CrossMark

PACAP Is Protective in a Rat Model of Retinopathy of Prematurity

Timea Kvarik[1,2] · Barbara Mammel[1,2] · Dora Reglodi[1] · Krisztina Kovacs[3] ·
Dora Werling[1] · Brigitta Bede[1] · Alexandra Vaczy[1] · Eszter Fabian[1] · Gabor Toth[4] ·
Peter Kiss[1] · Andrea Tamas[1] · Tibor Ertl[2] · Judit Gyarmati[2] · Tamas Atlasz[1,5,6]

Abstract The oxygen-induced retinopathy (OIR) is a well-established rodent model of retinopathy of prematurity (ROP), which is one of the most common causes of childhood visual impairment affecting preterm babies. Pituitary adenylate cyclase-activating polypeptide (PACAP) is known to have neuroprotective effects. Several studies have revealed the presence of PACAP and its receptors in the retina and reported its protective effects in ischemic and diabetic retinopathy. In this study, we investigated whether PACAP administration can influence the vascular changes in the rat OIR model. OIR was generated by placing the animals in daily alternating 10/50 oxygen concentrations from postnatal day (PD) 0 to PD14 then returned them to room air. Meanwhile, animals received PACAP or saline intraperitoneally or intravitreally from PD1 to PD8 or on PD11, PD14, and PD17, respectively. On PD19 ± 1, the retinas were isolated and the vessels were visualized by isolectin staining. The percentage of avascular to whole retinal areas and the number of branching points were measured. Change in cytokine expression was also determined. Intravitreal treatment with PACAP remarkably reduced the extent of avascular area compared to the non- and saline-treated OIR groups. Intraperitoneal PACAP injection did not influence the vascular extent. Retinal images of room-air controls did not show vascular alterations. No changes in the number of vessel branching were observed after treatments. Alterations in cytokine profile after local PACAP injection further supported the protective role of the peptide. This is the first study to examine the effects of PACAP in ROP. Although the exact mechanism is still not revealed, the present results show that PACAP treatment can ameliorate the vascular changes in the animal model of ROP.

Keywords Oxygen-induced retinopathy · Muller glial cell · Retina · Neovascularization

Introduction

Retinopathy of prematurity (ROP) is one of the leading causes of childhood blindness affecting babies born prematurely. Premature birth interferes with the normal retinal vasculogenesis leading to two pathological phases of ROP: in the first vasoobliterative phase, the vessel formation stops, small vessels obliterate, and retinal ischemia occurs. In the second vasoproliferative phase, new abnormal vessels start to form at the border of vascularized and avascular retina. These vessels are tortuous, dilated, and incapable of autoregulation; they can spread into the vitreous body, cause bleeding, and, in worst cases, traction of the retina occurs leading to blindness.

Despite being an intensively researched field of neonatology, the exact pathogenesis is still not revealed. Many causative factors have already been identified, including oxygen therapy. The first attempt to decrease the number of patients with ROP was to optimize the use of oxygen in neonatal care.

✉ Tamas Atlasz
attam@gamma.ttk.pte.hu

1 Department of Anatomy, PTE-MTA "Lendulet" PACAP Research Team, University of Pecs, Pecs, Hungary

2 Department of Obstetrics and Gynecology, University of Pecs, Pecs, Hungary

3 Department of Biochemistry and Medical Chemistry, University of Pecs, Pecs, Hungary

4 Department of Medical Chemistry, University of Szeged, Szeged, Hungary

5 Department of Sportbiology, University of Pecs, Ifjusag utja 6, Pecs 7624, Hungary

6 Janos Szentagothai Research Center, University of Pecs, Pecs, Hungary

Springer

Although neonatologists try to avoid the excessive use of oxygen, there are other risk factors which likely add to the etiology of the disease. Although numerous human and animal studies have tried to find the optimal treatment for the disease, still, no therapy has been proved to be effective without adverse effects (Beharry et al. 2016).

Pituitary adenylate cyclase-activating polypeptide (PACAP) is a neuropeptide first isolated from hypothalamic extracts. It was later shown, together with its receptors VPAC1, VPAC2, and PAC1, throughout the nervous system and in the peripheral organs (Clason et al. 2016; Girard et al. 2016; Vaudry et al. 2009). PACAP is a peptide with multiple functions; one of them is its general cytoprotective effect (Matsumoto et al. 2016). This has been demonstrated in a variety of systems in vitro and in vivo (Reglodi et al. 2015; Shioda and Nakamachi 2015; Somogyvari-Vigh and Reglodi 2004). One of the most well-studied neuroprotective actions of PACAP is its protective effect in the retina. After the demonstration of PACAP and its receptors in the retina, evidence on its retinoprotective efficacy was published in an optic nerve injury model (Seki et al. 1997, 2008). Subsequent studies have confirmed its retinoprotective efficacy in several models of retinopathy. In the adult retina, PACAP is protective in ischemic retinopathies induced by different techniques, such as ligation of the carotid arteries (Atlasz et al. 2007; Werling et al. 2014) and high intraocular pressure (Seki et al. 2011). PACAP has also been shown to be protective in models of other retinal pathologies, such as UV light-induced degeneration (Atlasz et al. 2011), NMDA-induced excitotoxicity (Wada et al. 2013) and streptozotocin-induced diabetic retinopathy (D'Amico et al. 2015; Szabadfi et al. 2014, 2016).

As ROP is a vasculoproliferative retinopathy, it is important to note that PACAP has been proven to influence both normal and pathological angiogenesis in several organs. Among others, it has been described that PACAP affects several angiogenetic factors in trophoblast and mammary epithelial cells (Csanaky et al. 2014; Horvath et al. 2014). Diabetic retinopathy also involves pathological vasculogenesis, which leads to degeneration of the neuronal retinal layers. In models of diabetic retinopathy, PACAP has been shown to ameliorate the diabetes-induced retinal degeneration at several levels (D'Amico et al. 2015; Szabadfi et al. 2014, 2016). Furthermore, in other retinopathies, PACAP has been found to counteract the injury-induced alterations in the cytokine profile, such as in ischemic retinopathy (Szabo et al. 2012).

PACAP, as a general neurotrophic molecule, also plays a role in the development of the retina. The necessity of a proper PACAPergic system has been shown during retinogenesis (Lang et al. 2010). PACAP is known to influence the phenotype of retinal dopaminergic cells during development (Borba et al. 2005). Given the neurotrophic and neuroprotective effects of PACAP, it is not surprising that the peptide has strong neuroprotective effects also in neonatal retinal injuries, such as monosodium glutamate-induced excitotoxic injury of the newborn retina (Babai et al. 2005; Kiss et al. 2011). Based on the strong protective effects of PACAP in both adult and neonatal retinas as well as its trophic effects on retinogenesis and its influence on vasculogenesis, we hypothesized that PACAP would be protective in a model of ROP, the most common vision-threatening condition of the newborn.

Materials and Methods

Experiments were performed on Sprague-Dawley rat pups of both sexes from birth to the age of 3 weeks. Animal housing and care and application of experimental procedures were in accordance with institutional guidelines under approved protocols (No: BA02/2000–15024/2011, University of Pecs following the European Community Council directive).

Oxygen-Induced Retinopathy

The oxygen-induced retinopathy (OIR) is a well-established rodent model of ROP. There are several different versions of this model, but the model that resembles most the human pathogenesis is the alternating OIR, where animals are exposed to daily alternating high and low oxygen concentration (Penn et al. 1994). Animals were kept in an oxygen chamber (Biosherix Ltd.) supplied by an oxygen sensor to monitor and maintain gas levels (ProOx P110, BioSpherix Ltd.) continuously. To induce OIR, litters of rat pups with their nursing mothers were exposed to daily alternating high ($50\% \pm 2$) and low ($10\% \pm 2$) oxygen concentration from PD1 to PD14, then they were returned to room air until PD18–20. Control litters were room air reared during the whole experiment (Penn et al. 1994).

Administration of PACAP38

PACAP38 solution was administered via two routes. One group received 1 mg/kg PACAP38 solution injected into the peritoneal cavity daily on the first week of life. In another group, as a local treatment, we injected 100 pmol/3 µl PACAP38 into the vitreous cavity of one eye on PD11, PD14, and PD17, while the other eye was given the same amount of vehicle. The dose of PACAP38 was chosen based on our earlier studies (Atlasz et al. 2010, 2016). The injections were performed with a 33-gauge syringe (Hamilton Company, USA) under anesthesia. The injection site was controlled through an operating microscope. The same treatment strategies were used on room-air control litters.

Immunohistochemistry

Immunohistochemistry for isolectin, a specific marker of vascular endothelial cells (Ismail et al. 2003), was performed as previously described by Connor and co-workers (Connor et al. 2009). Briefly, after anesthesia on PD19 ± 1, the eyes were removed and immediately placed into 4 % formalin solution for fixation at room temperature. After 1 h, the eyes were washed three times in phosphate buffer saline (PBS) and the retinas were isolated under dissecting microscope. To stain the retinal vasculature, 500-μl fluoresceinated isolectin solution (Isolectin GS-IB4 from Griffonia simpliciforia, Alexa Fluor 568 conjugate; Thermo Fischer Scientific Inc.) was added to the isolated retinas. After an overnight rocking in isolectin solution at room temperature, the retinas were rinsed three times in PBS. Finally, four incisions were made to flatten the retinas onto microscope slides and were covered with a coverslip with mounting media (Fluoromount, Sigma-Aldrich Co.). Immunohistochemistry was performed from 72 animals; detailed numbers are given in the "Results" section.

Assessment of Vessel Morphology

A trained observer blinded for the groups evaluated the percentage of avascular retinal territory, number of vessel branching, and clock hours with neovascular tufts. Measurements were executed by AdobePhotoshop CS6 and ImageJ softwares. Both central vascularized and peripheral non-vascularized areas were measured and the percentage of avascular areas was determined. To evaluate the branching of the vessels, the observer counted the branching points of the widest vessels in each quadrant of each retina. Retinal pictures were divided into 12 equal portions, the "clock hours" and the number of clock hours with neovascular tufts formation were given.

Results are represented in mean ± SEM. Statistical analysis was performed by analysis of variance with Fisher's post hoc test after Bartlett's test for equal variances using STATISTICA software (StatSoft Inc., Hungary).

Cytokine Array Analysis

After euthanasia on PD 19 ± 1, the eyeballs were removed and the retinas were carefully separated and quickly frozen in dry ice. Cytokine proteins were investigated from pooled tissue homogenates by semi-quantitative Rat Cytokine Antibody Array Kits (R&D Systems, Biomedica, Hungary). In these arrays, the sample proteins bind to selected captured antibodies spotted on nitrocellulose membranes. The kits contain all buffers, detection antibodies, and membranes necessary for the measurement. The arrays were performed as described by the manufacturer's protocol. Briefly, after blocking the membranes for 1 h and adding the detection antibody cocktail

for another 1 h at room temperature, the membranes were incubated with 1.5 ml tissue homogenates at 2–8 °C overnight on a rocking platform. After washing with buffer three times, membranes were incubated with horseradish peroxidase-conjugated streptavidin at room temperature and exposed to chemiluminescence detection reagent to develop X-ray films. The arrays were repeated four times. For data analysis, films were scanned and pixel densities of interested cytokines, selected by eye control, were measured by Protein Array Analyzer of ImageJ software and were normalized to the reference spots. To compare the possible differences between cytokine profiles of the different treatment groups, we determined the relative density change of the selected spots. Cytokine array was performed from 32 animals ($n = 8$ in each group).

Results

### Effect of PACAP on the Vessel Extension and Morphology

We evaluated whether systemic or local PACAP38 administration may alter the extension of vessels and cause change in vessel morphology in rats with OIR. To investigate the angiogenic effect of PACAP38 on retinal vascularization, we performed fluorescein isolectin staining and defined the nonperfused peripheral retinal area (Fig. 1).

Systemic, intraperitoneally ($n_{eye} = 42$) given PACAP38 (Fig. 1f) in a concentration of 100 μg/ml did not reveal any differences compared to the control ($n_{eye} = 47$) OIR retina (Fig. 1e) (18.08 ± 1.82 % vs 20.45 ± 1.51 %; $p = 0.28$) (Fig. 2). In contrast, intravitreal ($n_{eye} = 35$) treatment with PACAP injection (Fig. 1h), in a concentration of 100 pmol/3 μl, markedly reduced the extent of nonperfused area compared to that of the non-treated OIR group (13.25 ± 1.47 % vs 20.45 ± 1.51 %; $p = 0.0043$) and to the vehicle-injected ($n_{eye} = 19$) group (Fig. 1g) (13.25 ± 1.47 % vs 20.4 ± 2.58 %; $p = 0.029$) (Fig. 2). Retinal images of normoxia controls did not show vascular changes (Fig. 1a–d). As intraperitoneal PACAP injection did not influence vessel formation, we did not operate with this group for the further analyzes.

The neovascular phase of OIR is characterized by newly formed wide, tortuous vessels growing from preexisting ones, and the appearance of vascular tufts at the border of vascular and avascular area. One method to assess neovascularization is to give the number of clock hours containing vascular tufts. To evaluate branching, four vessels were selected in each retinal quadrant and their branching points were counted.

Control retinas did not develop abnormal vessel knots. Although we observed tuft formation in the OIR groups at the appropriate places, we did not find any difference between the groups (Fig. 3a). On counting the vascular branches,

Fig. 1 Representative pictures of isolectin GS-IB4 stained whole-mounted retina from control (**a**–**d**) and OIR animals (**e**–**h**) after intraperitoneal PACAP (**b**, **f**), intravitreal saline (**c**, **g**), or intravitreal PACAP (**d**, **h**) injections. *Scale bar* = 1 mm
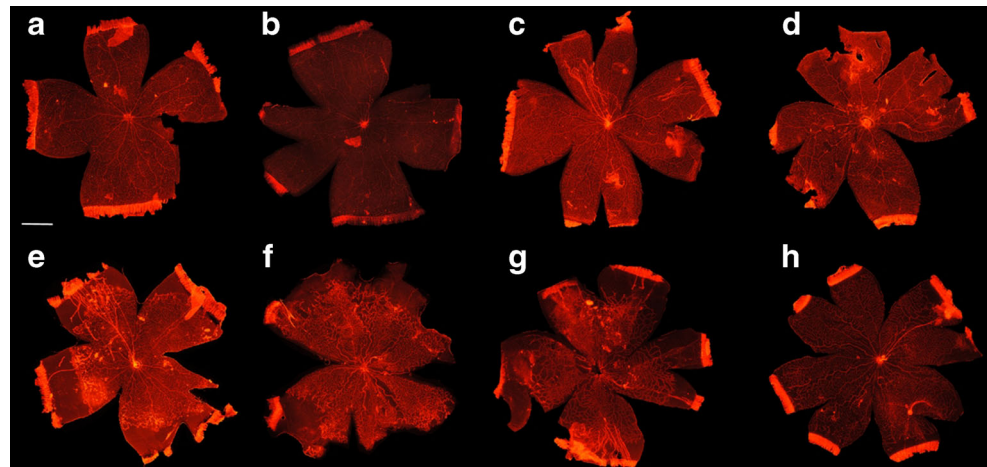
retinopathic eyes were found to develop more vessels than the controls, but PACAP treatment neither reduced nor increased their number (Fig. 3b).

Cytokine Protein Analysis

We compared the cytokine expressions of injected eyes to avoid the effect of the injection itself at the evaluation. In OIR ($n = 8$), the expression of several cytokines altered including proinflammatory, trophic, and adhesion factors: ciliary neurotrophic factor (CNTF), lipopolysaccharide-induced CXC chemokine (LIX), L-selectin, macrophage inhibitory protein (MIP-3a), tissue inhibitor of metalloproteinase (TIMP-1), and vascular endothelial growth factor (VEGF) showed elevation compared to the control retinas. After PACAP injection ($n = 8$), we observed a decrease in the expression of all the above mentioned cytokines and an increase in the expression of TIMP-1 (Fig. 4). Other cytokines did not show any marked difference.
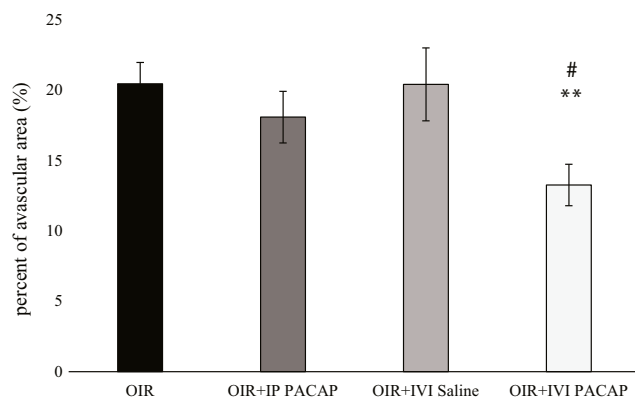

Fig. 2 Quantification of vasoobliterated retinal area given in percentage of total retinal area. Intravitreal PACAP treatment resulted in the reduction of avascular retina compared to the OIR and to the vehicle-injected group. Values are expressed in mean ± SEM, analyzed by ANOVA and Fisher's post hoc test. **OIR vs OIR + intravitreal PACAP $p < 0.005$; #OIR + intravitreal saline vs OIR + intravitreal PACAP $p < 0.05$. *OIR* oxygen-induced retinopathy, *IP* intraperitoneal, *IVI* intravitreal

Discussion

In the present study, we provided evidence for the retinoprotective effect of PACAP in a model of ROP in neonatal rats. We found that vascularized retinal area was increased in OIR animals receiving three intravitreal injections of 3 μl/100 pmol PACAP, whereas 7 days of intraperitoneal injection did not influence the vessel formation of the OIR retina. The cytokine expression of the OIR eyes was counteracted by local PACAP treatment. These present findings are in accordance with previous results showing retinoprotective effects of PACAP in numerous models of retinal degeneration (Atlasz et al. 2016). Our results further confirm the protective nature of the peptide in the newborn retina and they provide proof for an additional retinopathy where PACAP effectively counteracts lesions.

The main clinical approaches, controlled oxygen administration and laser photocoagulation, are directed towards slowing down the disease progression. In most severe cases, intravitreal anti-VEGF therapy is used (Hellström et al. 2013). As ROP is a leading cause of visual impairment in children, it is important to examine this promising retinoprotective agent in a rat model of this disease. PACAP has several actions during normal retinal development. It has been described that the specific PAC1 and VPAC receptors occur in the developing mammalian retina, including the retinal progenitor cells (Njaine et al. 2010). A shift in PAC1 receptor isoforms, null, hip, hop1, and hiphop1, has been reported during development. This shift may be responsible for the different roles exerted during differentiation of retinal neural elements, as it has been described for the development of other parts of the central nervous system (Denes et al. 2014; Yan et al. 2013). Also, PACAP controls the proliferation of retinal progenitor cells via the downregulation of cyclin D1 and the transcription factor Klf4, resulting in an anti-proliferative action during a given phase of development (Njaine et al. 2010, 2014). Our results show that not only during normal development does PACAP act as a neurotrophic factor but also during
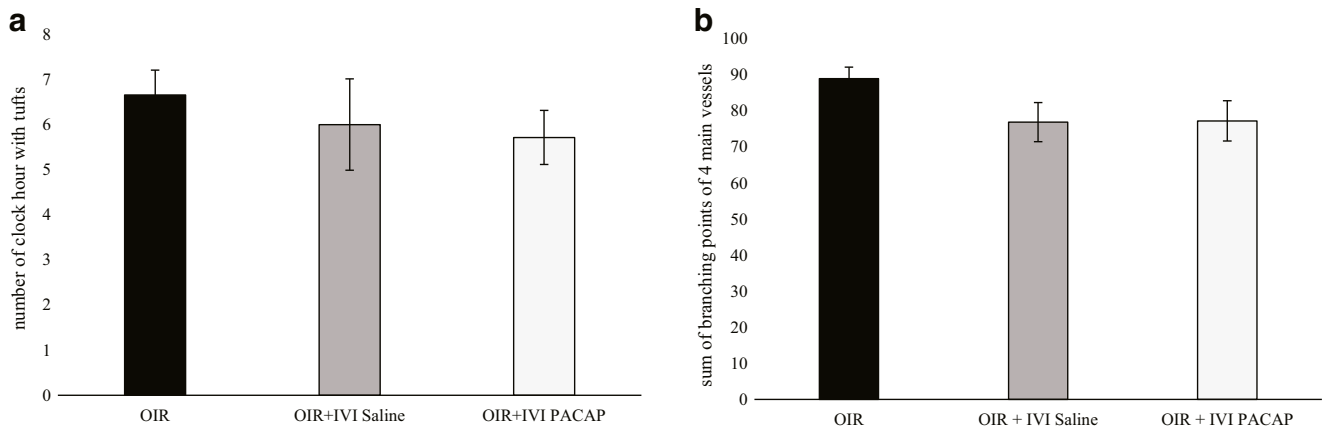
Fig. 3 Morphological analysis of vessels in OIR animals. (a) New vessel formation quantified as the number of clock hours with vessel tufts. (b) The number of branching points of the main vessels in each quadrant. PACAP treatment did not influence either the tuft formation or branching of vessels. Values are represented in mean ± SEM, analyzed by ANOVA with Fisher's post hoc test, $p < 0.05$. *OIR* oxygen-induced retinopathy, *IVI* intravitreal

pathological development, as it is in the case of ROP, where the peptide acts as a trophic factor providing protection against oxygen-induced damages.

Experiments with OIR animals contribute to the better understanding of the mechanism of the disease. In addition to the well-described vascular alterations, degeneration of the neural retina has already been identified. Moreover, current hypothesis of ROP pathophysiology suggests that neural damage antedates the abnormal vessel formation (Fulton et al. 2009). The lesion in OIR animals leading to vision impairment involve the loss of the inner plexiform layer, increased apoptosis in the inner nuclear layer,

astrocyte degeneration in the avascular area, and gliosis of Müller cells (Fulton et al. 2009; DeNiro et al. 2011; Coorey et al. 2012). The protective mechanism of PACAP involves several pathways, as shown in earlier studies on the retinoprotective effects of PACAP (Atlasz et al. 2010, 2016; Nakamachi et al. 2012). Lesion-induced increased apoptosis has been shown to be reduced by PACAP treatment in several models, such as in diabetic retinopathy and MSG-induced excitotoxic injury (Racz et al. 2006; Szabadfi et al. 2014). Inhibition of several pro-apoptotic signaling molecules like caspases, Jun-kinase, and apoptosis-inducing factor can be inhibited, while activation of
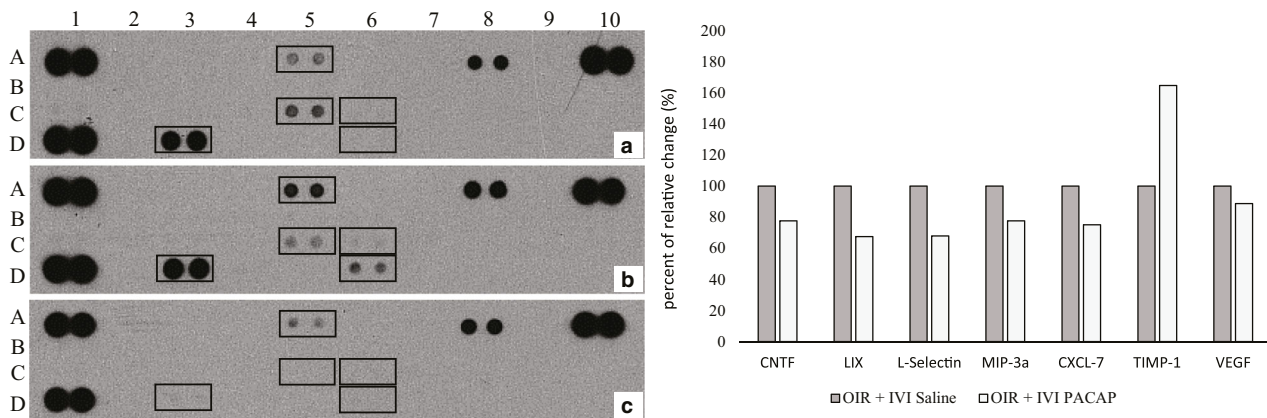
	1	2	3	4	5	6	7	8	9	10
A	PosC	CINC-1	CINC-2α/β	CINC-3	CNTF	Fractalkine	GM-CSF	sICAM-1	IFN-γ	PosC
B		IL-1α	IL-1β	IL-1ra	IL-2	IL-3	IL-4	IL-6	IL-10	
C		IL-13	IL-17	IP-10	LIX	L-Selectin	MIG	MIP-1α	MIP-3α	
D	PosC	RANTES	Thymus Chemokine	TIMP-1	TNF-α	VEGF			NegC	

Fig. 4 Representative rat cytokine array of saline-injected normoxia controls (a), saline-injected OIR (b), and PACAP-treated OIR retinas (c). Cytokines which showed changes on comparing the normalized densitometric values to the saline-injected OIR eyes were represented in the chart and quantified on the diagram. *OIR* oxygen-induced retinopathy, *IVI* intravitreal

anti-apoptotic factors—phosphoPKA, 14-3-3 protein, Bcl-Xl, etc.—can be triggered by PACAP treatment (Racz et al. 2006; Szabadfi et al. 2014). Regulation of these factors results in reduction of apoptotic neuronal cell death in the retina in several pathological conditions (Cheng et al. 2014; Endo et al. 2011; Wada et al. 2013).

The retinal glial cells play an important role in maintaining the microenvironment for retinal neurons and blood vessels. During angiogenesis, astrocytes ensure a template for endothelial cell migration, whereas Müller cells participate in the nourishment, protection, and communication of neurons (Fruttiger 2007). As a general response to retinal damage, gliosis of Müller cells appears. Several OIR studies have shown that these gliotic cells release inflammatory cytokines and VEGF leading to neovascularization (DeNiro et al. 2011; Coorey et al. 2012). Our previous studies have confirmed that PACAP prevents or ameliorates pathological Müller glial reaction in diabetic retinopathy, which is also associated with abnormal vessel growth in the retina (Szabadfi et al. 2014, 2016). In addition to the effects on apoptosis and Müller glial cell reaction, PACAP-induced actions on retinal inflammatory pathways can also partially account for its beneficial effects in OIR (Wada et al. 2013).

Whether the observed morphological amelioration is also reflected in functional improvement after PACAP treatment awaits further investigation, but based on our earlier studies in excitotoxic and ischemic lesions, the morphological protective action of PACAP is accompanied by functional protection, as measured by electroretinography (Varga et al. 2011; Danyadi et al. 2014).

In summary, our present study showed that intravitreal PACAP treatment has retinoprotective effect in oxygen-induced retinopathy, a rodent model of retinopathy of prematurity.

Compliance with Ethical Standards   Animal housing and care and application of experimental procedures were in accordance with institutional guidelines under approved protocols (No: BA02/2000–15024/2011, University of Pecs following the European Community Council directive).

References

Atlasz T, Babai N, Kiss P, et al. (2007) Pituitary adenylate cyclase activating polypeptide is protective in bilateral carotid occlusion-induced retinal lesion in rats. Gen Comp Endocrinol 153:108–114

Atlasz T, Szabadfi K, Kiss P, et al. (2010) Pituitary adenylate cyclase activating polypeptide in the retina: focus on the retinoprotective effects. Ann N Y Acad Sci 1200:128–139

Atlasz T, Szabadfi K, Kiss P, et al. (2011) Effects of PACAP in UV-A radiation-induced retinal degeneration models in rats. J Mol Neurosci 43:51–57

Atlasz T, Vaczy A, Werling D, et al. (2016) Neuroprotective effects of PACAP in the retina. In: Reglodi D, Tamas A (eds) Pituitary adenylate cyclase activating polypeptide—PACAP. Springer Nature, New York in press

Babai N, Atlasz T, Tamas A, et al. (2005) Degree of damage compensation by various PACAP treatments in monosodium glutamate-induced retina degeneration. Neurotox Res 8:227–233

Beharry KD, Valencia GB, Lazarro DR, Aranda JV (2016) Pharmacologic interventions for the prevention and treatment of retinopathy of prematurity. Semin Perinatol 40:189–202

Borba JC, Henze IP, Silveira MS, et al. (2005) Pituitary adenylate cyclase activating polypeptide (PACAP) can act as a determinant of the tyrosine hydroxylase phenotype of dopaminergic cells during retina development. Dev Brain Res 156:193–201

Cheng H, Ding Y, Yu R, Chen J, Wu C (2014) Neuroprotection of a novel cyclopeptide C*HSDGIC* from the cyclization of PACAP (1-5) in cellular and rodent models of retinal ganglion cell apoptosis. PLoS One 9:e108090

Clason TA, Girard BM, May V, Parsons RL (2016) Activation of MEK/ERK signaling by PACAP in guinea pig cardiac neurons. J Mol Neurosci 59:309–316

Connor KM, Krah NM, Dennison RJ, et al. (2009) Quantification of oxygen-induced retinopathy in the mouse: a model of vessel loss, vessel regrowth and pathological angiogenesis. Nat Protoc 4:1565–1573

Coorey NJ, Shen W, Chung SH, Zhu L, Gillies MC (2012) The role of glia in retinal vascular disease. Clin Exp Optom 95:266–281

Csanaky K, Doppler W, Tamas A, Kovacs K, Toth G, Reglodi D (2014) Influence of terminal differentiation and PACAP on the cytokine, chemokine and growth factor secretion of mammary epithelial cells. J Mol Neurosci 52:28–36

D'Amico AG, Maugeri G, Reitano R, et al. (2015) PACAP modulates expression of hypoxia-inducible factors in streptozotocin-induced diabetic rat retina. J Mol Neurosci 57:501–509

Danyadi B, Szabadfi K, Reglodi D, et al. (2014) PACAP application improves functional outcome of chronic retinal ischemic injury in rats—evidence from electroretinographic measurements. J Mol Neurosci 54:293–299

Denes V, Czotter N, Lakk M, Berta G, Gabriel R (2014) PAC1-expressing structures of neural retina alter their PAC1 isoform splicing during postnatal development. Cell Tissue Res 355:279–288

DeNiro M, Al-Mohanna FH, Al-Mohanna FA (2011) Inhibition of reactive gliosis prevents neovascular growth in the mouse model of oxygen-induced retinopathy. PLoS One 6:e222244

Endo K, Nakamachi T, Seki T, et al. (2011) Neuroprotective effect of PACAP against NMDA-induced retinal damage in the mouse. J Mol Neurosci 43:22–29

Fruttiger M (2007) Development of retinal vasculature. Angiogenesis 10:77–88

Fulton AB, Hansen RM, Moskowitz A, Akula JD (2009) The neurovascular retina in retinopathy of prematurity. Prog Retin Eye Res 28:452–482

Girard BM, Malley SE, Mathews MM, May V, Vizzard MA (2016) Intravesical PAC1 receptor antagonist, PACAP(6-38), reduces urinary bladder frequency and pelvic sensitivity in NGF-OE mice. J Mol Neurosci 59:290–299

Hellström A, Smith LE, Dammann O (2013) Retinopathy of prematurity. Lancet 26:1445–1457

Horvath G, Reglodi D, Brubel R, et al. (2014) Investigation of possible functions of PACAP in human trophoblast cells. J Mol Neurosci 54:320–330

Ismail JA, Poppa V, Kemper LE, et al. (2003) Immunohistologic labeling of murine endothelium. Cardiovasc Pathol 12:82–90

Kiss P, Atlasz T, Szabadfi K, et al. (2011) Comparison between PACAP- and enriched environment-induced retinal protection in MSG-treated newborn rats. Neurosci Lett 487:400–405

Lang B, Zhao L, McKie L, et al. (2010) GABAergic amacrine cells and visual function are reduced in PAC1 transgenic mice. Neuropharmacology 58:215–225

Matsumoto M, Nakamachi T, Watanabe J, et al. (2016) Pituitary adenylate cyclase-activating polypeptide (PACAP) is involved in adult mouse hippocampal neurogenesis after stroke. J Mol Neurosci 59:270–279

Nakamachi T, Matkovits A, Seki T, Shioda S (2012) Distribution and protective function of pituitary adenylate cyclase-activating polypeptide in the retina. Front Endocrinol (Lausanne) 3:145

Njaine B, Martins RA, Santiago MF, Linden R, Silveira MS (2010) Pituitary adenylyl cyclase-activating polypeptide controls the proliferation of retinal progenitor cells through downregulation of cyclin D1. Eur J Neurosci 32:311–321

Njaine B, Rocha-Martins M, Vieira-Vieira CH, et al. (2014) Pleiotropic functions of pituitary adenylyl cyclase-activating polypeptide on the retinal ontogenesis: involvement of KLF4 in the control of progenitor cell proliferation. J Mol Neurosci 54:430–442

Penn JS, Henry MM, Tolman BL (1994) Exposure to alternating hypoxia and hyperoxia causes severe proliferative retinopathy in the newborn rat. Pediatr Res 36:724–731

Racz B, Gallyas F Jr, Kiss P, et al. (2006) The neuroprotective effects of PACAP in monosodium glutamate-induced retinal lesion involves inhibition of proapoptotic signaling pathways. Regul Pept 37:20–26

Reglodi D, Renaud J, Tamas A, et al. (2015) Novel tactics for neuroprotection in Parkinson's disease: role of antibiotics, polyphenols and neuropeptides. Prog Neurobiol. doi:10.1016/j.pneurobio.2015.10.004

Seki T, Shioda S, Ogino D, Nakai Y, Arimura A, Koide R (1997) Distribution and ultrastructural localization of a receptor for pituitary adenylate cyclase activating polypeptide and its mRNA in the rat retina. Neurosci Lett 238:127–130

Seki T, Itoh H, Nakamachi T, Shioda S (2008) Suppression of ganglion cell death by PACAP following optic nerve transection in the rat. J Mol Neurosci 36:57–60

Seki T, Itoh H, Nakamachi T, et al. (2011) Suppression of rat retinal ganglion cell death by PACAP following transient ischemia induced by high intraocular pressure. J Mol Neurosci 43:30–34

Shioda S, Nakamachi T (2015) PACAP as a neuroprotective factor in ischemic neuronal injuries. Peptides 72:202–207

Somogyvari-Vigh A, Reglodi D (2004) Pituitary adenylate cyclase activating polypeptide: a potential neuroprotective peptide. Review. Curr Pharm Des 10:2861–2889

Szabadfi K, Szabo A, Kiss P, et al. (2014) PACAP promotes neuron survival in early experimental diabetic retinopathy. Neurochem Int 64:84–91

Szabadfi K, Reglodi D, Szabo A, et al. (2016) Pituitary adenylate cyclase activating polypeptide, a potential therapeutic agent for diabetic retinopathy in rats: focus on the vertical information processing pathway. Neurotox Res 29:432–446

Szabo A, Danyadi B, Bognar E, et al. (2012) Effect of PACAP on MAP kinases, Akt and cytokine expressions in rat retinal hypoperfusion. Neurosci Lett 523:93–98

Varga B, Szabadfi K, Kiss P, et al. (2011) PACAP improves functional outcome in excitotoxic retinal lesion: an electroretinographic study. J Mol Neurosci 43:44–50

Vaudry D, Falluel-Morel A, Bourgault S, et al. (2009) Pituitary adenylate cyclase activating polypeptide and its receptors: 20 years after the discovery. Pharmacol Rev 61:283–357

Wada Y, Nakamachi T, Endo K, et al. (2013) PACAP attenuates NMDA-induced retinal damage in association with modulation of the microglia/macrophage status into an acquired deactivation subtype. J Mol Neurosci 51:493–502

Werling D, Reglodi D, Kiss P, et al. (2014) Investigation of PACAP fragments and related peptides in chronic retinal hypoperfusion. J Ophthalmol 2014:563812

Yan Y, Zhou X, Pan Z, Ma J, Waschek JA, DiCicco-Bloom E (2013) Pro- and anti-mitogenic actions of pituitary adenylate cyclase-activating polypeptide in developing cerebral cortex: potential mediation by developmental switch of PAC1 receptor mRNA isoforms. J Neurosci 33:3865–3878