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The combination of GIP plus xenin-25 indirectly increases pancreatic polypeptide release in humans with and without type 2 diabetes mellitus

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ABSTRACT

Xenin-25 (Xen) is a 25-amino acid neurotensin-related peptide that activates neurotensin receptor-1 (NTSR1). We previously showed that Xen increases the effect of glucose-dependent insulinotropic polypeptide (GIP) on insulin release 1) in hyperglycemic mice via a cholinergic relay in the periphery independent from the central nervous system and 2) in humans with normal or impaired glucose tolerance, but not type 2 diabetes mellitus (T2DM). Since this blunted response to Xen defines a novel defect in T2DM, it is important to understand how Xen regulates islet physiology.

On separate visits, subjects received intravenous graded glucose infusions with vehicle, GIP, Xen, or GIP plus Xen. The pancreatic polypeptide response was used as an indirect measure of cholinergic input to islets. The graded glucose infusion itself had little effect on the pancreatic polypeptide response whereas administration of Xen equally increased the pancreatic polypeptide response in humans with normal glucose tolerance, impaired glucose tolerance, and T2DM. The pancreatic polypeptide response to Xen was similarly amplified by GIP in all 3 groups. Antibody staining of human pancreas showed that NTSR1 is not detectable on islet endocrine cells, sympathetic neurons, blood vessels, or endothelial cells but is expressed at high levels on PGP9.5-positive axons in the exocrine tissue and at low levels on ductal epithelial cells. PGP9.5 positive nerve fibers contacting beta cells in the islet periphery were also observed. Thus, a neural relay, potentially involving muscarinic acetylcholine receptors, indirectly increases the effects of Xen on pancreatic polypeptide release in humans.

1. Introduction

Pancreatic islet dysfunction, including impaired insulin secretion, is one of the hallmark features of type 2 diabetes mellitus (T2DM). Glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) are incretin hormones produced by enteroendocrine cells located in the proximal and distal intestine, respectively [1–3]. Both peptides are released into the circulation after meal ingestion in response to nutrients present in the lumen of the gut, but not to nutrients in the blood [3,4]. Circulating incretins then potentiate glucose-stimulated insulin secretion. Orally-derived glucose elicits a much greater insulin secretory response than comparable levels of intravenously administered glucose which is called the incretin effect. In addition to GIP and GLP-1, numerous neuropeptides and neurotransmitters regulate insulin release [5].

It has been known for many years that the incretin response, but not incretin release, is blunted in humans with T2DM [6,7]. In spite of this, exogenously administered GLP-1 remains active in T2DM and forms the rationale for incretin-based pharmacotherapies that increase GLP-1 receptor signaling [8,9]. Although it has been generally felt that the effects of GIP on insulin secretion are blunted in T2DM [10–12], we recently demonstrated that the *magnitude* of the insulin secretory response to exogenously administered GIP is similar in humans with normal glucose tolerance (NGT), impaired glucose tolerance (IGT), and T2DM [13]. Thus, humans with T2DM exhibit a blunted insulin secretory response to endogenously released, but not exogenously administered, GIP and GLP-1. The basis for this difference is unknown.

Xenin-25 (Xen) is a 25-amino acid neurotensin-related peptide originally reportedly produced by a subset of GIP-producing cells [14]. Although Xen is longer than the 13-amino acid neurotensin (Fig. 1A), only 6 (neurotensin) or 8 (Xen) C-terminal amino acids are required for biological activity. Both peptides require an unblocked C-terminal leucine for biological activity [15,16]. We previously showed that in mice, Xen increases the effects of GIP on insulin release but has little effect alone [17]. This in vivo response to Xen: 1) was not recapitulated with isolated islets, insulin-producing cell lines, or the in situ perfused pancreas; 2) was

Abbreviations: Alb, albumin alone; GLP-1, glucagon-like peptide-1; GIP, glucose-dependent insulinotropic polypeptide; G + X, GIP plus Xen; IGT, impaired glucose tolerance; ISR, insulin secretion rate; ivGGIs, intravenous graded glucose infusions; NTSR1, neurotensin receptor-1; NGT, normal glucose tolerance; PP, pancreatic polypeptide; PPR, pancreatic polypeptide response; T2DM, type 2 diabetes mellitus; Xen, xenin-25.

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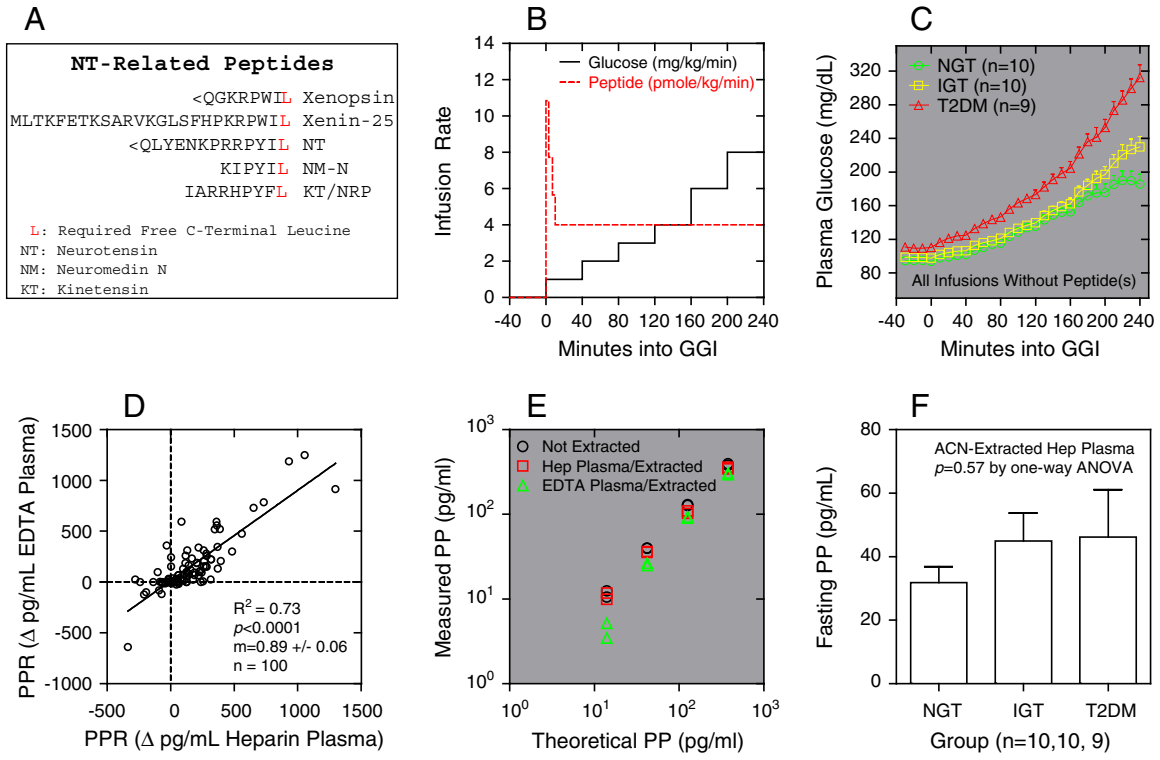

Fig. 1. Fasting PP levels are similar in humans with NGT, IGT, and T2DM. Panel A. Amino acid sequences for neurotensin-related peptides are shown. Note that the amino termini of neurotensin and xenopsin (<Q) contain a cyclic modification of glutamine. Panel B. Following a 10-hour overnight fast, subjects were administered ivGGIs at the indicated glucose and peptide infusion rates. Panel C. Plasma glucose levels were measured at the indicated times during the ivGGIs without peptide administration. Group mean ± SEM for each time point is shown for subjects with NGT (green circles), IGT (yellow squares), and T2DM (red triangles). Data are taken from ref. [13]. Panel D. Changes in PP levels from 0 to 40 min of the ivGGI were measured in EDTA and heparinized plasma during infusion with Alb, GIP, Xen, or the combination of G + X. Values measured in the heparin versus EDTA samples from 25 subjects are shown. Panel E. PP standards were prepared in buffer supplied by the manufacturer (black circles) or charcoal-stripped heparinized (red squares) or EDTA (green triangles) plasma. Only the plasma samples were extracted with acetonitrile before PP measurements. Measured values are plotted versus the theoretical concentration in the diluted standards. Panel F. Fasting PP levels were measured in heparinized plasma samples extracted with acetonitrile. Average PP levels ± SEM are shown for each group.

inhibited by atropine sulfate (crosses the blood–brain barrier) and atropine methyl bromide (does not cross the blood–brain barrier); and 3) was not associated with increased c-fos expression in regions of the brain involved in afferent and efferent signaling [17]. In contrast to Xen, carbachol potentiated the effects of GIP on insulin secretion in the in vitro/situ systems. Thus, Xen increases GIP-mediated insulin release in mice via a cholinergic relay in the periphery, possibly independent from parasympathetic neurons that innervate the islets. Interestingly, Kirchgessner and Gershon [18,19] have described an extensive network of myenteric neurons in the enteric nervous system that directly connect the stomach/duodenum to the pancreas. These interneurons function independently from the central nervous system and can modify pancreatic endocrine function. We have recently shown that Xen increases cytosolic free calcium levels in a subset of myenteric neurons isolated from guinea pig duodenum [15]. Thus, Xen-responsive neurons may regulate islet function.

We recently demonstrated using intravenous graded glucose infusions (ivGGIs) that Xen, in combination with GIP but not alone, rapidly and transiently increased insulin and glucagon secretion in humans with NGT and IGT, but not T2DM [13]. This response over the first 40 min of the ivGGIs occurred in the absence of significant changes in plasma glucose levels. Since this blunted response to Xen defines a novel defect in T2DM, it is important to understand how Xen regulates islet physiology. Several studies have reported that islets within the human pancreas are innervated by both cholinergic and non-cholinergic neurons [20–22] suggesting that as in mice, a neural relay could mediate the Xen signal to beta cells in humans. However, it has recently been reported that human islets are poorly innervated and islet-derived acetylcholine is released from alpha cells rather than neurons [23,24]. As a first step in understanding how Xen regulates islet function in humans, it is important to determine: 1) if Xen increases cholinergic input to islets in humans with and without T2DM and 2) which cells in the human pancreas express receptors for Xen.

Pancreatic polypeptide (PP) is a 36-amino acid peptide produced only by islet PP cells [25–27]. Classically, PP release in response to insulin-induced hypoglycemia has been used to indirectly assess cholinergic input to islets in humans. However, it has been shown that following an 8-hour fast, intravenous infusion of neurotensin also stimulates PP release in humans and this response is completely blocked by atropine [28]. Genetic and pharmacologic studies have shown that neurotensin receptor-1 (NTSR1) mediates the effects of Xen [15,29–33]. Our previous ivGGI study was conducted following a 10-hour fast and plasma glucose levels remained at basal levels for at least 40-min. Thus, the PP response (PPR) over this early portion of the ivGGI represents a surrogate measure for Xen-induced cholinergic input to islets. We now show that 1) the PP response to Xen is not impaired in humans with T2DM and 2) NTSR1 is expressed on pancreatic neurons but not on islet endocrine cells. Thus, a neural relay, potentially involving muscarinic acetylcholine receptors, indirectly increases the effects of Xen on islet endocrine cell function in humans.

2. Materials and methods

2.1. Study design and protocols

All protocols were approved by Washington University's Human Research Protection Office and the FDA (IND#103,374) and are registered with ClinicalTrials.gov (NCT00798915). Studies were performed in the