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PACAP is an Endogenous Protective Factor—Insights from PACAP-Deficient Mice

D. Reglodi · P. Kiss · K. Szabadfi · T. Atlasz · R. Gabriel · G. Horvath · P. Szakaly · B. Sandor · A. Lubics · E. Laszlo · J. Farkas · A. Matkovits · R. Brubel · H. Hashimoto · A. Ferencz · A. Vincze · Z. Helyes · L. Welke · A. Lakatos · A. Tamas

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Abstract Pituitary adenylate cyclase-activating polypeptide (PACAP) is a widespread neuropeptide with a diverse array of biological functions. Not surprisingly, the lack of endogenous PACAP therefore results in a variety of abnormalities. One of the important effects of PACAP is its neuroprotective and general cytoprotective role. PACAP protects neurons and other tissues against ischemic, toxic, and traumatic lesions. Data obtained from PACAP-deficient mice provide evidence that endogenous PACAP also has protective functions. Mice lacking PACAP are more vulnerable to different in vitro and in vivo insults. The present review summarizes data on the increased sensitivity of PACAP-deficient mice against harmful stimuli. Mice lacking PACAP respond with a higher degree of injury in cerebral ischemia, autoimmune encephalomyelitis, and axonal lesion. Retinal ischemic and excitotoxic injuries also produce increased cell loss in PACAP-deficient mice. In peripheral organs, kidney cell cultures from PACAP-deficient mice are more sensitive to oxidative stress and in vitro hypoxia.

D. Reglodi (✉) · P. Kiss · G. Horvath · A. Lubics · E. Laszlo · J. Farkas · A. Matkovits · R. Brubel · A. Vincze · A. Tamas
Department of Anatomy, PTE-MTA "Lendulet" PACAP Research Group, University of Pecs,
7624 Pecs, Szigeti u 12,
Hungary
e-mail: dora.reglodi@aok.pte.hu

K. Szabadfi · R. Gabriel
Department of Experimental Zoology and Neurobiology,
University of Pecs,
Pecs, Hungary

T. Atlasz
Department of Sportbiology, University of Pecs,
Pecs, Hungary

P. Szakaly
Surgery Clinic, University of Pecs,
Pecs, Hungary

B. Sandor
Department of Dentistry, Oral and Maxillofacial Surgery,
University of Pecs,
Pecs, Hungary

H. Hashimoto
Laboratory of Molecular Neuropharmacology,
Graduate School of Pharmaceutical Sciences, Osaka University,
Osaka, Japan

H. Hashimoto
Center for Child Mental Development, United Graduate School of Child Development, Kanazawa University and Hamamatsu University School of Medicine, Osaka University,
Osaka, Japan

H. Hashimoto
Department of Molecular Pharmaceutical Science,
Graduate School of Medicine, Osaka University,
Osaka, Japan

A. Ferencz
Department of Surgical Research and Techniques,
Semmelweis University,
Budapest, Hungary

Z. Helyes
Department of Pharmacology and Pharmacotherapy,
University of Pecs,
Pecs, Hungary

L. Welke
Department of Anatomy, Ross University School of Medicine,
Roseau, Dominica,West Indies

A. Lakatos
Department of Psychiatry and Human Behavior,
University of California, Irvine,
Irvine, CA, USA

In vivo, PACAP-deficient mice have a negative histological outcome and altered cytokine response in kidney and small intestine ischemia/reperfusion injury. Large intestinal inflammation, toxic lesion of the pancreas, and doxorubicin-induced cardiomyopathy are also more severe with a lack of endogenous PACAP. Finally, an increased inflammatory response has been described in subacute endotoxin-induced airway inflammation and in an oxazolone-induced allergic contact dermatitis model. In summary, lack of endogenous PACAP leads to higher vulnerability in a number of injuries in the nervous system and peripheral organs, supporting the hypothesis that PACAP is part of the endogenous cytoprotective machinery.

Keywords Knockout · Ischemia · Protection · Cytoprotective

Introduction

Pituitary adenylate cyclase-activating polypeptide (PACAP) was discovered as a hypothalamic neuropeptide stimulating adenylate cyclase in the pituitary (Miyata et al. 1989). Since its discovery, the distribution of PACAP has been described in the nervous system and in peripheral organs (Vaudry et al. 2009). PACAP acts on three main receptors: the specific PAC1 receptor and VPAC1 and VPAC2 receptors, which also bind vasoactive intestinal peptide with similar affinity. PACAP plays a role in a diverse array of physiological processes (Vaudry et al. 2009). Not surprisingly, the lack of endogenous PACAP therefore results in a variety of abnormalities. One of the most well-known and widely studied effects of PACAP is its cytoprotective action. PACAP is an antiapoptotic and anti-inflammatory peptide, with established protective effects in numerous cells and tissues (Abad and Waschek 2011; Reglodi et al. 2011, 2012; Seaborn et al. 2011; Vaudry et al. 2009). As PACAP is principally a neuropeptide with the highest concentrations in the nervous system, the first protective effects were shown in nerve cells both by in vitro and in vivo studies. The protective effects of PACAP are not restricted to the nervous system, however. PACAP has protective effects in several peripheral cells and organs, from cardiomyocytes to ischemic kidney injury. Most studies have utilized exogenous PACAP administration to prevent or attenuate tissue injury. The presence of PACAP in a wide variety of tissues has raised the question of whether PACAP also acts as an endogenous protective factor. One approach to this question was to study the changes in PACAP occurrence and expression upon injury. Indeed, several studies have provided evidence that PACAP is upregulated after nerve injuries (Somogyvari-Vigh and Reglodi 2004). These results suggest that PACAP is part of the endogenous protective mechanism, and the organism reacts to injury with elevated PACAP levels, as part of the restorative machinery. However, several damaging factors are also upregulated after injury. Even though the protective effects of PACAP imply the upregulation of protective pathways in case of postinjury elevated PACAP levels, recent studies from PACAP-deficient mice have provided the ultimate evidence for the endogenous protective function of PACAP. The neuroprotective and general cytoprotective effects of PACAP have been reviewed by several research groups (Somogyvari-Vigh and Reglodi 2004; Dejda et al. 2008; Ohtaki et al. 2008; Nakamachi et al. 2011; Reglodi et al. 2011, 2012; Seaborn et al. 2011). The aim of the present review is to summarize findings in PACAP-deficient mice, focusing in detail on studies related to tissue injury. The majority of the studies show that while the intact structure of most organs appears normal at macroscopical and light microscopical levels in PACAP-deficient mice, there is increased vulnerability to different types of injuries both in vitro and in vivo (summarized in Table 1).

PACAP-Deficient Mice Display Several Morphological, Biochemical, and Behavioral Abnormalities

As mentioned above, lack of endogenous PACAP leads to several biochemical and behavioral alterations. Briefly, PACAP-deficient mice have a decreased fertility rate (Shintani et al. 2002; Isaac and Sherwood 2008), higher mortality rate, partially due to their temperature sensitivity, respiratory abnormalities, metabolic changes, and increased insulin sensitivity (Gray et al. 2001; Hashimoto et al. 2001; Tanaka et al. 2004; Tomimoto et al. 2008; Wilson and Cummings 2008). PACAP plays an important role in processing light information (Hannibal 2006), and PACAP-deficient mice show changes in light-induced phase shift (Kawaguchi et al. 2003) and lack of light-induced elevation of renal sympathetic nerve activity and plasma corticosterone levels (Hatanaka et al. 2008; Hashimoto et al. 2009). Although monoaminergic transmitters (like serotonin and dopamine) do not show altered expression, monoamine turnover decreases with the lack of PACAP (Hashimoto et al. 2001; Ogawa et al. 2005).

Several behavioral abnormalities have also been described in mice lacking endogenous PACAP, including hyperactivity, explosive jumping behavior, and decreased anxiety (Hashimoto et al. 2001; Marquez et al. 2009). They show depression-like behavior, in line with the now well-established antidepressant role of PACAP, and altered pain responses, in accordance with the role of PACAP in pain processing (Mabuchi et al. 2004; Hashimoto et al. 2009; Sandor et al. 2010; Pinhasov et al. 2011; Markovics et al. 2012). PACAP also plays a role in the regulation of the stress axis, and so PACAP-deficient mice display several abnormal stress responses (Hamelink et al. 2002; Hashimoto et al. 2011; Gaszner et al. 2012; Stroth et al. 2011). For example, the failure to adequately counterregulate plasma glucose levels has been suggested to be due to impaired secretion of epinephrine, and thus, PACAP appears to function

Table 1 Summary of the increased vulnerability of PACAP-deficient mice in different types of cellular injuries

Tissue/cell and injury type	Sign of increased vulnerability in PACAP-deficient mice	Reference
Focal cerebral ischemia induced by middle cerebral artery occlusion	Mortality↑, infarct volume↑, brain edema↑, neurological symptoms↑, bcl-2↓, cytochrome c release into cytoplasm↑, differences in gene transcripts relation to neuroprotection	Chen et al. 2006; Ohtaki et al. 2006; Nakamachi et al. 2010
Retinal ischemia induced by bilateral common carotid artery occlusion	Worse histological outcome, thickness of retinal layers↓, number of cells in ganglion and inner nuclear layers↓	Szabadfi et al. 2012
Retina excitotoxicity induced by NMDA	Number of retinal ganglion cells↓, apoptotic markers↑	Endo et al. 2011
Experimental autoimmune encephalitis/model of multiple sclerosis, induced by myelin oligodendrocyte glycoprotein	Mortality↑, clinical symptoms↑, nervous tissue inflammation↑, expression of proinflammatory cytokines in spinal cord↑, expression of anti-inflammatory cytokines in spinal cord↓	Tan et al. 2009
Facial nerve crush injury	Axon regeneration↓, microglial reaction in facial motor nucleus↑, proinflammatory cytokine gene expression↑	Armstrong et al. 2008
Spinal cord injury	Injury size↑, neuronal functional recovery↓, degenerated nerve cell number↑	Tsuchikawa et al. 2011
Kidney, in vitro oxidative stress	Cell viability↓	Horvath et al. 2010a
Kidney, in vitro hypoxia	Cell viability↓	Horvath et al. 2010b
Kidney, in vivo ischemia/reperfusion injury	Histological scores of tissue injury↑, differences in cytokine expression, antioxidant superoxide dismutase↓	Szakaly et al. 2011
Small intestine, ischemia/reperfusion injury	Oxidative stress marker malondialdehyde↑, antioxidant superoxide dismutase and reduced glutathione↓, histological scores of injury↑	Ferencz et al. 2010a
Small intestine, cold ischemia	Oxidative stress marker malondialdehyde↑, antioxidant superoxide dismutase and reduced glutathione↓, histological scores of injury↑	Ferencz et al. 2010b
Large intestine, inflammation/model of colitis induced by dextran sulfate sodium	Clinical symptoms↑, histological signs of inflammation↑, interleukin-1beta, -6 and -12↑, interferon-gamma↑, development of colorectal tumor↑	Azuma et al. 2008; Nemetz et al. 2008
Pancreas, glucotoxicity and lipotoxicty	Glucose-induced calcium release↓, insulin secretion↓, oxidative stress marker uncoupled protein-2↑	Nakata et al. 2010; Sakurai et al. 2011a
Heart, doxorubicin-induced cardiomyopathy	Mortality↑, cardiac function↓, fibrosis↑, myocardial degenerative changes↑, ultrastructural abnormalities↑, reactive oxygen metabolites↑, number of apoptotic cells↑	Mori et al. 2010
Lung, endotoxin-induced subacute airway inflammation	Bronchial responsiveness↑, perivascular edema↑, peribronchial inflammation↑, interleukin-1beta↑, myeloperoxidase↑	Elekes et al. 2011
Skin, oxazolone-induced hypersensitivity/model for allergic contact dermatitis	Skin edema↑, inflammation↑, monocyte chemoattractant protein-1↑	Kemeny et al. 2010

as a stress-response peptide during metabolic stress (Hamelink et al. 2002).

There are a few studies demonstrating morphological alterations in PACAP-deficient mice. Cerebellar development has been demonstrated to be severely affected in animals lacking PACAP (Allais et al. 2007). Although the gross appearance and weight of the cerebellum does not show changes between wild-type and PACAP-deficient mice (Vaudry et al. 2005), histological analysis of the cerebellum shows that the thickness of the external granular layer is significantly reduced at postnatal day 4 and that of the internal granular layer at postnatal day 7 (Allais et al. 2007). Neuronal differentiation of the granule cells is delayed, while naturally occurring cell death is increased during ontogeny in PACAP-deficient mice (Allais et al. 2007). Another study has revealed abnormal axonal arborization in the dentate gyrus of these mice (Yamada et al. 2010). We have described that, although the microscopical structure of the inner ear is not altered in PACAP-knockout mice, the expression of calcium-buffering proteins and that of PAC1 receptor is significantly altered (Tamas et al. 2012). Recently, we have also shown that PACAP-deficient mice have an earlier onset of myelination in the brain, and based on these observations, the inhibitory effect of PACAP on myelination was proposed, possibly allowing time for axonal development, synapse formation, and thus neuronal plasticity (Vincze et al. 2011).

PACAP-Deficient Mice are More Vulnerable to Different Harmful Stimuli

Increased Vulnerability to Stressors in the Nervous System of PACAP-Deficient Mice

PACAP is now a well-known neurotrophic and neuroprotective peptide. Its protective effects in neurons have been shown against various harmful stimuli, such as 6-hydroxydopamine, ethanol, oxidative stress, and anisomycin (Somogyvari-Vigh and Reglodi 2004; Vaudry et al. 2009; Reglodi et al. 2011). Vaudry and coworkers have provided vast amounts of data for the neurotrophic and protective effects of PACAP in cerebellar granule cells (Botia et al. 2011; Seaborn et al. 2011). The potential protective role of endogenous PACAP is also supported by observations showing that PACAP and its receptors are altered after cerebral ischemia. For example, PACAP is strongly upregulated in cortical pyramidal cells after focal cerebral ischemia (Stumm et al. 2007) and in the dentate gyrus and CA1 field of the hippocampus after transient global cerebral ischemia (Shin et al. 2001; Riek-Burchardt et al. 2010). In a model of traumatic brain injury, a strong upregulation of PACAP mRNA was observed, particularly in the perifocal area (Skoglosa et al. 1999). Other nerve lesions have also been shown to induce PACAP upregulation in the respective ganglia or brainstem nuclei, such as in case of sciatic, facial, trigeminal, or sympathetic nerve transection and neuronal inflammation (Zhang et al. 1996; Larsen et al. 1997; Moller et al. 1997; Zhang et al. 1998; Zhou et al. 1999). In line with these studies showing the possible role of endogenous PACAP in protection against injuries, Vaudry and coworkers were the first to demonstrate that granule cells from PACAP-deficient mice react to cellular stressors with higher sensitivity (Vaudry et al. 2005). They found that elevated potassium, dibutyryl cAMP, and PACAP treatment itself promoted survival of granule cells, with no apparent differences between PACAP-deficient and wild-type mice. In control conditions, neuronal differentiation was also found to be identical. However, cells reacted with decreased survival in the presence of ethanol or oxidative stress induced by hydrogen peroxide (Vaudry et al. 2005). Incubation of granule cells with ethanol resulted in a significantly higher level of toxicity in cells from PACAP-deficient mice, which could be blocked by addition of exogenous PACAP. Similarly, oxidative stress by hydrogen peroxide resulted in a lower level of survival in granule cells of PACAP-deficient mice. Cell survival was decreased by approximately 20 % to both toxic agents in PACAP-deficient mice. These results clearly show that PACAP-deficient mice are more sensitive to cellular stressors and paved the path for subsequent experiments. The authors suggested that PACAP acts as an emergency response peptide supporting neuronal survival under sustained pathophysiological conditions (Vaudry et al. 2005). Recent results show that endogenous PACAP also plays an important role in the survival of newly generated adult hippocampal neurons induced by an enriched environment (Ago et al. 2011).

Brain Ischemia

The protective role of PACAP in global and focal ischemic neuronal death has been shown in several studies (Dohi et al. 2002; Reglodi et al. 2002; Tamas et al. 2002; Somogyvari-Vigh and Reglodi 2004; Ohtaki et al. 2008). These studies have provided evidence for the in vivo efficacy of PACAP in reducing cerebral infarct in focal ischemia and hippocampal neuronal death in global ischemia by approximately 50 %. Given the high concentration of endogenous PACAP in the brain, it was hypothesized that endogenous PACAP would protect the brain against ischemic insult. Indeed, Ohtaki et al. (2006) showed that infarct volume and neurological deficits were approximately 25 % higher in both homozygous and heterozygous PACAP-deficient mice with a middle cerebral artery occlusion, a model for stroke. There was no significant difference in these signs between heterozygous and homozygous animals, indicating that even the partial lack of PACAP increases the brain vulnerability to ischemia. The severity of focal ischemic damage was reduced by exogenous PACAP administration. It was also found that the ischemia-induced decrease in the antiapoptotic bcl-2 was further accentuated in

PACAP-deficient mice, while the cytochrome c released into the cytoplasm was increased compared to wild types (Ohtaki et al. 2006). The authors also found that this protection induced by PACAP was dependent on interleukin-6 (IL-6), which showed decreased immunoreactivity in heterozygous PACAP-deficient mice compared to wild-type controls. These results suggested that PACAP can decrease neuronal cell death induced by ischemic damage, partially via a signaling mechanism involving IL-6 (Ohtaki et al. 2006). Subsequent studies have confirmed the increased sensitivity of mice deficient in PACAP to cerebral ischemia. Heterozygous PACAP-deficient mice showed higher mortality (37 % mortality versus 16 %) and a 25 % increase in brain edema formation in a stroke model, without differences in cerebral blood flow (Nakamachi et al. 2010). Another research group also described that infarct volume and neurological deficits, evaluated as the number of walking faults, were significantly higher (10–15 %) in PACAP-deficient mice (Chen et al. 2006). Both the infarct volume and the level of neurological deficits could be ameliorated by treatment with exogenous PACAP. The authors also evaluated the changes in transcripts associated with cerebral ischemia using a cDNA microarray analysis. In wild-type mice, 228 known transcripts were upregulated, while one was downregulated, with a majority of transcripts upregulated at 24 h after ischemia (delayed response) and a smaller percentage upregulated at 1 h after ischemia (acute response) or at both times (sustained response). The analysis of the transcripts in PACAP-knockout mice showed that a larger percentage of the delayed-response genes required endogenous PACAP, indicating a more prominent role of endogenous PACAP in late responses. Among these transcripts are putative neuroprotective transcripts (Chen et al. 2006). The authors emphasized that although it is unlikely that all these transcripts are directly affected by PACAP, the peptide may trigger cell survival cascades leading to altered gene expression (Chen et al. 2006).

Retinal Ischemia and Excitotoxicity

Bilateral common carotid artery occlusion is a model of chronic cerebral hypoperfusion, leading also to ischemic retinal changes (Atlasz et al. 2007; Mester et al. 2009). Since PACAP had been demonstrated to be retinoprotective in ischemia-induced retinal degeneration (Atlasz et al. 2010), the question was raised whether the lack of endogenous PACAP would increase the degree of retinal damage. Indeed, 10 min of bilateral carotid artery occlusion followed by a 2-week reperfusion period resulted in a significantly worse histological outcome in PACAP-deficient mice, as shown by the thickness of the whole retina, the morphometric analysis of the individual retinal layers, and the cell numbers in the ganglion cell layer (Szabadfi et al. 2012). On average, there was a 25 % more reduction in the cell layers and ganglion cell number in PACAP-deficient mice. Exogenous PACAP administration could partially protect against retinal degeneration in PACAP-deficient mice. A recent study showed that even the partial lack of PACAP aggravates the death of retinal ganglion cells induced by NMDA toxicity using heterozygous PACAP-deficient mice (Endo et al. 2011). NMDA induced a reduction in retinal ganglion cell number and an increase in apoptotic markers. In heterozygous PACAP-deficient mice, 30–50 % had more severe cell loss after NMDA injection, and apoptotic cells appeared earlier. These effects could be reversed by exogenous PACAP administration (Endo et al. 2011). These results clearly show that endogenous PACAP reacts as a stress-response peptide, per se or by secondary mechanisms, that is necessary for endogenous protection against different retinal insults.

Experimental Autoimmune Encephalomyelitis

PACAP has also been shown to attenuate symptoms of autoimmune diseases, including experimental autoimmune encephalomyelitis, a model of multiple sclerosis (Kato et al. 2004). Tan and coworkers provided evidence that this protective effect is also present endogenously, since PACAP-knockout mice exhibited more severe clinical and pathological features of experimental autoimmune encephalomyelitis (Tan et al. 2009). Immunization with myelin oligodendrocyte glycoprotein (MOG35-55) resulted in an increased mortality of PACAP-knockout mice (30 % versus no mortality in the control group). The clinical symptoms peaked on day 14 and then declined in control animals, while symptoms continued increasing in PACAP-deficient mice, resulting in a higher average clinical score (more than 60 % higher in PACAP-deficient mice). The clinical manifestations were consistent with the histological findings of the inflamed nervous tissue showing a higher degree and more widespread inflammation in the knockout mice. The histological score was almost doubled in the PACAP-deficient mice compared to wild-type mice. In the spinal cord, expression of proinflammatory cytokine (TNF-alpha, IL-6, IFN-gamma, IL-12p35, IL-23p19, and IL-17) mRNAs was increased (ranging between 15 and 50 % increase). Similarly, enhanced mRNA expression was observed for proinflammatory chemokines and chemotactic receptors. On the other hand, anti-inflammatory cytokines (IL-4, IL-10, TGF-beta) showed a downregulation in the spinal cord. Regulatory T cells were reduced in lymph nodes and spinal cord, suggesting a regulatory function for PACAP in regulatory T cells' abundance after inflammation. Altogether, these results clearly show that PACAP is an endogenous protective peptide in a model of multiple sclerosis, providing immunological, pathological, and clinical protection (Tan et al. 2009; Abad and Waschek 2011).

Spinal Cord and Peripheral Nerve Injury

Mice lacking PACAP have also been shown to have impaired nerve regeneration and enhanced neuroinflammatory response in a motor nerve injury (Armstrong et al. 2008). In a model of a facial nerve crush injury, recovery of axon regeneration was significantly delayed in spite of no differences in motor neuron survival. The delayed regeneration was associated with an enhanced microglial reaction in the facial motor nucleus. Furthermore, increased levels (50–80 % increase) of proinflammatory cytokine gene expression (TNF-alpha, IL-6, and INF-gamma) in both the nucleus and the nerve crush site were observed, while mRNA for IL-4, a cytokine blocking macrophage activity, was reduced to less than 10 % of the level in wild-type mice (Armstrong et al. 2008).

Most recently, it has been reported that in heterozygous PACAP-knockout mice ($PACAP^{+/-}$), injury volume was larger, and the number of degenerated neuronal cells was significantly higher than in wild-type mice following contusion of the spinal cord. In addition, functional recovery score was lower, indicating that the lack of endogenous PACAP impairs spontaneous neuronal recovery (Tsuchikawa et al. 2011).

Increased Sensitivity to Harmful Stimuli in Peripheral Organs

Kidney

PACAP has protective effects in the kidney against various insults, including ischemia/reperfusion injury, myeloma light chain-induced nephropathy, cisplatin- and cyclosporine-induced nephrotoxicity (Li et al. 2008; Szakaly et al. 2008; Li et al. 2011; Reglodi et al. 2012). These results raised the question whether endogenous PACAP was also protective in kidney injuries. This was first tested in vitro, where primary kidney cell cultures derived from PACAP-deficient mice were exposed to oxidative stress by hydrogen peroxide (Horvath et al. 2010a). It was found that after 2 or 4 h of exposure to different concentrations of hydrogen peroxide, cell viability was significantly reduced in cultures of PACAP-knockout mice compared to those from control wild-type mice. Viability was approximately 30 % less, on average, in cells from PACAP-deficient mice than that of cells from wild-type mice. This increased vulnerability of kidneys from PACAP-deficient mice could be counteracted by exogenously given PACAP. Similar results were obtained in hypoxic injury in vitro: kidney cells isolated from PACAP-deficient mice were more susceptible to in vitro hypoxia induced by cobalt(II) chloride (Horvath et al. 2010b). These results show that endogenous PACAP protects against oxidative stress and hypoxia in the kidney and that PACAP may act as a stress sensor in renal cells. The in vitro hypersensitivity of renal cells from PACAP-deficient mice to oxidative stress has been subsequently demonstrated in vivo (Szakaly et al. 2011). In this study, PACAP-deficient mice underwent 45 or 60 min of renal ischemia followed by 2-week reperfusion. Mice lacking endogenous PACAP had a more negative histological outcome, with significantly higher histological scores for all tested parameters, such as degree of tubular dilation, Bowmann's capsule dilation, lymphocyte and macrophage infiltration, thyroidization, and the disappearance of the PAS-positive glycocalyx from under the brush border as measured on histological sections (Szakaly et al. 2011). The average histological score was about 40 % worse in PACAP-deficient mice after exposure to ischemia/reperfusion. In order to get insight into the mechanisms of the endogenous renoprotection by PACAP, tissue cytokine expression and the level of the endogenous antioxidant superoxide dismutase (SOD) were also determined after 60 min of ischemia/reperfusion. Cytokine expression was markedly different between wild-type and PACAP-deficient mice. In addition, the level of SOD was significantly, by 20 %, lower in PACAP-deficient animals after ischemia/reperfusion. These results show that the lack of endogenous PACAP leads to higher susceptibility to in vivo renal ischemia/reperfusion, further supporting the role of PACAP as an endogenous renoprotective peptide (Szakaly et al. 2011).

Small Intestine

Similar endogenous protective effects have been demonstrated in a small intestinal ischemia/reperfusion injury (Ferencz et al. 2010a). As PACAP had been shown to have beneficial effects given as an exogenous treatment in small bowel ischemia/reperfusion injury (Ferencz et al. 2009), the effects of endogenous PACAP have been tested in PACAP-deficient mice. In PACAP-knockout animals, tissue oxidative stress parameters, such as malondialdehyde (MDA), reduced glutathione (GSH), and SOD, were markedly changed after mesenteric small bowel ischemia. MDA increased significantly after 3 and 6 h of ischemia in both groups, but the increase was 10 and 25 % higher in the PACAP-deficient mice, respectively. In contrast, tissue concentration of the antioxidant GSH and activity of SOD significantly decreased with time in both groups. PACAP-deficient mice showed a 20–40 % greater decrease in these markers. Qualitative and quantitative histological results showed more destruction of the mucous, submucous layers, and crypts in knockouts compared to wild-type tissues. These processes correlated with the warm ischemia periods (Ferencz et al. 2010a). The protective effects of PACAP in small intestinal ischemic injury have highlighted the possibility of prolonged tissue conservation in experimental small intestinal transplantation. Indeed, addition of PACAP to standardized conservation solution prolonged the conservation period without injury (Ferencz et al. 2010b). Markers of oxidative stress were altered in PACAP-knockout animals in favor of increased oxidative insult. Namely, MDA levels were elevated, while

endogenous protective agents, SOD and GSH, were decreased in mice lacking PACAP. Furthermore, the tissue damage was also significantly worse as shown by detailed histological analysis in PACAP-deficient mice: mucosal, submucosal thickness, and depth of the crypts showed a 15–40 % more decrease in PACAP-deficient mice (Ferencz et al. 2010b).

Large Intestine

In the large intestine, the protective effects of endogenous PACAP have been shown in inflammatory diseases, such as animal models of colitis. Two groups, independently of each other and at the same time, reported similar results in dextran sulfate sodium-induced colon inflammation, a model of inflammatory bowel disease (Azuma et al. 2008; Nemetz et al. 2008). Azuma et al. (2008), using a short-term exposure (7 days) to dextran sulfate sodium, found that mortality increased by about 50 %, and the disease activity index determined by the weight loss, bleeding, and diarrhea was also markedly higher in mice lacking PACAP. Macroscopic examination revealed enhanced shortening of the large intestine in PACAP-deficient mice, to one-third of that of the wild-type mice. Although there was no difference in the structure of intact wild-type and PACAP$^{-/-}$ mice, histological scoring showed an increased number of infiltrating cells and crypt damage after colitis induction in PACAP-deficient mice in both the proximal and the distal colon parts. Furthermore, production of IL-1beta and IL-6 was upregulated by 50 % in the proximal colon of PACAP-deficient mice and that of interferon-gamma, IL-1beta, IL-6, IL-12, and keratinocyte-derived chemokine expression was upregulated in the distal colon by 50 % (Azuma et al. 2008). On the other hand, levels of anti-inflammatory cytokines, like IL-10, were only one-third of that of wild-type mice in the distal colon. Nemetz et al. (2008) described similar results using a longer exposure to dextran sulfate sodium (2 months). PACAP-deficient mice receiving normal water with no dextran sulfate sodium displayed no signs of inflammation and of colitis, suggesting that the lack of endogenous PACAP does not result in immunocompromisation leading to spontaneous development of colitis. After induction of colitis by exposure to dextran sulfate sodium, PACAP-knockout animals had more severe clinical symptoms and higher histological inflammation scores (50–70 % higher), restricted to the distal colon. In addition, induction of IL-1beta and IL-6 mRNAs was significantly higher (70 %) in PACAP-deficient mice. Interestingly, 60 % of PACAP-knockout mice developed colorectal tumors with aggressive-appearing pathology (Nemetz et al. 2008). Taken together, these data show that although the intact structure of the large intestine is not affected in PACAP-deficient mice, lack of endogenous PACAP leads to increased susceptibility to inflammation and inflammation-associated cancer development in the colon.

Pancreas

In the pancreas, PACAP occurs in the autonomic nerves and intrapancreatic ganglia (Sakurai et al. 2011a). It has been shown that PACAP protects pancreatic islets against oxidative stress induced by streptozotocin (Onoue et al. 2008). Not surprisingly, PACAP-knockout mice display increased sensitivity to glucotoxicity and lipotoxicity (Nakata et al. 2010; Sakurai et al. 2011a). High glucose or palmitate treatment markedly reduced the glucose-induced intracellular calcium release and insulin secretion in islets isolated from PACAP-deficient mice. Furthermore, the expression of uncoupled protein-2, which is upregulated in oxidative stress, was found to be expressed at twofold higher level in PACAP-deficient mice and further increased upon treatment with glucose or palmitate (Nakata et al. 2010). These data suggest that endogenous PACAP protects pancreatic beta cells from dysfunction induced by various insults (Sakurai et al. 2011a). In the exocrine pancreas, results regarding the effect of PACAP are somewhat conflicting. While overexpression of PACAP, as well as exogenous administration of pharmacological doses, aggravates cerulein-induced pancreatitis, PACAP-deficient mice react in an unusual manner to cerulein (Sakurai et al. 2011b). Approximately 60 % of PACAP-knockout mice showed severe hypothermia and died within 3 days after injection of cerulein. However, these mice exhibited less severe pancreatitis, as shown by elevations of serum pancreatic enzymes and histological abnormalities, probably due to the severe hypothermia (Sakurai et al. 2011b). There was no difference between the normothermic PACAP-deficient mice and their wild-type mates (Sakurai et al. 2011a). These results show that the lack of PACAP results in increased mortality following cerulein administration, and therefore PACAP may have protective effects also in pancreas, although the exact role of PACAP in pancreatitis remains to be further examined.

Heart

PACAP has also been shown to be protective in the heart. The peptide has been demonstrated to protect cardiomyocytes against oxidative stress, ischemia/reperfusion, and doxorubicin (Gasz et al. 2006; Racz et al. 2008, 2010; Alston et al. 2011). PACAP has also been found to reduce cardiac fibrosis (Sano et al. 2002). A recent study has revealed that this protective effect is also present endogenously (Mori et al. 2010). PACAP-deficient mice reacted with increased vulnerability to doxorubicin-induced cardiomyopathy. Homozygous PACAP-deficient mice showed a high mortality after doxorubicin treatment (only about 20 % survival), and heterozygous animals also displayed higher mortality (about 50 % survival rate) than wild type mice (90 % survival). Due to the observed high mortality rate of the homozygous mice, the rest of the experiments only included heterozygous (PACAP$^{+/-}$) animals.

Cardiac function was slightly, but significantly, lower at 10 days in these animals, as measured by echocardiography. Light microscopical histological analysis also revealed a one-third higher rate of fibrosis and degenerative changes in the myocardium, with a 50 % increase of the histological score together with a 50 % decrease of the myofibrillar diameter. Ultrastructural abnormalities were also more frequently observed in mice partially lacking PACAP, such as myofibrillar derangement and disruption and enlargement of subcellular organelles. Level of reactive oxygen metabolites, as a measure of oxidative stress, analyzed with a free radical electron evaluator, also showed a 30 % elevation in homozygous PACAP-deficient ($PACAP^{-/-}$) mice compared to controls. Finally, a sevenfold increase of apoptotic cells was detected in $PACAP^{+/-}$ mice. Continuous exogenous PACAP administration could attenuate the doxorubicin-induced changes in PACAP-deficient mice. Altogether, these results clearly show that not only exogenous but also endogenous PACAP has cardioprotective effects.

Lung

A recent study has revealed that mice lacking PACAP have an increased inflammatory response in endotoxin-induced subacute airway inflammation (Elekes et al. 2011). In this study, bronchial responsiveness as well as myeloperoxidase (MPO) activity was markedly increased at 6 and 24 h. Perivascular edema dominated the histological picture at 6 h, while remarkable peribronchial granulocyte accumulation, macrophage infiltration, and goblet cell hyperplasia were seen at 24 h. Airway hyperreactivity was significantly higher 24 h after endotoxin treatment, and inflammatory histopathological changes were more severe. MPO increase was almost double in $PACAP^{-/-}$ mice compared to the wild types at 6 h. These results provide evidence for a protective role for PACAP in endotoxin-induced airway inflammation and hyperreactivity.

Skin

PACAP-deficient mice also display increased inflammatory response and edema formation in allergic contact dermatitis (Kemeny et al. 2010). A delayed-type hypersensitivity reaction in the skin was induced by oxazolone, resulting in a 130 and 110 % swelling in wild-type mice after 24 and 48 h, respectively. This was slightly, but significantly, greater in PACAP-deficient mice after both 24 and 48 h (130 and 150 %, respectively). Histological analysis confirmed markedly increased edema in $PACAP^{-/-}$ mice, but the moderately enhanced inflammatory cell accumulation was not statistically significant compared with the wild types. There was no difference in myeloperoxidase activity of the ear homogenates. Elevation of monocyte chemoattractant protein-1, but not the levels of the other cytokines, was significantly higher (30 % higher) in the samples of the PACAP-deficient mice. These results suggest that PACAP exerts anti-inflammatory particularly edema-inhibiting effects in allergic contact dermatitis (Kemeny et al. 2010).

In summary, lack of endogenous PACAP leads to higher vulnerability in a number of injuries in the nervous system and peripheral organs while causing little or no marked changes under unchallenged circumstances. It is generally accepted that the protective effects of PACAP are mediated by a complex array of actions, including antiapoptotic, anti-inflammatory, and antioxidant effects. These mechanisms are supported also by the above studies, where lack of PACAP leads to increased apoptosis, inflammation, and oxidative stress, making the nervous system and peripheral organs more vulnerable to stressors. However, it is not known at the moment whether the lack of PACAP per se, secondary mechanisms or changes in the cellular protective machinery, or a combination of all is responsible for the observed increased sensitivity in PACAP-deficient mice. Numerous studies have shown that the addition of exogenous PACAP can alleviate some of the symptoms or decrease the level of cellular stress in both in vitro and in vivo models of injuries in PACAP-knockout mice. However, this still does not entirely prove that it is the lack of PACAP per se that is responsible for the increased vulnerability. PACAP is a widely accepted cytoprotective agent, which can be effective even in mice lacking endogenous PACAP. A recent study has pointed out a further possible mechanism to the endogenous protective role of PACAP. Ohtaki and coworkers have demonstrated that PACAP-knockout mice have a significantly higher level (approximately 20 %) of reactive oxygen metabolites, with a parallel decrease in biological antioxidant potential in the plasma (Ohtaki et al. 2010). However, this difference was only observed in aged PACAP-knockout mice. This observation supports the hypothesis that PACAP is part of the endogenous cytoprotective machinery and also shows that there are systemic changes affecting the entire organism, as plasma total antioxidant capacity was lower in PACAP-deficient mice. It also supports the observations that major differences are not present under all circumstances and can be provoked by challenges, such as tissue injury or aging. Further research has to determine the exact mechanisms responsible for the increased vulnerability as well as compensatory changes altered in case of PACAP deficiency. In any case, results show that the lack of endogenous PACAP leads to increased cellular stress and thus earlier onset of age-related changes and increased vulnerability to tissue injuries.

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