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Signal transduction by C-peptide: Effect on intracellular second messengers

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ABSTRACT

C-peptide is produced during insulin biosynthesis and present in equal amounts along with the insulin in circulation, it considering devoid of any biological activity for long time. Therefore, this review focused on cellular signaling effects of C-peptide. This peptide causes transmission of signals from exterior of cells to their interior by signal transduction process. The binding of C-peptide with G-proteins coupled receptors (GPCR) activate phospholipase C, which cleaves a membrane phospholipid to produces DAG and IP-3. DAG binds all members of the protein kinase C family, which, then become activated. IP-3 causes releasing of stored calcium into the cytoplasm, this mediated influx of extracellular calcium to intracellular leading to increase NO and cGMP. C-peptide in combination with insulin significantly enhances insulin receptor phosphorylation either by increase of kinases or decrease of phosphatases activity. C-peptide disaggregates insulin hexamer causing rapid appearance of insulin in plasma. Consequently, the interaction of C-peptide with GPCR influences many of intracellular processes including increase of glucose metabolism, phosphorylation/dephosphorylation, erythrocytes function, blood flow as well as cells growth and apoptosis. Nowadays, C-peptide is biochemically active peptide and it may be elicits insulin independent effect. Therefore C-peptide may be has beneficial role in treatment of diabetic associated complications.

KEYWORDS

C-peptide;
G-protein;
Signal transduction;
Second messengers.

OVERVIEW OF PROINSULIN CLEAVAGE

The proprotein convertase (PPC1), proprotein convertase (PPC2) as well as carboxypeptidase (CPE), involved in the production of C-peptide from proinsulin molecule[1]. This process occurs in calcium-rich acidic environment of pancreatic β-cells[2]. Both PPC1 and PPC2 are calcium-dependent enzymes[3], while, the CPE is zinc dependant, whereas zinc is found abundantly in the matrix of secretory granule in association with insulin[4]. These enzymes were synthesized as proproteins molecules like proinsulin and then processed and sorted to regulated pathways of secretion[5]. Both PPC1 and PPC2 were acted together to process proinsulin to insulin and C-peptide[6, 7].

The processing of the insulin precursor is primarily

sequential; the first cleavage at B chain/C-peptide junction is occurred under the effect of PPC1 to produce, des-31, 32-proinsulin, and then cleaved by PPC2 at A chain/C-peptide junction. While, the basic amino acids residues at C-terminal were removed by aid of CPE. Retention of the C-peptide is required for correct disulfide bridges formation between A and B chains of insulin, afterword the C-peptide removed[8]. See Figure 1.

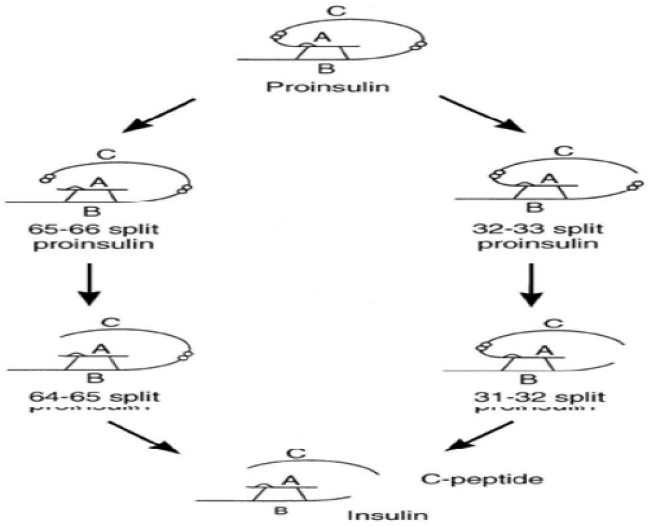

Figure 1: Processing of C-peptide and insulin from proinsulin. Numbers refer to amino acid residues of proinsulin numbered from the N- terminus[8].

Defect of proinsulin processing

The defect in the processing of proinsulin into insulin and C-peptide leads to elevation of proinsulin/insulin ratio. This defects may be due to decrease in the activities of PPC1[9] or PPC2[10] as well as inactivation of CPE[11]. Furthermore, mutations in the insulin gene resulted in abnormal proinsulins that are not normally processed into fully active insulin[12]. These abnormalities were associated with type 2 diabetes (T2D) and/or impaired glucose tolerance.

In these cases, there is a relative increase in proinsulin / insulin ratio. Patients with T2D might be apparently hyperinsulinemic, owing to proinsulin cross reactivity, but are deficient in mature insulin. Also, the cause of hyperproinsulinemia in T2D is thought to be a pancreatic β-cell defect[13].

Ravelli *et al.*[14], demonstrated that impaired glucose tolerance might be related to abnormal levels of proinsulin and insulin resistance. Additionally, Gabbay[15], described familial hyperproinsulinemia, an autosomal dominant defect in which C-peptide remains attached to A chain and in which Arginine 65 has been replaced.

Structure of C-peptide

C-peptide, a cleavage product of the proinsulin molecule, has long been regarded as biologically inert. The number of amino acids in C-peptide is ranged from 30 to 35 amino acids according to species[16]. Human C-peptide consists of 31 amino acid residues 5 of them are acidic residues but it lacks the basic residues. C-peptide is devoid of detectable stable secondary structure, but the N-terminal 11 amino acids residues form α-helical structure[17].

The region of positions 13–25, containing five glycine residues, is in parts lacking in some mammals, and not have a stable secondary structure. The C-terminal five amino acids residue (27–31) highly conserved residues and found to possess biological activity[18]. TABLE 1, represented the structure of C-peptide from different species.

TABLE 1 : Amino acids sequence of proinsulin C-peptide among some species according one letter abbreviation of amino acids.

Species	Amino acids sequence
Human	EAEDLQV– – GQVELGGGPGAGSLQPLALEGSLQ
Bovine	EVEGPQV– –GALELAGGPGAGG– – – – – LEGPPQ
Mouse	EVEDPQV– –EQLELGGSPG– – DLQTLALEVARQ
Rat	EVEDPQV– – PQLELGGGPEAGDLQTLALEVARQ
Pig	EAENPQA– –GAVELGGGLGG– – LQALALEGPPQ
Sheep	EVEGPQV– –GALELAGGPG– – – – – AGGLEGPPQ
Horse	EAEDPQV– – GEVELGGGPGLGGLQPLALAGPQQ

Role of C- peptide in insulin maturation

Preproinsulin carries a signal peptide that directs the peptide chain of preproinsulin to the interior of the endoplasmic reticulum(ER). In the ER the N-terminal signal sequence of preproinsulin is cleaved and the resulting proinsulin molecule undergoes folding and disulfide bond formation[19].

At the Golgi apparatus proinsulin is packaged into secretory granules and converted to insulin and C-pep-

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tide by PPCs. C-peptide and insulin are stored in mature secretory vesicles, afterword they, co-secreted in equimolar amounts[20]. The mature insulin is stored in the form of zinc-containing hexamers until secretion[1].

C-peptide plays an important role in insulin folding, by providing optimal orientation of sulfhydryl groups for inter and intra disulfide bond formation between A and B chains of insulin[21, 22]. Beside this role, C-peptide maintains the C-peptide/insulin A-chain junction in proinsulin structure, which is a recognition site for PPC2[23]. Figure 2 depicts the role of C-peptide in processing and secretion of proinsulin molecule.

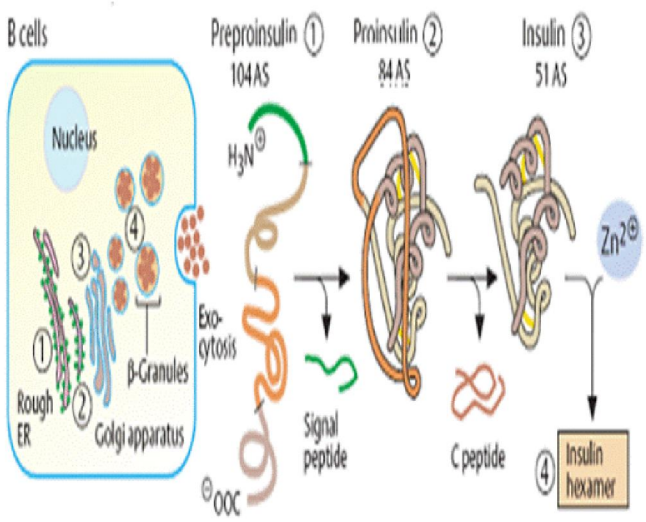

Figure 2 : Removal of signal peptide from proinsulin molecule as well as sorting and packaging insulin and C-peptide.

C-peptide receptors

Upon binding of C-peptide with G-protein receptors (GPCR) several enzymes were activated including Na^+/K^+-ATPase, eNOS as well MAPK[24]. These receptors for C-peptide present in renal tubular cells, skin fibroblasts and endothelial cells[25]. The presence of such receptor was demonstrated by using of G-proteins inhibitors. Whereas, treatment of the cells with pertussis toxins (PTX) as G-protein inhibitors abolishing the interaction between C-peptide and receptors[26]. Also, most of the intracellular signaling of this peptide blocked by pre-incubation of the cells with PTX, these observations indicated that involvement of GPCR in C-peptide signaling[27].

Signal transduction by C-peptide

The messages transmitted by hydrophilic signaling substances were sent to the interior of the cell by membrane receptors. The binding of signaling molecules with the outside of the cell trigger a new second signal on the inside of the cells. In the interior of the cell, this secondary signal influences the activity of enzymes, ion channels, metabolism and cytoskeleton as well as activation or inhibition of transcription factors.

Binding of C-peptide with GPCR activate phospholipase C (PLC), provoke increasing of second messengers like diacylglycerol (DAG), and inositol trisphosphate (IP-3)[27]. Furthermore, C-peptide provokes a prompt elevation of intracellular calcium as one of strong second messengers[28]. This leads to stimulation of eNOS in renal tubular and endothelial cells[29]. In addition to, C-peptide stimulates phosphorylation of protein kinase C (PKC)[30, 31].

As well, exposure of the cells to C-peptide induced the activation of one or more components of the mitogen activated protein kinases (MAPKs) cascade in a concentration-dependent manner, which increase transcription factor expression[32]. Furthermore, C-peptide has also been found to induce the mimic effects of insulin in muscle cells[33]. The following figure shows the binding of C-peptide to a cell membrane receptor that is coupled to a PTX-sensitive G-protein.

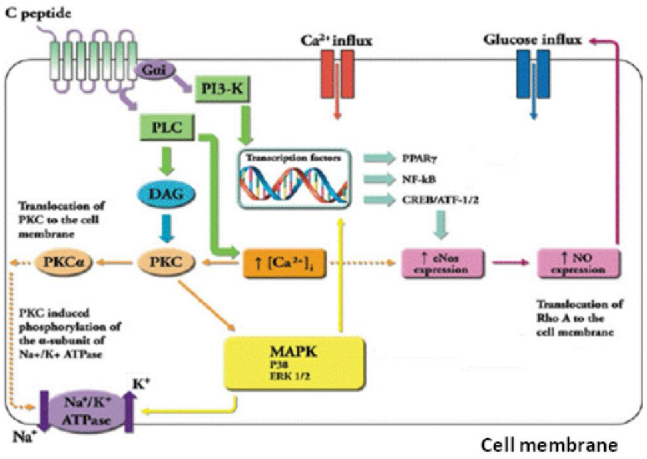

Figure 3 : Overall signalling transduction and second messenger's activation by C-peptide[27].

Effect of C-peptide on phosphorylation/ dephosphorylation

Phosphorylation/ dephosphorylation process plays an important role in the regulation of enzymes activity at post translational level. This biochemical process is under control of protein tyrosine kinase (PTK), in con-

junction with protein tyrosine phosphatase (PTP)[34]. PTP is a negative regulator in the insulin-stimulated signaling pathway. Therefore, the lacking of PTP activity increased sensitivity towards insulin[34]. Conversely, protein tyrosine kinase required for phosphorylation, is chromium dependent[35].

Meyer *et al.*,[36], demonstrated that there is an increase in ATP release from the erythrocytes by chromium-activated C-peptide. C-peptide complexed with chromium, therefore the amount of glucose transported into the erythrocyte increased, this indicated that C-peptide inhibition of PTP activity. Moreover, J?gerbrink *et al.*,[37], reported that C-peptide inhibit intracellular PTP activity, this increased autophosphorylation of the insulin receptor.

Activation of Na^+/K^+-atpase by C-peptide

The Na^+/K^+-ATPase protein complex utilizes energy released from the hydrolysis of ATP to drive the counter-transport of Na^+ and K^+ ions across the plasma membrane. It has been demonstrated that C-peptide cause's phosphorylation of the Na^+/K^+-ATPase α-subunit in rat's kidney medullary thick ascending limb tubules[38].

In diabetes, Na^+/K^+-ATPase activity is decreased in various tissues, and this reduction is thought to be one of the factors responsible to the development of diabetic complications[39]. Kidney tubules are a rich source of Na^+/K^+-ATPase and C-peptide stimulates this pump in rat tubular segments[40].

Reduced erythrocyte Na^+/K^+-ATPase activity in T1D patient's results in impaired deformability and increased blood viscosity[41] and is strongly linked to microvascular complications. Complete C-peptide deficiency as in T1D patients and in insulin-treated patients with T2D, erythrocyte Na^+/K^+-ATPase activity is lower than in healthy controls[42]. Injection of C-peptide into T1D patients corrects this Na^+/K^+-ATPase activity.

Effects of C-peptide on nitric oxide

It has been reported that that supplementation of C-peptide to diabetic animal models and to T1D patients results in concentration dependent increases of microvascular blood flow[43]. This effect attributed to increase the nitric oxide (NO) level either by activation of endothelial nitric oxide synthase (eNOS) by calcium dependent mechanism or by induction eNOS gene expression[44]. Additionally, the interaction between C-peptide and GPCR increases the tissues perfusion by activation of the NO system[27]. The C-peptide-mediated influx of extracellular calcium leads to increased NOS activity, NO and cGMP production, vasodilation, and blood flow which beneficial in endothelial cells[45].

In neuronal cells, C-peptide in combination with insulin significantly enhances insulin receptor (IR) phosphorylation and activation of downstream signals, might underlie the effects of C-peptide on neurite outgrowth. The combined vascular and neurotrophic effects of C-peptide are thought to contribute to its observed beneficial effects in diabetic poly neuropathy[45].

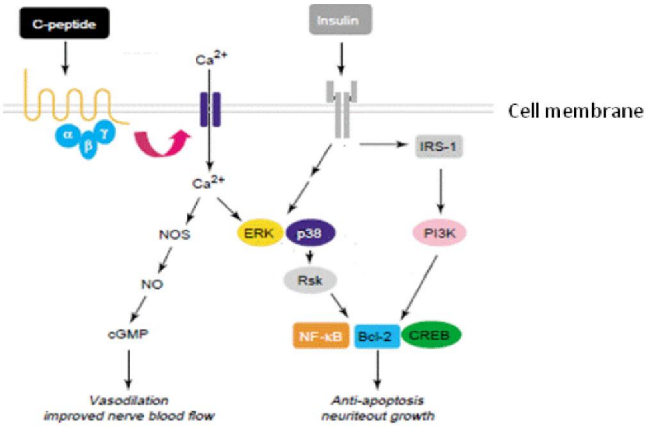

Figure 4 : In neuronal and endothelial Cells, C-peptide signals are transduced via pertussis-toxin sensitive G-protein-coupled receptor (GPCR) that couples positively to calcium signaling[45]

Effects C-peptide on calciumþ levels

C-peptide increases the intracellular level of calcium[46], This is also true for the C-terminal pentapeptide of C-peptide. This was demonstrated by previous study reported that, addition of PTX prevents the effects of C-peptide on intracellular calcium levels of both full-length C-peptide and of the C-terminal pentapeptide which suggest that G-protein-dependent effects are upstream of the influx of calcium[28]. Furthermore, addition of the calcium chelator to the medium abolishes the effects of C-peptide on intracellular calcium levels, suggesting that the increase of intracellular calcium is mediated by influx of extracellular of such ion [32, 45].

Effects of C-peptide on cell growth

MAPKs are important regulator of many cellular

processes, such as mitosis, metabolism, cell survival, gene expression as well as programmed cell death (apoptosis)[47, 48]. Claire and Nigel[27], reported that C-peptide activates MAPKs after binding to GPCR leading to activation of PLC as well as PKC isoforms. In addition to increased intracellular levels of DAG and calcium[49, 50]. These processes have been linked to activation of cAMP-response-element-binding protein (CREB) and activating transcription factor 1 (ATF1) as one transcription factors[49].

Tsimaratos *et al.*[31], similarly demonstrated PKCα- 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, PKCs and promotes translocation of the low-molecular-weight GTP binding protein from the cytoplasm to the membrane[31].

Effect on transcriptional factors

C-peptide causes activation and DNA binding with several transcription factors, also, C-peptide is reported to decreased surface expression of the cell adhesion molecules P-selectin and intercellular adhesion molecule-1 on vascular endothelium, thereby inhibiting leucocytes endothelium interactions[51].

A stimulatory effect of C-peptide on cell proliferation has been reported for renal tubular[52]. Also, such peptide enhances anti-apoptotic effect of insulin on neuroblastoma cells grown at a high glucose concentration[53]. Additionally, C-peptide exposure resulted in activation of PI-3-K and p38 MAPK leading to enhanced expression and translocation of nuclear factor kappa B (NFκB). As well as, C-peptide has been found to protect against tumor necrotic factor-α (TNF-α) mediated apoptosis of renal tubular cells[54].

Insulin like action of C-peptide

There C-peptide signaling pathways mediated by activation of MAPKs as well as PI-3-K are similar signaling follow after insulin-insulin receptor interactions[55]. Therefore, C-peptide exhibited insulin-like actions though increasing muscle glucose transport[33]. It has been founded that C-peptide at level (0.3–3 nM) activated insulin receptor through increase phosphorylation of tyrosine in the insulin receptor. Moreover, submaximal concentrations of insulin and C-peptide were additive in effect[56].

Mass spectrometry analysis revealed that insulin hexamers in solution became undetectable in the presence of C-peptide. This may be due to C-peptide binding with insulin causes disaggregation of insulin. Accordingly, subcutaneous injection of mixture of C-peptide and an insulin in T1D patients result in a rapid appearance of insulin in plasma and stimulation of glucose utilization compared with injection of insulin alone[57]. Insulinomimetic like signalling of C-peptide demonstrated this in figure.

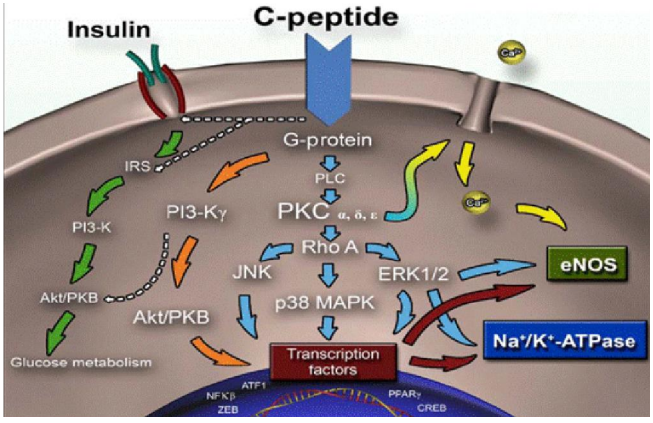

Figure 5 : Insulin like signaling of C-peptide. Dashed lines indicate insulinomimetic signalling demonstrated in muscle cells. GTPγS, guanosine triphosphate γS; JNK, c-Jun N-terminal kinase; PPARγ, peroxisome proliferator activated receptor γ; ZEB, zinc finger homeodomain enhancer binding protein[58].

CONCLUSION

C-peptide elicits insulin-independent biological effects through binding with GPCR receptors. Some enzymes like PTP, PI-3-K, MAPK, GSK3, Na^+/K^+-ATPase and eNOS were activated by C-peptide receptors interaction, this indicating that it has signaling effect. C-peptide causes disaggregation of insulin, therefore it increase the availability of biologically active monomeric insulin.

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