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THE EFFECTS OF PITUITARY ADENYLATE CYCLASE ACTIVATING POLYPEPTIDE IN RENAL ISCHEMIA/REPERFUSION

A Review

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Pituitary adenylate cyclase activating polypeptide (PACAP) is a multifunctional neuropeptide occurring in the nervous system as well as in the peripheral organs. Beneficial action of PACAP has been shown in different pathological processes. The strong protective effects of the peptide are probably due to its complex modulatory actions in antiapoptotic, anti-inflammatory and antioxidant pathways. In the kidney, PACAP is protective in models of diabetic nephropathy, myeloma kidney injury, cisplatin-, gentamycin- and cyclosporin-induced damages. Numerous studies have been published describing the protective effect of this peptide in renal ischemia/reperfusion. The present review focuses on the ischemia/reperfusion-induced kidney injury and gives a brief summary about the results published in this area.

Keywords: Kidney – ischemia/reperfusion – PACAP – renoprotection

INTRODUCTION

Pituitary adenylate cyclase activating polypeptide (PACAP) is a neuropeptide of the secretin/glucagon/VIP peptide family and consists of 38 or 27 amino acids (PACAP38 and PACAP27, respectively). PACAP occurs in all organs and one of the best known effects are its cytoprotective actions [50]. The effects of PACAP are mediated by G-protein-coupled receptors: the specific PAC1 receptor and VPAC1 and 2 receptors, which also bind VIP with similar affinity [23]. In accordance with the widespread distribution, PACAP exerts pleiotropic effects [50]. Among others, PACAP has protective effects in pathological conditions of the nervous system, such as models of neurodegenerative diseases, ischemia or traumatic nerve injuries [33, 47]. Similar effects have been shown in non-neuronal tissues, such as in the cardiovascular, respiratory, gastrointestinal and immune system and in endocrine organs against various kinds of harmful stimuli [50]. It is generally accepted that the protective function of PACAP is mediated by the antiapoptotic, anti-inflammatory and antioxidant effects: PACAP inhibits proapoptotic signaling at several levels, while it stimulates antiapop-

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totic pathways. PACAP also inhibits several proinflammatory cytokines and it also elevates the antioxidant capacity [41, 42, 50].

In accordance with the cytoprotective effects in peripheral organs, numerous studies reported on the nephroprotective effect of this peptide. This nephroprotective effect has been shown in animal models of diabetic nephropathy, cisplatin-, cyclosporin-, gentamycin-induced nephrotoxicity, myeloma nephropathy and ischemia/reperfusion-induced kidney injury [34]. In case of gentamycin-induced kidney injury, repeated use of PACAP reduced the increased TNF-α level [3, 27]. In a rat model of diabetic nephropathy, iv. PACAP suppressed the increase of the profibrotic cytokine TGF-β and that of the proinflammatory cytokine TNF-α. It counteracted the weight gain of the kidneys and the enlargement of the glomeruli. In PACAP-treated rats the protein level measured in the urine was lower [3, 27]. Furthermore, another study described that PACAP treatment reduced the severity of the histological alterations in diabetic kidneys and decreased the expression of several cytokines [5]. In cyclosporin A-induced kidney damage both *in vivo* and *in vitro* studies proved the protective effect of PACAP [21]. PACAP reduced TGF-β1 expression, the rise of LDH-levels, decreased apoptosis and the production of oxygen reactive species, suppressed the increased expression of the major extracellular matrix components, ameliorated the tubulointerstitial damage in the kidney and nearly normalized serum creatinine level [21]. In cisplatin-induced kidney injury the administration of PACAP reduced the increased level of blood urea nitrogen and serum creatinine, decreased apoptosis and the severity of the renal morphological changes. Increase of the level of TNF-α both in the plasma and in the kidney could be counteracted by PACAP treatment [28, 30]. Several studies have reported the protective effect of PACAP in myeloma nephropathy [2, 3, 25–27]. Among others, PACAP was able to reduce TNF-α and IL-6 expression as well as the morphological damage of tubular cells. A human case report showed that iv. PACAP resulted in reduction of free lambda light chains in the urine [26]. It is also known that PACAP increases blood flow of the kidneys and stimulates renin secretion through the activation of PAC1 receptor [11, 13].

Ischemia/reperfusion-induced injuries represent a major problem in the clinical practice. Microvascular perfusion failure, no-reflow and tissue hypoxia play a role in the pathomechanism of this damage despite reperfusion and reoxygenation. Reperfusion following ischemia induces an inflammatory response. Inflammatory cells (such as macrophages and neutrophils) taking part in this response produce cytokines, chemokines, oxygen free radicals and lipid mediators that contribute to the development of the injury. Apoptosis and necrosis are also involved in this process [31]. Regarding the kidney, some surgical interventions, kidney transplantation and numerous disorders are accompanied by ischemia/reperfusion-induced injury. There are several studies dealing with the effect of PACAP in kidney damage caused by ischemia/reperfusion, briefly summarized in the present review.

Effect of exogenous PACAP in ischemia/reperfusion-induced kidney injury

Cytoprotective effects of PACAP in ischemia/reperfusion-induced injuries both in the nervous system and the peripheral organs have been reported in several studies. This protective effect was first described in global and focal cerebral ischemia [32, 42, 48]. Subsequently, the protective effects of PACAP were reported in ischemic injury of the retina [4] and in ischemia/reperfusion-induced damages of peripheral organs, such as in intestinal, cardiac and liver ischemia/reperfusion [8, 20, 39].

The first study about the role of PACAP in ischemia/reperfusion-induced kidney injury was reported by Riera and his coworkers [38]. They showed that administration of PACAP in continuous infusion (160 pmol/h 7 days long) starting before or 6 h after warm ischemia attenuated the tubulointerstitial damage in the kidney and decreased the serum creatinine level. They also showed that PACAP increased the plasma cAMP-level, the serum interleukin-6 level and decreased the indicators of inflammatory cell infiltration. Based on these results it can be expected that the protective effect of PACAP in the kidney during ischemia/reperfusion is mediated, at least partially, by the anti-inflammatory effect of this peptide. Subsequently, our research group described that a single iv. injection of PACAP attenuated the tubular damage, ameliorated the survival of the experimental animals and improved the ischemic tolerance of the kidney after different times of ischemia/reperfusion [44]. Control animals had decreased survival time and their kidneys showed multifocal acute tubular atrophy after 45 min ischemia, while 60 min ischemia resulted in premature death with severe multifocal tubular atrophy in the kidneys in all control rats. In contrast, PACAP-treated animals survived with mild histological changes following 45 min ischemia. PACAP-treated rats had higher survival rate after 60 min ischemia and kidneys showed only moderate focal tubular alterations. All control rats died before the termination of ischemia in case of 75 min ischemia, whereas PACAP-treated animals survived longer (5–10 days after the ischemia), with the kidneys showing severe focal tubular atrophy.

Regarding the protective mechanisms, it is suggested that PACAP acts through a combination of antiapoptotic, anti-inflammatory and antioxidant actions. Apoptosis plays an important role in ischemia/reperfusion-induced kidney injury [40]. The antiapoptotic effect of PACAP in the kidney had already been proven in cisplatin- and in cyclosporin-induced kidney injury [21, 28]. We found a significant decrease of the antiapoptotic Bcl-2 after renal ischemia/reperfusion, reversed by PACAP treatment [15]. Cytokines and chemokines also play an important role in the development of kidney injury after ischemia/reperfusion [6, 18, 19, 49]. PACAP decreased proinflammatory cytokines, like TNF-α and IL-6, in different pathological conditions in the kidney [27]. The anti-inflammatory effect of PACAP was also confirmed in renal ischemia/reperfusion [15]. Increased expression of some cytokines was reduced by preoperative PACAP treatment, such as fractalkine (chemokine ligand 1), sICAM-1 (soluble intercellular adhesion molecule-1), L-selectin, RANTES (regulated upon activation normal T cell expressed and secreted), CNTF (ciliary neurotrophic factor),

MIP-3α (macrophage inflammatory protein-3α), and TIMP-1 (tissue inhibitor of metalloproteinase-1) [15]. Another study has described the decrease of the superoxide dismutase (SOD) activity after 60 min ischemia and reperfusion in the kidney [17]. This decrease could be counteracted by a single iv. PACAP injection. The results are in accordance with the findings of earlier studies, where the changes of SOD activity were measured in intestinal ischemia/reperfusion [8]. The protective effect of PACAP has also been shown in oxidative stress-induced kidney injury *in vitro* [17]. Hydrogen peroxide-induced oxidative stress resulted in the decrease of primary rat kidney cell survival, that could be dose-dependently attenuated by PACAP treatment.

The protective effect of PACAP in a mouse model of ischemia/reperfusion-induced kidney injury has also been proven [29]. Intraperitoneal administration of PACAP resulted in attenuated tubular alterations and neutrophil accumulation, in addition, apoptosis was also decreased. Blood urea nitrogen, serum creatinine, plasma and kidney homogenate TNF-α level were lower in PACAP-treated animals. Toll-like receptors, which are important mediators of innate immunity in the kidney, were also shown to participate in the protective effects of PACAP [22, 29].

Presence of PACAP and its receptors in the kidney

The presence of PACAP38 both in rat and mouse kidneys has been revealed by mass spectrometry and radioimmunoassay [7, 14]. PACAP38- and PACAP27-like immunoreactivity could be observed both in the cortex and medulla with the former being stronger [1, 7, 45]. We also showed that PACAP38- and PACAP27-like immunoreactivity detected in the kidney sensitively reacted to ischemia/reperfusion [45]. Measurements were performed after 60 min ischemia and 1, 6, 24 h reperfusion. In the cortex, an acute decrease of PACAP was followed by a marked increase on the intact side, which was not observed on the ischemic side. In the medulla, changes could be observed only on the clamped side, where PACAP38-like immunoreactivity showed a significant increase. Although the exact mechanism of these changes is not known yet, it can be suggested that PACAP increased due to its protective effect. Similar upregulation of PACAP has been found earlier in response to other insults [42, 52]. The longer and more expressed increase of PACAP in the medulla may explain the higher resistance against ischemia. The presence of PACAP receptors has also been shown in the urinary system, intensively expressed in kidney cortical tubular epithelial cells [7, 12, 23, 24, 36, 37, 51]. The localization of the receptors on the tubular epithelial cells can explain the attenuated tubular damage in case of PACAP treatment.

The effect of endogenous PACAP in ischemia/reperfusion-induced kidney injury

It is suggested that endogenous PACAP functions as a protective agent against different harmful stimuli [35, 50]. Several studies have found that PACAP deficient mice

Table 1
Summary of the protective effects of PACAP in renal ischemia-reperfusion

Reference	Mode of investigation		Observed changes
Riera et al. [38]	*in vivo*	administration of PACAP	decreased tubulointerstitial damage, lower serum creatinine level, reduced myeloperoxidase (MPO) activity and CD45+ cell number, increased IL-6 level
Szakaly et al. [44]	*in vivo*	administration of PACAP	reduced tubulointerstitial damage, decreased mortality
Horvath et al. [15]	*in vivo*	administration of PACAP	increased Bcl-2 level, decreased level of several proinflammatory cytokines
Horvath et al. [14]	*in vivo*	lack of endogenous PACAP	decreased cell survival in H_2O_2-induced oxidative stress
Horvath et al. [16]	*in vivo*	lack of endogenous PACAP	decreased cell survival in $CoCl_2$-induced *in vitro* hypoxia
Li et al. [29]	*in vivo*	administration of PACAP	attenuated tubular damage, lower level of serum creatinine and blood urea nitrogen, decreased apoptosis, reduced neutrophil infiltration, decreased expression of the markers TNF-α, MyD88, TLR4, TRAF6, TRIF, IRF3
	in vivo		decreased apoptosis, reduced expression of MCP-1, IL-6, MIP-2, MyD88, TLR4, TRAF6, TRIF, IRF3 in primary mouse renal proximal tubule cells
Horvath et al. [17]	*in vivo*	administration of PACAP	increased cell survival in primary rat kidney cell culture following H_2O_2-induced oxidative stress
	in vivo		increased SOD activity and GSH level
Szakaly et al. [46]	*in vivo*	lack of endogenous PACAP	more severe histological outcome, higher level of numerous proinflammatory cytokines, reduced SOD activity
Khan et al. [22]	*in vivo*	administration of PACAP	reduced expression of kidney injury biomarkers, lower level of serum creatinine, decreased apoptosis, reduced neutrophil infiltration and tubular damage, suppressed protein levels of TLR-associated cytokines, reverse of the changes of the expression of several TLR-related genes

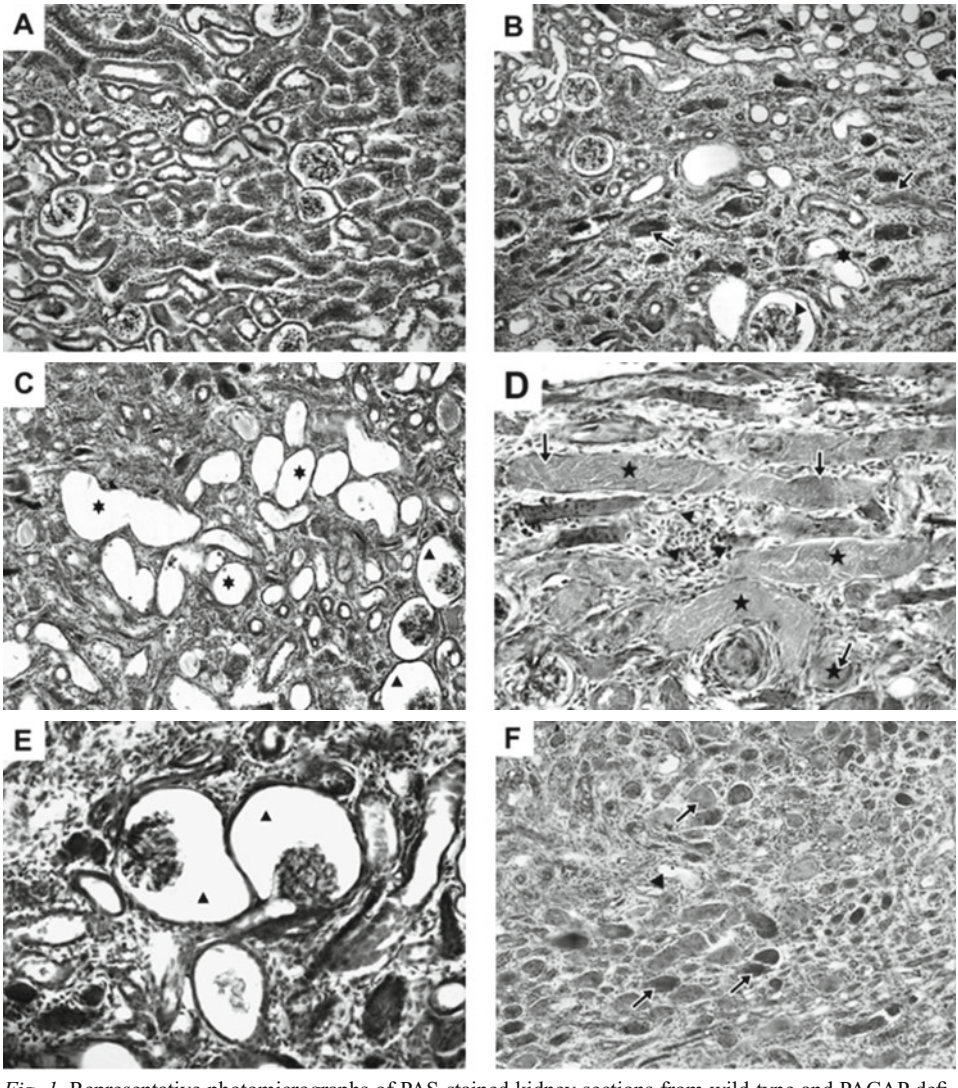

Fig. 1. Representative photomicrographs of PAS-stained kidney sections from wild-type and PACAP deficient mice. A: Cortex of a control (sham-operated) wild-type mouse. Both the Malpighian corpuscles and the tubuli are intact, the PAS+ glycocalyx layer can be clearly seen. B: Cortex of a wild-type mouse after 45 min ischemia and reperfusion. Some tubuli filled with cylinders can be observed (thyreoidisation) (arrows). The triangle represents a mildly dilated Bowman's capsule. Moderate tubular dilatation can be also seen (asterisk). C: The tubuli are widened in the cortex of a PACAP deficient mouse after 60 min ischemia and reperfusion (asterisks). The absence of the PAS-positive glycocalyx is seen on the luminal surface of the tubuli. Dilatation of the Bowman's capsule can be also observed (triangle). D: Cortex of a PACAP deficient mouse following 45 min ischemia and reperfusion. The tubuli are dilated and the lining epithelial cells are strongly flattened (arrows). Almost all of the tubuli show thyreoidisation (asterisks). The connective tissue contains a lot of PAS-stained macrophages (arrowheads). E: Extremely enlarged Bowman's capsules in the cortex of a PACAP deficient mouse after 60 min ischemia and reperfusion are shown (triangle). F: Thyreoidisation (arrows) in the kidney of a wild-type mouse following 60 min ischemia and reperfusion

react more sensitively to various stressors [35], including ischemic lesions. PACAP deficient mice displayed larger infarcted area in a model of focal cerebral ischemia and the extent of the ischemic retinal lesion was also increased [43]. There are similar findings in peripheral tissues in ischemic lesions. A greater damage could be observed in PACAP deficient mice in case of intestinal ischemia while intact small intestine of deficient mice was similar to that of wild-type mice [9, 10]. We have also reported such findings in case of the kidneys of PACAP deficient mice [46]. Although histological evaluation showed no difference between the kidneys of intact wild-type and PACAP deficient mice, there was more severe tissue damage after ischemia/reperfusion in PACAP deficient mice. Here we show a representative example of the increased vulnerability of PACAP deficient mice. Kidneys of PACAP deficient mice showed more severe morphological outcome after 45 or 60 min ischemia and 2 weeks reperfusion (Fig. 1). This is reflected in all tested histopathological parameters (dilatation of the Bowman's capsule, tubular dilatation, thyreoidisation, lymphocyte- and macrophage infiltration, damage of the glycocalyx layer) [46]. We also determined cytokine expression and found an increased expression of several cytokines in PACAP deficient mice after ischemia/reperfusion, such as C5a, macrophage inflammatory protein-2 (MIP-2), monocyte chemoattractant protein-1 (MCP-1), tissue metalloproteinase inhibitor-1 (TIMP-1), B-lymphocyte chemoattractant (BLC) [46]. Lower activity of the endogenous antioxidant SOD could be measured in control (sham-operated) PACAP deficient mice compared to the control wild-type mice. The activity of SOD decreased both in wild-type and PACAP deficient mice following ischemia/reperfusion. The extent of this decrease correlated to the baseline level was significant only in the PACAP deficient mice [46]. Based on the results can be concluded that endogenous PACAP has an effect on the antioxidant/scavenger system, and that PACAP deficient mice are more vulnerable to harmful stimuli.

Nephroprotective effect of endogenous PACAP in oxidative stress-induced kidney injury has also been revealed *in vitro.* Primary kidney cell cultures derived from PACAP deficient mice exposed to oxidative stress or *in vitro* hypoxia had decreased cell viability [14, 16] compared to the cultures of wild-type mice. This reduced cell viability could be counteracted by exogenously given PACAP38.

In summary, the published results have revealed that both the exogenous and the endogenous PACAP have a protective effect in renal ischemia/reperfusion *in vivo* as well as *in vitro* (summarized in Table 1). The antiapoptotic, anti-inflammatory and antioxidant effects of the peptide can all play an important role in this protective effect.

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