JACOBS PUBLISHERS

OPEN ACCESS

Jacobs Journal of Neurology and Neuroscience

Research Article

Envisioning PACAP-Based Therapy for Alzheimer's Disease.

Pengcheng Han[1]*, Jiong Shi[1], Mary T. Lucero[2,3]

[1]Dignity Health St. Joseph Hospital and Medical Center, 350 W. Thomas Rd, Phoenix, AZ 85013

[2]University of Utah School of Medicine, 30 N. 1900 E. Salt Lake City, UT 84132

[3]American University of the Caribbean School of Medicine, One University Rd, Cupecoy, St. Maarten, Netherland Antilles

*Corresponding author: Dr. Pengcheng Han, Dignity Health St. Joseph Hospital and Medical Center, 350 W. Thomas Rd, Phoenix, AZ 85013, USA, Email: Pengcheng.Han@dignityhealth.org

Received: 07-29-2014

Accepted: 08-01-2014

Published: 08-25-2014

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 AdenylateCyclase 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 AdenylateCyclase 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 adenylatecyclase, 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 I and 2 (VPAC1 and VPAC2). Among these, PAC1 is specific to PACAP and is the major PACAP receptor in brain tissue. The activation ofPAC1 receptors activates G-proteins. Depending on the splice variant of PACAP and the subtype of G-protein to which the receptor is coupled, PACAP activateseither the Adenylate Cyclase (AC)- cyclic adenosinemonophosphateresponse (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 levelswere reduced in frontal cortex, medial temporal cortex, entorhinalcortex, 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 [AdenylateCyclase 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 Amyloidand 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 (Aβ) areconsidered 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 Aβ, 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 APPα (sAPPα), 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 Aβ and an increase of sAPPα[47].This suggests PACAP may shift the APP processing bias towards α-secretaseand 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 (GSK3β) 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 GSK3β [7] and stimulates PP2A [10]. PACAP may likely reduce Tau hyperphosphorylation by activating cAMP-PKA, thus inhibiting GSK3β 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 Aβ load is an attractive approach for AD therapy. We showed that Aβ42 oligomers at a concentration of 0.5 μM reduced 24 hr survival rate in cultured primary cortical neurons, and PACAP enhances the neuronal survival rate in the presence of Aβ42 (20). It was also observed that PACAP (50 nM) protects pheochromocytoma cell line (PC12 cells) from Aβ 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, andcognitive improvement is the surrogate marker for evaluating the effectiveness of therapies[56]. The earliest clue of PACAP's effects on memory came fromamnesiac (amn) gene mutant flies [13]. Amn gene encodes an invertebrate homolog of vertebral PACAP. PACAP is also essential for associative memory in Lymnaeastagnalis, 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 ADclinical 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 enhancesnovel 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 (TNFα, 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 TNFα-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 GSK3β, 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.

Acknowledgements

P. Han is supported by Arizona Alzheimer's Disease Consortium Pilot grant, Barrow Neurological Foundation, and UA-BNI joint translational neuroscience award.

References

1. Amin FM, Hougaard A, Schytz HW, Asghar MS, Lundholm E et al. Investigation of the pathophysiological mechanisms of migraine attacks induced by pituitary adenylatecyclase-activating polypeptide-38. Brain. 2014, 137: 779-794.

2. Beal MF Mitochondrial dysfunction in neurodegenerative diseases. Biochim Biophys Acta. 1998, 1366: 211-223.

3. Bik W, Wolinska-Witort E, Pawlak J, Skwarlo-Sonta K, Chmielowska M et al. PACAP 38 as a modulator of immune and endocrine responses during LPS-induced acute inflammation in rats. J Neuroimmunol. 2006, 177: 76-84.

4. Bonilla E, Tanji K, Hirano M, Vu TH, DiMauro S et al. Mitochondrial involvement in Alzheimer's disease. BiochimBiophysActa. 1999, 1410: 171-182.

5. Bourgault S, Chatenet D, Wurtz O, Doan ND, Leprince J et al. Strategies to convert PACAP from a hypophysiotropicneurohormone into a neuroprotective drug. Curr Pharm Des. 2011, 17: 1002-1024.

6. Cohen JR, Resnick DZ, Niewiadomski P, Dong H, Liau LM et al. Pituitary adenylyl cyclase activating polypeptide inhibits gli1 gene expression and proliferation in primary medulloblastoma derived tumorsphere cultures. BMC Cancer. 2010, 10: 676.

7. Cohen P, Frame S. The renaissance of GSK3. Nat Rev Mol Cell Biol. 2001, 2: 769-776.

8. Dejda A, Jolivel V, Bourgault S, Seaborn T, Fournier A et al. Inhibitory effect of PACAP on caspase activity in neuronal apoptosis: a better understanding towards therapeutic applications in neurodegenerative diseases. J Mol Neurosci. 2008, 36: 26-37.

9. Delgado M, Abad C, Martinez C, Juarranz MG, Leceta J et al. PACAP in immunity and inflammation. Ann N Y Acad Sci. 2003, 992: 141-157.

10. Dodge-Kafka KL, Bauman A, Mayer N, Henson E, Heredia L et al. cAMP-stimulated protein phosphatase 2A activity associated with muscle A kinase-anchoring protein (mAKAP) signaling complexes inhibits the phosphorylation and activity of the cAMP-specific phosphodiesterase PDE4D3. J Biol Chem. 2010, 285: 11078-11086.

11. Driscoll I, Troncoso J. Brain resilience and plasticity in the face of Alzheimer pathology. Curr Alzheimer Res. 2011, 8: 329.

12. Eikelenboom P, Rozemuller AJ, Hoozemans JJ, Veerhuis R, van Gool WA. Neuroinflammation and Alzheimer disease: clinical and therapeutic implications. Alzheimer Dis AssocDisord 14 Suppl. 2000, 1: S54-61.

13. Feany MB, Quinn WG. A neuropeptide gene defined by the Drosophila memory mutant amnesiac. Science. 1995, 268: 869-873.

14. Garcia-Sierra F, Mondragon-Rodriguez S, Basurto-Islas G. Truncation of tau protein and its pathological significance in Alzheimer's disease. J Alzheimers Dis. 2008, 14: 401-409.

15. Giunta S, Castorina A, Bucolo C, Magro G, Drago F et al. Early changes in pituitary adenylatecyclase-activating peptide, vasoactive intestinal peptide and related receptors expression in retina of streptozotocin-induced diabetic rats. Peptides. 2012, 37: 32-39.

16. Gong CX, Iqbal K. Hyperphosphorylation of microtubule-associated protein tau: a promising therapeutic target for Alzheimer disease. Curr Med Chem. 2008, 15: 2321-2328.

17. Graeber MB. Genetics of neuroinflammation in Alzheimer disease. Neurogenetics. 1999, 2: 135-136.

18. Han P, Lucero MT. Pituitary adenylatecyclase activating polypeptide reduces A-type K+ currents and caspase activity in cultured adult mouse olfactory neurons. Neuroscience. 2005, 134: 745-756.

19. Han P, Lucero MT. Pituitary adenylatecyclase activating polypeptide reduces expression of Kv1.4 and Kv4.2 subunits underlying A-type K(+) current in adult mouse olfactory neuroepithelia. Neuroscience. 2006, 138: 411-419.

20. Han P, Tang Z, Yin J, Maalouf M, Beach TG et al. Pituitary adenylatecyclase-activating polypeptide protects against beta-amyloid toxicity. Neurobiol Aging. (2014a), 35: 2064-2071.

21. Han P, Liang W, Baxter LC, Yin J, Tang Z et al. Pituitary adenylatecyclase-activating polypeptide is reduced in Alzheimer disease. Neurology. 2014b, 82: 1724-1728.

22. Hattori S, Takao K, Tanda K, Toyama K, Shintani N et al. Comprehensive behavioral analysis of pituitary adenylatecyclase-activating polypeptide (PACAP) knockout mice. Front Behav Neurosci. 2012, 6: 58.

23. He C, Chen F, Li B, Hu Z. Neurophysiology of HCN channels: from cellular functions to multiple regulations. Prog Neurobiol. 2013, 112: 1-23.

24. Hoffman DA, Magee JC, Colbert CM, Johnston D. K+ channel regulation of signal propagation in dendrites of hippocampal pyramidal neurons. Nature. 1997, 387: 869-875.

25. Horvath G, Racz B, Reglodi D, Kovacs K, Kiss P et al. Effects of PACAP on mitochondrial apoptotic pathways and cytokine expression in rats subjected to renal ischemia/reperfusion. J Mol Neurosci. 2010, 42: 411-418.

26. Johnston D, Hoffman DA, Magee JC, Poolos NP, Watanabe S et al. Dendritic potassium channels in hippocampal pyramidal neurons. J Physiol. 2000, 525 Pt 1: 75-81.

27. Kanekar S, Gandham M, Lucero MT. PACAP protects against TNFalpha-induced cell death in olfactory epithelium and olfactory placodal cell lines. Mol Cell Neurosci. 2010, 45: 345-354.

28. Kovacech B, Novak M. Tau truncation is a productive posttranslational modification of neurofibrillary degeneration in Alzheimer's disease. Curr Alzheimer Res. 2010, 7: 708-716.

29. Liu F, Liang Z, Gong CX. Hyperphosphorylation of tau and protein phosphatases in Alzheimer disease. Panminerva Med. 2006, 48: 97-108.

30. Lombard DB, Zwaans BM. SIRT3: as simple as it seems? Gerontology. 2013, 60: 56-64.

31. Macdonald DS, Weerapura M, Beazely MA, Martin L, Czerwinski W et al. Modulation of NMDA receptors by pituitary adenylatecyclase activating peptide in CA1 neurons requires G alpha q, protein kinase C, and activation of Src. J Neurosci. 2005, 25:11374-11384.

32. Manecka DL, Mahmood SF, Grumolato L, Lihrmann I, Anouar Y. Pituitary adenylatecyclase-activating polypeptide (PACAP) promotes both survival and neuritogenesis in PC12 cells through activation of nuclear factor kappaB (NF-kappaB) pathway: involvement of extracellular signal-regulated kinase (ERK), calcium, and c-REL. J Biol Chem. 2013, 288: 14936-14948.

33. Mao SS, Hua R, Zhao XP, Qin X, Sun ZQ et al. Exogenous administration of PACAP alleviates traumatic brain injury in rats through a mechanism involving the TLR4/MyD88/NF-kappaB pathway. J Neurotrauma. 2012, 29: 1941-1959.

34. Matsuyama S, Matsumoto A, Hashimoto H, Shintani N, Baba A. Impaired long-term potentiation in vivo in the dentate gyrus of pituitary adenylatecyclase-activating polypeptide (PACAP) or PACAP type 1 receptor-mutant mice. Neuroreport. 2003, 14: 2095-2098.

35. McGeer PL, McGeer EG, Yasojima K. Alzheimer disease and neuroinflammation. J Neural Transm Suppl. 2000, 59: 53-57.

36. Metcalfe MJ, Huang Q, Figueiredo-Pereira ME. Coordination between proteasome impairment and caspase activation leading to TAU pathology: neuroprotection by cAMP. Cell Death Dis. 2012, 3: e326.

37. Michel S, Itri J, Han JH, Gniotczynski K, Colwell CS. Regulation of glutamatergic signalling by PACAP in the mammalian suprachiasmatic nucleus. BMC Neurosci. 2006, 7: 15.

38. Miyata A, Arimura A, Dahl RR, Minamino N, Uehara A et al. Isolation of a novel 38 residue-hypothalamic polypeptide which stimulates adenylatecyclase in pituitary cells. Biochem Biophys Res Commun. 1989, 164: 567-574.

39. Musiek ES, Holtzman DM. Origins of Alzheimer's disease: reconciling cerebrospinal fluid biomarker and neuropathology data regarding the temporal sequence of amyloid-beta and tau involvement. Curr Opin Neurol. 2012, 25: 715-720.

40. Nakata M, Yada T. [Physiological and therapeutic roles of PACAP in glucose metabolism and diabetes]. Nihon Yakurigaku Zasshi. 2004, 123:267-273.

41. Nakata M, Yada T. PACAP in the glucose and energy homeostasis: physiological role and therapeutic potential. Curr Pharm Des. 2007, 13: 1105-1112.

42. NIH Alzheimer's Disease Research Summit offers research recommendations. 2012.

43. Nonaka N, Farr SA, Nakamachi T, Morley JE, Nakamura M et al. Intranasal administration of PACAP: uptake by brain and regional brain targeting with cyclodextrins. Peptides. 2012, 36: 168-175.

44. Ojaimi J, Byrne E. Mitochondrial function and alzheimer's disease. Biol Signals Recept. 2001, 10: 254-262.

45. Pirger Z, Laszlo Z, Hiripi L, Hernadi L, Toth G et al. Pituitary adenylatecyclase activating polypeptide (PACAP) and its receptors are present and biochemically active in the central nervous system of the pond snail Lymnaeastagnalis. J Mol Neurosci. 2010, 42: 464-471.

46. Qin X, Sun ZQ, Dai XJ, Mao SS, Zhang JL et al. Toll-like receptor 4 signaling is involved in PACAP-induced neuroprotection in BV2 microglial cells under OGD/reoxygenation. Neurol Res. 2012, 34: 379-389.

47. Rat D, Schmitt U, Tippmann F, Dewachter I, Theunis C et al. Neuropeptide pituitary adenylatecyclase-activating polypeptide (PACAP) slows down Alzheimer's disease-like pathology in amyloid precursor protein-transgenic mice. FASEB J. 2011, 25: 3208-3218.

48. Reglodi D, Kiss P, Lubics A, Tamas A. Review on the protective effects of PACAP in models of neurodegenerative diseases in vitro and in vivo. Curr Pharm Des. 2011, 17: 962-972.

49. Ressler KJ, Mercer KB, Bradley B, Jovanovic T, Mahan A et al. Post-traumatic stress disorder is associated with PACAP and the PAC1 receptor. Nature. 2011, 470: 492-497.

50. Samant SA, Zhang HJ, Hong Z, Pillai VB, Sundaresan NR et al. SIRT3 deacetylates and activates OPA1 to regulate mitochondrial dynamics during stress. Mol Cell Biol. 2013, 34: 807-819.

51. Sambamurti K, Kinsey R, Maloney B, Ge YW, Lahiri DK. Gene structure and organization of the human beta-secretase (BACE) promoter. FASEB J. 2004, 18: 1034-1036.

52. Seaborn T, Masmoudi-Kouli O, Fournier A, Vaudry H, Vaudry D. Protective effects of pituitary adenylatecyclase-activating polypeptide (PACAP) against apoptosis. Curr Pharm Des. 2011, 17: 204-214.

53. Sun QQ, Prince DA, Huguenard JR. Vasoactive intestinal polypeptide and pituitary adenylatecyclase-activating polypeptide activate hyperpolarization-activated cationic current and depolarize thalamocorticalneurons in vitro. J Neurosci. 2003, 23: 2751-2758.

54. Tabet N, Mantle D, Orrell M. Free radicals as mediators of toxicity in Alzheimer's disease: a review and hypothesis. Adverse Drug React Toxicol Rev. 2000, 19: 127-152.

55. Tan YV, Abad C, Lopez R, Dong H, Liu S, Lee A, Gomariz RP, Leceta J, Waschek JA. Pituitary adenylyl cyclase-activating polypeptide is an intrinsic regulator of Treg abundance and protects against experimental autoimmune encephalomyelitis. Proc Natl Acad Sci U S A. 2009, 106: 2012-2017.

56. Veroff AE, Bodick NC, Offen WW, Sramek JJ, Cutler NR. Efficacy of xanomeline in Alzheimer disease: cognitive improvement measured using the Computerized Neuropsychological Test Battery (CNTB). Alzheimer Dis AssocDisord. 1998, 12: 304-312.

57. Watson MB, Nobuta H, Abad C, Lee SK, Bala N, Zhu C, Richter F, Chesselet MF, Waschek JA. PACAP deficiency sensitizes nigrostriatal dopaminergic neurons to paraquat-induced damage and modulates central and peripheral inflammatory activation in mice. Neuroscience. 2013, 240: 277-286.

58. Yaka R, He DY, Phamluong K, Ron D. Pituitary adenylatecyclase-activating polypeptide (PACAP(1-38)) enhances N-methyl-D-aspartate receptor function and brain-derived neurotrophic factor expression via RACK1. J Biol Chem. 2003, 278: 9630-9638.

59. Yang K, Lei G, Jackson MF, Macdonald JF. The involvement of PACAP/VIP system in the synaptic transmission in the hippocampus. J Mol Neurosci. 2010, 42: 319-326.