involved. While it is possible that spinal cord section prevents sympathoadrenal input to the liver, resulting in an impairment of catecholamine-mediated increases of hepatic glucose output, it is difficult to suggest another parasympathetically mediated counterregulatory mechanism, other than increased glucagon secretion, which could contribute to glucose recovery from hypoglycemia.

In preliminary studies employing these surgical ablations, we have demonstrated a dominant role for the autonomic nervous system as opposed to lowered plasma glucose levels in the glucagon response to severe insulin-induced hypoglycemia in dogs (99). However, further studies are necessary to critically test the hypothesis of redundant autonomic mediation of the glucagon response to moderate hypoglycemia.

C. Effects of glucopenia-induced autonomic activation on insulin secretion

1. Parasympathetic activation. Few studies have examined the effects of glucopenia-induced parasympathetic activation on insulin secretion. Although cholinergic activation normally stimulates insulin secretion (Fig. 2), during the hypoglycemia induced by insulin injection, the parasympathetic effects on insulin secretion are presumably minimized because, at low ambient glucose levels, the B cell would be relatively unresponsive to vagal stimulation-induced acetylcholine release (186), as it is to other secretagogues (187). In contrast, when 2-DG is used to induce glucopenia, the hyperglycemia resulting from the glucopenic stress might be expected to potentiate the insulin response to cholinergic activation, and, in some experiments, insulin levels increase during 2-DG-induced glucopenia. In mice, atropine markedly reduces the increase of plasma insulin levels after 2-DG injection (188), demonstrating cholinergic mediation. In dogs, the small increase of insulin levels that follows a moderate dose of 2-DG is unaffected by atropine (60).

The candidate parasympathetic neuropeptides, VIP and GRP, are also stimulatory to insulin release (Fig. 2), but, again, the low glucose levels during insulin-induced hypoglycemia presumably would minimize their effects.

2. Sympathoadrenal activation. Evaluation of the effect of sympathetic activation on insulin secretion during insulin-induced hypoglycemia is complicated by the technical problems of measuring endogenous insulin in the presence of large amounts of exogenous insulin. Measurement of plasma C-peptide, which is released in equimolar quantities with endogenous insulin (28), provides a solution. Indeed, decreased plasma C-peptide levels have been observed during insulin-induced hypoglycemia (26, 189). However, the mechanism of this suppression is not well understood. In certain cases, this decrease of insulin secretion may not be due to sympathoadrenal activation, or even to a direct effect of hypoglycemia on the B cell itself, because suppression of C-peptide is also observed during a hyperinsulinemic clamp when plasma glucose levels are maintained by glucose infusion (189). These data suggest that exogenous insulin is capable of directly suppressing endogenous insulin release.

Other experiments do suggest a sympathetic contribution. In one study, endogenous insulin responses to the insulin secretagogue, tolbutamide, were measured during insulin-induced hypoglycemia. The investigators waited until the exogenous insulin levels had decreased to levels low enough that the endogenous responses to tolbutamide were detectable. They found that the α -adrenergic antagonist, phentolamine, increased the endogenous insulin response to tolbutamide (190), suggesting that, in these studies, the sympathetic nervous system was activated and had been suppressing the insulin response.

Miller and associates (191) provided the most convincing evidence for the suppression of insulin secretion by sympathetic nerves during hypoglycemia with their cross-perfusion experiments. In these studies, the pancreas of a small dog was cross-perfused with blood from a large donor dog. Glucose was allowed to fall in the systemic circulation of the small dog, resulting in central glucopenia, while its pancreas was perfused with blood containing a slightly hyperglycemic level of glucose. When the splanchnic nerves of the dog with the perfused pancreas were severed, an increase of insulin secretion from the perfused pancreas was observed, indicating that these nerves had been directly suppressing insulin release.

The use of 2-DG to produce glucopenia circumvents the problems with the use of exogenous insulin (see above). In baboons, 2-DG injection inhibits plasma insulin levels, and the α -adrenergic antagonist, phentolamine, reverses this inhibition (160). In mice, 2-DG

increases plasma insulin levels, but this increase is potentiated by phentolamine (188). The interpretation of these adrenergic blocking experiments is complicated, however, because selective α -adrenergic antagonism results in enhanced and unopposed β -stimulation of insulin secretion. Therefore it is difficult to assess the net effect of the simultaneous α - and β -adrenergic agonism that presumably occurs during sympathoadrenal activation without performing experiments with combined and complete α - and β -adrenergic blockade.

Experiments that do not rely on adrenergic antagonists also suggest an impairment of insulin release during glucopenic stress produced by 2-DG. A reduction of the acute insulin response to glucose is reversed by adrenalectomy in monkeys (192), and in rats (193), suggesting mediation by epinephrine. Other experiments in rats suggest a role for pancreatic sympathetic nerves. In these experiments, the drug, guanethidine, which markedly reduces the release of norepinephrine from noradrenergic nerve terminals, but not that of epinephrine from the adrenal medulla (194), prevents the inhibition of plasma insulin after 2-DG injection (195).

Other studies suggest impairment of insulin secretion without defining the mechanism. In man (45, 196) and dogs (171), plasma insulin levels do not increase during 2-DG-induced glucopenia despite increased plasma glucose levels, which suggests relative, rather than absolute, inhibition of B cell secretion.

Although many consider catecholamines to be the mediators of this impaired insulin secretion, the candidate neuropeptides, galanin and NPY, can also inhibit insulin release (Fig. 2). However, it has not been established that these neuropeptides are, in fact, released from pancreatic nerves during glucopenic stress.

In summary, the moderate or marked sympathoadrenal activation accompanying glucopenic stress usually results in relative or absolute inhibition of insulin release. The contribution of sympathoadrenal activation to the impairment of insulin release during insulin-induced hypoglycemia is more difficult to demonstrate because both hypoglycemia itself and exogenous insulin can inhibit insulin secretion.

IV. Conclusions

Although hypoglycemia *per se* can directly influence glucagon and insulin secretion, as has been most clearly demonstrated *in vitro*, the autonomic activation that accompanies glucopenia *in vivo* makes a greater contribution to the changes of pancreatic hormone secretion than traditionally thought. This view is supported by evidence of multiple and redundant autonomic input to the islet during moderate glucopenic stress and the dif-

ficulty of completely blocking that input in order to clearly reveal the full autonomic contribution. Specifically, we conclude: 1) The parasympathetic input to the pancreas is activated by modest glucopenia and may be maximal at moderate glucopenia. 2) The sympathoadrenal input to the pancreas is minor until moderate glucopenia and then increases progressively with the severity of the stress. 3) This differential activation of the two branches of the autonomic nervous system may lead to situations in which the autonomic component of the glucagon response to modest glucopenia is mediated predominantly by the parasympathetic nervous system, to moderate glucopenia is mediated by both branches, and to marked glucopenia is mediated predominately by the sympathoadrenal system. 4) During moderate glucopenic stress, the effect of autonomic activation on the islet may be redundant, thus providing an explanation for the difficulty of demonstrating an autonomic contribution to islet responses by blocking only one branch of the autonomic nervous system. New experiments, which are specifically designed to eliminate the redundant autonomic input to the islet, are needed to critically test this hypothesis and to determine the actual contribution of the autonomic nervous system to the pancreatic hormone responses during moderate glucopenic stress.

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