

REVIEW

Cellular and physiological effects of C-peptide

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ABSTRACT

In recent years, accumulating evidence indicates a biological function for proinsulin C-peptide. These results challenge the traditional view that C-peptide is essentially inert and only useful as a surrogate marker of insulin release. Accordingly, it is now clear that C-peptide binds with high affinity to cell membranes, probably to a pertussis-toxin-sensitive G-protein-coupled receptor. Subsequently, multiple signalling pathways are potently and dose-dependently activated in multiple cell types by C-peptide with the resulting activation of gene transcription and altered cell phenotype. In diabetic animals and Type 1 diabetic patients, short-term studies indicate that C-peptide also enhances glucose disposal and metabolic control. Furthermore, results derived from animal models and clinical studies in Type 1 diabetic patients suggest a salutary effect of C-peptide in the prevention and amelioration of diabetic nephropathy and neuropathy. Therefore a picture of Type 1 diabetes as a dual-hormone-deficiency disease is developing, suggesting that the replacement of C-peptide alongside insulin should be considered in its management.

INTRODUCTION

Diabetes is a disease of increasing worldwide public health importance. It is associated with the unique micro-angiopathic complications of nephropathy, neuropathy and retinopathy and, in addition, individuals with diabetes suffer accelerated atherosclerosis with greatly increased risks of myocardial infarction, stroke and limb loss due to peripheral vascular disease [1,2]. Although rigorous glycaemic control may reduce the burden of diabetes-associated morbidity, DCCT (Diabetes Control and Complications Trial) [3] showed that, even with intensive insulin treatment to achieve blood glucose concentrations close to normal, a substantial proportion of patients still develop complications. These clinical observations suggest that factors other than hyperglycaemia may contribute to the development of complications in diabetes.

The human proinsulin connecting peptide (C-peptide) is a 31-amino-acid cleavage product of insulin biosynthesis (Figure 1) that has until recently been regarded as a biologically inert molecule, serving merely to link and stabilize the A- and B-chains of the insulin molecule, thus enabling correct folding and interchain disulfide bond formation [4]. The release of C-peptide into the bloodstream at a concentration equimolar with that of insulin makes it a useful marker of insulin release because, unlike insulin, C-peptide is subjected to negligible first-pass metabolism by the liver. Appreciation of C-peptide structural variability and lack of sequence conservation between species [5] has led to a general consensus that C-peptide lacks physiological effects and the belief that it serves no other role beyond that described in insulin biosynthesis. For these reasons, traditional clinical interest in C-peptide has been restricted to its use as a surrogate marker of insulin release.

Key words: C-peptide, G-protein-coupled receptor, proinsulin, Type 1 diabetes, Type 2 diabetes.

Abbreviations: ATF, activating transcription factor; COX-2, cyclo-oxygenase-2; CREB, cAMP-response-element-binding protein; DAG, diacylglycerol; ERK, extracellular-signal-regulated kinase; GFR, glomerular filtration rate; GPCR, G-protein-coupled receptor; IGF, insulin-like growth factor; JNK, c-Jun N-terminal kinase; MAPK, mitogen-activated protein kinase; NF- κ B, nuclear factor κ B; NOS, NO synthase; eNOS, endothelial NOS; OK, opossum kidney; PI3K, phosphoinositide 3-kinase; PKC, protein kinase C; PLC, phospholipase C; PPAR, peroxisome-proliferator-activated receptor; PTX, pertussis toxin; STZ, streptozotocin; TGF β , transforming growth factor β ; TNF, tumour necrosis factor; TNFR, TNF receptor.

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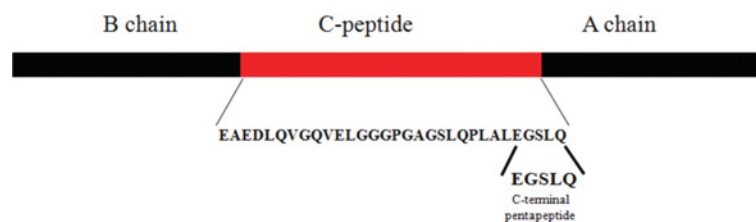


Figure 1 Linear structure of human proinsulin showing the A- and B-chains of insulin separated by C-peptide

The amino acid sequence of C-peptide is shown highlighting the C-terminal pentapeptide that is particularly important in membrane interactions and signalling.

Over the past decade, however, this dogma has been challenged. A series of studies demonstrating specific binding of C-peptide to cell membranes, a variety of cell signalling and physiological actions evoked by C-peptide, and a protective role for C-peptide in diabetic complications has suggested an active hormone-like role for C-peptide and highlighted a therapeutic potential for C-peptide in diabetes.

CELL MEMBRANE INTERACTIONS AND SIGNALLING EFFECTS OF C-PEPTIDE

Binding of C-peptide to cell membranes

To exert hormone-like activity, C-peptide must have a receptor. Specific displaceable binding of C-peptide to pancreatic islet β -cells was initially described by Flatt et al. [6]. Ido and co-workers [7] found improvements in nervous and vascular function in diabetic rats treated with various derivatives of C-peptide, but suggested they did not bind in a stereospecific manner to a receptor. Instead, it was postulated that cellular actions resulted from poorly defined C-peptide–membrane interactions linked to a conserved mid-portion sequence of the molecule, but independent of its direction or chirality [7].

Using Na^+/K^+ -ATPase activity as a functional readout, C-peptide fragment activity was studied in rat kidney tubule segments [8]. The rat C-peptide C-terminal pentapeptide EVARQ elicited 100% of the activity of intact C-peptide, whereas the remaining portion of the molecule was completely inactive. Furthermore, the corresponding region of human C-peptide (EGSLQ) elicited 75% activity (Figure 1). The glutamic acid and glutamine residues at positions 1 and 5 respectively, are generally conserved in mammals, and similar well-defined functional C-terminal sequences are also found in other hormones such as gastrin and cholecystokinin.

Overall, the studies of the C-terminal pentapeptide of C-peptide reveal properties typical of a peptide ligand interacting with a specific receptor. In contrast, mid-C-peptide sequences only partially recapitulate the activity of the intact molecule and exhibit different properties. No activity relating to this region was observed

with the des-(27–31)-C-peptide, but several non-natural D-amino-acid-containing sequences showed some activity that was reduced with fragments greater than nine amino acids in length [8]. This behaviour is incompatible with a peptide–receptor interaction, but reminiscent of the non-specific interaction of C-peptide with plasma membranes postulated by Ido et al. [7].

Clearly, the balance of activity provided by these two regions of the C-peptide molecule *in vivo* may be important, but is poorly understood. However, efficient C-peptide signalling requires the presence of conserved glutamic acid residues at positions 3, 11 and 27, and the presence of helix-promoting residues in the N-terminal segment [9]. Together with the results relating to its C-terminus, the overall picture of the structure–activity relationship of the C-peptide molecule is one of a three-part structure, where the terminal sections are involved in functional interactions, with the mid-region forming a joining segment [9].

High-affinity specific binding of fluorophore-labelled C-peptide to human cell membranes has been demonstrated using sensitive fluorescence correlation microscopy that permits the measurement of membrane interactions at the single molecule level in sub-femtolitre volumes [10]. The maximal number of binding sites, approx. 1000–1500 per cell, was found on renal tubular cells. Half-maximal occupation of binding sites was seen at 0.3 nmol/l C-peptide, and full occupation at 0.9 nmol/l C-peptide. Co-addition of excess intact C-peptide and C-terminal pentapeptide, but not scrambled C-peptide, insulin and IGF (insulin-like growth factor)-I or IGF-II, was able to displace binding. Pre-incubation with PTX (pertussis toxin) also significantly inhibited C-peptide binding [10].

These findings provide strong support for the existence of a specific GPCR (G-protein-coupled receptor) for C-peptide that interacts with the C-terminal pentapeptide region of the molecule, and is fully saturated within the physiological C-peptide concentration range (0.9 nmol/l). Thus, in the presence of normal pancreatic β -cell function, these receptors should be fully occupied by prevailing C-peptide concentrations and, therefore, no further response may be expected with exogenous administration. This feature may explain the absence of

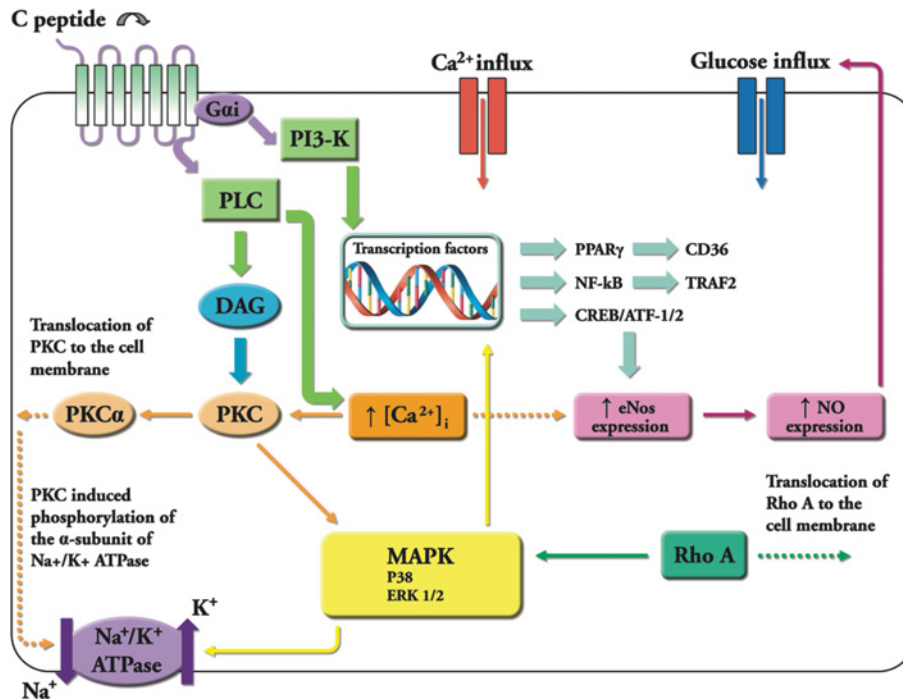


Figure 2 Schematic representation of cellular signalling effects of C-peptide

Available evidence suggests that C-peptide binds to a cell membrane receptor that is coupled to a PTX-sensitive G-protein. Activation of this GPCR stimulates both PLC and PI3K. PLC activation evokes an increase in $[Ca^{2+}]_i$, resulting in the concomitant activation of both eNOS and PKC, the latter of which, together with increased *de novo* synthesis of DAG, stimulates the activation of the Na^+/K^+ ATPase via PKC α translocation to the cell membrane. Stimulation of the MAPK pathway also results in both increased Na^+/K^+ ATPase activity and activation of various transcription factors. Together with the elevated PI3K levels, MAPK-induced transcription factor expression is increased and effects, including reduced apoptosis, and increased eNOS and CD36 levels, are observed. For abbreviations, see text.

a response when C-peptide is administered to healthy subjects.

Currently, the identity of the C-peptide receptor remains elusive. Attempts at identification using gene cloning and proteomic strategies have so far been unsuccessful [11], although it remains possible that alternative methodology may be more successful in the future. Research effort in this area is well justified and urgently needed.

Regulation of Na^+/K^+ -ATPase

The ubiquitous Na^+/K^+ -ATPase protein complex uses energy released from the hydrolysis of ATP to drive the counter-transport of Na^+ and K^+ ions across the plasma membrane. In diabetes, Na^+/K^+ -ATPase activity is demonstrably decreased in numerous tissues, and this deficient activity is thought to contribute to the development of diabetic complications [12]. Kidney tubules are a rich source of Na^+/K^+ -ATPase and C-peptide stimulates this pump in rat tubular segments [13], where mid-region peptides evoked partial activity, but C-terminal tetra- and penta-peptides elicited full activity, proving as potent as full length C-peptide [8,14]. Broadly similar to the results of Ido et al. [7] described above,

these findings support further the concept of a specific C-peptide–receptor interaction with downstream signalling.

Reduced erythrocyte Na^+/K^+ -ATPase activity in Type 1 diabetic patients results in impaired deformability and increased blood viscosity [15], and is strongly linked to microvascular complications. In Type 1 diabetic patients and complete C-peptide deficiency, and in insulin-treated patients with Type 2 diabetes, erythrocyte Na^+/K^+ -ATPase activity is consistently lower than in healthy controls [16]. Infusion of C-peptide into Type 1 diabetic patients corrects this Na^+/K^+ -ATPase activity, maximal effects being achieved with plasma C-peptide levels of approx. 3.5 nmol/l [17]. Similar improvements in nerve Na^+/K^+ -ATPase activity and function in diabetic rats have also been observed [7,18].

The molecular mechanisms by which C-peptide regulates Na^+/K^+ -ATPase are now becoming clear. In rat kidney medullary thick ascending limb tubules, physiological concentrations of C-peptide cause a rise in intracellular $[Ca^{2+}]_i$, translocation of Ca^{2+} -dependent PKC (protein kinase C) to the plasma membrane and phosphorylation of the Na^+/K^+ -ATPase α -subunit [19]. These effects are sensitive to PTX, suggesting a direct relationship between Na^+/K^+ -ATPase activity and C-peptide acting via a GPCR (Figure 2).

Effects on eNOS [endothelial NOS (NO synthase)]

Administration of C-peptide to diabetic animal models and to Type 1 diabetic patients results in concentration-dependent increases in microvascular blood flow to muscle, skin and kidney. This vasodilatory effect may result from C-peptide-induced NO release from endothelial cells [20–22]. These C-peptide-elicited effects on NO are PTX-sensitive, and result both from Ca^{2+} -dependent activation of eNOS and induction of eNOS gene transcription and protein expression [22,23]. Hence signalling downstream of C-peptide–GPCR interactions may mediate increases in tissue perfusion due to activation of the NO system of potential benefit in the context of diabetic microvascular complications.

MAPKs (mitogen-activated protein kinases)

MAPKs link cell-surface receptors or chemical and physical stresses to key regulatory targets within cells [24] by phosphorylating target substrates and, thus, regulating key cellular processes, such as mitosis, metabolism, cell survival, gene expression and apoptosis [25,26]. The MAPK family comprises three subfamilies: ERK (extracellular-signal-regulated kinase) 1 and 2, JNK (c-Jun N-terminal kinase) 1, 2 and 3, and p38 MAPK (α , β , γ and δ).

Several reports document an ability of C-peptide to activate MAPKs. Kitamura et al. [27] examined the impact of C-peptide on MAPK signalling in the Swiss 3T3 mouse embryonic fibroblast cell line and found that human C-peptide induced both a time- and concentration-dependent (evident at 1 pmol/l and maximal at 1 nmol/l) activation of ERK1/2, an effect abolished by treatment with PTX. This effect was not replicated in all cell types nor was it reproduced by either retrosequenced or all-D-amino-acid C-peptide.

C-peptide-induced phosphorylation of both ERK1/2 and p38 MAPK has now been described in numerous cell lines, including mouse lung capillary endothelial (LEII) cells [28], OK (opossum kidney) proximal tubular cells [29], human renal tubular cells (HRTC) [30] and L6 rat skeletal myoblasts [31]. This activity has been linked to activation of CREB (cAMP-response-element-binding protein) and ATF1 (activating transcription factor 1) transcription factors [28], and downstream of these to eNOS gene transcription.

In OK proximal tubular cells, C-peptide potently activates ERK, exhibiting a ‘bell shaped’ dose–response curve, with maximal activation at 300 pmol/l, and Akt with a sigmoidal dose–response, where maximal activity is evoked by 5 nmol/l C-peptide [29]. Increases in intracellular [Ca^{2+}] are seen in response to 5 nmol/l C-peptide in OK proximal tubular cells with consequent stimulation of PKC α . C-peptide is also a functional

mitogen in this cell type, stimulating significantly increased cell proliferation [29].

Tsimaratos et al. [19] similarly demonstrated PKC α - and ERK1/2-dependent activation of Na^+/K^+ -ATPase by C-peptide in isolated kidney tubular segments. Furthermore, in human kidney proximal tubular cells, C-peptide activates ERK1/2, JNK, PKC ϵ and PKC δ , and promotes translocation of the low-molecular-mass GTP-binding protein Rho A from the cytoplasm to the membrane [30]. These phenomena are themselves dependent upon upstream activation of PLC (phospholipase C). All these events are PTX-sensitive.

The pathways by which C-peptide activates MAPKs can now be described with some precision and involve: (i) C-peptide binding to a PTX-sensitive GPCR; (ii) activation of PLC; (iii) increased DAG (diacylglycerol) and intracellular Ca^{2+} levels stimulating several PKC isoforms; (iv) PKC-dependent activation and translocation of RhoA to the plasma membrane; and (v) phosphorylation and activation of MAPKs (Figure 2).

Activation of transcription factors by C-peptide

Changes in cell phenotype consequent upon alterations in cell signalling are frequently accompanied by changes in gene transcription. Activation of MAPKs by 1 nmol/l C-peptide is associated with phosphorylation and activation of the transcription factors CREB, ATF1 and ATF2 in LEII cells [28]. Enhanced expression and translocation of NF- κ B (nuclear factor κ B), and expression of the anti-apoptotic Bcl-2 protein is seen in neuroblastoma cells after C-peptide treatment [32]. In Swiss 3T3 fibroblast cells, C-peptide activates the transcription of COX-2 (cyclo-oxygenase-2) via NF- κ B [33].

Using OK cells, we carried out detailed studies of transcription factor activation [34,35] concentrating on PPAR γ (peroxisome-proliferator-activated receptor γ) and NF- κ B in response to both C-peptide and insulin. PPAR γ is a member of the nuclear hormone receptor family and the target of the insulin-sensitizing thiazolidinediones used in the treatment of Type 2 diabetes [36]. We found with respect to PPAR γ that: (i) both C-peptide and insulin transactivated PPAR γ [34]; (ii) C-peptide (EC_{50} 4 nmol/l) was more potent than insulin (EC_{50} 10 nmol/l) in this regard; (iii) both agents evoked similar phosphorylation of PPAR γ following activation of PI3K (phosphoinositide 3-kinase); (iv) as a consequence of PPAR γ activation, both C-peptide and insulin increased the transcription of the PPAR γ -regulated gene CD36 [34]; and (v) only the effects of C-peptide were attenuated by PTX, and its signalling receptor system was fundamentally different from that of insulin. Together these findings illustrate a new mechanism by which C-peptide and insulin could

interact, regulating glycaemia and the expression of PPAR-controlled genes involved in metabolism and the control of inflammation.

To investigate whether C-peptide may protect kidney proximal tubular cells in diabetic nephropathy, the influence of C-peptide on TNF α (tumour necrosis factor α)-mediated tubular effects was determined [35]. TNF α is a pleiotropic peptide cytokine and an important contributor to the development of diabetic nephropathy [36–41]. It elicits a wide spectrum of cellular responses, including differentiation, proliferation, inflammation and cell death, via its interaction with two members of the TNFR (TNF receptor) family TNFR1 and TNFR2 [42].

TNF α markedly reduced OK cell viability and induced apoptosis [35], but this was preventable by insulin or C-peptide. Both insulin and C-peptide activated NF- κ B, with insulin demonstrating a typical sigmoidal dose-response maximal at 100 nmol/l, and C-peptide a 'bell shaped' dose-response curve maximal at 5 nmol/l and declining thereafter. Although as in previous studies PTX prevented this C-peptide effect, the presence of a G $_{\alpha i}$ -linked GPCR was additionally revealed by the ability of C-peptide to stimulate GTP[S] (guanosine 5'-[γ -thio]triphosphate) binding to G $_{\alpha i}$ in OK cell membranes. The ability of C-peptide to prevent TNF α -induced apoptosis was associated with the induction, via NF- κ B, of survival genes, such as TRAF2 (TNFR-associated factor 2) (Figure 2) [35].

Therefore, *in vitro*, C-peptide regulates the transcription of protective genes in the face of pathophysiological stimuli implicated in the development of diabetic nephropathy. The evidence suggests that C-peptide possesses unique signalling capabilities acting via a G $_{\alpha i}$ -coupled GPCR and may potentially exert protective effects in Type 1 diabetes where endogenous C-peptide is completely deficient.

PHYSIOLOGICAL EFFECTS OF C-PEPTIDE

C-peptide and glucose handling

Soon after the mechanism of insulin biosynthesis was elucidated, studies addressed the possibility that C-peptide may contribute to the regulation of glucose metabolism. However, inconsistent results nourished the belief that C-peptide lacked significant biological activity [43]. Nonetheless, interest in C-peptide and glucose handling persisted, and Zierath et al. [44,45] showed that C-peptide dose-dependently stimulated glucose transport by human skeletal muscle *in vitro* by a pathway shared partly with insulin.

In diabetic rats, supra-physiological concentrations of C-peptide prolonged the hypoglycaemic actions of insulin [46] and, later, using the euglycaemic clamp technique in STZ (streptozotocin)-induced diabetic rats, physiological concentrations of rat C-peptide were found

to significantly augment the disposal and metabolic clearance rates for glucose [47]. This result was largely blocked by the NOS inhibitor L-NMMA (N^G-monomethyl-L-arginine), suggesting that these actions of C-peptide were mediated by NO. These effects were undetectable in healthy rats.

The effects of C-peptide on glucose utilization have also been examined in short-term studies in Type 1 diabetic patients. When subjected to euglycaemic clamping, these patients display a 25% increase in glucose turnover when C-peptide is infused to a circulating concentration of approx. 0.8 nmol/l [48]. In agreement with the hypothesis that a C-peptide receptor is fully saturated at 0.9 nmol/l (see above), no further increase in glucose turnover was observed when C-peptide levels were further elevated to 2.1 nmol/l. Improvement in whole-body glucose utilization under these conditions was due to increased muscle uptake rather than reduced hepatic synthesis [49]. Similarly in a study of 13 Type 1 diabetic patients, or matched controls, studied over a 2 h period under euglycaemic clamp conditions, C-peptide infusion significantly increased glucose utilization in both groups [50].

Despite the finding of short-term effects of C-peptide on glucose handling, long-term effects are less clear. In one study, C-peptide was administered for 1 month, together with insulin, to nine Type 1 diabetic patients, whereas controls received insulin alone. Fructosamine and HbA_{1c} (glycated haemoglobin) levels were significantly lower after 1 month in the C-peptide group [51]. Conversely, in a 3 month study of 21 Type 1 diabetic patients, the subcutaneous administration of C-peptide in addition to regular insulin led to no significant improvement in glycaemic control compared with controls who received insulin alone [52].

C-peptide and renal function in diabetes

Renal complications in diabetes can be devastating. Diabetic nephropathy is a syndrome comprising albuminuria, falling GFR (glomerular filtration rate), hypertension and cardiovascular disease. Worldwide, diabetic nephropathy is now the single commonest registered diagnosis at entry into renal replacement therapy programmes [53] and represents the leading cause of end-stage renal disease in the U.S.A. and Europe. Diabetic nephropathy in Type 1 diabetes evolves in a predictable manner. Early in the course of Type 1 diabetes, renal hypertrophy and hyperfiltration, sometimes with low-grade proteinuria, are characteristically observed. These abnormalities are largely reversible with treatment and, thereafter, a period of clinical latency ensues. Later, microalbuminuria indicative of incipient nephropathy may develop, and later still established nephropathy becomes manifest as persistent heavy proteinuria with a declining GFR, characteristic glomerular pathological appearances and developing renal tubulointerstitial

fibrosis. These hallmark histopathological appearances result from a series of interconnected renal cellular events that occur in the presence of persistently high glucose, such as increased flux of polyols and hexosamines, formation of ROS (reactive oxygen species), activation of PKC, secretion of TGF β (transforming growth factor β), altered expression of cyclin kinases, increased matrix proteins and decreased matrix-degrading enzymes and metalloproteinases [54].

In a series of studies of early renal changes in Type 1 diabetes, C-peptide has been shown to exert a salutary effect. Motivated by the observations that: first, insulin treatment in patients with newly diagnosed Type 1 diabetes improves but often fails to normalize renal hyperfiltration, and secondly that patients with Type 2 diabetes who have maintained endogenous insulin and C-peptide secretion generally do not develop glomerular hyperfiltration, Johansson et al. [48] examined the possibility that C-peptide may regulate renal functional alterations, such as GFR, in diabetes. These investigators made detailed renal haemodynamic assessments in Type 1 diabetic patients during short-term (60 min) infusions of C-peptide compared with controls given 0.9% saline. They found that C-peptide administration was accompanied by a significant 7% fall in GFR from 143 ± 3 to 133 ± 4 ml \cdot min $^{-1}$ \cdot 1.73 m $^{-2}$, whereas the GFR of controls did not change after 60 min of 0.9% saline infusion. Extending these observations in a randomized double-blind study in Type 1 diabetes, these investigators demonstrated further a 6% reduction in GFR and a 55% reduction in proteinuria after 1 month of C-peptide and insulin compared with insulin alone [51]. Similarly, in a somewhat longer 3 month study in microalbuminuric Type 1 diabetic patients, C-peptide administration was associated with a reduction in urinary albumin excretion from 58 to 34 μ g/min [52].

These findings in patients have also been paralleled in animal studies. In STZ rats with early diabetes, physiological levels of C-peptide attenuate glomerular hypertrophy, glomerular hyperfiltration and proteinuria [55–58]. Indeed, in a ‘head to head’ study with an ACEI (angiotensin-converting enzyme inhibitor), C-peptide was as effective as captopril in reducing glomerular hyperfiltration in STZ rats [59]. At a later time point (4 weeks) in STZ diabetic rats given C-peptide, these kidney functional improvements were accompanied by preserved glomerular structure with significantly reduced hypertrophy and matrix accumulation [59].

In STZ diabetic mice, C-peptide also reduced urinary albumin excretion, together with glomerular expression of the pro-fibrotic cytokine TGF β and type IV collagen [60]. When mouse glomerular podocytes were exposed to C-peptide, TGF β -induced expression of mRNAs for type IV collagen and PAI1 (plasminogen-activator inhibitor 1) were blocked [60]. Most recently, micropfusion studies of isolated glomeruli from alloxan-induced

diabetic mice have revealed that C-peptide constricts afferent glomerular arterioles when applied via the vessel lumen [61]. The effect was only minimal in glomeruli similarly isolated from normoglycaemic animals.

Although afferent arteriolar vasoconstriction may explain the beneficial effects of C-peptide on early glomerular haemodynamic alterations and microalbuminuria in diabetes, the available results indicate that the effects of C-peptide in diabetic nephropathy may extend beyond this to the amelioration of TGF β -mediated pathways of scarring and fibrosis. What are needed now are more long-term studies of C-peptide administration in animal models of diabetes to study the effects on renal fibrosis and excretory function, together with clinical studies to explore the potential renoprotective effects of C-peptide in human diabetes.

A further clue to the importance of C-peptide in protecting the kidney from diabetic nephropathy comes from the results of combined islet–kidney transplantation. Persistent islet function in patients after this procedure is associated with improved survival of the renal allograft, and there is a clear positive association between renal graft function and continued C-peptide secretion irrespective of glycaemic control [62]. Faced with these clinical observations, it is tempting to suggest that continued ambient exposure of the transplanted kidney to islet-transplant-derived C-peptide is responsible for these improvements in renal outcome [63].

C-peptide and nerve function

Diabetic neuropathy is a prominent and debilitating complication of longstanding diabetes resulting from a combination of metabolic, vascular and hormonal factors as a consequence of hyperglycaemia. Studies in diabetic rodents have suggested that C-peptide may prevent neuronal dysfunction by improving endoneurial blood flow and nerve Na $^{+}$ /K $^{+}$ -ATPase activity [64]. Studying the spontaneously diabetic BB/Wor rat with polyneuropathy, Zhang et al. [65] reported that C-peptide administration dose-dependently improved nerve conduction velocities. The protective effect of C-peptide in this model was accompanied by beneficial alterations in a variety of neurotrophic factors and receptors, and was most marked if the agent was continuously administered by an osmo-pump [66].

In a short-term study of 12 Type 1 diabetic patients and clinical signs of autonomic neuropathy, treatment with C-peptide in addition to insulin was accompanied by increased respiratory heart rate variability, suggestive of improved autonomic function [52]. Using a randomized double-blind placebo-controlled design, Ekberg et al. [67] studied the effect of C-peptide supplementation on nerve function in 26 asymptomatic Type 1 diabetic patients of relatively short (approx. 10 years) duration and subclinical neuropathy. At baseline, the diabetic subjects had significantly impaired sensory nerve

conduction velocity compared with controls, but in the C-peptide-treated group a significant improvement in this parameter was evident after only 6 weeks of treatment, and by 3 months the deficit was corrected by 80%. In a subsequent larger multi-centre study of similar design in Type 1 diabetic patients (duration of diabetes approx. 30 years) with established clinical neuropathy, C-peptide treatment resulted in significant and substantial improvements in neuropathic parameters as determined by both neurophysiological measurement and clinical examination [68].

C-PEPTIDE IN TYPE 2 DIABETES

The balance of evidence strongly points to the bioactivity of C-peptide, and it is not difficult to envisage how its replacement may elicit biological effects when C-peptide is completely deficient in Type 1 diabetes. Indeed, all clinical studies of C-peptide have been carried out in Type 1 diabetic patients. Type 2 diabetes is associated with insulin resistance and as the disease evolves patients exhibit elevated insulin and C-peptide concentrations. Indisputably many Type 2 diabetic patients develop nephropathy and neuropathy in the face of increased circulating C-peptide levels; however, given these high ambient levels of C-peptide in Type 2 diabetic patients, and bearing in mind the experimentally derived affinity of C-peptide-binding sites (see above), it is likely that any cognate receptor would be fully occupied and potentially down-regulated.

Important differences also exist between the complications observed in Type 1 and Type 2 diabetes. Neuropathy in Type 1 diabetes progresses more predictably and quickly, and is associated with typical myelin sheath and axonal derangements not present in Type 2 diabetes [69]. With respect to diabetic nephropathy, the predictable evolution of renal disease observed in Type 1 diabetes (described above) is less well documented in Type 2 diabetes. In addition, whereas diabetes-specific renal lesions are found in all Type 1 diabetic patients with nephropathy, renal morphology in Type 2 diabetes is much more heterogeneous with prominent arteriosclerosis and ischaemic nephropathy [70]. In Type 1 diabetic nephropathy, chronic hyperglycaemia beginning in the first 2 decades of life is usually the only evident cause of kidney disease. On the other hand, patients with Type 2 diabetes are generally over the age of 40 and have evidence of age-related glomerulosclerosis, together with other propagators of renal disease, such as hypertension, obesity and dyslipidaemia. Thus nephropathy in Type 2 diabetes reflects a heterogeneous combination of kidney diseases precipitated by a mixture of mechanisms that may modify and overwhelm the typical renal responses to hyperglycaemia and the features of pure diabetic nephropathy [71]. Consequently, it has been suggested

that, in terms of responses to treatment, Type 1 and Type 2 diabetic patients should be considered separately.

Attention has been drawn to a possible role for C-peptide in the development of vascular inflammation and atherosclerosis in Type 2 diabetes. This concern is based on observations that C-peptide deposits may be found co-localized with CD4⁺ lymphocytes in early atherosclerotic lesions in Type 2 diabetic patients, but not in similar vascular lesions in non-diabetics [72]. Subsequent studies demonstrated the chemotactic activity of C-peptide for CD4⁺ lymphocytes *in vitro* [73]. Although these findings are weakened because circulating C-peptide levels were not measured in the diabetic subjects, they suggest that elevated C-peptide in Type 2 diabetes may contribute to vascular dysfunction. Other work has also shown that C-peptide may stimulate smooth muscle cell proliferation [74], but this finding was contradicted by other authors [75,76]. Furthermore, contrary to these findings that C-peptide may be a key mediator in the development of vascular inflammation and atherosclerosis in Type 2 diabetes, studies by Luppi et al. [77] have recently demonstrated both an anti-inflammatory and potential anti-atherogenic role for C-peptide through a reduction in the expression of several biochemical markers of endothelial dysfunction in HAECs (human aortic endothelial cells) [77].

SUMMARY AND CONCLUSIONS

We believe the evidence that C-peptide possesses biological activity is now so strong that Type 1 diabetes should be regarded as a dual-hormone-deficiency disease. In this case, it appears reasonably intuitive to advocate replacement of C-peptide in addition to insulin in Type 1 diabetes, and indeed some clinical trial evidence supports this view [52,67,68]. The situation in Type 2 diabetes is more complex and requires further study, particularly in the light of work indicating that prolonged elevation of C-peptide in insulin-resistant individuals may be associated with pro-atherogenic actions. Importantly though, these findings do not negate a role for C-peptide replacement in deficiency states. Numerous hormones display a physiological window of activity where circulating concentrations within the window are associated with health, but both low and high levels are associated with adverse systemic effects. L-Thyroxine is a good example of this paradigm [78].

C-peptide replacement in Type 1 diabetes promises to provide an important new therapeutic tool to reduce the misery of diabetic complications. Longer-duration clinical trials of C-peptide treatment are now needed in Type 1 diabetes, along with more studies of the intricacies of C-peptide actions in Type 2 diabetes. Work to identify the C-peptide receptor is urgently required in order to better define its function as a therapeutic target and the pharmacology of C-peptide bioactivity.

FUNDING

The authors' research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

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Received 1 September 2008/1 October 2008; accepted 15 October 2008
Published on the Internet 2 March 2009, doi:10.1042/CS20080441