

## Invited Mini Review

## Neuroprotective roles of pituitary adenylate cyclase-activating polypeptide in neurodegenerative diseases

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**Pituitary adenylate cyclase-activating polypeptide (PACAP) is a pleiotropic bioactive peptide that was first isolated from an ovine hypothalamus in 1989. PACAP belongs to the secretin/glucagon/vasoactive intestinal polypeptide (VIP) superfamily. PACAP is widely distributed in the central and peripheral nervous systems and acts as a neurotransmitter, neuromodulator, and neurotrophic factor via three major receptors (PAC1, VPAC1, and VPAC2). Recent studies have shown a neuroprotective role of PACAP using *in vitro* and *in vivo* models. In this review, we briefly summarize the current findings on the neurotrophic and neuroprotective effects of PACAP in different brain injury models, such as cerebral ischemia, Parkinson's disease (PD), and Alzheimer's disease (AD). This review will provide information for the future development of therapeutic strategies in treatment of these neurodegenerative diseases. [BMB Reports 2014; 47(7): 369-375]**

## INTRODUCTION

PACAP is a protein encoded by the *ADCYAP1* gene in humans. PACAP was isolated from ovine hypothalamus and named after its ability to stimulate cAMP formation in rat anterior pituitary cells (1). PACAP exists in a 27- and a 38-amino acid form (PACAP27 and PACAP38, respectively) processed from a prohormone precursor (2). Based on its amino acid composition, PACAP belongs to secretin/glucagon/vasoactive intestinal polypeptide (VIP) superfamily. The sequence homology of PACAP from protochordates to mammals suggests the conservation of important biological functions during evolution. Three G protein coupled seven transmembrane re-

ceptors (GPCR), PAC1, VPAC1, and VPAC2, have been cloned and identified as major PACAP receptors. PAC1 receptor acts as a PACAP-selective receptor, whereas VPAC1 and VPAC2 receptors have equal affinities for PACAP and VIP. Several PAC1 receptor isoforms are generated from alternative splicing of the N-terminal extracellular domain and the C-terminal cytoplasmic intracellular loop (ic3) (3-5). Cell-type specific expression of PACAP receptors determines relative ligand-binding potency and distinct patterns of intracellular signaling pathways (6). All PACAP receptors are coupled to adenylate cyclases (ACs) and increase intracellular concentrations of 3',5'-cyclic adenosine monophosphate (cAMP). The PAC1 receptor isoform can also be coupled to phospholipase C $\beta$  (PLC $\beta$ ) and produce inositol phosphate by activating IP3 receptor-mediated Ca<sup>2+</sup> mobilization (7).

## NEUROTROPHIC AND NEUROPROTECTIVE ACTIVITIES OF PACAP

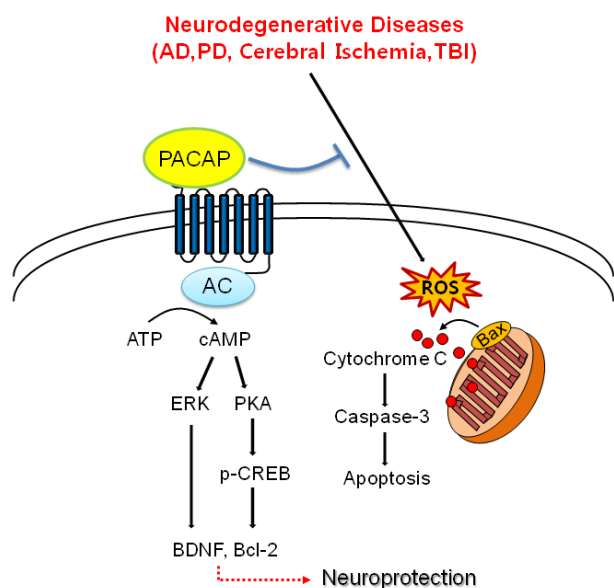
PACAP is expressed throughout the central nervous system (CNS), such as in the hypothalamus, hippocampus, cerebellum, and substantia nigra (8, 9). In the peripheral nervous system (PNS), PACAP is expressed in sensory neurons, sympathetic preganglionic neurons, and parasympathetic ganglionic neurons (10). The widespread distribution of PACAP indicates that the peptide has pleiotropic functions in the nervous system. PACAP has been shown to function as a neurohormone, a neurotransmitter, and a neurotrophic factor. In the developing CNS, PACAP acts as a neurotrophic factor, promoting cell survival and differentiation in various cells, including cerebellar granule cells, dorsal root ganglion cells, and cortical neuroblast (11-13). The neurotrophic effects of PACAP can be modulated according to splice variants of the PAC1 receptor expressed in development. In mature brain, PACAP also inhibits apoptotic cell death and promotes survival and regeneration under various pathological conditions. In cultured cells, PACAP is known to promote the survival of rat cortical neurons against glutamate-induced toxicity (14). PACAP increases the survival of dopaminergic neurons against 6-hydroxydopamine-induced neurotoxicity (15). In differentiated PC12 cells and primary sympathetic neurons, PACAP also prevents serum and NGF withdrawal-induced cell death (16-19). The

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**Fig. 1.** Schematic representation summarizing the mechanism concerning neuroprotective actions of PACAP in neurodegenerative diseases. PACAP binding with PAC1 receptors activates adenylate cyclase (AC)-linked signal transduction pathway. PACAP triggers the anti-apoptotic transcriptional target gene expression. PACAP also inhibits apoptotic signaling responses, including ROS generation, mitochondrial Bax and cytochrome C release, and subsequent caspase-3 activation.

neurotrophic and neuroprotective effects of PACAP are mediated by direct or indirect mechanisms (20). In most studies, the neurotrophic and neuroprotective actions of PACAP occur through the activation of the cAMP-protein kinase A (PKA) pathway (16, 21). Additionally, PACAP can influence the mitogen activated protein kinase (MAPK) pathway (22, 23). A direct protective effect of PACAP on neurons is often accompanied by the inhibition of caspase-3, a key apoptotic enzyme (24). Induction of transcriptional target gene expression, such as BDNF, mediates the neuroprotective action of PACAP in rat cortical neurons (25). In some cases, PACAP inhibits the expression of proapoptotic factors, such as Bcl-2-associated X protein (Bax), and activates the phosphatidylinositol 3'-OH kinase (PI3K) pathway (26, 27) (Fig. 1). Indirectly, PACAP mediates neuroprotective actions by modulating glial cells to provide neurotrophic support and control of inflammatory responses (28). PACAP induces astroglial cells, which have large numbers of PACAP receptors, to release interleukin-6 (IL-6) in ischemia *in vivo* to protect neurons (29, 30).

## PACAP AND NEUROPATHOLOGY

### Cerebral ischemia

Decreased blood flow to the brain causes decreases in oxygen and glucose, resulting in cerebral ischemia or stroke. Total loss

of blood flow to the brain causes global ischemia while local interruption due to cerebral artery occlusion causes focal cerebral ischemia (31). PACAP has significant neurotrophic and neuroprotective effects after stroke. PACAP can cross the blood-brain barrier (BBB) and injection of PACAP prevent ischemic neuronal damage in transient global and focal cerebral ischemia (32). Application of PACAP intracerebroventricularly or intravenously in a model of transient global ischemia prevented the ischemic death of rat CA1 neurons, even if administration was delayed until 1 day after the ischemic event (33). Systemic administration of PACAP also effectively decreased infarct volume in a rat model of focal ischemia and ameliorated neurological deficits when administration began 4 h after middle cerebral artery occlusion (MCAO), a mouse model of stroke (34). Additionally, PACAP-deficient mice show more vulnerability following MCAO (15). The infarct volumes and neurological deficits were greater in PACAP-deficient mice than in the wild-type mice. Studies comparing transcriptome alterations during ischemic insult in wild type and PACAP deficient mice suggest the possible involvement of *Ier3*, met-enkephalin, substance P, and neurotensin expression in its neuroprotective effects (15). PACAP-deficient mice exhibit higher cytoplasmic cytochrome c levels and lower Bcl-2 expression than wild-type mice, indicating that the PACAP acts on the mitochondrial apoptotic pathway to inhibit caspase-9 and subsequent caspase-3 activation (30). PACAP also activates the DNA repair function of apurinic/apyrimidinic endonuclease 1 (APE1) (35). Stroke is categorized as acute, subacute, and chronic depending on the period. During the acute period (variable from a few minutes to hours), impaired adenosine triphosphate (ATP) production, loss of  $\text{Na}^+\text{-K}^+$  pump activity, glutamate bursts, and increases in intracellular  $\text{Ca}^{2+}$  concentration occur, leading to excitotoxicity in neurons. In the subacute periods (a few hours to a few days), neurons and microglial cells are activated and produce reactive oxygen species (ROS) and inflammatory cytokines. In the chronic period (a few days after), neurons die apoptotically and mitochondria are the structures in this process. PACAP may act on several of these processes for neuroprotection. For example, PACAP can protect against glutamate-induced cytotoxicity and excitotoxic concentrations of glutamate stimulate PACAP expression (14, 36). PACAP inhibits ROS-induced cell death in several cell types (37, 38). Furthermore, PACAP decreases the neuroinflammatory response and attenuates microglial activation (39, 40).

### Traumatic brain injury

Traumatic brain injury (TBI), physical damage to the brain, is a major factor leading to death and chronic disability in individuals under the age of 45 years worldwide (41, 42). Pathological evidence suggest that TBI involves a complex neurodegenerative process, which includes many pathways (43). Studies have shown neuroprotective effects of PACAP in different models of TBI. Moderate TBI in rat brain induces changes in the mRNA expression of PACAP and the PAC1 re-

ceptor in the cortex and hippocampus (44). The upregulation of endogenous PACAP and its receptors and the protective effect of exogenous PACAP after different central and peripheral nerve injuries show the important function of PACAP in the neuronal regeneration (45). In a rat model of TBI induced by central fluid percussion, PACAP treatment significantly reduced the diffusion of axonal injury and protected the corticospinal tract (46). PACAP promotes neural restoration through enhanced neurogenesis, angiogenesis, and neuroprotective effects in TBI (47). In a weight-drop model of TBI, microinjection cerebroventricularly before TBI significantly improved motor and cognitive dysfunction, attenuated apoptosis, and decreased brain edema (48).

The inflammatory response is a common pathological reaction to brain trauma like other neuronal diseases (49). The cerebral inflammatory response to TBI activates macrophages/microglia, neurons, and astrocytes, and increases the release of inflammatory mediators, such as interleukin-1 $\beta$  (IL1 $\beta$ ) and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) (50). PACAP has immunomodulatory properties and can inhibit production of TNF- $\alpha$  from microglia activated by lipopolysaccharide (LPS) *in vitro* (51, 52). Exogenous administration of PACAP alleviates TBI in rats through a mechanism involving the TLR4/MyD88/NF- $\kappa$ B pathway (48). Thus, PACAP exerts a neuroprotective effect by inhibiting a secondary inflammatory response in microglia and neurons (48). TBI induces T cell-mediated immune suppression in both animal and clinical studies (53). PACAP inhibits the expression of IL-12, thereby suppressing T cell proliferation (53). Although cerebral ischemia and TBI have differing pathogenesis, they may also share some common pathways, including excitotoxicity, ROS generation, nitric oxide production, elevated Ca<sup>2+</sup> levels, and apoptosis (54, 55).

### Parkinson's disease

Parkinson's disease (PD) is characterized by motor movement disorders due to damage to or destruction of dopaminergic neurons in the substantia nigra (SN) (56). In addition to the motor impairment, cognitive and behavioral disturbances may also arise in the disease. Several animal models have been developed to study the pathogenesis of PD. In particular, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is a widely used neurotoxin to produce experimental models of PD. MPTP inhibits the mitochondrial respiratory chain, causing energy depletion and dopaminergic neuronal loss in the SN (57, 58). In this model, the pretreatment of MPTP-intoxicated mice with PACAP improved memory impairment in the test session of the spatial reference version of the water maze (59).

Unilateral lesion of the dopaminergic cells with the 6-hydroxydopamine (6-OHDA) is also commonly used for the generation of PD rodent models (60). Injection of PACAP into 6OHDA-induced lesions in the SN can effectively reduce dopaminergic neurodegeneration in the SN and ventral tegmental area and improve behavioral symptoms (61, 62). PACAP effectively protects dopaminergic nigrostriatal neurons from apop-

toxis (61). Moreover, PACAP-treated animals show less severe acute neurological symptoms and a more rapid amelioration of behavioral deficits than wild-type animals (61). PACAP also protects PC12 cells from apoptosis induced by rotenone, which is thought to provoke PD by interrupting mitochondrial complex I activity (63). PACAP protects dopaminergic neurons against rotenone-, 6-OHDA-, and MPP<sup>+</sup>-induced toxicity in cell culture (64, 65). PACAP protects SH-SY5Y dopaminergic cells in salsolinol (SALS)-induced PD models (66).

Recent advances in PD pathology suggest that diverse cellular and molecular events, including oxidative stress, microglia-mediated inflammation, as well as apoptotic mechanisms, are likely to be involved in the neurodegenerative process (67). The neuroprotective effect of PACAP is mediated by inhibition of ROS production by microglial cells (68). In mesencephalic cultures, pretreatment with PACAP protects dopaminergic neurons against 6-OHDA-induced neurotoxicity (64). Moreover, PACAP increases the number of tyrosine hydroxylase (TH) immunoreactive neurons, and enhances dopamine uptake. Because PACAP also acts as a neuromodulator, regulating synaptic transmission, the neuroprotective actions of PACAP in PD may occur through the regulation of dopamine release. Consistent with this, PACAP induces catecholamine release from adrenal chromaffin cells, sympathetic neurons, and neurosecretory cells by elevating intracellular Ca<sup>2+</sup> concentrations (69-71). Neuroprotective effects of PACAP in MPTP-induced PD mouse models involve the modulation of K(ATP) subunits and D2 receptors in the striatum (72). The neuroprotective effects of PACAP affect not only dopaminergic neurotransmission but also cholinergic neurotransmission, balancing the dopamine-acetylcholine systems in the basal ganglia neuronal pathway (72). PACAP can also act on the MPTP-altered expression of proteins, such as the mTOR anti-apoptotic and RNA-dependent protein kinase (PKR) apoptotic pathways of translational control (TC) (73, 74).

### Alzheimer's disease

Deposition of amyloid  $\beta$  peptide (A $\beta$ ) is a central process leading to the development of Alzheimer's disease (AD) (75). A $\beta$  is produced by the proteolytic cleavage of the amyloid precursor protein (APP) with sequential cleavages by a group of enzymes termed  $\alpha$ -,  $\beta$ -, and  $\gamma$ -secretases. ADAM family (a disintegrin- and metalloproteinase-family enzyme) acts an  $\alpha$ -secretase and  $\beta$ -site APP-cleaving enzyme 1 (BACE1) acts as a  $\beta$ -secretase. The  $\gamma$ -secretase is a complex of enzymes, composed of presenilin 1 or 2 (PS1 or PS2), nicastrin, and anterior pharynx defective and presenilin enhancer 2 (76). Proteolytic cleavage of APP by  $\alpha$ -secretase precludes formation of amyloidogenic peptides and leads to the release of soluble N-terminal APP fragments (sAPP $\alpha$ ) with neurotrophic and neuroprotective properties. Several reports suggest the neuroprotective action of PACAP is mediated by stimulating  $\alpha$ -secretase activity (77). In the brain of the APP[V717I] AD transgenic mouse model, PACAP treatment results in an enhancement of the non-amy-

loidogenic pathway of APP processing and in improved cognitive function (78). Treatment of SK-N-MC neuroblastoma cells, which express endogenous PAC1 receptors, with PACAP shows enhanced secretion of sAPP $\alpha$  versus untreated cells (77). The activation of the  $\alpha$ -secretase activity in cells endogenously expressing PAC1 receptor indicates that physiological receptor levels are sufficient to mediate this response (77). Moreover, stably overexpressing functional PAC1 receptors in HEK cells strongly stimulates  $\alpha$ -secretase cleavage of APP (77). A comparative analysis of cortical gene expression profiles showed that PACAP was significantly downregulated in several AD mice models and in the human AD temporal cortex, supporting the physiological relevance of PACAP in AD (79). PACAP is neuroprotective, but brain uptake is limited by an efflux component, such as peptide transport system-6 (PTS-6) (80). In the SAMP8 AD mouse model, PACAP with anti-sense-PTS shows improved cognition by inhibiting the peptide efflux pump (80). There is increasing evidence for the involvement of a key lipid carrier, apolipoprotein (ApoE) in AD (81). A mouse deficient in ApoE serves as a useful *in vivo* model to study development and degeneration (82). VIP, a PACAP family member, shows protection from developmental retardation and memory deficits in ApoE-deficient mice (83). In rat PC12 cell cultures, PACAP also shows a potent neuroprotective effect over a long period at a very low concentration of A $\beta$ -induced cytotoxicity (84). The enzyme caspase-3 is involved in the signaling pathways for this neurotrophic effect of PACAP.

## CONCLUSIONS

PACAP shows significant neuroprotective potential resulting from its neurotrophic and anti-apoptotic effect in various *in vivo* and *in vitro* models. *In vivo*, PACAP is a peptide and is metabolized mainly by dipeptidyl peptidase IV (DPP IV), a ubiquitous amino-terminal dipeptidase (85). Thus, metabolically stable PACAP analogs or derivatives may represent promising drug candidates. In support of this, a metabolically stable PACAP derivative, acetyl-[Ala<sup>15</sup>, Ala<sup>20</sup>]PACAP38-propylamide, which behaves as a super-agonist of the PAC1 receptor, is being developed (86). To avoid side effect such as migraine, it will be necessary to determine the lowest dose of PACAP needed in animal models. Moreover, strategies to target the delivery of the PACAP to the tissues of interest may also need to be developed (87). Based on published data, PACAP may become useful a therapeutic agent in many neurological disorders characterized by neurodegeneration, such as cerebral ischemia, TBI, PD, and AD.

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