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Research Article

Envisioning PACAP-Based Therapy for Alzheimer's Disease.

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Abstract

Alzheimer's disease (AD) is the most common cause of dementia in elderly adults. With the growing number of people living to an older age, there is an urgency to better understand elements of the pathogenic pathway, discover agents that target these elements, and establish their roles in the treatment and prevention of AD. AD pathogenesis is contributed by a complex of molecular events involving abnormal accumulation of β amyloid, Tau protein hyperphosphorylation, mitochondrial dysfunction, synaptic dysfunction and inflammation. National Institute of Health Alzheimer's Disease Research Summit in 2012 recommended diversification of therapeutic targets in future research. Our recent work found that Pituitary Adenylate Cyclase Activating Polypeptide (PACAP) is significantly reduced in post-mortem brain tissues obtained from AD patients. PACAP is intrinsically expressed in mammals and is considered to be a potent neurotrophic and neuroprotective peptide. While the preclinical studies applying PACAP to treat Alzheimer's disease are only but a few, a significant number of studies indirectly point to the promises of PACAP-based therapy. In this review, we summarize the evidence of PACAP deficit in Alzheimer's disease, the potential effects of PACAP on β -amyloid, Tau protein, mitochondrial function, cognitive function and neuroinflammation. We discuss the promises and challenges associated with PACAP-based therapy.

Keywords: PACAP; Alzheimer's Disease; Mitochondria; Cognitive Function; Neuroinflammation

Introduction

In response to multiple failures in therapeutic clinical trials for Alzheimer's disease (AD) in the past decade, the National Institute of Health (NIH) -National Institute of Aging (NIA) Alzheimer's Disease Research Summit in 2012 recommended diversification of therapeutic targets in future research (NIH, 2012). The Summit recommendation also emphasized the importance of cross validation of a potential target in both patients and animal models, as an essential component for translational study. Our recent work found that Pituitary Adenylate Cyclase Activating Polypeptide (PACAP) is significantly reduced in post-mortem brain tissues obtained from AD patients (21). Could restoration of normal PACAP levels prevent or delay the AD pathogenesis? In this mini-review, we summarize

the evidence documenting the relevance of PACAP in Alzheimer's disease and analyze the promises and challenges of PACAP based therapy for AD.

PACAP in the nervous system

PACAP was initially isolated from bovine pituitary gland by Arimura and colleagues 25 years ago (38). This molecule increases cyclic AMP by activating adenylate cyclase, and hence named as Pituitary Adenylate Cyclase Activating Polypeptide (PACAP). It is characterized as a small peptide with 38 amino acids in full length or 27 amino acids in the short version. Subsequent research found PACAP expression is widely distributed through multiple brain regions and peripheral organs. While acknowledging the importance of PACAP in multiple organs, here we only focus our discussion on PACAP in the brain. The predominant form in mammal CNS is PACAP38, albeit PACAP27 exists in relative minor proportions. Both forms of

PACAP activate PACAP-specific receptor type 1 (PAC1), but also Vasoactive Intestine Peptide (VIP) receptor type 1 and 2 (VPAC1 and VPAC2). Among these, PAC1 is specific to PACAP and is the major PACAP receptor in brain tissue. The activation of PAC1 receptors activates G-proteins. Depending on the splice variant of PACAP and the subtype of G-protein to which the receptor is coupled, PACAP activates either the Adenylate Cyclase (AC)-cyclic adenosine monophosphate response (cAMP)-Protein Kinase A (PKA) pathway or Phospholipase C (PLC)-Protein Kinase C (PKC) pathway. Through these complicated intracellular signalling pathways, PACAP promotes neurogenesis and differentiation during neurodevelopment, and PACAP inhibits apoptosis and provides neuroprotection under various toxic stimuli. The neuroprotective properties of PACAP provides the rationale for using PACAP to treat neurodegenerative disorders (48), although this goal has not yet reached the clinical stage. PACAP shares a similar sequence with VIP, which also shows protective effects but is beyond the scope of this article.

PACAP deficit in Alzheimer's Disease

We recently reported a PACAP deficit in Alzheimer's disease [21]. We measured PACAP levels from post-mortem brain tissues obtained from AD patients and age-matched cognitively normal subjects. PACAP protein and expression levels were reduced in frontal cortex, medial temporal cortex, entorhinal cortex, visual cortex and in cerebrospinal fluid (CSF). This reduction could not simply be attributed to diffusive cell loss because (1) we normalized PACAP levels to protein quantity and PACAP levels remained low even after adjusting for any protein differences; and (2) PACAP mRNA [Adenylate Cyclase Peptides A (ADCYPA) gene] transcription was reduced in these brain areas. CSF comparisons revealed that PACAP reduction is relatively specific to AD; PACAP deficiency was not seen in Parkinson's disease dementia or frontotemporal dementia. In addition, we observed PACAP reduction in triple transgenic AD mice [20]. The PACAP deficit in both human AD and AD mice establishes a strong rationale to develop PACAP based therapy. Indeed, we are encouraged by numerous previous studies by us and others pointing to the promises of PACAP therapy in AD. We analyse these studies in the following sections.

The effect of PACAP on β Amyloid and Tauopathy

The pathogenesis of AD is a complex process involving multiple intertwining factors including beta amyloid plaques and neurofibrillary tangles. Among these, the production, oligomerization, and deposition of beta amyloid ($A\beta$) are considered to be the initiating event, because amyloid precursor protein (APP) transgenic mice have cognitive deficits. Amyloid precursor protein (APP) is cleaved sequentially by β -secretase (BACE1) and gamma-secretase, producing small peptide $A\beta$, which self-associates into soluble oligomers, molecules toxic to neurons. Further aggregating oligomers may eventually precipitate to form amyloid plaques, a hallmark for AD pathology. [39]. In contrast, α -secretase cleaves APP at a

different site, producing soluble APP α (sAPP α), which is not harmful. A desirable therapeutic strategy is to inhibit BACE1 while enhancing α -secretase. Intranasally injected PACAP to APP transgenic mice showed a reduction of $A\beta$ and an increase of sAPP α [47]. This suggests PACAP may shift the APP processing bias towards α -secretase and away from β -secretase. However, there remains no direct evidence showing that PACAP changed the expression or activity of these APP processing enzymes. Nevertheless, BACE1 gene has an upstream cAMP element-binding protein (CREB) cis-regulatory sequence implicating an unknown modulatory role of cAMP-PKA on BACE1 transcription [51].

The neurofibrillary tangles (NFT) formed by Tau protein aggregates are contributed by excessive Tau phosphorylation [16] and abnormal proteolytic truncation [14]. The hyperphosphorylation is a result of excessive kinase activity and/or reduced phosphatase activity [29]. Glycogen synthase kinase 3 beta (GSK3 β) is one of the most well characterized Tau kinases and Protein Phosphatase 2A (PP2A) is the key phosphatase [29]. The intracellular signalling of cAMP-PKA inactivates GSK3 β [7] and stimulates PP2A [10]. PACAP may likely reduce Tau hyperphosphorylation by activating cAMP-PKA, thus inhibiting GSK3 β activity and stimulating PP2A. However, there remains no direct evidence supporting this hypothesis. Besides Tau hyperphosphorylation, truncation of Tau protein at Aspartate (D421) site by caspase-3 results in the fragments that are most significantly associated with pathological NFT formation and cognitive deficits [14, 28]. PACAP27 reduces Tau truncation by diminishing caspase activity [36].

The effect of PACAP on mitochondrial function and brain resilience

Mitochondrial dysfunction is a crucial event in AD pathogenesis [2,4]. Instead of producing constant and sufficient ATP, dysfunctional mitochondria fail to keep oxidative respiration within the physiological demand and consequently leads to over production of oxygen free radicals and cellular apoptosis [44,55]. Multiple studies had shown PACAP inhibits mitochondrial Bcl-2 (B-cell lymphoma 2) and caspase activity [8,15,25,52] which is directly inducing irreversible apoptosis. Our work focused on the upstream mitochondrial respiratory chain, primarily on complex I [20]. PACAP enhances mitochondrial capacity without hastening the respiratory speed, thus providing sufficient energy but not increasing free radicals. This mitochondrial enhancing effect was partially attributed to an upregulation of Sirt3, a member molecule of SIRTUIN family, which is an important deacetylating enzyme enhancing mitochondrial activity [20,30,50].

Interestingly, some patients had characteristic amyloid plaques and neurofibrillary tangles in post-mortem brain without antemortem cognitive deficits, which is explained by "neuronal resilience" [11]. Enhancing neuronal resilience

in the presence of A β load is an attractive approach for AD therapy. We showed that A β 42 oligomers at a concentration of 0.5 μ M reduced 24 hr survival rate in cultured primary cortical neurons, and PACAP enhances the neuronal survival rate in the presence of A β 42 (20). It was also observed that PACAP (50 nM) protects pheochromocytoma cell line (PC12 cells) from A β induced toxicity [11,32]. Thus, PACAP may be an effective neuroprotective agent to enhance neuronal resilience.

The effect of PACAP on cognitive function

Cognitive impairment is the primary clinical diagnostic criteria for Alzheimer's disease, and cognitive improvement is the surrogate marker for evaluating the effectiveness of therapies [56]. The earliest clue of PACAP's effects on memory came from amnesiac (amn) gene mutant flies [13]. Amn gene encodes an invertebrate homolog of vertebral PACAP. PACAP is also essential for associative memory in *Lymnaea stagnalis*, a prototype model for studying the molecular mechanism of simple associative learning [45]. PACAP or PAC1 receptor knockout mice showed significant cognitive and memory impairment [34]. As PACAP is an important factor in neurogenesis, cell differentiation and endocrine functions, the complete PACAP or PAC1 receptor knockout inevitably compromised neurodevelopment. The cognitive impairment of PACAP knockout is most likely a consequence of insufficient early development, and is by no means an authentic model for senile degeneration like AD. Indeed, PACAP knockout mice showed severe locomotive impairment, social activity abnormality and anxiety-like behavior [22]. Although these psychiatric phenotypes may be associated with Alzheimer's disease in the late stage, they are not the primary intrinsic AD clinical manifestations. PACAP knockout mice may not be helpful in elucidating AD type memory deficit unless we develop brain-specific and age-specific conditional knockout mice in the future.

Nevertheless, intranasal administration of PACAP enhances novel object recognition in SAMP8 mice [43] and APP transgenic mice [47] two commonly used strains for AD models. This suggests that PACAP enhances memory under both physiological and pathological conditions. As we reported PACAP decreases in AD, others reported PACAP increases in posttraumatic stress disorder (PTSD) [49]. Interestingly, PACAP seems to correlate with a spectrum of memory from PTSD to AD, because PTSD is an excessive memory of a detrimental life event supposed to be forgotten within 1-3 months while AD is a lost memory of recent events supposed to be retained. In this way, both animal and human studies point out the essential role of PACAP in memory.

The memory enhancing effect of PACAP may be mediated by multiple molecular mechanisms. For example, PACAP enhance N-methyl-D-aspartate receptor (NMDA-R) channel current. This enhancement was attributed to NMDA-R type 2A (NR2A) subunits through activation of PLC/PKC/Pyk2/Src signalling pathway [31,59] and NMDA-R type 2B (NR2B) subunits through the PKA

signalling pathway [58]. Regardless of the detailed mechanism, PACAP enhances calcium influx through NMDA channels. In addition, 10 nM to 100 nM PACAP is optimal for enhancing AMPA current in suprachiasmatic nucleus [37]. We measured an average of 2 ng/ml PACAP in nondemented human CSF, which is equivalent to approximately 0.5 nM if we assume the predominant component is full length PACAP38 (MW. ~4KD). Thus, PACAP likely stimulates both NMDA and AMPA currents at this physiological concentration. In addition to the effects on the synaptic glutamate receptors, PACAP may affect intrinsic voltage gated ion channels. For example, PACAP inhibits Hyperpolarization-activated cyclic nucleotide-gated (HCN) channels [53,23]. Our previous work showed that PACAP reduces A-type potassium (K) channel [18,19]. Given that A-type K channel (KA) is a gate keeper to filter out dendritic AP back propagation, reducing KA may facilitate synaptic transmission [24,26].

The effect of PACAP on Neuroinflammation

Regional inflammatory reactions in the brain, especially around amyloid plaques, have been identified as an exacerbating factor for AD pathogenesis [12,17,35]. PACAP is a potent anti-inflammatory peptide (3,9). It downregulates the expression of proinflammatory factors (TNF α , IL-2, IL-1, IL-6, IL-12, MIF, iNOS) but upregulates anti-inflammatory cytokine IL-10, and favors different ion toward protective T2 subtype [9]. In CNS specifically, PACAP knockout mice showed stronger microglial activation [57] whereas PACAP protected against ischemic/hypoxic injury via inhibition of microglia activation [46]. Using PACAP knockout mice to induce experimental autoimmune encephalomyelitis (EAE, a model for multiple sclerosis) resulted in a more severe demyelination and stronger inflammatory activity compared to EAE induced using wild type mice [55]. In addition to the downregulation of proinflammatory factors via transcriptional modulation, PACAP also enhances the neuronal resistance to TNF α -induced insults via activation of both PLC and AC pathways (27). Taken together, the combined effect of anti-inflammation and neuroprotection may be well utilized in AD therapy.

The advantages and challenges of PACAP based therapy

PACAP based therapy is advantageous over current available therapy. First, PACAP is a highly potent neuroprotective factors proven effective in multiple in vitro and in vivo experiments [48]. Second, PACAP addresses multiple steps in AD pathogenesis and enhances memory function, based on our analysis above. These effects may provide a synergistic therapy to AD. Third, PACAP has a therapeutic effect for diabetes [40,41] and chronic brain injury [33], the two important risk factors for AD. Fourth, unlike Nerve Growth Factor (NGF) or Brain-derived Nerve Factor (BDNF), PACAP does not pose a risk for tumorigenesis. Instead, PACAP prevents brain tumors by promoting cell differentiation [6].

However, there remain significant challenges to applying PACAP therapy to clinical application. Only 0.1% fraction of intravenous injected PACAP reached the brain tissue[5]. This is not a failure to cross blood brain barrier. In fact, PACAP as a small peptide has high diffusion rate across BBB. Rather, PACAP had been metabolised and degraded in plasma before reaching the brain. Although this small fraction into brain is sufficient to induce a neuroprotective effect, a transient surge in blood PACAP may have serious side effects. For example, intravenous injection of PACAP induced headache in patients with a clear history of migraine[1]. It remains unclear whether PACAP induces similar degrees of headache in migraine-free human subjects, but it is advisable to be cautious. Thus, targeting PACAP into the brain without increasing blood PACAP is a technical challenge. Fortunately, intranasal administration of PACAP in rodent AD models has been successful. Using 131I-labeled PACAP as a tracer, Nonaka et al showed that 1-2% of intranasal PACAP reached the brain tissue and distributed among all the lobes with only slight variation. Only 2% PACAP reach plasma[43]. PACAP does not induce harm on olfactory epithelium but instead provides additional protective effects (18, 19).

Conclusion

In summary, PACAP deficit is apparent in Alzheimer's disease. PACAP reduces the production of beta-amyloid, reduces the activity of GSK3 β , prevents the irreversible Tau protein cleavage by inhibiting caspase activation, and enhances mitochondrial respiratory function and neuronal resilience in the presence of beta-amyloid toxicity. PACAP enhances long term memory by functionally boosting synaptic function through modulating AMPA, NMDA, HCN and potassium channels. In addition, PACAP addresses multiple risk factors associated with AD. Although the mechanism and time course for PACAP depletion in AD patients is unknown, replacement of lost PACAP may slow disease progression. Intranasal administration of PACAP in two rodent AD models showed significant enhancement of cognitive function. Taken together, PACAP-based therapy is likely a promising novel approach for AD therapy.

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