

Multifaces of Pituitary Adenylate Cyclase-Activating Polypeptide (PACAP): From Neuroprotection and Energy Homeostasis to Respiratory and Cardiovascular Systems

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Abstract

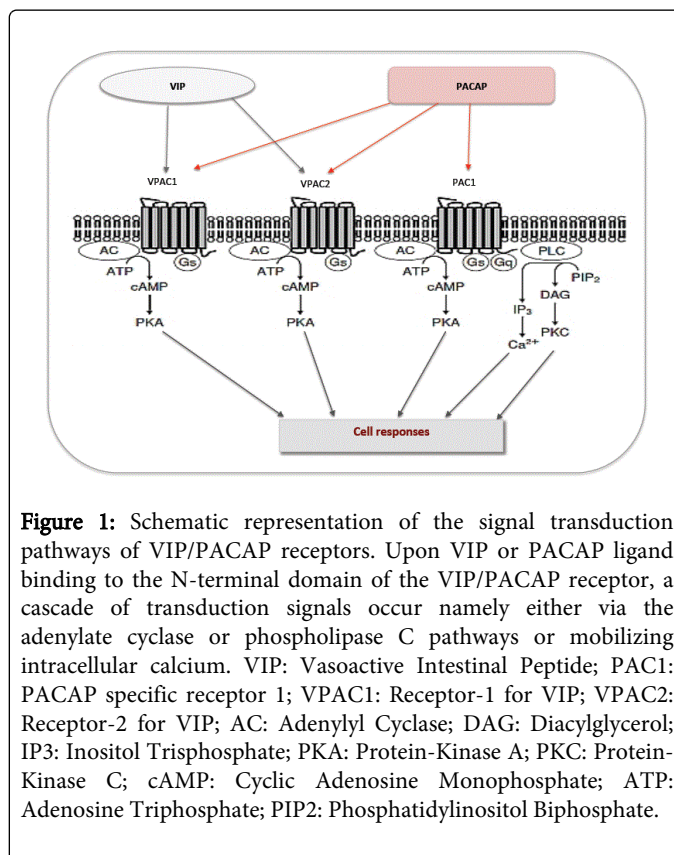
Pituitary adenylate cyclase-activating polypeptide (PACAP) belongs to the secretin/glucagon/vasoactive intestinal peptide (VIP) family and is one of the most highly conserved neuropeptides. The effects of PACAP are mediated through three G-protein coupled receptors: PAC1R, which has specific affinity for PACAP, and VPAC1 and VPAC2 that have equal affinity for both PACAP and VIP. PACAP and PAC1R are widely expressed and distributed throughout the body, including the central nervous system, the gastro-intestinal tract, the endocrine pancreas, the respiratory and cardiovascular systems. With this widespread tissue distribution, PACAP has been shown to be a pleiotropic peptide exerting a range of physiological functions. Within the body, PACAP serves as a neurotransmitter, neuromodulator, neurotrophic factor, neuroprotectant, secretagogue, and neurohormone. In this present review, we provide current insight on the role of PACAP in neuroprotection, its role in energy homeostasis and the impact PACAP may have on respiratory and cardiovascular disease. We conclude with an outlook for the future of PACAP-related research.

Keywords: PACAP; Neuroprotection; Energy homeostasis; Respiratory system; Cardiovascular system

Introduction

Pituitary adenylate cyclase-activating polypeptide (PACAP) is a neuropeptide originally isolated from ovine hypothalamic tissue by Arimura and coworkers in 1989 based on its capacity to activate adenylate cyclase to produce cyclic AMP in rat pituitary cells [1-3]. This neuropeptide belongs to the secretin/glucagon/vasoactive intestinal peptide (VIP) superfamily, and exists in two amidated forms from the same precursor, PACAP38 (38-amino acid residues) and PACAP27. Since its discovery, extensive research has been dedicated to understanding the biological role of PACAP. PACAP has been detected in all vertebrates studied so far and is one of the most highly conserved neuropeptides [4].

PACAP binds to the PACAP specific receptor 1 (PAC1R), and the receptors for VIP, VPAC1 and VPAC2. The PAC1R, of which there are at least nine variant forms, is specific for PACAP, whereas the other two receptors, VPAC1 and VPAC2, bind both PACAP and VIP [5-7]. The PAC1R belongs to the class II family of G protein-coupled receptors that trigger mainly adenylate cyclase activation through G_s protein subunits [8]. Alternatively, PAC1R is also capable of coupling to G_q proteins to activate the phospholipase C (PLC) pathways leading to increased inositol triphosphate (IP₃) turnover and a rise in intracellular calcium concentrations (Figure 1) [9,8].



PACAP is a neuropeptide acting as a neurotransmitter, neuromodulator, or neurotrophic factor. PACAP and its receptors are widely expressed and distributed throughout the central nervous system (CNS) and in various peripheral organs, including endocrine glands (adrenal, pancreas, ovaries, and testes), the gastro-intestinal tract, and the respiratory and cardiovascular systems [10]. Consistent with this widespread distribution, PACAP has been found to exert pleiotropic physiological functions. This review article presents an update on PACAP research and its known involvement in a variety of physiological and pathophysiological processes based on neuroprotective effects, its homeostatic control of energy metabolism and its impact on respiratory and cardiovascular systems. We conclude by discussing its possible potential for future therapeutic applications.

PACAP and its neuroprotective effects

Soon after its discovery and characterization, the distribution of PACAP in the CNS of mammals was investigated [11]. Although PACAP mRNA expression is widespread throughout the CNS, the most abundant population of PACAP-containing neurons in the brain is found in the hypothalamus [12,13]. Moreover, using *in situ* hybridization and immunocytochemistry, some extra hypothalamic regions, including the hippocampus, cerebral cortex, striatum, nucleus accumbens, amygdala and substantia nigra abundantly express both PAC1R and PACAP (Table 1) [12,14,15], suggestive of the involvement of the peptide in the neuronal functions [16].

Brain structures	mRNA
Olfactory bulb	++
Cerebral cortex	++
Amygdala	++
Hippocampal formation	+
Thalamus	++
Hypothalamus	
Arcuate nucleus	++
Mediobasal hypothalamus	++
Ventromedial nuclei	+++
Paraventricular nucleus	++
Cerebellum	-/++
Brainstem	-/++

Table 1: Localization and relative abundance of PACAP mRNA in the rat brain by *in situ* hybridization as denoted by: high (+++), moderate (++), low (+), very low (-).

Neurodegenerative disorders, including the most common type of dementia, Alzheimer's disease (AD), and the most frequent movement disorder, Parkinson's disease, are morphologically characterized by progressive neuronal cell death. Recently, apoptosis and inflammation, through the action of cytotoxic factors (TNF- α and IL -1), have been implicated as a general mechanism in the degeneration of selective neuronal populations [17,18]. PACAP exerts potent neuroprotective effects, through the activation of PAC1R, by reducing apoptosis, both

in vitro and *in vivo*, in rodent models of Alzheimer's, Huntington's, and Parkinson's diseases and traumatic brain and spinal cord injuries [3,16,19]. In PC12 cells, a useful cell model for neuronal differentiation, PACAP promotes cell survival by attenuating beta-amyloid-induced toxicity through reducing caspase-3 activity [20]. PACAP also protects PC12 cells from apoptosis induced by rotenone, a mitochondrial complex I inhibitor [21], known to be involved in the pathogenesis of Parkinson's disease, which is characterized by a progressive loss of dopaminergic neurons in the substantia nigra. Moreover, in a rat model of Parkinson's disease, it has been shown that pretreatment with PACAP protects 50% of dopaminergic neurons and improves behavioral deficits [22].

Another pathological state associated with apoptosis and commonly found in diabetic patients is retinal neurodegeneration, the latter is also closely linked to apoptosis [23]. Diabetes-associated hyperglycemia is a key initiator of retinal damage and the mechanism of retinal cell death in diabetic retinopathy includes apoptosis. Recently, it has been demonstrated that intraocular PACAP injection markedly attenuated diabetic retinal injury by increasing the levels of several anti-apoptotic markers (p-Akt, p-ERK, p-ERK2, PKC, Bcl-2), while decreasing the levels of the pro-apoptotic markers (p-p38MAPK, caspases) [24], suggesting that PACAP mitigates diabetic retinopathy by protecting against apoptosis.

PACAP is also able to modulate the inflammatory response associated with many neurodegenerative diseases, by inhibiting both chemokine and pro-inflammatory cytokine (NFkB, TNF- α , IL-1) production [25,26]. Therefore, PACAP is able to exert anti-apoptotic, as well as anti-inflammatory effects, two mechanisms involved in the pathogenesis and the progression of neurodegenerative diseases. In addition to its protective effects on neuronal loss during neurodegenerative disorders, accumulating evidence implicates PACAP as an important regulator of neuronal cell death after ischemia [27]. Given that PACAP can cross the blood-brain barrier by a saturable mechanism [28], it has been demonstrated that intravenous injection of a very low concentration of exogenous PACAP suppresses neuronal cell death in rat global and local brain ischemia models [29-31]. However, the mechanisms underlying the neuroprotection effects of PACAP in these models are not fully elucidated. Recently, Ohtaki et al. [32] have demonstrated that after ischemia, PACAP decreases neuronal cell death by suppressing cytochrome c release. PACAP mediates such release phosphorylating extracellular signal-regulated kinase (ERK) directly and signal transducer and activator of transcription 3 (STAT 3) indirectly via IL-6 release. ERK and STAT3 increase and phosphorylate Bcl-2, an anti-apoptotic protein, suppressing cytochrome c release from the mitochondria to the cytoplasm, thereby preventing neuronal cell death.

Another example of PACAP's role as a neuroprotective peptide is the attenuation of cell apoptosis in a rat model of spinal cord injury [33]. PACAP also was found to enhance the neuroprotective ability of human mesenchymal stem cells (hMSCs) used to repair injured spinal cord tissue [34]. Additionally, administration of PACAP mitigated oxidative stress and tissue damage in a small-bowel autotransplantation model [35]. The upregulation of endogenous PACAP and its receptors and the protective effect of exogenous PACAP after the different neuronal injuries highlighted above, show the important function PACAP can play in neuronal regeneration and suggest PACAP may be a promising therapeutic agent in the treatment of neuronal injuries.

Further works to elucidate the precise mechanisms underlying the neuroprotective effects of PACAP are thus warranted. However, the therapeutic potential of PACAP for the treatment of CNS injuries or neurodegenerative diseases has many challenges including its low bioavailability. Once circulating in the blood, PACAP is subjected to rapid degradation by the endogenous peptidase, dipeptidyl peptidase IV (DPP-IV). It has been established that the half-life of PACAP38 injected into mice or human is between 2 and 10 minutes [36,37]. This poor plasma/serum stability will hamper the therapeutic potential of PACAP. To circumvent such metabolic instability, PACAP could be injected simultaneously with a DPP-IV inhibitor, which is known to extend some of the effects of PACAP [38]. Alternatively, new strategies such as chemical modifications to increase the metabolic stability of PACAP while preserving its biological activity could be explored as new areas of pharmacological research.

PACAP regulates the stress response

In addition to its neuroprotective effects, evidence has shown PACAP as a master regulator of central and peripheral stress responses required to restore and maintain homeostasis [recently reviewed in 39,40]. PACAP has been shown to regulate catecholamine production and release and is required for sustained epinephrine release in response to metabolic stress [41]. PACAP also modulates the hypothalamic-pituitary-adrenal (HPA) axis in response to psychological stress by regulating the secretion of corticosterone [42]. Roman et al. [43] have recently demonstrated that chronic variate stress exposure in rodents increases PACAP and PAC1R transcript expression in the bed nucleus of the stria terminalis (BNST) of the limbic structure. Furthermore, acute PACAP injections can stimulate anxiety-like behavior and heightened corticosterone release while these stress-induced behavioral responses were attenuated with chronic inhibition of BNST PACAP signaling by continuous infusion with the PAC1R antagonist. Based on these findings, PACAP receptor antagonists could have therapeutic relevance in preventing hyperactivity of the HPA axis and offering protection against stress-associated behavioral and endocrine defects.

PACAP's actions on energy homeostasis

Results from a number of studies using either injection of peptides or gene deletion have highlighted a role for PACAP in the regulation of energy homeostasis including appetite, thermogenesis, body mass and endocrine parameters [44,45]. There is now strong evidence that PACAP is involved in the control of feeding behavior [46]. Hypothalamic nuclei such as ventromedial nucleus (VMN), arcuate nucleus (ARC) and paraventricular nucleus (PVN), the key feeding centers in which major peripheral energy signals are directly sensed and integrated [47] heavily express PACAP and PAC1R [48], suggesting that PACAP may be critical for the regulation of feeding behavior and body weight. Intracerebroventricular (icv) injection of PACAP into the VMN or PVN decreases food intake in rodents [44,49]. However, unlike VMN injection, only PACAP injections into the PVN affect meal patterns by producing significant reductions in meal size, duration, and total time spent eating [48]. The neurons expressing the classical feeding related neuropeptides such as proopiomelanocortin (POMC) and neuropeptide Y (NPY) express the PAC1R [50,51]. It has been reported that icv administration of PACAP to food-deprived mice causes a dose-dependent reduction of food consumption during the first 3 h post PACAP injection along with increases in energy expenditure [44,48]. One hour after PACAP

administration, mRNA expression of POMC was significantly increased in the ARC, with no changes in NPY expression, indicating that hypophagia induced by central administration of PACAP is mediated, at least in part, through activation of the hypothalamic melanocortin system [44].

Based on its secretory property on exocrine and endocrine cells, PACAP induces a concentration-dependent relaxation of gastric smooth muscles [52,53], causing a decrease of gastric motility and a delay in stomach emptying [54] suggesting a satiety effect of PACAP. The satiety induced by the delay in stomach emptying could be the mechanism by which PACAP suppresses appetite. In the gastrointestinal tract, PACAP also stimulates the release of some regulatory peptides including somatostatin and PYY that may contribute to the anorexic effects of PACAP peptide.

Lower body weight and decreased fat mass under normal temperature housing conditions have been observed in PACAP null mice [55]. This reduction of body weight and adiposity was not associated with reduced food intake but instead was accompanied by a thermogenic defect [55]. Deletion of PACAP in mouse results in a temperature sensitive phenotype, whereby PACAP null pups display reduced survival at lower housing temperature with most pups dying suddenly in their second postnatal week of life [56]. Shortly thereafter it was shown that an increase in housing temperature of just three degrees (to 24°C) improved postnatal survival dramatically; suggesting PACAP plays an important role in thermoregulation [57]. Tanida et al. [58] have showed increased sympathetic nervous system (SNS) activity innervating the brown adipose tissue (BAT) and increased body temperature following a PACAP injection in rat. The thermogenic role of PACAP [59] in BAT is supported by the observation that PACAP null mice have decreased norepinephrine in this organ [57]. Despite the changes in sympathetic outflow to brown adipose tissue, the mass and histology of the interscapular BAT depot did not differ from those of the control wild-type mice [57]. Therefore, the precise underlying mechanism by which PACAP null mice are cold intolerant remains to be understood and require future studies.

In addition, Gray et al. [56] reported that PACAP null mice that died prematurely (postnatal day 7-12) showed increased lipid deposition in the liver, heart, and skeletal muscles, suggesting abnormal lipid metabolism in the PACAP null mice. The presence of PACAP mRNA and PAC1R has been detected in most endocrine glands. Furthermore, PACAP is expressed in pancreatic β cells and autonomic nerve terminals innervating the pancreas, suggesting a role for the peptide in pancreas function. As previously reviewed, PACAP seems to be much more potent than other regulatory peptides in stimulating pancreatic hormone secretion [60,61]. *In vitro*, PACAP potently stimulates insulin secretion from β cells, in clonal isolated mouse and rat islets as well as in the perfused rat pancreas [62,63]. Also *in vivo*, a clear stimulation of insulin secretion is evident from studies in mice [64]. PACAP also stimulates glucagon secretion from perfused rat pancreas [65] and *in vivo* in mice [66]. Of particular interest is that PACAP stimulates both insulin and glucagon secretion in humans [67]. Due to its potent insulinotropic effect, studies using PACAP receptor antagonists, mice lacking PACAP or mice with specific overexpression of PACAP in the pancreatic β cell have been undertaken to further examine the role of PACAP in islet function [60,67]. Interestingly, mice with genetic deletion of PACAP have impaired glucose-stimulated insulin secretion [68] and a PAC1R antagonist inhibits insulin secretion [69], while mice overexpressing PACAP in pancreatic β cells display increased insulin secretion in

response to an oral or intraperitoneal glucose load [70]. Moreover, chronic PACAP-signaling deficiencies cause a host of carbohydrate disturbances that result in decreased insulin and blood glucose levels in fasted and fed animals under both chow and high fat/high sucrose diets [55]. However, at postnatal day 5 (P5) fasting plasma insulin levels were increased while blood glucose levels were decreased in PACAP null pups as compared to while type controls [56]. These observations reflect the important role played by PACAP in glucose and insulin homeostasis. In support of a potent insulinotropic role of this peptide, PACAP-based therapy may be a strategy for novel treatment of type 2 diabetes. However, the use of PACAP in clinic may be dampened by its stimulatory effect on glucagon secretion in human.

Effects of PACAP on the Respiratory System

Given the localization of PACAP and PAC1R to nuclei involved in the regulation of respiratory system, PACAP signaling likely plays an important role in lung function. PACAP has been localized in nerve fibers innervating the lung, is a potent bronchodilator and causes marked vasodilation of pulmonary blood vessels [71]. As mentioned above, PACAP-knockout mice appear normal at birth but show a high mortality with sudden death at ~ 2 weeks of age. Raising the ambient temperature of the room in which the mother and litter are housed can substantially decrease the mortality of PACAP deficient animals highlighting the thermoregulatory effect of PACAP. Unlike most fatal congenital abnormalities in which the highest rate of mortality is apparent immediately after birth, mortality of PACAP-deficient animals does not peak until the second week [56,57]. These phenotypic hallmarks reliably produce the spectrum and heterogeneity of traits that closely reflect the phenotype of sudden infant death syndrome (SIDS) in human. First, SIDS deaths peak during the third month of life, not immediately after birth, and secondly, thermal stress has been implicated as a causal factor in SIDS as SIDS rates are higher in winter months than summer season in northern climates [72,73]. Moreover, mutations in the PACAP gene are possible risk factors in SIDS in a subset of African-American cases [74]. Thus, PACAP-knockout mice display a SIDS-like phenotype, although the underlying physiological mechanism is unknown.

Currently, there are three tenable hypotheses for why a PACAP-signaling deficiency causes an increased susceptibility to neonatal death: (i) the metabolic hypothesis whereby PACAP-signaling deficiency causes metabolic disturbances (thermoregulatory defect) leading to wasting and death [57]; (ii) the pulmonary hypertension hypothesis whereby PACAP-signaling deficiency leads to pulmonary hypertension and right heart failure [75]: PAC1-deficient mice had abnormalities associated with pulmonary hypertension, including chronic hypoxemia, increased systolic right ventricular pressure, right ventricular enlargement, a 30% reduction in the density of the pulmonary capillary bed and an increase in wall thickness of small pulmonary arteries (an observation also made in SIDS victims; [76]; and (iii) the breathing defect hypothesis whereby PACAP-signaling deficiency causes breathing defects resulting in chronic hypoxemia and atrioventricular block [77]. It has been reported that PACAP^{-/-} mice display significantly reduced ventilation during baseline breathing and show blunted responses to hypoxia [77]. Interestingly, hypoxia induced respiratory arrest in neonate PACAP^{-/-} mice may be the cause of the sudden death of PACAP null mice at 2 weeks of age [78]. Endogenous PACAP plays a role as a respiratory regulator linked with the catecholaminergic system in the medulla oblongata; disruption of this system is responsible for causing the blunted

responses to hypoxia that may be involved in the SIDS-like phenotype of PACAP null mice [78]. These reports suggest that the PACAP null mouse is a good model for SIDS. Thus, information from rodent models with pathophysiological traits mimicking those found in human SIDS would be of great interest. Also, whether or not PACAP gene mutations predispose human infants to SIDS is unknown and this opens new directions for future research.

Effects of PACAP on the Cardiovascular System

The presence of PACAP and its PAC1R in cardiac tissue and blood vessels [79-81] suggests that the peptide may play an important role in the cardiovascular functions. However, one area where a role for PACAP is not well studied is its cardiovascular regulatory effect. According to recent data, the peptide is present in the cardiovascular system and has various distinct effects. PACAP has been demonstrated to exhibit protective effects against *in vitro* ischemia/reperfusion-induced apoptosis in cardiomyocytes [82,83] and oxidative stress-induced apoptosis in endothelial cells [84]. Intravenous injection of PACAP provokes a substantial increase in heart rate and enhances the contractile ventricular force [85,86]. In rat, PACAP knockdown-induced tachycardia is abolished by the β -adrenoreceptor antagonist propranolol, indicating that PACAP can stimulate norepinephrine release from sympathetic nerve terminals subserving the heart [87]. In addition, a report from Otto et al. [75] demonstrated that the absence of PAC1R in mice causes right heart failure after birth, demonstrating the crucial importance of PAC1-mediated signalling for the maintenance of normal cardiovascular function during early postnatal life. This *in vivo* finding could lead one to speculate that the increased neonatal death noticed in PAC1 deficient mice is not only related to respiratory problems but also to rapidly developing heart failure.

PACAP has also been found to stimulate the production of vascular endothelial growth factor, which plays an important role in angiogenesis [86]. During cold exposure, 60% of all energy expended by a mouse is done in BAT through high rates of oxidative metabolism that ultimately produces heat [89,90]. To allow such high rates of oxidative metabolism to be maintained during exposure to cold, BAT requires a substantial blood supply to provide adequate nutrients and oxygen and to carry away waste products and heat. This requirement for increased blood supply is met by induction of extensive angiogenesis within BAT during exposure to cold [91]. As PACAP is a known non-classic regulator of angiogenesis [92], the highly cold-sensitive phenotype of PACAP deficient mice may be related to decreased angiogenesis in their BAT.

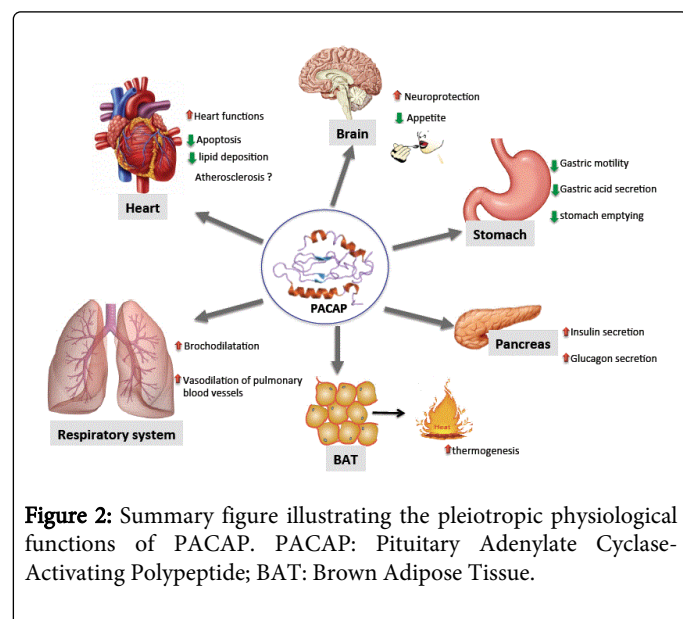
Cold stress is also associated with cardiovascular consequences such as increased heart rate to augment energy availability to thermogenic organs such as BAT. As PACAP induces tachycardia via the SNS, it is possible that impairment of cold-induced thermogenesis in PACAP null mice relates to a decrease in heart rate that reduces the nutrient and oxygen availability to BAT. Future studies will be required to explore this hypothesis. In addition, an early report from Gray et al. [56] showed that PACAP null pups that die prematurely had increased lipid deposition in metabolically active cells, including cardiomyocytes of the heart and hepatocytes of the liver. This huge amount of lipid accumulation observed in the heart of PACAP null mice early in postnatal life suggests that PACAP may have a cardioprotective effect.

To our knowledge, there is no *in vivo* study looking at the role of PACAP on cardiovascular health. By using cultured vascular endothelial cells (EC) and smooth muscle cells (SMC) as model,

Chang [93] found that PACAP significantly (i) increased the production of anti-atherosclerotic substances by EC, (ii) inhibited the proliferation of SMC and (iii) reduced the production of lipid peroxide by EC and SMC in hyperlipid conditions, suggestive of anti-atherosclerotic effects of PACAP. In addition, a recent *in vitro* study has demonstrated that ApoE receptor 2 and LDL receptors expression were significantly augmented in dorsal root ganglion (DRG)-derived cells co-cultured with PACAP-treated 3T3-L1 cells [94], a finding that again supports potential anti-atherosclerotic properties of PACAP. The accumulation of lipids within arteries remains to be the initial impulse for the pathogenesis of atherosclerosis; however, both inflammation and oxidative stress are also considered to play a critical role in this process. As mentioned above, the anti-inflammatory and anti-oxidative properties of PACAP may also confer an anti-atherosclerotic effect to this peptide. Taken together, these findings and observations indicate that PACAP exerts major regulatory effects on the cardiovascular system, suggesting there may be therapeutic potential for PACAP or PAC1R agonists for the treatment of cardiovascular diseases such as heart failure and atherosclerosis.

Conclusion and Future Direction

This review provides current insight on the role of PACAP in many biological functions (summarized in Figure 2).



The availability of knockout mouse models, mice with tissue-specific overexpression of PACAP and the development of PACAP agonists/antagonists have allowed for a much deeper understanding of the crucial role PACAP plays in neurodevelopment, energy metabolism, and the cardiovascular and respiratory systems. In this review, we have discussed literature that relates the role of PACAP in neuroprotection, energy metabolism, cardiovascular and respiratory systems. However, the molecular mechanisms by which PACAP exerts its effects are not completely understood. Indeed, very little is known about the role of PACAP plays in respiratory and cardiovascular diseases. Future research aimed at understanding how this highly conserved peptide regulates such diverse systems will not only enhance our understanding of PACAP biology but may also identify new therapeutic targets for the management of neurological, metabolic, respiratory or cardiovascular disease.

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