Cyclic Glycine-Proline Is a Bioactive Peptide

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Contributor: Jian Guan , Fengxia Li , Dali Kang , Tim Anderson , Toni Pitcher , John Dalrymple-Alford , Paul Shorten , Gagandeep Singh-Mallah

The reversible binding of IGF-1 to IGF binding protein (IGFBP)-3 regulates the amount of bioavailable, functional IGF-1 in circulation. Cyclic glycine-proline (cGP), a metabolite from the binding site of IGF-1, retains its affinity for IGFBP-3 and competes against IGF-1 for IGFBP-3 binding. Thus, cGP and IGFBP-3 collectively regulate the bioavailability of IGF-1. The molar ratio of cGP/IGF-1 represents the amount of bioavailable and functional IGF-1 in circulation.

cyclic glycine-proline (cGP) | ageing brain | stroke | Insulin-like growth factor-1 | cogntive function | hypertension | dementia | neurodegeneration

1. Formation of Endogenous cGP

Free IGF-1 is not enzymatically stable [1]. The enzymatic breakdown of IGF-1 at its N-terminal forms des-N-(1-3) IGF-1 (des-IGF-1) in plasma and brain tissue [2][3]. As a major binding site for IGFBPs, the loss of the N-terminal tripeptide, Glycine-Proline-Glutamate (GPE) reduces the binding affinity of des-IGF-1 for IGFBPs [4]. IGFBPs treatment reduces the formation of GPE by increasing IGF-1 binding [5]. The different rotation in the prolyl–glycine bond determines the trans or cis isoform GPE, with a constant 4:1 ratio of trans/cis isoforms in circulation [6]. Both isoforms of GPE are also enzymatically unstable [7][8]. The cis isoform of GPE forms cGP through cyclisation after the enzymatic cleavage of glutamate [6]. By evaluating the amount of GPE in plasma and brain tissues in rats, Baker and others described the enzymatic degradation of GPE [8]. Unlike the linear isoforms of GPE, such a cyclic structure may render cGP resistant to enzymatic breakdown and become more lipophilic for better tissue uptake [6][9].

2. cGP Is a Bioactive

Following the isolation of cGP from rat brain tissue [10], the neurological function of cGP and its analogues have been extensively examined by different researchers. Gudasheva et al. reported the efficacy of cGP and its analogues in protecting the brain from ischemic brain injuries and improving cognitive function [11][12][13]. Experimental research shows that both GPE and cGP exhibit pharmacological effects similar to those of IGF-1. For example, the administration of GPE, or cGP at a dose equimolar to those of IGF-1 protects the brain from hypoxic-ischemic injury in the same experimental setting in rats [7][14][15][16]. As a transient intermediate between IGF-1 and

cGP, the biological effects of GPE may be mediated through cGP. cGP is a small, lipophilic, enzymatic stable peptide. It is orally bioavailable with effective tissue (brain) uptake [16][17][18][19].

As part of its pharmaceutical development, a structural analogue of cGP, cyclic Gly-2allyl-Pro, protects the brain from ischemic injury, 6-OHDA-induced motor deficit, and scopolamine-induced acute memory impairment [15][20][21]. Clinical trials of cGP analogues showed promising outcomes for treating developmental neurological conditions [22][23][24][25], including Rett syndrome and Fragile X syndrome [22][26][27]. The neuroprotective effects of cGP have been shown to be associated with promoting neurogenesis, synaptic function, and vascular remodeling, and inhibiting inflammation, apoptosis, and vascular damage. However, these changes could be a result, rather than the cause, of reduced brain damage. Several receptors have been suggested to mediate the biological function of GPE and cGP; for example, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor, γ-aminobutyric acid, *N*-methyl-D-aspartate receptor, and metabotropic glutamate receptors [11][28][29][30], but the results are inconclusive. It is also known that the N-terminal of IGF-1 does not interact with the IGF-1 receptors [30][31]. The investigations into the mechanism of cGP led to the discovery of its role in regulating IGF-1 function and its association with age-related neurological conditions.

3. Mode of Action of cGP

Recent research results have provided the initial evidence for the mode and mechanism of cGP action, in which cGP action is mediated by normalizing IGF-1 bioavailability, and thus its function. The effects of cGP in IGF-1 function are first evaluated by examining the survival/growth and tube formation of human endothelial cells [32]. The results from several experimental paradigms reveal that treatment with cGP can either promote, maintain, or inhibit IGF-1-induced cell survival/growth when IGF-1 treatment alone fails to stimulate, or moderately or highly stimulates, cell survival/growth [32]. The presence of IGF-1 is essential for cGP to be effective in experimental settings. The overexpression or knockdown plasmids of IGF-1 receptors has further confirmed that the efficacy of cGP in endothelial cells is mediated via IGF-1 and is a result of the regulated IGF-1 effect [32]. To maintain the homeostasis of IGF-1 function, cGP stimulates IGF-1 function when IGF-1 function is insufficient, or inhibits IGF-1 function when IGF-1 is overly promoted, without altering the function of IGF-1 within a physiological range. The different effects of cGP on endothelial cell survival/growth are dependent on its concentration relative to that of IGF-1, in which a higher cGP/IGF-1 molar ratio leads to a stimulatory effect, whereas a lower cGP/IGF-1 ratio results in an inhibitory effect. cGP does not alter IGF-1-induced cell growth/survival when the concentration of cGP is similar to that of IGF-1 [32]. Experimental studies have also demonstrated vascular effects of IGF-1 [33][34], cGP [32], or GPE, a transient intermediate between IGF-1 and cGP [35][36] in preventing vascular damage and improving vascular remodeling.

As a metabolite from a major binding site of IGF-1, cGP retains the binding affinity to IGFBP-3, thus competing with IGF-1 for the binding of IGFBP-3. Concentration dependent competitive binding between cGP and IGF-1 to IGFBP-3 is evaluated using an in vitro peptide–peptide interaction assay [32]. When IGFBP-3 is incubated with different concentrations of cGP and IGF-1 in differing ratios, a higher ratio of cGP/IGF-1 increases the percentage of unbound/total IGF-1, whereas a lower ratio of cGP/IGF-1 reduces the percentage of unbound/total IGF-1 [32][37].

These data suggest that the cGP can interfere with IGF-1 binding to IGFBP-3, and the ratio of cGP/IGF-1 represents the amount of free IGF-1 [32]. This hypothesis has been subsequently confirmed by experimental studies and clinical observations.

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