Emerging therapeutic potential for xenin and related peptides in obesity and diabetes

Sarah L. Craig, Victor A. Gault, Nigel Irwin*

SAAD Centre for Pharmacy and Diabetes, University of Ulster, Coleraine, Northern Ireland, BT52 1SA, UK

*Address correspondence and reprint requests to Dr. Nigel Irwin, SAAD Centre for Pharmacy and Diabetes, University of Ulster, Coleraine, Northern Ireland, BT52 1SA, UK

Email: n.irwin@ulster.ac.uk

Tel: ++44 (0) 28 70 124574

Fax: ++44 (0) 28 70 323939
Abstract

Xenin-25 is a 25 amino acid peptide hormone co-secreted from the same enteroendocrine K-cell as the incretin peptide glucose-dependent insulino tropic polypeptide (GIP). There is no known specific receptor for xenin-25, but studies suggest that at least some biological actions may be mediated through interaction with the neurotensin receptor. Original investigation into the physiological significance of xenin-25 focussed on effects related to gastrointestinal transit and satiety. However, xenin-25 has been demonstrated in pancreatic islets and recently shown to possess actions in relation to the regulation of insulin and glucagon secretion, as well as promoting beta-cell survival. Accordingly, the beneficial impact of xenin-25, and related analogues, has been assessed in animal models of diabetes-obesity. In addition, studies have demonstrated that metabolically active fragment peptides of xenin-25, particularly xenin-8, possess independent therapeutic promise for diabetes, as well as serving as bioactive components for the generation of multi-acting hybrid peptides with antidiabetic potential. This review will focus on continuing developments with xenin compounds in relation to new therapeutic approaches for diabetes-obesity.

Keywords: Xenin-25; glucose-dependent insulino tropic polypeptide (GIP); insulin secretion; satiety; diabetes
1. **INTRODUCTION**

Xenin-25, the human counterpart of the amphibian peptide xenopsin,\(^1\) is a naturally occurring 25 amino-acid peptide hormone originally identified by Feurle and colleagues from isolates of human gastric duodenal and jejunal mucosa (Table 1).\(^2\) Subsequent studies further demonstrated that xenin-25 can also be extracted from the gastric mucosa of various other animal species, including rat, rabbit, dog and pig.\(^3\) In humans, it was later revealed that xenin-25 is synthesised and secreted from a subpopulation of chromogranin A-positive enteroendocrine K-cells.\(^4\) Xenin-25 is structurally related to neurotensin (Table 1), and it is believed that the peptide may mediate some of its biological effects through activation of neurotensin receptors.\(^5\) However, there is evidence indicating that some biological actions of xenin-25 are independent of neurotensin receptors,\(^6\) although a specific xenin-25 receptor has not yet been identified. A highly conserved precursor of xenin-25, namely proxenin, has been described with sequence homology to yeast and mammalian alpha coat protein (COPA).\(^7\) Interestingly, the 35 N-terminal amino acid residues of COPA are identical to proxenin, and treatment of proxenin with pepsin releases biologically active xenin-25.\(^8\)

2. **BIOLOGICAL ACTIVITY OF XENIN-25**

Well established biological actions of xenin-25 include effects on gastrointestinal transit rate and gastric emptying,\(^2,9,10\) appetite suppression\(^11-14\) as well as pancreatic endocrine and exocrine secretions.\(^2,14,15\) These actions are considered in more detail below.

2.1 **GASTROINTESTINAL TRANSIT**

Xenin-25 is known to delay gastric emptying in rodents and humans (Figure 1),\(^9,13\) thought to be linked to the presence of neurotensin receptors on nerve fibres in longitudinal stomach muscle.\(^13\) The action of xenin-25 in both the small and large intestines of guinea pigs has been
extensively studied. As such, a biphasic response to xenin-25 is elicited in the jejunum resulting in a small relaxation followed by a large contraction, thought to be due to a neurokinetic excitatory effect, along with muscarinic, purinergic and tachykinin-related mechanisms (Figure 1). In the colon, xenin-25 induces a myokinetic relaxation effect, involving Ca\(^{2+}\)-dependent potassium channels and the P2-purinoceptor. Distinct differences in the action of xenin-25 on guinea pig intestine suggest that there are neurokinetic and myokinetic receptor variants responsible for this xenin-25 effect. Furthermore, Clemens and co-workers reported that xenin-25 relaxes rat ileum, and that this effect is mediated by an apamin-sensitive neurotensin-type receptor. In dogs, xenin-25 has a dose-dependent neurotensin-like effect on gall bladder contractions, with non dose-dependent effects on jejunum contractions (Figure 1). However, it has been suggested that effects of xenin-25 on the intestine may not be mediated entirely through interaction with neurotensin receptors, indicating the presence of other receptors that regulate xenin-25 activity, as discussed above. More recently, the ability of xenin-25 to stimulate gastrointestinal transit has been demonstrated to be absent following Roux-en-Y gastric bypass surgery, signifying the importance of the duodenum in this regard. Indeed, xenin-25 is known to modulate interdigestive and postprandial duodenojejunal motility in humans, even at relatively low infusion doses of 40 pmol/kg for 10 min. Xenin-25 is also known to inhibit the secretion of pentagastrin-stimulated gastric acid, which could also play a role in the modulation of overall gastric transit.

### 2.2 APPETITE SUPPRESSION

Xenin-25 acts as a satiety factor and controls energy intake, in harmony with the knowledge that circulating xenin-25 concentrations increase after a meal (Figure 1). Indeed, xenin-25 regulates energy balance in children, where its overall affect may be altered by both obesity

This article is protected by copyright. All rights reserved.
and intestinal inflammation.\textsuperscript{24} Other studies have confirmed clear anorexigenic effects of xenin-25 in chicks\textsuperscript{10} and rodents.\textsuperscript{11,12,23,25} Effects of xenin-25 on feeding may be directly linked to aforementioned actions on gastrointestinal transit.\textsuperscript{9,13} As such, the rate of gastric emptying was reduced by 93\% in xenin-25 treated mice, and linked to decreased food consumption.\textsuperscript{9} However, it has also been suggested that xenin-25 induced anorexia in chicks is mediated centrally, through direct effects on the ventromedialis hypothalami.\textsuperscript{26} Similarly, Kim and Mizuno\textsuperscript{9} demonstrated that the appetite suppressive effects of xenin-25 are partly related to the activation of cells in the nucleus of the solitary tract. Xenin-25 may also impact feeding through modulation of neurotensin receptor related signalling pathways in the hypothalamus.\textsuperscript{12,27} that are independent of the action of both melanocortin and leptin. Activation of these hypothalamic neurotensin receptors is believed to result in stimulation of extracellular signal regulated kinase (ERK) activity to control food intake.\textsuperscript{28} However, this effect of xenin-25 to suppress appetite via ERK pathways is debated by others.\textsuperscript{27} Indeed, IL-1 signalling has been identified as another potential central pathway through which xenin-25 modulates energy balance.\textsuperscript{29} It has also been proposed that myenteric neurons can mediate the effects of xenin-25 on feeding, as well as those on gastric emptying, gall bladder contractions and gut motility.\textsuperscript{30} More recently, a direct effect of xenin-25 on lipid metabolism and adipose tissue has been described including reduced lipogenesis and increased lipolysis (Figure 1),\textsuperscript{31} which could also be linked to notable effects of xenin-25 on energy turnover.

2.3 PANCREAS

Early studies noted that xenin-25 stimulated secretions from the exocrine pancreas.\textsuperscript{2} In addition, the same research group later revealed that xenin-25 also induced secretion of pancreatic-derived endocrine hormones in dogs, including pancreatic polypeptide, insulin and glucagon (Figure 1).\textsuperscript{21} This effect was later confirmed in isolated mouse islets,\textsuperscript{15} where xenin-
8, a naturally occurring bioactive fragment of xenin-25 that displays essentially the same biological action profile as the parent peptide (Table 1), was also shown not to regulate somatostatin release. This would imply that the effect of xenin-25 on glucagon and insulin release is mediated via a direct effect on pancreatic alpha- and beta-cells, respectively. Others suggest that xenin-25 does not directly act on beta cells, but instead stimulates acetylcholine release from non-ganglionic cholinergic neurons to activate beta-cell muscarinic receptors. In contrast, it has been reported that cholinergic pathways are not involved in the stimulatory effects of xenin-25 on pancreatic secretions. In addition, Taylor et al. have demonstrated that the insulinotropic actions of xenin-25 do not appear to be mediated through binding to neurotensin receptors in mice.

More recent research has highlighted the potential of xenin-25 as a direct independent insulinotropic agent, along with an ability to potentiate the insulin-releasing and glucose-lowering effects of the incretin hormone, glucose-dependent insulinotropic peptide (GIP). Furthermore, related studies have revealed that xenin-25 enhances pancreatic beta-cell proliferation in both rodent and human beta-cells (Figure 1). Since type 2 diabetes mellitus is characterised by both beta-cell loss and reduced bioactivity of GIP, this offers hope for xenin-25 as a potential new therapeutic option for people currently living with the condition. Although, it should be noted that there is a suggestion that xenin-25 may inhibit secretion of glucagon-like peptide-1 (GLP-1), which would be expected to be detrimental in terms of antidiabetic activity, although more research is required to confirm this finding.

3. **XENIN-25 AS AN ANTIDIABETIC AGENT**

Several studies reveal that under both normal and type 2 diabetic conditions, xenin-25 can induce insulin release and potentiate the biological actions of GIP. However, the
main barrier in terms of exploiting xenin-25 therapeutically relates to its short biological half-life, due to enzymatic degradation.\textsuperscript{32} Thus, as was the case for clinical exploitation of GLP-1, enzyme-resistant forms of xenin-25 have been generated and characterised, and shown to possess clear therapeutic promise (Table 1). As such, a fatty acid derivatised form of xenin-25, namely xenin-25[Lys\textsubscript{13}PAL], has recently been synthesised and fully characterised, with hypothesised antidiabetic effects (Table 1).\textsuperscript{36} A follow-up study employing chronic administration of xenin-25[Lys\textsubscript{13}PAL] to high fat fed diabetic mice confirmed this suggestion, as treated mice presented with augmented insulin secretion, improved glucose homeostasis, enhanced tissue insulin sensitivity and partial restoration of normal islet morphology.\textsuperscript{37} Moreover, knowledge that serine-like proteases are primarily responsible for xenin-25 degradation,\textsuperscript{32} led to the generation of a modified xenin-25 analogue where Lysine (Lys) and Arginine (Arg) amino acid residues were substituted for Glutamine (Gln), to yield xenin-25-Gln (Table 1).\textsuperscript{38} Xenin-25-Gln was enzyme resistant and retained biological activity, with chronic administration in high fat fed and \textit{ob/ob} mice resulting in enhanced metabolic control, increased GIP sensitivity and improved circulating lipid profile.\textsuperscript{38} Together these observations underline the potential antidiabetic potential offered by stable, potent and long-acting forms of xenin-25.

4. XENIN-25 FRAGMENT PEPTIDES

Although stable xenin-25 analogues have beneficial effects in preclinical models of diabetes-obesity, therapeutic attractiveness could be enhanced through use of truncated and bioactive peptide fragments. This would make peptide synthesis easier and less expensive, as well as facilitating possible non-injectable peptide drug delivery.\textsuperscript{43,44} In addition, recent research has highlighted the possibility of linking together different bioactive peptide fragment domains, to create multi-targeting hybrid peptides.\textsuperscript{45-47} In this regard, the C-terminal octapeptide of xenin-
25, namely xenin-8, has long been recognised as a naturally-occurring bioactive form of xenin-25. As such, xenin-8 has previously been demonstrated to possess clear effects on pancreatic endocrine cells, including potent release of insulin. A detailed study has since revealed the degradation profile of xenin-25 in mouse plasma, including evidence of the following C-terminally truncated metabolites; xenin 9-25, xenin 11-25, xenin 14-25 and xenin 18-25, where xenin 18-25 represents xenin-8 (Table 1). Of these xenin-25 metabolites, only xenin-8 possessed biological activity. Interestingly, xenin-8 has no effect on satiety, which may suggest a change in passage through the blood-brain barrier of this fragment xenin-25 peptide as compared to the parent molecule. More intriguingly, the C-terminal octapeptide of xenin-25-Gln, named xenin-8-Gln (Table 1), was demonstrated to retain all the gluco-regulatory and antidiabetic actions of the full-length stable analogue. This observation led to the recent generation of a novel GIP/xenin hybrid peptide, (D-Ala\(^2\))GIP/xenin-8-Gln, that combined an enzymatically stable form of the 14 amino acid bioactive N-terminal region of GIP, with xenin-8-Gln. (D-Ala\(^2\))GIP/xenin-8-Gln retained xenin and GIP like biological actions, and markedly improved glucose tolerance, insulin resistance, GIP bioactivity as well as enhancing pancreatic beta-cell area in high fat fed diabetic mice. Whilst further studies are necessary for complete functional characterisation of (D-Ala\(^2\))GIP/xenin-8-Gln, initial observations are favourable. Furthermore, xenin-6 (xenin 20-25) has also been characterised as a biologically active fragment of xenin-25, possessing notable insulinotropic actions (Table 1). Interestingly, introduction of a reduced pseudopeptide bond between the Lys\(^20\) and Arg\(^{21}\) amino acid residues in xenin-6, further enhanced bioactivity. Thus, analogues of xenin-6, or related hybrid peptides, may yet hold promise in the treatment of diabetes. In addition, given that related insulin-releasing peptides, such as xenopsin, neurotensin and caerulein, possess an N-terminal pyroglutamic acid (pGlu) residue that appears to prolong biological half-life, this
approach may also prove useful for enhancing bioactivity of xenin-25 and associated fragment peptides.

5. CONCLUSION

Despite the plethora of currently available drugs for people with type 2 diabetes, there is a real need to develop novel pharmacologic approaches to improve clinical outcomes. Xenin-25 based therapeutics could represent one such opportunity. Early work characterising the impact of xenin-25 on gut motility and energy turnover, coupled with more recent studies highlighting positive effects on metabolic control, demonstrate beneficial and potentially translatable effects. In addition, the use of shorter bioactive forms of xenin-25, to create unimolecular polypharmacy options for diabetes, appear to be positive and merit investigation. Taken together, further studies into the therapeutic potential of xenin-25, and related peptides, for diabetes are fully warranted.

Acknowledgments

The authors work on xenin peptides has been supported by the European Foundation for the Study of Diabetes (EFSD), Invest Northern Ireland, SAAD Trading and Contracting Company, Department of Education and Learning (DEL) Northern Ireland and University of Ulster strategic research funding.

Conflict of interest statement

VAG and NI are named on patents filed by the University of Ulster for exploitation of incretin-based drugs and other peptide therapeutics.
Ethical statement

There are no ethical issues related to this review article.

References


This article is protected by copyright. All rights reserved.


29. Kim ER, Xu Y, Mizuno TM. Impaired suppression of feeding by the gut hormone xenin in type I interleukin-1 receptor-deficient mice. *Behav Brain Res*. 2014;261:60-64.


Table 1: Amino acid sequences of xenin-25 as well as its related stable analogues and naturally occurring fragment peptide

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Amino acid sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xenopsin</td>
<td>pGLU-GLY-LYS-ARG-PRO-TRP-ILE-LEU-OH</td>
</tr>
<tr>
<td>Xenin 18–25 (Xenin-8)</td>
<td>H-HIS-PRO-LYS-ARG-PRO-TRP-ILE-LEU-OH</td>
</tr>
<tr>
<td>Xenin 18–25 Gln</td>
<td>H-HIS-PRO-GLN-GLN-PRO-TRP-ILE-LEU-OH</td>
</tr>
<tr>
<td>Xenin 20-25 (Xenin-6)</td>
<td>H-LYS-ARG-PRO-TRP-ILE-LEU-OH</td>
</tr>
</tbody>
</table>
Figure 1. Schematic showing the main biological actions of xenin-25. Effects of xenin-25 on adipose, brain, gallbladder, gut and pancreas are considered.