# Dual-action peptides: a new strategy in the treatment of diabetes-associated neuropathy

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**Peripheral neuropathy is one of the most common and debilitating complications of type 1 and type 2 diabetes mellitus. Recent studies have shown that several small, non-neural peptides possess neurotrophic activity and exert beneficial effects on nervous system function in experimental and clinical diabetes. Two of these, C-peptide and islet neogenesis-associated protein peptide, are derived from pancreatic proteins and use related signal transduction mechanisms. Derivatives of erythropoietin possess similar properties in the nervous system. As a group, these peptides are of increasing interest as leads to potential new approaches in the treatment of diabetes-associated neuropathies and other neurodegenerative conditions. This review addresses the recent advances made with these peptides in the context of diabetic neuropathy, and highlights similarities and differences in their mechanisms of action from the perspective of combination therapy.**

The diabetes epidemic, along with the secondary complications of the disease, will be among the most pressing challenges facing health care systems globally over the next decade. The most common complication associated with diabetes is diabetic polyneuropathy (DPN), a disorder affecting the peripheral and autonomic nervous systems in 50–90% of individuals with type 1 or type 2 diabetes. The American Medical Association has recently reiterated the importance of early diagnosis in the management of DPN [1]. However, early recognition is difficult. Although signs of DPN (e.g. nerve conduction abnormalities) can be detected clinically as early as a few years after the initial diagnosis of diabetes [2], DPN is asymptomatic in approximately half of all cases. Typically, symptoms are reported by patients many years into the progression of the disease. As a consequence, DPN is underdiagnosed [3] and treatment strategies based solely on the rigorous control of blood glucose levels [4,5] can be unsuccessful because of patient noncompliance, the extent of nerve damage at the time of diagnosis or factors other than hyperglycemia that can contribute to the development of DPN (see Box 1). A new strategy is necessary that directly addresses the underlying nerve damage in established DPN. The recent discovery of a group of 'dual action' peptides – non-neural peptides with neurotrophic properties in addition to their non-neural functions – is a step in this direction. Two of these peptides [C-peptide and the islet neogenesis-associated protein (INGAP) peptide] are derived from the pancreas, and were of initial interest because of their effects on glucose transport (C-peptide) and homeostasis (INGAP). These effects might have a role in the recently discovered therapeutic benefits of these peptides on DPN in experimental and clinical diabetes [6–8]. By contrast, the beneficial effects of erythropoietin (EPO) and its derivatives on DPN are not related to control of blood glucose levels. The potential of these peptides in the treatment of DPN is likely to prove especially effective when applied in combination with current antidiabetic therapies or in optimized delivery systems.

## Limitations of symptomatic therapies for DPN

The most prevalent form of DPN is a chronic sensorimotor neuropathy of the peripheral nerves, with the most common symptoms being a spontaneous burning or deep aching pain of the lower limbs as well as defects in the perception of thermal stimuli [9]. Consequently, a major focus of pharmaceutical research continues to be the development of therapeutics that manage the painful symptoms of DPN. The tricyclic antidepressants amitriptyline and imipramine are the most commonly prescribed symptomatic treatments

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for DPN [10]. Their inhibitory effects on monoamine reuptake are thought to also inhibit nociceptive pathways, but their use is limited by their unfavorable side-effects and high overdose profiles. Next generation antidepressants that address these problems, such as mixed inhibitors of serotonin and norepinephrine (SNRIs), have shown benefit in managing pain in DPN. An extended release formulation of venlafaxine (Effexor XR®, Wyeth), in which the drug diffusion rate is controlled by the capsule coating to prolong the release and thus the effect of the drug, appeared effective in combating pain in Phase II clinical trials for DPN in type 1 and type 2 diabetes [11]. Another agent, duloxetine (Cymbalta®, Lilly), received FDA approval in September 2004 for the alleviation of pain in DPN [12]. The anticonvulsant gabapentin (Neurontin®, Pfizer) is also frequently prescribed for painful DPN, and its successor pregabalin (Lyrica®, Pfizer), which exhibits more favorable side-effect profiles, was approved by the FDA in December of 2004 for the treatment of neuropathic pain in DPN. The beneficial effects of anticonvulsants against pain in DPN are thought to involve the modulation of sodium channels, which are altered in the peripheral nerves in DPN. However, a major limitation of all these symptomatic treatments for DPN is that they have little – if any – impact on the actual progression of the disorder. As DPN progresses, the risk of initially unperceived foot ulcers or injuries increases in the affected individuals, leading to 'diabetic foot' and amputations in the most severe cases [1]. The importance of developing strategies to directly correct the nerve damage caused by diabetes is therefore obvious.

## Failure of neurotrophin peptide-based therapies for DPN

Based on the hypothesis that deficient neurotrophic support of peripheral sensory neurons contributes to the pathogenesis of DPN [13], early attempts to correct the nerve damage in diabetes involved the administration of members of the neurotrophin family. Nerve growth factor (NGF) was the first to be tested and showed efficacy in several Phase II clinical trials for DPN, albeit with dose-limiting hyperalgesic side effects [14]. However, pivotal Phase III studies failed to reproduce the effects observed in earlier studies, possibly due to changes in the formulation (slightly different concentration of acetate and sodium chloride) and concentration (2.0 mg/ml in Phase II compared with 0.1 mg/ml in Phase III) of the recombinant human NGF, and thus inadequate levels of delivery [14,15]. In later studies, brain-derived neurotrophic factor (BDNF), which also showed early promise as a treatment for DPN, failed to demonstrate efficacy in clinical trials [16]. As shown for NGF and BDNF, neurotrophin-3 (NT-3) has shown encouraging results in treating neuropathy associated with experimental diabetes [17–19], but clinical trials have not been initiated. The failures of the neurotrophin trials highlight the limitations of monotherapies in treating a disorder as complex as DPN, and point to the fact that combination therapies might be more appropriate for this disorder [20]. This view is in accord with the current consensus that, in addition to decreased neurotrophic support for sensory neurons, multiple other factors contribute to the pathogenesis of DPN, including oxidative stress, microvasculature damage, metabolic abnormalities and the formation of advanced glycation end products (AGEs) [21] (Figure 1). The most promising strategies for the successful treatment of DPN are likely to be those that aim to correct at least the most influential of these defects, which can vary between individuals due to the heterogeneity of DPN. In this regard, the dual-action peptides (peptides that affect more than one target, probably by different mechanisms) have drawn attention because of the wide-ranging nature of their actions.

**BOX 1**

### Diabetic polyneuropathy

Diabetic polyneuropathy (DPN) is a family of sensory and autonomic nerve disorders that is one of the most common secondary complications of type 1 and type 2 diabetes. The most common form of DPN is a distal symmetric polyneuropathy of the sensory nerves, which affects 50–90% of individuals with long-term diabetes. A principal feature of DPN is a 'dying-back' phenomenon that involves progressive degeneration of the peripheral sensory nerves. Common symptoms of DPN include spontaneous burning pain of the lower limbs and allodynia (pain provoked by normally non-painful stimuli). Diagnosis of DPN requires the the presence of two or more clinical signs or symptoms, and underdiagnosis is frequent because of the asymptomatic nature of DPN in approximately half of all cases. The loss of insulin (a neuronal trophic factor) in type 1 diabetes and the high blood glucose levels (which can cause nerve damage) in both type 1 and type 2 diabetes are believed to contribute to the development of DPN. The exact pathogenesis is not fully understood, but is generally thought to be complex and involve several interrelated factors including oxidative stress, microvasculature damage, decreased neurotrophic support, advanced glycation end-product formation, and metabolic abnormalities.

## New peptide-based therapies for DPN: the dual-action peptides

### *C-peptide*

C-peptide is the 31-amino-acid peptide that connects the A and B chains of proinsulin – the insulin precursor molecule. Cleavage of C-peptide from proinsulin leads to exposure of the C terminus of the insulin β chain, and to subsequent conformational changes required for the binding of insulin to its receptor. Although C-peptide is secreted in a 1:1 ratio with insulin, it has a longer plasma half-life (20–30 min compared with 3–5 min for insulin) [22] and has been used as a marker of residual β-cell function in type 1 diabetes. Besides its role in the proper folding of insulin, C-peptide exerts no direct effects on blood glucose levels, and was originally thought to be biologically inert. A lack of specific biological action was also suggested by the proportion of amino acid residues that varied (and thus did not likely contribute to a specific action) between human and mouse C-peptide (35% amino acid residue variability between the human and mouse peptide sequence) versus that of other comparable, biologically active peptides involved in pancreatic function, such as insulin (8% variability), glucagon (0% variability), or IGF-1 (6% variability) [23]. However, it is now recognized that C-peptide exerts beneficial effects on neural (corrects sensory dysfunction), vascular (improves microvascular blood flow) and renal (reduces glomerular hyperfiltration) tissues in diabetes, and these effects are attributed to the Glu27 residue and possibly the Glu3 and Glu11 residues [24], which are conserved across species.

The first demonstration of the biological activity of C-peptide was the stimulation of glucose transport in human skeletal muscle [25]. The non-additive effects of the combination of C-peptide and insulin observed in this study suggested that C-peptide

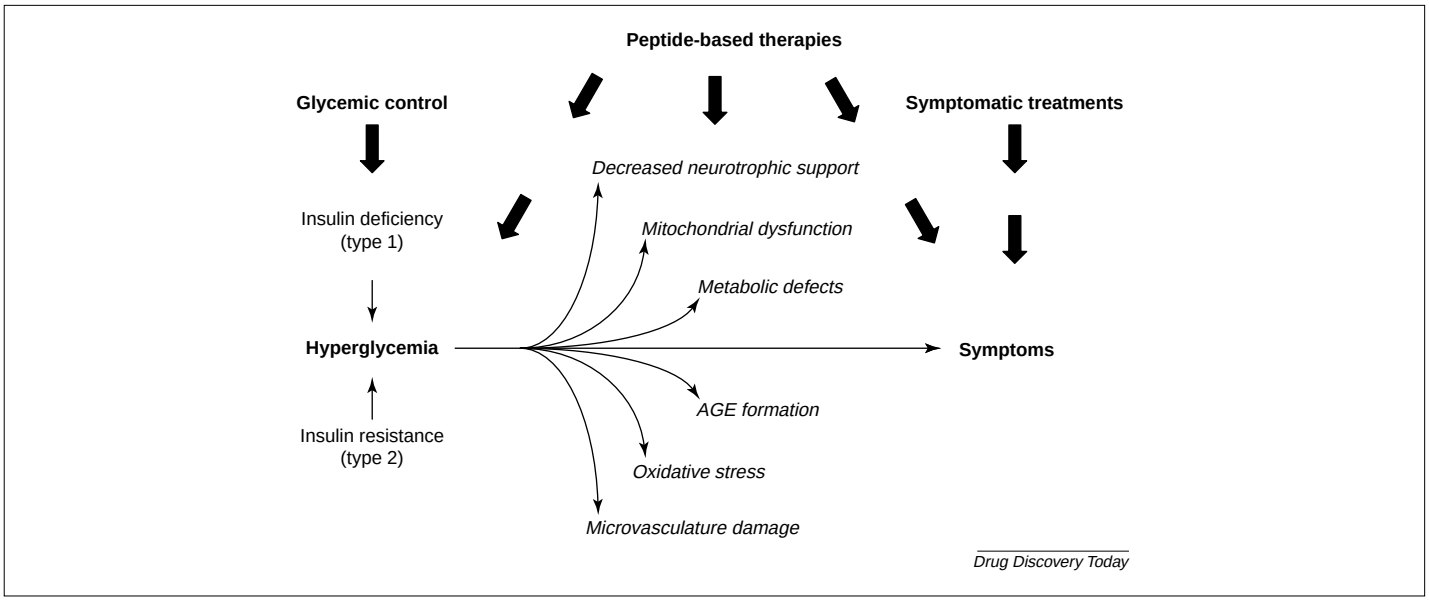


**FIGURE 1**

**Major strategies in the treatment of diabetic peripheral neuropathy**. Defects in insulin and blood glucose levels are the primary insults in the pathogenesis of diabetic peripheral neuropathy (DPN). Consequently, multiple damaging phenomena arise in the peripheral nervous system, including decreased neurotrophic support from Schwann cells, mitochondrial dysfunction, metabolic defects, formation of advanced glycation end-products (AGEs), oxidative stress, and damage to the nerve microvasculature. As the damage from these phenomena typically occurs well before symptoms are noticeable, strategies based solely on glycemic control are insufficient to treat DPN. In addition, symptomatic strategies are effective in alleviating pain, but have little if any effect on the steady progression of the disorder. Thus, new strategies such as peptide-based therapies that directly address the underlying nerve damage in DPN are among the most promising candidate molecules for the treatment for DPN. Among the dual-action peptides, INGAP peptide also exerts effects on blood glucose levels and, as a group, these peptides have much potential to correct nerve damage and improve the symptoms of DPN.

uses insulin-mediated systems. C-peptide was subsequently shown to mimic some of the effects of insulin on glycogen synthesis and amino-acid uptake [26], and to exert insulin-like effects in various neural cell types (Box 1). It affords neuroprotection against apoptosis that occurs in hippocampal neurons in BioBreeding/Worcester (BB/W) type 1 diabetic rats [27] and stimulates cell proliferation and neurite outgrowth in human neuroblastoma cells [28]. Furthermore, C-peptide appears to have several targets that lead to its effects in DPN. For example, C-peptide stimulates $Na^+$ and $K^+$ ATPase activity in peripheral nerves in diabetic animals [29], which points to the potential ability of C-peptide to ameliorate the abnormalities in ion fluxes across cell membranes that are characteristic of DPN. C-peptide also promotes vasodilation in endothelial cells through stimulation of nitric oxide synthase (NOS) activity [30,31], suggesting that C-peptide provide not only neurotrophic support but also improve microvasculature defects in DPN. There is also evidence from preclinical studies that C-peptide administration improves peripheral nerve conduction velocity and reduces axonal degeneration in BB/W type 1 diabetic rats [6]. In a Phase II clinical trial involving 49 subjects with type 1 diabetes of 5–15 years' duration and clinically identified but asymptomatic DPN, C-peptide was administered subcutaneously for three months. This regimen resulted in a significant correction of the initial deficit in sensory nerve conduction velocity in the diabetic group compared with placebo, although the conduction velocity in the diabetic group was still within the normal range before the start of treatment. [7]. Further investigations are warranted to evaluate the benefits of C-peptide for use in treating DPN, especially because it is not clear whether conduction velocity changes actually contribute at all to the symptoms experienced by diabetic patients.

### *Mechanism of action and methods of stabilization*

The failure of radioligand binding assays to detect a receptor for C-peptide suggested that C-peptide clusters on cell membranes to form a pore or ion channel in a manner similar to that of antibiotic peptides [32]. However, the central glycine-rich segment of C-peptide confers a considerable structural flexibility [33], and subsequent work has demonstrated that each molecule of C-peptide assumes a random coil structure in solution [34]. These studies also showed that C-peptide fails to associate stably with lipid membranes or vesicles, suggesting that a non-receptor-mediated membrane interaction is not involved. Recently, fluorescence correlation spectroscopy demonstrated specific ligand–receptor interactions for C-peptide where the traditional radioligand binding assays failed [35]. Several lines of evidence suggest that the C-peptide receptor is a cell surface G-protein-coupled receptor (GPCR) that is positively coupled to $Ca^{2+}$ signaling [26,36]. The neuroprotective effects of C-peptide have been linked to mitogen-activated protein kinase (MAPK) pathways (Figure 2), specifically an upregulation of p38 and a downregulation of c-Jun N-terminal kinase (JNK), leading to increased transcription of the pro-survival molecules Bcl-2 and nuclear factor κ enhancer binding protein (NF-κB) [28,37]. These signaling events occur only when C-peptide and insulin are applied in combination, as does an enhancement of phosphorylation of the insulin receptor (IR) but not the insulin-like growth factor 1 (IGF-1) receptor. Taken together, these findings suggest that C-peptide exerts its beneficial effects in DPN by augmentation of insulin signaling in a manner analogous to, but distinct from, that of IGF-1. Co-administration of C-peptide and insulin might provide additional benefits in DPN compared with insulin alone in type 1 diabetic individuals.

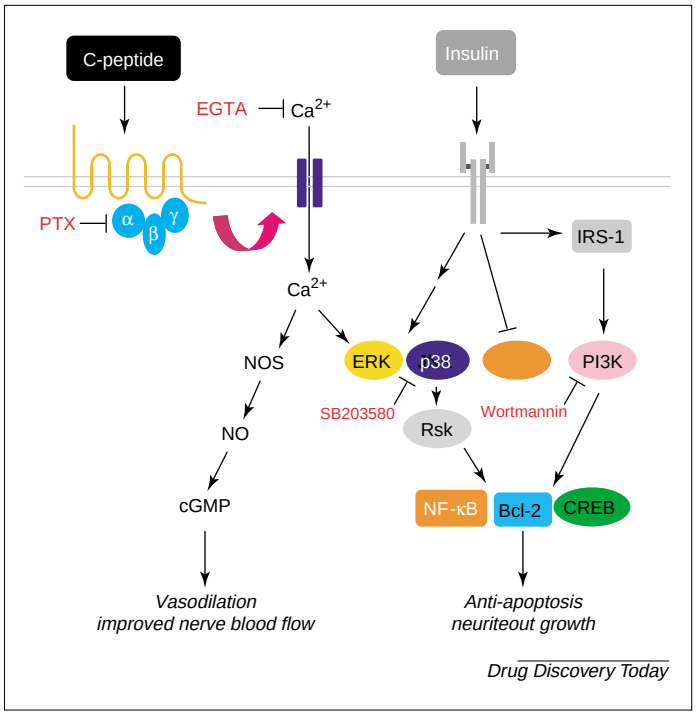


**FIGURE 2**

**Proposed signaling mechanism for C-peptide in neuronal and endothelial cells.** C-peptide signals are transduced via an unidentified pertussis-toxin-sensitive G-protein-coupled receptor (GPCR) that couples positively to $Ca^{2+}$ signaling [36]. The C-peptide-mediated influx of extracellular $Ca^{2+}$ leads to increased NOS activity, NO and cGMP production, vasodilation, and blood flow [23]. C-peptide also exerts neuroprotective and neurotrophic effects. In neuronal cells, C-peptide in combination with insulin significantly enhances insulin receptor (IR) phosphorylation, phosphatidylinositol-3-kinase (PI3K) activity, and nuclear factor κ enhancer binding protein (NF-κB) and Bcl-2 expression [77]. Upregulation of p38 activity and downregulation of c-Jun N-terminal kinase (JNK) activity are also involved in the anti-apoptotic effects, whereas enhancement of cAMP response-element-binding (CREB) activity might underlie the effects of C-peptide on neurite outgrowth. In endothelial cells, C-peptide enhances phosphorylation of extracellular-regulated kinase (ERK) and p38/Rsk, but does not appear to modulate JNK activity [78] The combined vascular and neurotrophic effects of C-peptide are thought to contribute to its observed beneficial effects in DPN. Adapted, with permission, from [23] and [77].

Abbreviations: EGTA, ethylene glycol bis(2-aminoethyl ether)-N,N,N′N′-tetraacetic acid; IRS-1, insulin receptor substrate-1; PTX, pertussis toxin; Rsk, p90 ribosomal S6 kinase.

The short plasma half life of C-peptide (20–30 min) suggests that it is sensitive to physiological degradation, similar to other candidate therapeutic peptides for diabetes. Strategies taken to improve the bioavailability of these small peptides could be suitable for C-peptide (Table 1). For example, the plasma half life of glucagon-like peptide 1 (GLP-1), a 31-amino-acid gastrointestinal peptide with neurotrophic effects, can be extended considerably by attachment of a polyethylene glycol (PEG) moiety (PEGylation) [38], N-acetylation [39] or conjugation of GLP-1 to serum proteins such as albumin (CJC-1131, ConjuChem) [40]. However, bioequivalence and biosafety remain principal concerns in such strategies, a fact underscored by the recent announcement that the Phase II clinical trial of DAC™–GLP-1 for diabetes has been put on hold due to insufficient safety margins as revealed by the three-month toxicity studies (www.goodmedia.com/equicom/conjuchem/web/presspop.cfm?newsID=4347).

**TABLE 1**

**Potential strategies for increasing the stability of peptide-based therapeutics for diabetic polyneuropathy[a]**

| | | |
|---|---|---|
| Amino acid modification | GLP-1[b]; INGAP peptide[c] | [55,67,68] |
| Endogenous carrier conjugation | GLP-1[d] | [40,69] |
| Liposome incorporation | EPO | [70] |
| Mimetic generation | Somatostatin; GLP-1 | [71–73] |
| N-acetylation | GLP-1 | [39] |
| PEGylation | Exendin-4; GLP-1 | [38,74,75] |
| Peptidase inhibition | NAAG[e]; GLP-1[f] | [72,76] |

[a]Abbreviations: EPO, erythropoietin; GLP-1, glucagon-like peptide 1; INGAP, islet neogenesis-associated protein; NAAG, *N*-acetyl-aspartyl-glutamate.
[b]D-amino acid substitution led to decreased GLP-1 receptor binding and loss of insulinotropic effects.
[c]Covalent attachment of poly-L-lysine tail allows for formation of stable complexes with charged polymers while retaining neurotrophic effects.
[d]Conjugation to albumin.
[e]NAAG stability was increased by carboxypeptidase II inhibition.
[f]GLP-1 stability was increased by dipeptidyl peptidase IV inhibition.

### *Islet neogenesis-associated protein peptide*

INGAP peptide is a synthetic pentadecapeptide that comprises the biologically active portion (amino acids 104–118) of INGAP. The native protein was originally identified as a regeneration factor expressed in the acinar tissue of partially obstructed rodent pancreata, and thought to be induced in response to diabetes [41]. Subsequent work demonstrated the expression of INGAP in the pancreata of normal hamsters, and its possible protective role against diabetes was proposed [42]. Preclinical studies have shown that INGAP, as well as INGAP peptide, are capable of inducing the formation of new islets of Langerhans in the pancreas and that chronic administration of INGAP peptide can reverse hyperglycemia in streptozotocin (STZ)-induced type 1 diabetic mice [43]. In addition, in an islet transdifferentiation paradigm, INGAP peptide induced the conversion of duct-like epithelial structures to islet-like structures [44]. More recent work suggests that INGAP peptide also stimulates insulin secretion *in vitro* [45]. The strategy of INGAP peptide administration is markedly distinct from that of other pharmacological approaches to diabetes in that it aims to create new functional islets of Langerhans [46,47] rather than to stimulate or protect the existing islet mass [48], as do GLP-1, antioxidants and other current type 2 antidiabetic agents. Phase II clinical trials of INGAP peptide for type 1 and type 2 diabetes have recently been completed, the results of which are still forthcoming.

INGAP peptide appears to have multiple targets and exerts multiple effects in the peripheral nervous system. It enhances the regrowth of neurites in axotomized dorsal root ganglia (DRG), which contain the cell bodies of sensory axons [49]. Administration of INGAP peptide to STZ-induced type 1 diabetic mice corrects sensory dysfunction and upregulates the expression of proteins involved in nerve regeneration, such as tubulin and actin, in an insulin-independent manner [8]. These proteins might conceivably contribute supportive trophic functions that would help axons withstand the pathological events underlying DPN. In addition, evidence from *in vitro* and *in vivo* studies suggest the beneficial effects of INGAP peptide in the DRG involve enhancement of mitochondrial function [8,49]. The combined neurotrophic effects of INGAP peptide, in addition to its euglycemic effects in chronic

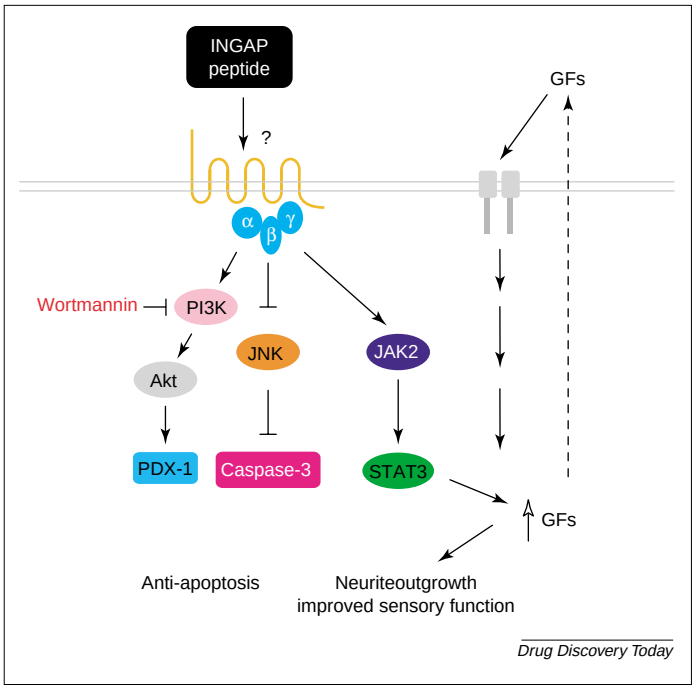


**FIGURE 3**

**Proposed signaling mechanism for INGAP peptide in neuronal and pancreatic cells.** As with C-peptide, islet neogenesis-associated protein (INGAP) peptide is thought to signal through an as yet unidentified G-protein-coupled receptor (GPCR). INGAP peptide activates phosphatidylinositol-3-kinase (PI3K)/Akt and pancreatic duodenal homeobox 1 (PDX-1) in the process of conversion of duct-like epithelial structures into islet-like structures in culture [79]. These changes are accompanied by downregulation of c-Jun N-terminal kinase (JNK) phosphorylation and caspase-3 activity. In sensory ganglia, INGAP peptide stimulates the Janus kinase 2–signal transducer and activator of transcription 3 (JAK2–STAT3) axis when administered to streptozotocin-induced diabetic mice [8]. An indirect effect on neurons via enhancement of Schwann cell-derived growth factors has been proposed [49]. Abbreviation: GFs, growth factors.

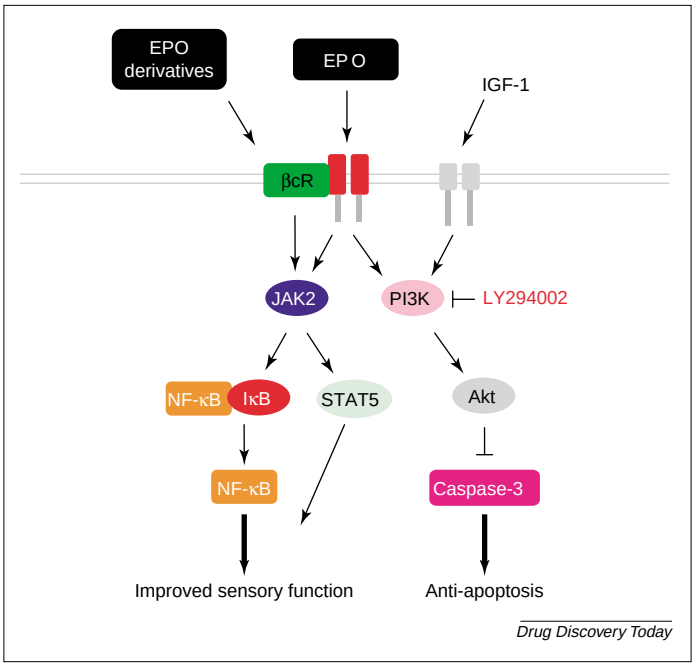


**FIGURE 4**

**Proposed signaling mechanism for erythropoietin and its derivatives.** The classical effects of erythropoietin (EPO) are transduced through the EPO receptor, a cytokine receptor expressed on both red blood cells as well as neurons and Schwann cells [57], leading to activation of the Janus kinase 2–signal transducer and activator of transcription 5 (JAK2–STAT5) axis. EPO also exerts neuroprotective effects, which involve synergism with insulin-like growth factor 1 (IGF-1) to activate phosphatidylinositol-3-kinase (PI3K)/Akt and decrease caspase-3 activity in cortical neurons [80]. In addition, JAK2-mediated phosphorylation of IκB allows nuclear factor κ enhancer binding protein (NF-κB) to translocate to the nucleus and transcribe genes involved in the neuroprotective effects. EPO and its non-erythropoietic derivatives of EPO have also been shown to partially improve DPN in rodents [62] [65], but the derivatives do not bind to the EPO receptor. Rather, EPO derivatives mediate their protective effects by signaling through the common β receptor, which associates with the EPO receptor in a heteroreceptor complex [81]. Abbreviation: βcR, common β receptor.

treatment paradigms, could make it particularly effective in treating DPN in type 1 and type 2 diabetes.

### *Mechanism of action and methods of stabilization*

A cellular receptor for INGAP peptide has not yet been identified, although some insight is provided by the close homology that INGAP shares with the Reg proteins, the gene products of the 'regenerating genes' (Regs) that are involved in various pancreatic, neural, and gastrointestinal tissue functions [50]. The receptor for Regs in islets is a cell-surface receptor with a single transmembrane region, a short intracellular N-terminal region and a relatively long C-terminal region [51]. Whether or not the putative receptor for INGAP in nervous tissue or islets is similar to the Reg receptor remains to be clarified. It has been proposed that INGAP peptide can affect sensory neurons indirectly in the DRG by stimulating Schwann cell (SC)-derived trophic factors like NGF [49], transforming growth factor-β (TGF-β) and – possibly – IGF-1 (Figure 3). Downstream signaling events include enhancement of adenylate cyclase activity and cyclic AMP (cAMP)-dependent activation of protein kinase A (PKA) [52], although non-PKA-dependent pathways also appear to be involved in the neuritogenic effects of INGAP peptide (unpublished observations). When administered to diabetic mice, INGAP peptide stimulates the Janus kinase 2–signal transducer and activator of transcription 3 (JAK2–STAT3) axis and upregulates tubulin and actin expression in the DRG, with no observed effects on the phosphorylation of extracellular-regulated kinase (ERK) or phosphatidylinositol-3-kinase (PI3K)–Akt. These findings are in contrast to reports of a PI3K–pancreatic duodenal homeobox 1 (PDX-1)-dependent mechanism of action of INGAP peptide in pancreatic tissue, but are in agreement with results indicating that axonal regeneration is predominantly mediated by the JAK2–STAT3 signaling in adult sensory neurons [53]. Further understanding of INGAP peptide-mediated signal transduction in the sensory nervous system will be important in developing effective combination therapies for DPN. In this regard, recent work reporting that combining antioxidants with AGE inhibitors leads to increased expression of INGAP in diabetic hamsters is of particular interest [54].

One of the strategies that we have taken to enhance the stability and thus bioavailability of INGAP peptide is the covalent attachment of a poly-5-lysine tail to the C-terminus of the peptide. This modification allows for the formation of INGAP peptide complexes with micelle-forming charged polymers [55] – such complexes should be more resistant to cellular degradation processes. Early tests are showing that the C-terminal modification does not

confer toxicity, and that the biological effects of INGAP peptide on neurite outgrowth in the DRG are retained.

### *Erythropoietin derivatives*

The discovery of the neuroprotective properties of erythropoietin (EPO), a 34 kD protein associated with hematopoiesis and with no known effects on glycemia, has been one of the more surprising findings in the DPN arena in recent years. In addition to its location on erythrocytes, EPO receptor (EPOR) expression is found in central neurons, astrocytes and glia [56], as well as in sensory neurons and SCs within the DRG [57], suggesting that EPO has various cellular targets. In central neurons, EPO affords protection against focal ischemia [58]. These effects of EPO are mediated by JAK2–STAT5 activity, as well as by crosstalk between the JAK2–STAT5 and JAK2–NF-κB axes (Figure 4). It has recently been shown that JAK2 activity also leads to activation of ERK and PI3K/Akt in cortical and striatal neurons subjected to ischemia [59]. However, clinical DPN is a dying-back neuropathy characterized by an axonal degeneration that precedes the loss of neuronal cell bodies, and cell death prevention strategies relevant to neuroprotection in the central nervous system might not be directly applicable to DPN and other peripheral axonopathies [60]. However, in the case of EPO, distinct central neuroprotective mechanisms and peripheral axonoprotective mechanisms have been described. Following axonal injury, nitric oxide is released from sensory neurons, which triggers the release of EPO from SCs [61]. The action of SC-derived EPO on neuronal EPORs initiates gene transcription programs that hinder axonal degeneration. This suggests that EPO might be beneficial in DPN, and recent work has indeed demonstrated that EPO can prevent and partially correct DPN in STZ-induced diabetic rats [62]. The mechanisms involved here probably include a JAK2-mediated pathway, because JAK2 activation is observed in neurons and SCs of the DRG after EPO administration in rodent models of nerve crush [63]. One possibility is that EPO-induced activation of JAK2 is coupled to STAT3, as occurs in INGAP peptide-treated diabetic mice [8].

One of the limitations of the proposed usefulness of EPO in treating DPN is its potential for inducing excess red blood cell production [64], a phenomenon observed in diabetic rats receiving long-term EPO treatment [62]. Strategies taken to address this concern include the development of carbamylated derivatives of EPO that lack erythropoietic properties but retain their axonoprotective effects in DPN as well as in other neuropathies [65]. Another potential concern common to all protein-based therapeutics is the development of autoantibodies to exogenously administered EPO or EPO derivatives [66]. Thus, future directions for EPO research in DPN could include the development of small peptides based on the biologically active portion of EPO, as well as the development of strategies to protect such peptides against cellular degradation.

## Conclusion

Although early detection is advocated as being essential to the effective management and treatment of DPN, this is difficult because of the often asymptomatic nature of the disorder in its early stages. Furthermore, this problem is compounded by frequent clinical underdiagnosis and the reporting of symptoms typically only many years into the progression of the disease. By that time, multiple damaging phenomena (neurotrophic deficiency, oxidative stress and metabolic abnormalities) that result from the initial defects in insulin and blood glucose levels make correction of hyperglycemia alone inadequate to treat DPN. New strategies are necessary that directly address the underlying nerve damage in established DPN, and in this regard the dual-action peptides, C-peptide and INGAP peptide, and EPO derivatives, which exert multiple corrective effects in DPN, are among the most promising candidate molecules for the first effective treatment for DPN.



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