Title

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Journal

Diabetes Nutrition and Metabolism, 15(5)

ISSN

0394-3402

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Publication Date

2002-10-01

Peer reviewed

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Autonomic mechanism and defects in the glucagon response to insulin-induced hypoglycaemia

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INTRODUCTION

The induction of hypoglycaemia by the injection of insulin is well known to stimulate the secretion of glucagon. However, despite over 25 years of investigation, the mechanism mediating this specific glucagon response is still debated. Three major hypotheses have been advanced. The first can be called the "A-Cell Hypothesis" and it is the most straightforward. It posits that low glucose acts directly on the A-cell of the pancreatic islet to stimulate glucagon release. Evidence in favor of this hypothesis has been reviewed by Gerich (1) and includes the demonstration that the isolated pancreas increases its secretion of glucagon when perfused by a low glucose solution. Evidence against this hypothesis includes the failure of low glucose to stimulate glucagon release from isolated A-cells (2). The second major mechanism, the "B-Cell Hypothesis", is slightly less direct. It posits that hypoglycaemia (and the concomitant hyperinsulinaemia) suppresses insulin secretion from the islet B-cell which had been tonically restraining the glucagon release from the islet A-cell. In this scheme, the glucagon response to insulin-induced hypoglycaemia (IIH) is then due to a disinhibition of the A-cell, secondary to the suppression of endogenous insulin. Evidence in favor of this hypothesis has been reviewed by Samols *et al* (3) and

Diab. Nutr. Metab. 15: 318-323, 2002.

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Key words: Sympathetic, Type 1 diabetes, parasympathetic.

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includes the demonstration that exogenous insulin inhibits glucagon secretion and the anecdotal evidence that the glucagon response to IIH in Type 1 diabetic patients is lost when these patients lose their residual B-cell function (4). Evidence against this hypothesis includes the failure of suppression of endogenous insulin *per se*, to stimulate the glucagon secretion (5). The third major mechanism, the "Autonomic Hypothesis", is the most indirect. It posits that hypoglycaemia, perceived either by central (6) or peripheral (7) glucose receptors, activates the autonomic nervous system which in turn stimulates the islet A-cell to release glucagon. Evidence in favor of this mechanism has been reviewed by us previously (8-10) and is outlined in the remainder of this article. Evidence against this hypothesis includes findings that single autonomic lesions have minimal effects on the glucagon response to IIH (8) and that there is an apparent recovery of the glucagon response to IIH in patients with Type 1 diabetes mellitus (T1DM) who have received pancreas transplants (11).

AUTONOMIC INPUTS TO THE ISLET

There are three autonomic inputs to the islet. They are: 1) circulating epinephrine whose release is under sympathetic control, 2) islet sympathetic nerves, and 3) islet parasympathetic nerves (Fig. 1). Walter Cannon, the father of American physiology, was the first to demonstrate, in the late 1920's, that epinephrine was released from the adrenal medulla in response to IIH. Following the development of the radioenzymatic assay from plasma catecholamines in the 1970's, the epinephrine response to IIH has been confirmed countless times. Gerich was the first to demonstrate that epinephrine stimulates glucagon secretion in humans (12). In the 1970's, indirect evidence appeared that the parasympathetic nerves of the pancreas were also activated during IIH (13). This evidence was based on the secretion of pancreatic polypeptide (PP) from the islet F cell, which is very sensitive to cholinergic stimulation (13). Thus, the ability of atropine to block the PP response to IIH strongly suggested that the parasympathetic nerves of the pancreas are activated during IIH. Electrical stimulation of the parasympathetic nerves also stimulates glucagon secretion (14, 15). Likewise, a number of groups in the 70's and 80's showed that electrical stimulation of sympathetic nerves increased glucagon (16). In the 1990's, Havel *et al* (17) and Dunning *et al* (18) showed that the sympathetic nerves to the pancreas were activated during severe IIH by measuring an increase in the spillover of norepinephrine (NE) from dog pancreas. In 2000, our group developed a method for assessing the activity of the abdominal sympathetic nerves which is useful in much smaller animals. It involves the expression of the early immediate gene product, Fos, in the nucleus of the principal ganglionic cells of the celiac ganglion (Fig. 2) (19). This ganglion projects post-ganglionic sympathetic nerves to the pancreas.

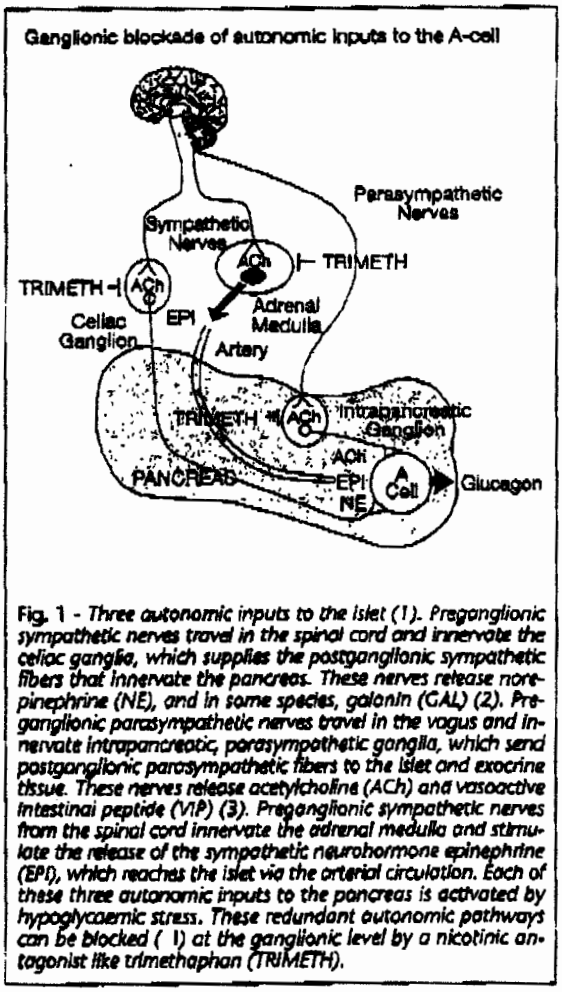

Fig. 1 - *Three autonomic inputs to the islet (1). Preganglionic sympathetic nerves travel in the spinal cord and innervate the celiac ganglia, which supplies the postganglionic sympathetic fibers that innervate the pancreas. These nerves release norepinephrine (NE), and in some species, galanin (GAL) (2). Preganglionic parasympathetic nerves travel in the vagus and innervate intrapancreatic, parasympathetic ganglia, which send postganglionic parasympathetic fibers to the islet and exocrine tissue. These nerves release acetylcholine (ACh) and vasoactive intestinal peptide (VIP) (3). Preganglionic sympathetic nerves from the spinal cord innervate the adrenal medulla and stimulate the release of the sympathetic neurohormone epinephrine (EPI), which reaches the islet via the arterial circulation. Each of these three autonomic inputs to the pancreas is activated by hypoglycaemic stress. These redundant autonomic pathways can be blocked ( ) at the ganglionic level by a nicotinic antagonist like trimethaphan (TRIMETH).*

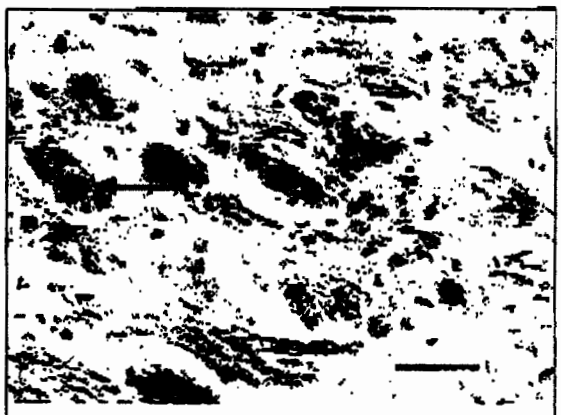

Fig. 2 - *Fos expression in the nuclei (←) of principal ganglion cells of the celiac ganglia (CG) in response to the ip injection of insulin. Scale bar=72 µm. From (19), with permission.*

AUTONOMIC MEDIATION

Thus, by the mid 90's, it was established that hypoglycaemia activated all three autonomic inputs to the islet and that each input, by itself, was capable of stimulating glucagon secretion. Therefore, it was logical to hypothesize that this autonomic activation was a major mechanism mediating the glucagon response to IIH. However, earlier work in humans had shown that interference with one or two of these three autonomic inputs failed to significantly alter the glucagon response to IIH (8). Together those data suggested either that there was a different mechanism in humans *vs* animals or that there was a redundant autonomic mediation of the glucagon response. In 1989, we proposed this redundant autonomic mediation as a way to reconcile these negative data with the autonomic hypothesis (8). We then proceeded to design experiments to test the autonomic hypothesis, with redundant autonomic mediation in mind. These experiments were therefore designed to block all three autonomic inputs to the islet either surgically or pharmacologically. Using the former approach, we were able to demonstrate not only autonomic mediation of the majority of the glucagon response to IIH but also that selective activation of pancreatic sympathetic nerves was capable of mediating most of the glucagon response (20). However, since these studies were done in animals under anesthesia and with severe hypoglycaemia, the question of the autonomic contribution in studies of unanesthetized humans during less severe hypoglycaemia remained. Therefore, a pharmacologic approach was used in humans to block all three autonomic pathways. Since each pathway involved the release of acetylcholine and its action on a nicotinic receptor, a single nicotinic antagonist, trimethaphan, could achieve such blockade (Fig. 1). These experiments, done in humans at clamped hypoglycaemia, in the presence or absence of trimethaphan, showed conclusively that impairing autonomic activation during IIH markedly impaired the glucagon response to IIH (21). Thus, although these studies do not rule out important roles for a direct stimulating effect of hypoglycaemia on the A-cell (the A-Cell Hypothesis) or for the suppression of insulin to augment glucagon secretion during hypoglycaemia (the B-Cell Hypothesis), they do convincingly demonstrate an important role for activation of the autonomic nervous system in mediating the glucagon response to IIH (the Autonomic Hypothesis).

SYMPATHETIC DEFECT IN TYPE 1 DIABETES MELLITUS

Since our evidence (9, 10) and those of others (6, 22) suggested that the glucagon response to IIH is autonomically mediated in non diabetic individuals and since this glucagon response is lost in Type 1 diabetes mellitus (T1DM), we hypothesized (10) that there may be autonomic defects in T1DM that contribute to the loss of this specific glucagon response. This possibility had not been seriously considered before for two reasons. First, previous discussions had focused the field on either the A-Cell Hypothesis (23, 24) or the B-Cell Hypothesis (3, 25). Second, the autonomic defects known to occur in T1DM, such as classical Diabetic Autonomic Neuropathy (DAN), occur well after the loss of the glucagon response to IIH in T1DM. Thus, if an autonomic defect does contribute to the loss of the glucagon response to IIH seen in T1DM, it may be restricted to one of the autonomic inputs of the islet and may therefore have easily been missed in earlier studies.

Given our interest and experience in studying the sympathetic innervation of the islet, we looked for defects in this autonomic input, using an accepted animal model of autoimmune T1DM, the BB diabetic rat. To identify islets, we used immunohistochemical staining for glucagon on their rim. To identify sympathetic nerves in the islet core, we used immunohistochemical staining for vesicular monoamine transporter 2 (VMAT2)

in nerve fibers. VMAT2 is a transporter expressed on the synaptic vesicles of sympathetic nerves. (26). To validate that this new technique could detect a loss of sympathetic fibers within the islet, we treated the animals with 6-hydroxydopamine (6-OHDA). We found that 6-OHDA treatment eliminated VMAT2-positive fibers both within the islet and in the exocrine pancreas. To quantify this denervation, we determined the area occupied by VMAT2-positive fiber segments with the islet. This area was negligible in the islets of 6-OHDA-treated animals. We next applied this method to determine if there was a loss of islet sympathetic nerves in BB diabetic rats, using BB non-diabetic rats as controls. We found a marked decrease in the area of VMAT2-positive nerve fibers within the islets of BB diabetic rats. We also found that this loss was restricted to the islets and occurred within two weeks after the presentation of T1DM (27). We have therefore named it early sympathetic islet neuropathy (eSIN), to distinguish it from DAN which occurs late in this disease.

SUMMARY AND CONCLUSIONS

In summary, this article briefly reviews the evidence that three separate autonomic inputs to the islet are capable of stimulating glucagon secretion and that each is activated during IIH. We have reviewed our evidence that these autonomic inputs mediate the glucagon response to IIH, both in non-diabetic animals and humans. Finally, we outline our new preliminary data suggesting an eSIN in an autoimmune animal model of T1DM.

We conclude that the glucagon response to IIH is autonomically mediated in non-diabetic animals and humans. We further suggest that at least one of these autonomic inputs, the sympathetic innervation of the islet, is diminished in autoimmune T1DM. These data raise the novel possibility that an autonomic defect contributes to the loss of the glucagon response to IIH in T1DM.

ACKNOWLEDGEMENTS

This research was supported by the Medical Research Service of the Department of Veterans Affairs and the National Institute of Diabetes and Digestive and Kidney Disease Grants (DK-12829, DK-12047, and DK-50154). The Authors thank their colleagues, particularly the Metabolism Research Group in Seattle, for both their scientific criticism and for their encouragement during the development of the ideas contained herein. The Authors also thank Diane Herring for help preparing the manuscript and Huy Tran for help with the figures.

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DISCUSSION

Question from the floor: It's a very elegant presentation. I have a couple of questions: the BB rat, I think, is a unique model of diabetes in which the glucagon concentrations are very low; in most models, the glucagon concentration is actually high, like the streptozotocin rat. So my question is how do you deal with this issue? It is very elegant work but is the BB rat really the same as human Type 1 diabetes?
Gerald Taborsky: This gets down to a very important point about Type 1 diabetes. There is evidence, not mine but from the work of others, that the glucagon response to insulin-induced hypoglycaemia is lost in human Type 1 diabetes. However, it seems to be lost when the last of the residual B cell function is lost. The BB diabetic rat shares this characteristic with humans but other animal models of Type 1 diabetes, like the streptozotocin diabetic rat, do not. We ourselves have not actually measured responses in the BB diabetic rat but if I recall correctly, its responses to arginine are either normal or elevated, not reduced. Further, I believe that the pancreatic content of glucagon is normal in the BB diabetic rat. For these reasons, I believe that there is no general problem with glucagon in the BB diabetic rat and that the BB diabetic rat is a rather good model of human Type 1 diabetes.

Question from the floor: You have told us over the years, with your beautiful work, that there are three autonomic pathways controlling glucagon secretion. Now you show us that one of these pathways may be deficient. What about the others? I mean how do you explain this?
Gerald Taborsky: This is the crux of my problem: Can a deficiency in just one autonomic pathway actually cause an impaired glucagon response to insulin-induced hypoglycaemia? It is pretty clear, at least early on in Type 1 diabetes, that the adrenal medullary response is relatively normal. Therefore, we are forced to postulate that the redundancy of the autonomic inputs that we have demonstrated in normal animals and humans is either altered in diabetic animals or that there is some other mechanism that suppresses the glucagon response. This view is reinforced by our unpublished observations that denervation of pancreatic sympathetic nerves alone, in a non-diabetic animal, does not impair the glucagon response. Thus, although I do believe that we do deserve some credit for discovering a new, specific form of neuropathy in Type 1 diabetes, we have yet to demonstrate its impact on this specific glucagon response.

Question from the floor: Do you think it is possible that the loss of sympathetic or autonomic innervation is associated with the loss of B cells? Further, is it possible that the denervation observed is specific to the B cells, not to the A cells?