RD Lawrence Lecture 2017 Incretins: the intelligent hormones in diabetes

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Abstract

The incretin hormones glucose-dependent insulino tropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) have attracted considerable scientific and clinical interest due largely to their insulin-releasing and glucose-lowering properties. Indeed, GLP-1-based therapies are now key treatment options for many people with diabetes worldwide. In contrast, GIP-based agents have yet to reach the clinic based primarily on the impaired insulino tropic action of...
GIP observed in people with diabetes. Nevertheless, GIP is a key physiological regulator of insulin secretion and stable forms of GIP show much promise in rodent models to alleviate diabetes–obesity. Recent studies suggest that GIP may have an important role to play in a combination therapeutic approach or bioengineered with other gut peptides. Moreover, recent experimental studies indicate that incretins also exert pleiotropic effects in regions of the brain associated with learning and memory, thereby supporting preclinical data demonstrating that incretin-based drugs improve cognitive function. This review article, based on the RD Lawrence Lecture presented at Diabetes UK Annual Professional Conference (2017), provides a brief overview of incretins with a major focus on GIP, the development of designer GIP analogues, and how these molecules can improve cognition. Thus, incretins can be considered as ‘the intelligent hormones’ and may hold the key to successfully treating the alarming rise in neurodegenerative disorders.

Highlights

- GIP is a major physiological regulator of insulin secretion and β-cell function.
- Development of DPP-4-resistant GIP agonists and reversal of β-cell desensitization may reveal therapeutic potential for GIP.
- Bioengineered GIP, GLP-1 and glucagon dual- and triple-acting molecules provide enhanced metabolic outcomes.
- GIP and GLP-1 improve learning and memory processes in preclinical models such as Alzheimer’s disease.
- Further clinical studies are now required to determine potential of incretin hormones to treat neurodegenerative disorders.
Introduction

A series of classical experiments conducted during the early-to-mid 1960s revealed that important factors within the gut played a pivotal role in the postprandial release of insulin [1,2]. Indeed, these studies demonstrated the incretin effect, that is, the augmentation of insulin secretion observed when the same amount of glucose is administered orally vs intravenously. Soon afterwards, Unger and Eisentraut proposed the term entero-insular axis, which described all the gut factors that contributed to enhanced insulin secretion following ingestion of a meal [3]. In the late 1970s, Creutzfeldt suggested that the entero-insular axis encompassed nutrient, neural and hormonal signalling, and that two fundamental criteria were required for a hormone to be classified as an incretin [4]. First, an incretin must be released in response to nutrients, particularly carbohydrates, and second, an incretin must stimulate insulin release at physiological concentrations. Only two hormones currently fulfil both these criteria and are considered bona fide incretins: glucose-dependent insulintropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1). Even though both GIP and GLP-1 possess powerful insulin-releasing and glucose-lowering properties, only GLP-1 has been developed into a successful monotherapy for people with Type 2 diabetes. Several GLP-1 receptor agonists currently approved for use include: Exenatide (Byetta), Lixisenatide (Lyxumia), Liraglutide (Victoza), Exenatide QW (Bydureon), Dulaglutide (Trulicity) and Albilglutide (Eperzam). Further information on GLP-1 receptor agonists can be found elsewhere [5]. The remainder of this article focuses on the sister incretin GIP, which was presented as part of the RD Lawrence Lecture at Diabetes UK Annual Professional Conference in 2017.
**Glucose-dependent insulinotropic polypeptide**

GIP was first isolated in the late 1960s from a crude porcine cholecystokinin extract, where it was originally shown to inhibit histamine-induced gastric acid secretion from canine stomach [6]. In keeping with its biological property, the peptide was named gastric inhibitory polypeptide (GIP). However, as subsequent studies were conducted, it was revealed that physiological concentrations of GIP could stimulate glucose-induced insulin secretion from pancreatic β cells [7]. Hence, the hormone was renamed ‘glucose-dependent insulinotropic polypeptide’, retaining the original acronym GIP. Interestingly, the first published sequence of GIP was shown to comprise 43 amino acid residues, but subsequent studies established that the original 43 amino acid sequence contained an additional glutamine residue after position 29 (see Fig. 1 for GIP1–42 amino acid sequence) [8].

GIP is produced by enteroendocrine K cells and its secretion is regulated largely by carbohydrate and fat [9]. The peptide is derived from a GIP precursor comprising a putative signal peptide (21 amino acids), N-terminal peptide (30 amino acids), GIP coding region (42 amino acids) and C-terminal peptide (60 amino acids) [10]. Processing to the full-length (42 amino acids) GIP peptide in K cells occurs via prohormone convertase (PC) 1/3 (see Fig. 1). It is important to note that recent studies have suggested that the GIP precursor can also be cleaved by PC2 resulting in a C-terminally truncated form of GIP known as GIP(1–30) [11]. Although studies in our laboratory indicate that the truncated GIP(1–30) exhibits full bioactivity compared with native GIP in rodents, further studies are needed to clarify the precise physiological and/or pathophysiological role of GIP(1–30) in humans. GIP binds to the specific high-affinity GIP receptor, a G-protein-coupled receptor, which is located in a number of cells and tissues throughout the body. Within the β cell, the major signalling pathway whereby GIP stimulates glucose-induced insulin secretion is through stimulation of adenyl cyclase and resultant increase in cyclic adenosine monophosphate (cAMP) [12].

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However, GIP also influences a number of multiple up-stream events during the complex process of beta cell insulin secretion and can elicit more distal effects on insulin exocytosis. Although GIP has typically been regarded as an incretin hormone (and regulator of glucose-induced insulin secretion), GIP receptor mRNA has been detected in a number of extrapancreatic tissues. The GIP receptor is found in adipose tissue, small intestine, adrenal cortex, lung, pituitary, heart, testis, bone and brain [13]. In addition to its well-established insulin-releasing properties, GIP stimulates the growth, differentiation, proliferation and survival of pancreatic β cells [12]. As well as direct effects on β cells, GIP possesses other glucose-lowering extrapancreatic actions (e.g. inhibition of hepatic glucose production) [14]. With regards to adipocytes, GIP increases lipoprotein lipase activity, stimulates lipogenesis, enhances fatty acid and glucose uptake, and augments insulin-induced fatty acid incorporation [15]. In bone, GIP increases the proliferation of osteoblasts and inhibits osteoclast resorptive activity, thus enhancing bone strength and quality [14]. More recently, GIP has been shown to exert neuroprotective properties by protecting synapse function and numbers, stimulating neuronal proliferation, decreasing β-amyloid plaque formation in the cortex and reducing neuronal chronic inflammation response [16]. Therefore, GIP may exhibit therapeutic potential for various degenerative diseases, including diabetes, obesity, osteoporosis and neurodegeneration.

**Limitations of using GIP therapy**

Even though GIP may hold therapeutic promise, the drive to generate GIP-based agents has proved problematic due to two major limitations. First, the biological activity of GIP is relatively short-lived (~ 5 min) due primarily to proteolytic degradation by the ubiquitous enzyme dipeptidyl peptidase-4 (DPP-4) which rapidly inactivates GIP by removing the N-terminal dipeptide (Tyr1–Ala2) [15]. Furthermore, GIP (and its metabolites) are rapidly
eliminated from the body through renal clearance. The second, and arguably the most unattractive drawback, is the fact that the insulin-releasing properties of GIP are severely diminished in people with Type 2 diabetes [17]. However, it is important to note that the reduced incretin effect observed in Type 2 diabetes has been shown to extend beyond GIP and encompass several factors including GLP-1 [18]. The precise reasons underlying the diminished incretin effect in Type 2 diabetes is still unclear, but recent studies suggest an early β cell defect that is present prior to development of full-blown Type 2 diabetes [19]. Importantly, several rodent and human studies have indicated that reduction in hyperglycaemia using established glucose-lowering agents can restore GIP-mediated insulin secretion in Type 2 diabetes [20]. Thus, the insulinoceptive actions of GIP are not lost in Type 2 diabetes and can be revived, highlighting that GIP may still hold therapeutic potential.

**Designer GIP analogues**

Two main strategies to avoid the rapid inactivation of GIP (and GLP-1 as well) have been the development of DPP-4 inhibitors (which prevent GIP and GLP-1 degradation) and bioengineering of DPP-4 resistant analogues of GIP. As the action of DPP-4 is not confined to GIP alone, the most obvious pharmacological approach is to generate N-terminally modified GIP peptides (or analogues) that are resistant to DPP-4-mediated degradation. In fact, over many years, our laboratory has championed the development of designer GIP analogues, and has conducted extensive basic and preclinical studies that have identified a substantial series of structurally modified GIP peptides (Table 1) [21].

Our early studies focused on structurally modifying the N-terminus of GIP at the tyrosine in position 1 (Tyr1) through, for example, the addition of an acetyl group, or amino acid substitution of alanine at position 2 (Ala2). Here, we highlight that such chemically modified forms of GIP exhibited greatly or moderately increased DPP-4 stability and in vitro
biological actions in terms of both cAMP production and acute insulin secretion. Perhaps somewhat surprisingly to us at first, Tyr1-modified analogues of GIP appeared to be the most potent, which we attributed to superior resistance to DPP-4 degradation. By capping the N-terminus of GIP, the DPP-4 enzyme was not able to access the cleavage site (Ala2–Glu3 peptide bond). Acute in vivo studies using obese diabetic ob/ob mice revealed that, in general, Tyr1-modified GIP analogues were noticeably better at stimulating insulin release and lowering glucose concentrations compared with Ala2-substituted GIP analogues. Strategically, we utilized the Aston ob/ob mutant mouse as our preferred preclinical model as it represents an extensively studied form of spontaneous obesity and diabetes, exhibiting hyperphagia, marked obesity, moderate hyperglycaemia and severe hyperinsulinaemia [22]. Thus, positive data with Tyr1-modified analogues in ob/ob mice were particularly encouraging, given the severity of insulin resistance and the defective β cell function, including poor response to native GIP. Of particular note, the N-acetylated form of GIP, N-AcGIP, appeared to perform the best in the range of parameters examined (Table 1).

As noted earlier, GIP (and related analogues) undergo rapid renal clearance, and so we realized that preventing DPP-4 degradation alone would not be sufficient to generate efficacious longer-acting forms. One possible means by which to delay renal clearance is to encourage plasma protein binding through peptide acylation through the incorporation of a fatty acid moiety [23]. Thus, we designed and evaluated a series of second-generation GIP analogues (Table 2) [21]. All acylated GIP analogues contained a fatty acid moiety attached to the epsilon side-chain of lysine either at position 16 (Lys16) or 37 (Lys37), with or without additional N-terminal modification. Preliminary experiments clearly indicated that all acylated peptides, regardless of fatty acid moiety or site of attachment, exhibited enhanced metabolic stability and improved biological activity both in vitro and in vivo. More comprehensive studies using repeated daily injections of acylated GIP analogues in ob/ob
mice significantly decreased circulating glucose concentrations and glycated haemoglobin, and improved glucose tolerance, glucose-mediated insulin response and insulin sensitivity, clearly highlighting the ability of acylated GIP analogues to improve diabetes control. Of all the acylated GIP peptides tested, C-14 (myristic acid) acylated peptides appear to offer much more protracted and improved biological properties over their C-16 (stearic acid) and C-18 (palmitic acid) counterparts [24]. However, more detailed studies examining other fatty acid moieties, site of attachment and importance of additional linker molecules in GIP would be useful to ascertain the best lead candidate.

It is important to note that acylation is not the only method that could be utilized to slow renal clearance of peptides and/or proteins. Several other potential approaches involve attaching peptides to large molecules such as polymers or carbohydrates, or fusion to plasma proteins such as albumin or immunoglobulin (IgG) fragments. Some examples that relate specifically to the sister incretin GLP-1 include: conjugation of ATIII-binding pentasaccharides [25], albumin–exendin-4 conjugate (CJC-1134-PC) [5] and recombinant GLP-1 fused genetically to human albumin (albiglutide) [5]. However, another common approach is PEGylation, in which polyethylene glycol (PEG) polymer chains are attached to peptides/proteins. PEGylation has several potential advantages including decreased immunogenicity and limited penetration of the blood–brain barrier. In our laboratory, we developed a novel mini-PEGylated GIP compound whereby we conjugated a 145 Da PEG residue to the C-terminus of GIP. Mini-PEGylated GIP displayed enhanced metabolic stability and markedly improved hyperglycaemia, glucose tolerance and insulin secretion in mice fed a high-fat diet compared with the native hormone [26]. Other modified GIP analogues based on both GIP(1–42) and GIP(1–30) sequences have also been generated and tested in a range of preclinical models, such as [d-Ala2]GIP, and collectively these stable GIP analogues show promise for the treatment of diabetes (reviewed in Finan et al. [27]).

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Therapeutic role for GIP in human Type 2 diabetes

To date, there is a distinct lack of published human studies using GIP (or modified forms) in a therapeutic context. So, the question remains, where could GIP fit as an anti-diabetes agent, if at all? It seems unlikely that a GIP-based agent could ever be utilized as a stand-alone monotherapy. However, strategies that could re-sensitize the impaired insulin-releasing action of GIP, may well be the most likely scenario for a GIP-based therapy. One example of an approach to restore the defective GIP action in Type 2 diabetes has come from recent observations using xenin-based compounds. Xenin is a 25 amino acid peptide co-secreted with GIP from K cells that can significantly potentiate the insulin-releasing activity of GIP. However, like many regulatory gut peptides, xenin is also susceptible to degradation, making therapeutic use of the native form unlikely. Thus, several stable xenin and hybrid GIP–xenin analogues have been generated, and shown to exhibit improved biological properties over the native peptide. Importantly, stable xenin analogues augment the action of GIP in cellular and animal models [28,29]. However, teasing out the molecular mechanisms underlying xenin-based peptide action is not straightforward as there is no known ‘xenin’ receptor, so further detailed studies characterizing these beneficial actions are required.

Another major clue as to where a GIP therapy might fit has emerged from observations that several types of gastric bypass surgeries can cure people with Type 2 diabetes. Such beneficial actions to improve metabolic control in these individuals are independent of marked weight loss and although the precise mechanisms underlying Type 2 diabetes remission are unknown and still somewhat controversial, altered secretion of gut hormones (including GIP) play a major role [30]. Thus, there undoubtedly appears to be an intricate relationship between gut hormones and it is conceivable that pharmacologically manipulating more than one gut peptide-mediated signalling pathway will provide superior therapeutic efficacy. In recent years, there has been a major push towards so-called ‘polypharmacy’ and
the development of dual- and triple-acting peptide therapies for diabetes. Recent preclinical studies have shown that co-administration or combination therapy with analogues of GIP and other gut hormones and/or analogues (e.g. GLP-1) leads to improved metabolic outcomes [31,32]. However, the development of combination therapies would not be perceived as being straightforward, especially concerning optimization of the most effective dose and suitable pharmaceutical formulation. It is not surprising then that attempts to bioengineer GIP-based agonists into a single molecule comprising GLP-1 activity (and other peptides such as glucagon and oxyntomodulin) are well underway [33–37]. Furthermore, new data in people with Type 2 diabetes show that the GIP/GLP-1 receptor agonist, RG7697, improved glycaemic control and reduced body weight, and is generally safe and well tolerated [38]. We certainly await further emerging data in this exciting area of GIP pharmacology.

**Novel actions of incretin hormones in the brain**

It is well known that people with diabetes can suffer from a range of complications including retinopathy, cardiovascular disease, nephropathy and neuropathy. There is also growing evidence to suggest that diabetes (and obesity) can increase the risk of developing cognitive decline and dementia, including Alzheimer’s disease [39]. As one might expect, unravelling the precise underlying mechanisms connecting diabetes–obesity and impaired cognition are not easily achieved, however, there is evidence to indicate that insulin resistance, oxidative stress and inflammation are likely contributing factors [39]. For example, several reports have shown that insulin signalling is impaired in both animal models and people with Alzheimer’s disease [40]. Therefore, this raises the question as to what molecules could potentially re-sensitize the insulin-signalling pathway within key brain areas responsible for learning and memory. Although treatment with insulin itself might seem a possibility, and studies have shown that insulin exerts beneficial effects in improving several features of
neurodegeneration, systemic administration of insulin would increase the risk of hypoglycaemia [41]. However, incretin hormones could offer an alternative approach to enhance insulin-signalling without the risk of unwanted hypoglycaemic episodes.

So what evidence is there to suggest a potential role for incretin hormones to positively influence cognitive function in diabetes–obesity? Incretin receptors are located in various brain regions, including the hippocampus and cortex, which are important regions involved in learning and memory processes [16]. Importantly, incretin hormones are able to access the brain as they can cross the blood–brain barrier by passive diffusion [41]. Reports from both cellular and animal experiments have shown that incretin hormones exert a number of beneficial effects in the brain including protection of neurons from oxidative stress, enhancing neurogenesis and reducing apoptosis and chronic inflammation [42]. A number of in vivo studies using models of diabetes–obesity have been utilized to investigate actions of incretin hormones on cognition and behaviour. For instance, several studies conducted in our laboratory have employed the novel object recognition task, a well-established behavioural test that exploits the ability of a rodent to explore a novel object over a familiar object. Using this approach, we, and others, have shown that recognition memory is severely impaired in animal models of diabetes–obesity, including high-fat feeding [43,44] (Fig. 2). Moreover, incretin hormones significantly improve recognition memory and, importantly, these actions are not attributable to hypermoteric activity or anxiety, thus suggesting a direct effect [43,45,46] (Fig. 2). The argument for a direct effect of incretin hormone action in the brain, at least in part, is further strengthened by the fact that other insulin sensitizers such as metformin do not improve cognition even against a backdrop of improved insulin sensitivity [47]. Thus, it appears that part of the beneficial actions of incretin hormones on cognitive function stems from direct actions on incretin receptors in the brain itself. Furthermore, mice that lack GIP or GLP-1 receptors exhibit impaired recognition memory as they fail to
discriminate between novel and familiar objects compared with controls [42]. Even though the precise mechanisms underlying positive actions of incretin hormones (and mimetics) on cognitive function are not well understood, one major line of evidence points towards improved hippocampal synaptic plasticity [43].

Hippocampal synaptic plasticity is considered an important neurochemical basis for learning and memory formation and it involves long-lasting changes in the efficiency of processing information at synaptic sites. An important tool in measuring the strength of synaptic connections between hippocampal neurons is long-term potentiation. Studies have shown that deficits in long-term potentiation are closely correlated with impairments in hippocampal-dependent memory formation [48]. Indeed, long-term potentiation induction and maintenance in the hippocampus is severely compromised in animal models of diabetes–obesity (e.g. high-fat feeding, ob/ob, and streptozotocin-induced), indicative of impaired cognitive function [46]. Moreover, incretin hormones not only enhance hippocampal synaptic plasticity and memory formation, but they can also protect synapses from the detrimental effects of β-amyloid on hippocampal synaptic plasticity [49]. These observations are supported in incretin receptor knockout mice, as they display a significant reduction in hippocampal synaptic plasticity [16]. A large number of genes and proteins are involved in cognitive processes underlying memory and learning. Some of the key ones include: neurotrophic receptor tyrosine receptor kinase 2 (NTRK2), sirtuin 1 (SIRT1), synaptophysin (SYP); mammalian target of rapamycin (mTOR) and vascular endothelium growth factor (VEGF). Studies in our laboratory have shown that treatment of high-fat fed mice with incretin hormones leads to increased hippocampal expression of NTRK2, SIRT1, SYP, mTOR and VEGF [47]. Understandably, this is just a very small snapshot and more detailed genome-wide expression profiling and confirmatory protein blotting data could reveal further insights.
Immunohistochemistry has been used to assess the hippocampal expression of key proteins such as glial fibrillary acidic protein (GFAP), doublecortin (DCX) and 8-oxoguanine which act as markers for inflammation, neurogenesis and oxidative stress, respectively. Importantly, high-fat fed mice treated with incretin hormones exhibit markedly reduced oxidative stress and inflammation, and enhanced neurogenesis [47]. Indeed, even though we have considered the cognitive-enhancing benefits of incretin hormones in preclinical models of diabetes–obesity, it is important to highlight that such beneficial actions also extend to other models such as Alzheimer’s disease. One example of an Alzheimer’s disease mouse model is the APPswe/PS1 deltaE9 (APP/PS1) mutant, which develops β-amyloid plaques from around 5–6 months, and progressive age-related memory impairments from around 7 months. Key observations in APP/PS1 mice treated with incretin hormones include: protection of memory and synaptic plasticity, increased synapse numbers, reduced β-amyloid plaque load, reduction in total amyloid precursor protein (APP), aggregated β-amyloid and inflammation, and reduced expression of IRS-1 pSer616 [42]. Data are now emerging showing beneficial and additive effects of dual GLP-1/GIP agonists over mono-agonists in models of Alzheimer’s disease [50]. Collectively, all these data demonstrate that incretin hormones show promise for improving cognitive function, not only as an added benefit to people being treated for diabetes, but as a potential drug treatment for other forms of dementia such as Alzheimer’s disease. Indeed, several clinical trials are ongoing and we await the outcome of these studies with much interest [42].

**Conclusions**

Over the past number of years, the field of incretin biology has evolved. Once traditionally viewed to act largely in the regulation of postprandial insulin secretion, emerging data highlight that there is more to incretin hormones than meets the eye. GIP, often viewed as the
neglected twin of GLP-1, is now actively being pursued and developed as a novel diabetes therapeutic in the form of a bioengineered dual-acting molecule with GLP-1. Furthermore, both incretin hormones show promise in improving cognitive function with clinical trials now underway in people with Alzheimer’s disease using established GLP-1 receptor agonists. Incretin hormones really are the ‘intelligent hormones’, and as an enthusiast in the incretin field, I look forward with much excitement to learn of many more unexpected benefits.

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**Competing interests**

None declared.

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FIGURE 1 Processing of GIP precursor to full-length GIP(1–42) in intestinal K cells.

In intestinal K cells the enzyme PC1/3 cleaves the GIP precursor between positions 51 and 93 to generate full-length GIP(1–42).

FIGURE 2 Effects of Liraglutide on recognition index in mice with high-fat dietary-induced obesity and insulin resistance. Animals received twice-daily injections of Liraglutide (200 µg/kg bw; subcutaneously) or saline vehicle for 28 days. Normal control mice were included for comparative purposes. Following treatment, an object recognition test (5 min) was performed using two familiar objects during an acquisition phase (A) and following introduction of a novel object four hours later (B). The recognition index (RI) is displayed, which is percentage time spent exploring the novel vs. familiar object. (C) The left-hand side illustrates a photograph of the arena with a novel and familiar object and a high-fat-fed mouse treated with Liraglutide. The right-hand side shows a representative track which clearly displays two surrounding areas in the arena used by the software to analyse the object exploration events. Values are means ± SEM. *P < 0.05 compared with high-fat (HF) saline group. Reproduced with permission from Porter et al., Diabetes Obes Metab 2010; 12: 891–899 [43].
<table>
<thead>
<tr>
<th>Peptide</th>
<th>In vitro DPP-4 half-life (h)</th>
<th>Max in vitro insulin response (% GIP max)</th>
<th>In vivo glucose AUC (% GIP max response)</th>
<th>In vivo insulin AUC (% GIP max response)</th>
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Data reproduced from Gault et al. [15]

Ac, acetyl; AUC, area under the curve; Fmoc, 9-fluorenlymethoxycarbonyl; GIP, glucose-dependent insulinotropic polypeptide; Pal, palmitic acid; pGlu, pyroglutamyl.
Table 2 Series of fatty acid derivatized glucose-dependent insulinotropic polypeptide analogues tested *in vitro* and *in vivo*

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Data reproduced from Kerr et al. [24]

Ac, acetyl; AUC, area under the curve; GIP, glucose-dependent insulinotropic polypeptide; MYR, myristic acid; PAL, palmitic acid; STE, stearic acid.
Figure 1

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Figure 2

A
Aquilation phase

B
Trial phase

C

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