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·Review·

Carboxypeptidase E (NF-α1): a new trophic factor in neuroprotection

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Carboxypeptidase E (CPE) is a prohormone-processing enzyme and sorting receptor that functions intracellularly. However, recent studies have demonstrated that CPE acts as a trophic factor extracellularly to up-regulate the expression of a pro-survival gene. This mini-review summarizes the roles of CPE in neuroprotection and the implications for neurodegenerative diseases.

Keywords: carboxypeptidase E; NF-α1; neuroprotection; stress; neurodegenerative disease

Introduction

Carboxypeptidase E (CPE) was first identified as a prohormone-processing enzyme[1, 2] that cleaves the C-terminally-extended basic residues (arginine and/or lysine) from peptide intermediates to produce bioactive neuropeptides and peptide hormones[3]. Since then, CPE has been found to possess various non-enzymatic activities. It is a regulated secretory pathway (RSP) sorting receptor[4] and targets pro-BDNF (brain-derived neurotrophic factor) to the regulated secretory pathway[5], but not nerve growth factor (NGF)[6]. Moreover, the cytoplasmic tail of CPE drives bi-directional transport of BDNF vesicles and its secretion in hippocampal neurons[7] and mediates the localization of synaptic vesicles to the pre-active zone in hypothalamic neurons[8]. Recent studies suggest that CPE is a new trophic factor that functions independently of its enzymatic activity. Here, we review the role of CPE in neuroprotection and the implications for neurodegenerative diseases.

CPE Expression in Brain Is Modulated by Stress

The first evidence that CPE functions as a neuroprotective protein came from correlative studies showing that it is up-regulated after stress. In the hippocampal CA1 and CA3 regions and in the cortex, increased levels of CPE mRNA and protein occur after 15 min of transient global ischemia followed by 8 h of reperfusion[9]. Moreover, Zhou *et al.* showed that in mice lacking an active CPE protease, a sublethal episode of focal cerebral ischemia results in abundant TUNEL-positive cells in the ischemic cortex, in contrast to only a few in the ischemic cortex of wild-type mice, suggesting that neurons are more susceptible to cell death in the absence of CPE[10]. CPE gene expression is up-regulated in the amygdala of rats exposed to cat odor, a stressor that induces anxiety-like behavior[11]. Also, CPE protein and mRNA are significantly elevated in the mouse CA3 region after mild chronic restraint stress[12]. This form of stress in mice also results in elevation of the pro-survival Bcl-2 protein/mRNA and p-AKT levels in the hippocampus, while CPE knockout (KO) mice[13] show a decrease[12]. Thus the up-regulation of CPE during stress contributes to neuronal survival. In contrast to the increased CPE, the offspring of pregnant ewes subjected to aversive interactions with human handlers show a decrease in CPE concomitant with abnormal dendritic spine density and morphogenesis in the prefrontal cortex and hippocampus[14]. This is consistent with a report showing that CPE KO mice exhibit abnormal dendritic arborization and spine morphology in these areas[15], demonstrating that

CPE plays a role in normal cytoarchitecture and neuronal function in these brain regions. Supporting evidence came from the finding that CPE is a binding partner for nitric oxide synthase 1 adaptor protein, a protein involved in the regulation of dendritic patterning in hippocampal neurons[16].

CPE Knock-out Mice Have Neurological Deficits

Since CPE has been implicated in neuroprotection, one would expect that a lack of CPE in the brain would result in neurodegeneration and behavioral abnormalities. Indeed, CPE KO mice display memory deficits as revealed by the Morris water maze, object preference, and social transmission of food preference[17], and show no evoked long-term potentiation (which is required for memory and learning) in hippocampal slices. Neonatal CPE KO mice also exhibit a significant delay in eye opening, which reflects a developmental delay in the central nervous system (Cawley *et al*., unpublished data). In addition, *Cpe*$^{fat/fat}$ mutant mice lacking CPE exhibit anxiety- and/or depression-like behaviors[18]. CPE KO mice at 4 weeks of age or older, but not at 3 weeks, exhibit marked degeneration of the CA3 region which normally expresses high levels of CPE[17]. The neurodegeneration in CPE KO mice was initially thought to be a developmental defect. However, a recent study[19] suggests that this is due to weaning, because the hippocampus is intact in 4-week-old CPE KO mice that have not yet weaned, but weaning of CPE KO mice at 2 or 3 weeks of age, which involves maternal separation (emotional stress) and ear-tagging and tail-snipping for genotyping (physical stress), each results in degeneration of the CA3 neurons by 3 and 4 weeks. Interestingly, daily treatment with carbamazepine, an antiepileptic agent, in 2-week-old CPE KO mice for 2 weeks prevents the neurodegeneration, despite the weaning process at 3 weeks[19]. Therefore, emotional and physical stress in early life lowers the seizure threshold and exacerbates the degeneration of susceptible neurons in the CA3 region in the absence of the neuroprotective protein, CPE.

CPE Acts as a Trophic Factor to Promote Neuronal Survival

The animal model studies discussed above along with *ex vivo* studies suggest that CPE is a neuroprotective protein. For example, primary cultured hippocampal neurons from CPE KO mice are more prone to die in culture than those from wild-type littermates[20]. Also, low-potassium-induced apoptosis is significantly increased in CPE$^{+/-}$ cerebellar granule neurons (CGNs) in comparison to CPE$^{+/+}$ CGNs, indicating that CPE plays a neuroprotective role in this type of neuron as well as hippocampal neurons[21]. More direct evidence came from a study showing that transduction of an adenovirus carrying CPE into primary cultured hippocampal neurons, causing over-expression of CPE, protects against hydrogen peroxide-induced neurotoxicity[17]. Although the mechanism was unknown, CPE was assumed to function intracellularly to process some precursor protein that had neuroprotective activity. However, a recent study suggests that CPE acts extracellularly as a neuroprotective trophic factor, independent of its enzymatic activity. Extracellular CPE functions by signaling through the ERK and Akt pathways to up-regulate the expression of the pro-survival protein Bcl-2 and inhibit caspase-3 activation, indicating that it confers neuroprotection against cell death by modulating mitochondrial energetics[20]. CPE also protects hippocampal neurons against cell death induced by oxidative stress and glutamate neurotoxicity. In addition, it promotes the long-term survival of embryonic hippocampal neurons from CPE KO mice in culture[20]. Since the pattern of the CPE-induced activation of the ERK and Akt signaling pathways is similar to classic trophic factors such as BDNF and NGF, an alternative name was given to CPE, “Neurotrophic Factor-α1” (NF-α1), indicating its functions as a trophic factor. In addition to primary cultured neurons, a recent study further found that secreted NF-α1 protects PC12 cells, a pheochromocytoma cell line, against starvation- and hypoxia-induced cell death[22].

CPE in Neurodegenerative Diseases

Since CPE can function as a trophic factor, it is not surprising to find that aberrations of its expression and/or distribution occur in neurodegenerative diseases. In contrast to normal human cortex where CPE is preferentially localized in dendrites and perikarya, cortices from patients with Alzheimer’s disease (AD) show a high accumulation of CPE in dystrophic neurites surrounding amyloid beta. Of note, a similar accumulation of CPE occurs in a mouse model of AD[23]. This pattern of accumulation is similar to

trophic factors and neuropeptides such as galanin. The overexpression of galanin in AD brains has been shown to promote neuronal survival[24] and exogenous galanin has neuroprotective effects in a rodent model of AD[25]. Thus we hypothesize that the accumulation of CPE in dystrophic neurites in AD is a self-defense mechanism to delay the onset and progression of AD. Interestingly, a CPE mutant named "QQ CPE", has been found in the cortex of a patient with AD[3]. Cell biological studies demonstrated that QQ CPE is synthesized but fails to be secreted when transfected into neuro2a cells, a neuroendocrine cell line. In addition, co-expression of wild-type and QQ CPE results in the degradation of both forms and a reduction in the secretion of wild-type CPE, indicating that the mutant acts in a dominant-negative manner[3]. Overexpression of QQ CPE by adenovirus transduction in rat hippocampal and cortical neurons results in increased levels of CHOP (C/EBP homologous protein), a transcription factor induced by endoplasmic reticulum stress-induced apoptosis, decreased levels of Bcl-2, and increased cytotoxicity and neuronal death[3]. Hence, neurons expressing QQ CPE may lack the neuroprotective functions of CPE and this may lead to neurodegenerative diseases, including AD, with aging. Indeed, analysis of CPE proteolytic activity in Brodmann's area 21 of normal and AD patients postmortem shows changes in the activity of both soluble and membrane forms of CPE, suggesting changes in the levels of CPE protein in the AD patients[26]. In addition, in cathepsin B and L double-knockout mice, a model of neuronal ceroid lipofuscinoses with early-onset neurodegeneration, CPE is increased >10 folds[27], presumably to compensate for the lack of the two enzymes. However, given the new finding that CPE has trophic properties, this 10-fold increase is likely to protect neurons from further degeneration. In addition, in experimental autoimmune encephalomyelitis (EAE)[28], a mouse model of multiple sclerosis, a trait locus for EAE has been mapped to the *Cpe* gene on chromosome 8, while microarray data from the inflamed spinal cord of EAE mice shows a decrease in CPE concomitant with an increase in the severity of the disease[29].

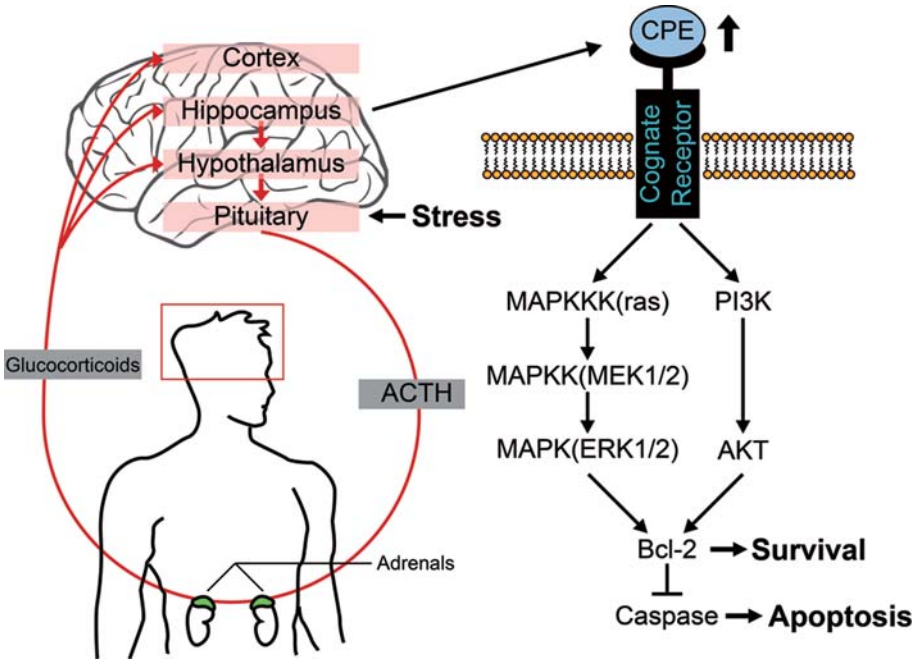

Fig. 1. Pathways for CPE-mediated neuronal survival during stress. During stress, ACTH is released into the circulation from the pituitary, which then stimulates glucocorticoid release from the adrenals. Glucocorticoids are then transported to the hippocampus which enhances the expression and secretion of CPE. CPE binds to a cognate receptor in hippocampal neurons to activate ERK and AKT signaling pathways which then mediate the upregulation of expression of Bcl-2, an anti-apoptotic protein. Bcl-2 inhibits the activation of caspases to prevent cell death and promote cell survival.

Conclusions

Here we have reviewed recent evidence that CPE is a new trophic factor and is involved in neurodegenerative diseases. CPE joins the ranks of other important neurotrophins such as BDNF and NGF, and plays pivotal roles in neuroprotection and neuronal survival during stress-induced apoptosis (Fig. 1) and neural development, given a recent study showing that CPE is highly expressed in neural stem cells[30]. Indeed, CPE is a negative regulator of proliferation in adult neural stem cells[30]. In addition, the mechanisms underlying the neuroprotection by CPE deserve further investigation, such as identifying the receptor to which it binds to activate the ERK and Akt signaling pathways (see Fig. 1). CPE could directly bind to a cognate receptor to function as a trophic factor, or act as a binding partner to activate the downstream signaling pathways. One example of the latter is that CPE forms a complex with the Wnt3a ligand and the Frizzled receptor to inhibit the wnt signaling pathway[31]. In addition, the molecular domain of CPE, which is responsible for the neuroprotective effects, needs to be explored for therapeutic use in drug design. Although many efforts have been made to understand and cure neurodegenerative diseases, successful treatment is still lacking, and the available therapies provide only symptomatic improvement. CPE is an emerging and a promising therapeutic target for neurodegenerative diseases. Encouragingly, clinical trials have shown that neurotrophic factors are potentially effective in treating AD[32]. Thus, continued investigations into the function of CPE/NF-α1 as a new trophic factor are warranted.

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