Protective role of different neuropeptides in retinal injuries

Ph.D. thesis

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1 Introduction

1.1 Anatomy of the eyeball

The organ of vision is a complex sensory organ which consists of eyeball and accessory structures. The eyeball is composed of three structural layers. The outer or fibrous layer includes the sclera and the cornea. The middle or vascular layer includes the choroid, the ciliary body, and the iris. The layers of the eye and the lens serve as boundaries for three chambers within the eye. The chambers are the following: anterior-, posterior- and vitreous. The anterior- and posterior chamber is filled by aqueous humor, which is a transparent watery fluid. Normally the aqueous humor flows from the posterior chamber around the iris and to the anterior chamber. Then it drains out through the trabecular meshwork to the canal of Schlemm and finally to the aqueous veins. The innermost layer of the eyeball is the retina, which is a photosensitive layer. The light is focused to the retina by optic system of the eye, which induces several electric and chemical responses. We can distinguish ten histological layers within the vertebrate retina. The layers from inside to outside are the following:

Inner limiting membrane (ILM) – composed of the basal lamina of Müller's cells

Nerve fiber layer (NFL) – contains processes of ganglion cells that lead from the retina to the brain

Ganglion cell layer (GCL) – contains the cell bodies of ganglion cells

Inner plexiform layer (IPL) – contains the processes of horizontal, amacrine, bipolar and ganglion cells that connect to each other

Inner nuclear layer (INL) – contains the cell bodies of horizontal, amacrine, bipolar and Müller's cells

Outer plexiform layer (OPL) – contains the processes of photoreceptors and processes of the horizontal, amacrine and bipolar cells that connect to them

Outer nuclear layer (ONL) – contains cell bodies of photoreceptors

Outer limiting membrane (OLM) – the apical boundary of Müller's cells

Photoreceptor layer (PL) – contains the outer and inner segments of photoreceptors

Pigment epithelium (PE) – the outer layer of the retina, actually not part of the neural retina.

The layers of the rodent retina are similar to human retina.

1.2 Bilateral carotid communis occlusion (BCCAO)

In the retina, ischemic injury can be induced by ligating both common carotid permanently. This model is one of the most common ischemic model which can cause morphological changes

in the retina. Retinal ischemia is a major cause of visual impairment, which is responsible for several retinal disorders.

1.3 Glaucoma

Glaucoma refers to a group of optic neuropathies. The most common form is open-angle glaucoma, which is a progressive condition that develops by the blockage of the aqueous humor drainage system leading to intraocular hypertension. The increased intraocular pressure will cause the loss of the retinal ganglion cells and their axons. Today, treatments are limited to moderate the intraocular pressure elevation; however, retinal degeneration continues to progress at a slower rate. There is an emerging need for therapeutic agents that can prevent apoptosis and exert neuroprotective effect.

Various animal models have been approved that mimic the ocular changes associated with glaucoma. In these models, we can investigate the pathophysiology and test the efficiency of promising treatments. Even though in some ways the anatomy and physiology of rodent eyes differ from primates, they serve as the best inducible glaucoma model systems. In rats the aqueous humor produced in the ciliary body, flow through the trabecular meshwork and gets collected in the Schlemm's canal, similarly to humans. Finally, fluid enters the episcleral drainage veins. The physiological intraocular pressure levels (approximately 10 mmHg) are maintained by the dynamic balance of the aqueous humor inflow and outflow. Injected microbeads get stuck in the intracellular space of the trabecular meshwork leading to impaired aqueous outflow and finally inducing ocular hypertension.

1.4 PACAP and VIP

Pituitary adenylate cyclase-activating polypeptide (PACAP) is an endogenous neuropeptide first isolated as a hypothalamic peptide in two biologically active forms (PACAP1-38 and PACAP 1-27). It is the most conserved member of the secretin/glucagon/VIP family and it exerts diverse biological actions. PACAP acts on G-protein coupled receptors, namely PAC1, VPAC1, and VPAC2. Since its discovery, it has become evident that PACAP has strong neuroprotective effects in several in vivo and in vitro models. PACAP has a widespread occurrence in the body and a broad array functions.

Vasoactive intestinal peptide (VIP) is another member of the secretin/glucagon/VIP superfamily. It is a 28 amino acids peptide first reported in 1970, which is now known as a neurotransmitter, neuromodulator, neurotrophic and neuro-protective factor widespread in the peripheral and central nervous system. VIP acts on the same 3 receptors as PACAP. VIP and

PACAP bind both VPAC1 and VPAC2 receptors with similar affinity and PACAP binds PAC1 receptor with higher affinity than VIP. VIP is also a multifunctional peptide which has neuroprotective effects in various in vitro and in vivo injury models.

Both PACAP and VIP exert protective effects in several tissues. VIP has stronger anti-inflammatory effects, while PACAP is a more potent antiapoptotic peptide. Both are attracting great interest as new sources of potential therapeutics.

1.5 TAT

TAT peptide is an 11-amino acid cell-penetrating peptide derived from the human immunodeficiency virus type 1 (HIV). TAT is a kind of protein transduction domain with an ability to efficiently traverse cellular membranes, either alone or with additional molecular cargo. TAT can not only transfer different types of molecules (peptides, large molecular proteins, DNAs, etc.) into a variety of cell types, but TAT is also able to bring the linked molecules across many biological barriers, including the blood-brain barrier, mucosal barrier, and lung respiratory epithelium in vivo.

1.6 Optical coherence tomography and electroretinography for studying the retina

Optical coherence tomography (OCT) is one of the most frequently used diagnostic techniques in today's clinical ophtalmology, rapidly becoming the standard of care in retinal diagnostics. It is a non-invasive high-resolution optical imaging technology based on the interference between the signal from an object under investigation and a local reference signal. OCT can produce a real-time, cross-section image of the investigated object.

Electroretinography (ERG) is a diagnostic test that measures the electrical activity of the retina in response to a light stimulus. ERG may be used to assess photoreceptors, horizontal cells, bipolar cells and their interaction. The a-wave represents the photoreceptor response; the b-wave is generated by cells in the inner retina, such as ON bipolar and Müller cells. ERG is a clinically useful technique for the interpretation of the diagnosis of retinal disease.

2 Aims

Previous studies have shown that intravitreally administrated PACAP has retinoprotective effects in different retinopathies. We have proven that PACAP, given in a form of eye drops, is able to pass through the ocular barriers to reach the retina and exert retinoprotective effects in ischemic retinopathy. The cell-penetrating peptide TAT can enhance the traversing ability of TAT-bound peptides through the biological barriers and cellular membranes. We hypothesized that TAT-bound PACAP and VIP could be more effective in exerting neuroprotective effects.

I. The first part of our experiment was to investigate the potential retinoprotective effects of PACAP-TAT and VIP-TAT administered in eye drops following bilateral carotid artery occlusion (BCCAO)-induced retinopathy.

II. The second half of our experiment was to investigate the effects of PACAP1-38 eye drops in microbead-induced glaucoma model.

3 Research I – Investigation of the role of PACAP-TAT and VIP-TAT in ischemic retinopathy

3.1 Materials and methods

BCCAO model and the eye drops treatment

Adult male Wistar rats were housed in the animal facility in individual cages in a 12 h light-dark cycle with food and water ad libitum. Animal housing, care, and application of experimental procedures were in accordance with institutional guidelines under approved protocols (No: BA02/2000-26/2017). Under isoflurane anesthesia, common carotid arteries were exposed on both sides through a midline incision and then ligated with a 3–0 filament. A group of animals (sham group) underwent all steps of the operating procedure except ligation of the carotid arteries. Immediately following the operation, the right eye of the animal was treated with PACAP-TAT and VIP-TAT (1 μg/drop). Animals were treated for five consecutive days, twice a day with one drop of the drug, under brief isoflurane anesthesia (max. 5 mins).

Histological analysis

After fourteen days of the BCCAO operation, rats were killed and the eyes were processed for histology. First, the eyes were fixed in 4% paraformaldehyde and embedded in Durcupan ACM resin. Retinas were then cut at 2 μm and stained with toluidine blue dye. Sections were mounted in DPX medium. The following parameters were measured: the thickness of OLM-ILM, the width of different retinal layers (ONL, OPL, INL, IPL), the number of cells/100 μm section length in the GCL, and number of cells/500 μm^2 in the outer (ONL) and inner nuclear (INL) layers.

Statistics

Results are presented as mean±SEM. Statistical analyses were made using the two-way ANOVA test followed by Fisher- and Bonferroni's post hoc test.

3.2 Results

The BCCAO operation caused significant thickness reduction in all retinal layers compared to sham animals. The most marked reduction in thickness was found in OPL and IPL and as a consequence, the thickness of OLM-ILM was significantly less than in control retinas. The

PACAP-TAT and VIP-TAT administration alone in sham animals did not cause any changes in the retinal thickness. PACAP-TAT or VIP-TAT eye drops caused significant amelioration in all retinal layers compared to the sham group. The thickness of the major retinal layers was significantly larger than the degenerated ones. This was especially conspicuous in the OPL, which almost disappeared in several BCCAO-induced degenerated retinas and was preserved in PACAP-TAT or VIP-TAT-treated animals. BCCAO led to a significant cell loss in the ONL, INL and GCL. Eye drops with PACAP-TAT counteracted the effects of the BCCAO in all nuclear layers. The cell numbers in the GLC/100 μm, in the ONL/500 μm^2, and in the INL/500 μm^2 were significantly higher compared to the BCCAO-induced degenerated retinas. VIP-TAT administration also led to reduced cell loss in almost all nuclear layers, except in the ONL/500 μm^2.

3.3 Discussion

In the present study, we demonstrated the efficacy of TAT-bound PACAP and VIP peptides to reach the retina and exert a retinoprotective effect in a model of ischemic retinopathy in rats. The retinoprotective effects of PACAP are well-documented in several different retinopathy models. Intravitreal injections of PACAP have been shown to lead to robust retinoprotective effects in various models of retinal injuries. The protective effects have been demonstrated to affect all neuronal cell types. PACAP is not only affects the neurons and glial cells of the retina leading to retinoprotection, but also helps to preserve the integrity of the blood-retinal barrier and protects the retinal pigment epithelial cells against oxidative stress injury, a process important in preservation of the outer barrier of the retina.

VIP has also been shown to have effects on the visual system according to some studies, although most results point to its involvement in photic neuronal transmission rather than its trophic effects. VIP is an important neuromodulator along the visual transmission pathways, not only in the retina, but all the way to the cortex where has an influence on the visual information processing. Regarding retinoprotection, few studies have indicated that VIP may also exert trophic effects in certain retinal injuries. Among others, VIP has been shown to protect retinal ganglion cells against excitotoxic injury in vitro. VIP also protected against ischemia-reperfusion injury induced by ophthalmic vessel ligation, where both systemic and intravitreally administered VIP could decrease the oxidative stress as shown by the reduced malondialdehyde levels. This could preserve the histological structure, which is in accordance with our present findings. Our earlier study, using the same hypoperfusion model, showed that

intravitreal VIP administration led to retinal morphological amelioration, but only at doses ten times higher than PACAP. In the present study, we show a similar degree of protection, using TAT-bound VIP. VIP's actions include not only direct effects, but also indirect effects, through stimulation of activity-dependent neurotrophic protein (ADNP) and its short fragment NAP, with highly potent neuroprotective effects. Both ADNP and NAP exerted strong protection against a variety of stress factors. In the retina, NAP protects against laser-induced retinal damage, decreases hypoxia-inducible factor levels in a model of diabetic retinopathy, prevents apoptotic cell death, and promotes neuronal growth after hypoxia-induced injury. VIP also affects autonomic reflexes and choroidal blood flow, which eventually affects retinal blood supply. Applying VIP on the ocular surface in form of eye drops has so far been shown to exert local effects on the cornea.

Regarding ischemic injury, PACAP has been shown to be protective in most cell layers affected in BCCAO-induced retinal ischemia. VIP was previously proven to be ten times less effective: intravitreal 100 pmol/5 ul VIP, in contrast to the same dose of PACAP, led to no ameliorating effect on the retinal structure. However, 1000 pmol/5ul intravitreal VIP produced a protective effect. In form of eye drops, VIP was not effective alone. However, in our present study, we confirm that VIP bound to TAT peptide could effectively traverse the ocular barriers and exert a neuroprotective effect in the retina. PACAP-TAT did not prove to have significantly higher retinoprotective efficacy than untagged PACAP, but VIP exerted much stronger retinoprotective effects when bound to TAT. These results were consistent with our previous report that TAT with similar structure to PACAP(28–38) endowed VIP with higher affinity for PAC1-R. As for PACAP38, the tagging with TAT at the C-terminus of PACAP38 would be redundant and interfere with the receptor binding. This may be the reason why TAT tagging had some negative effects on PACAP38's activity on the activation of PAC1-R. Also, as VIP has been implicated in a variety of other ocular diseases as a possible therapeutic approach, our results with topical applications leading to retinoprotection may open new therapeutic approaches.

In summary, our present study provides evidence, for the first time, that topical administration of PACAP-TAT and VIP-TAT attenuate ischemic retinal degeneration via the PAC1 receptor presumably due to a multifactorial protective mechanism.

4 Research II – Investigation of protective effects of PACAP eye drops in glaucoma model

4.1 Materials and methods

Animals, microbead injection and treatment

Adult male Sprague–Dawley (SD) rats (n = 50) weighing 300–500 g were used in this experiment. Animals were maintained under a 12 h light/dark cycle and fed and watered ad libitum. All the procedures were approved by the Animal Welfare Committee of the University of Pecs (BA02/2000-16/2017). Rats were divided randomly into four experimental groups: (i) PBS + vehicle (Systane (S)) n = 8; (ii) PBS + PACAP1-38 (P) n = 8; (iii) microbeads + vehicle (S) n = 17; and (iv) microbeads + PACAP1-38 n = 17. Fluorescent polystyrene microbeads were injected into the anterior chamber of both eyes by Hamilton syringe. In the control groups, eyes received an injection with the same volume of PBS. Two weeks after the injection, we repeated the same procedure. After the microbeads injection, the eyes were treated with Systane solution or PACAP1-38 eye drops (1 μg/drop). Intraocular pressure (IOP) changes were measured in both eyes with a rebound tonometer.

OCT and ERG examination

OCT imaging was performed 1 day before the microbeads injections. The pupils were dilated using eye drops that contained 0.01% atropine. During the procedure, we applied artificial tears to protect the corneal surface. Images of the retina were collected before and 8 weeks after the injections.

ERG responses were recorded from both eyes 1 day before and 8 weeks after microbeads injection. Before the measurement, animals were dark-adapted overnight (>12 h) and all set-up preparations were performed under dim red light. Rats were placed on a heating pad and ERGs were recorded by active electrodes from the corneal surface and the reference electrodes were placed subcutaneously on the head. The following parameters were measured: amplitude of the a-wave and the amplitude of the b-wave.

Histological analysis

After 8 weeks of the microbead injection, rats were killed and the eyes (n=32) were processed for histology. First, the eyes were fixed in 4% paraformaldehyde and embedded in Durcupan ACM resin. Retinas were cut at 2 μm and stained with toluidine blue dye. Sections

were mounted in DPX medium. The following parameters were measured: the thickness of OLM-ILM and the number of cells/100 μm section length in the GCL.

Immunohistochemistry

For immunohistochemistry, retinas (n=32) were fixed in 4% paraformaldehyde. 15–17 μm thin sections were cut on gelatin-coated slides with cryostat. Sections were incubated overnight at 4°C with primary polyclonal antibodies: mouse anti-Brn3a and rabbit anti-GFAP diluted in 1:200 in antibody diluting buffer. Immunoreactivity was detected with Alexa Fluor-594, donkey anti-mouse and Alexa Fluor-488, donkey anti-rabbit diluted 1:400 in PBST. After washing, cell nuclei were stained with propidium iodide. Slides were coverslipped with Fluoroshield and microphotographs were made by Nikon Eclipse 80i fluorescence microscope. Photographs were further processed with the Adobe Photoshop CS6 program. Fold change of GFAP-positive area was measured by ImageJ software.

Retinal whole mounts

Eyes (n=36) were fixed in 4% paraformaldehyde. After washing, retinas were removed and four small cuts were made. Primary mouse anti-Brn3a was diluted in PBST and incubated overnight at 4 ˚C. Immunoreactivity was visualized with Alexa Fluor-594 donkey anti-mouse diluted 1:400 in PBST. After washing, the slides were mounted with Fluoroshield mounting medium. Brn3a-positive RGCs were counted in 4 regions, each of area 50.000 μm^2. Counting was managed by ImageJ. Images were analyzed with Nikon Eclipse 80i epifluorescence microscope. Photographs were also further processed with the Adobe Photoshop CS6 program.

Statistics

Statistical comparisons were made using the two-way ANOVA followed by Fischer's (histology; ERG; GFAP; Brn3a whole-mount) and Bonferroni's (IOP; Brn3a section) post hoc analysis. Data are presented as means ± SEM. Differences with $p < 0.05$ were considered significant.

4.2 Results

Effect of PACAP eye drops on IOP

In control situations, we did not detect any changes in the IOP. In the Beads+S group, IOP increase developed during the observation period, while topical PACAP1-38 administration attenuated the elevation of the IOP in the microbead-injected retinas. We found more than 50%

elevation of IOP in both groups receiving beads already one week after the microbeads injection. On week 3, a significant difference started to develop between the two microbead-injected groups. This tendency was observed during the 8 weeks. The differences were statistically significant starting from week 3, throughout the observation period. In the PBS-injected control groups, IOP levels remained close to the baseline on week 8. In the microbead-injected vehicle-treated eyes, IOP showed a significant elevation in contrast to the PACAP1-38-treated eyes.

Effects of PACAP1-38 eye drops treatment on histological changes of the retina

PACAP1-38 administration in PBS-injected animals did not result in any alterations in the retinal layers. In vivo 3D OCT retinal images supported our histological findings. Retinal layers in microbead-injected animals showed signs of severe degeneration compared to the PBS controls. A significant reduction was detected in the OLM–ILM thickness in microbead-injected group. The number of cells in the GCL/100 μm was also significantly decreased. Topical administration of PACAP1-38 led to significant protection in the retina. The microbead-injected PACAP1-38-treated retinas had a more preserved structure compared to the vehicle-treated retinas and resulted in a significantly better preserved whole retinal distance between the OLM–ILM. Quantitative morphometric analysis demonstrated that the loss in the number of cells in the GCL was also prevented in the PACAP1-38-treated groups.

Effects of PACAP1-38 treatment on immunohistochemical changes

PBS-treated retinas did not show any remarkable immunofluorescent changes in either the vehicle-treated or the PACAP1-38 eye drops groups. Significant GFAP upregulation was detected following microbeads injection in the retinas in the Beads+S group. Expression was more intense in the inner retinal layers compared to the PACAP1-38-treated retinas. Increased IOP resulted in massive loss of the Brn3a immunopositivity in RGCs (Beads+S) compared to the control eyes. Glaucomatous retinas receiving PACAP1-38 eye drops showed significantly smaller reduction in RGC cell number. To further confirm this quantitative observation, surviving RGCs were also counted in whole-mount retinas. No significant differences were detected in PBS-injected groups. A reduced number of RGCs were observed in glaucomatous eyes compared to the retinas in the PACAP1-38-treated group. We found that the decrease in Brn3a expression was counteracted by topical PACAP1-38 treatment.

Protective effect of PACAP1-38 eye drops on visual responses after ocular hypertension

ERG was recorded 12 h after dark adaptation. In control situations, ERG waves were similar in the PBS+S and PBS+P retinas. In the glaucomatous vehicle-treated group, the light responses significantly decreased. However, in the glaucomatous PACAP1-38-treated eyes, the waveforms were almost the same as in the PBS-injected groups. The scotopic a- and b-waves in the PBS-injected eyes were similar in the PBS+S and in the PBS+P ones. We observed significant reduction of the a- and b-wave amplitudes in the Beads+S group compared to the PBS+S-treated animals. ERGs showed significant functional protection after PACAP1-38 administration in the microbead-injected eye.

4.3 Discussion

Glaucoma is a complex disease that is far from being completely understood. In our present study, we could stably reproduce the retinal ganglion cell death induced by high IOP. Using the microbead-induced model, we proved that the neuropeptide PACAP was able to prevent the marked increase in the intraocular pressure and the significant ganglion cell loss.

PACAP has well-documented neuro- and general cytoprotective effects, including protective actions in several retinopathies. Among others, PACAP has been shown to reduce injuries in models of different retinopathies. A difficulty of systemic treatment of retinopathies with PACAP is that the peptide has a short half-life in the serum due to the rapid degradation by the dipeptidyl-peptidase IV enzyme. Most studies demonstrating the retinoprotective effects of PACAP, therefore, applied intravitreal treatment. We have previously shown that PACAP, given in form of eye drops, is able to pass the ocular barriers and reaches the retina in sufficient concentration to induce protective effects in a model of ischemic retinopathy. The principal finding of our present study is that PACAP1-38, delivered as eye drops, has a protective role in microbead-induced glaucoma.

Similarly to human glaucoma, the elevation of IOP in this rat model can lead to the loss of retinal ganglion cells. Normal IOP values were recorded around 10–12 mmHg, similar to those described by studies using Sprague–Dawley rats. In the glaucomatous eyes, beads induced the blockage of the trabecular meshwork leading to IOP elevation. In our present study, we were able to reproduce this elevation in IOP after the injections and showed that PACAP1-38 eye drops treatment could prevent this increase. This was an unexpected positive finding of the present study. Although the exact mechanism is not known yet, the IOP-lowering effect of PACAP can be an additional protective factor in glaucoma. Aqueous humor (AH) production

and flow is a very tightly regulated process influenced by numerous factors and structures. It is not known yet how PACAP affects AH production and/or flow, but other cAMP-inducing substances have been described to have IOP-lowering effects. One of the possible mechanisms can occur through the cAMP level, as cAMP plays a critical role in the regulation of AH production and outflow. Moreover, PACAP can reduce the small GTPase RhoA which has a role in the regulation of trabecular meshwork.

Our histological findings are also in accordance with those of others. Microbeads injections induced histological changes between the two limiting layers, similar to other retinal injuries, such as LPS-induced retinal inflammation. PACAP1-38 eye drops preserved the normal retinal structure and prevented the ganglion cell loss investigated by routine histology and the specific Brn3a immunohistochemical labeling. Müller glial cells are known to be over-activated in various injuries. Müller cells are known to have PAC1 receptor and PACAP can exert several effects on them. Among others, PACAP has been shown to influence inflammatory cytokine (IL-6) expression in Müller cells. The activation of Müller cells could be confirmed in our present study, demonstrating more intense GFAP labeling in hypertensive conditions, while in the PACAP-treated group, GFAP positivity was limited only to the end feet of the glial cells, in concordance with previous findings. Seki et al. (2011), using another model for increased IOP with saline injection, have already demonstrated the protective effects of intravitreal injections of PACAP in the retina, focusing on the ganglion cell death. They suggested that PACAP1-38 can induce different signaling pathways depending on the concentration. Our findings support their results that PACAP1-38 has neuroprotective effects in hypertension-induced glaucoma. Our present results further confirm these earlier findings in a model more closely resembling the pathophysiological mechanisms of human glaucoma. In addition, we could show protective effects not only in the ganglion cell layer but also in other layers and in Müller cells, and could also confirm that the morphological improvement is associated with functional amelioration. Above all, we could provide evidence for the protective effects of PACAP in this model using a non-invasive eye drops application of PACAP1-38.

To investigate whether the morphological amelioration by PACAP treatment is also reflected in functional improvement, we performed ERG measurements. Here, we confirmed that PACAP treatment could also prevent the deterioration in visual function detected in the Beads+S group. This observation is in accordance with previous findings in ischemic retinopathy. Although the two pathomechanisms differ, they also share some common features, as vascular dysregulation has also been described in glaucoma. We observed several functional alterations in the Beads+S group proving that low-to-moderate elevation of IOP is necessary to

induce experimental glaucoma in rodents. In contrast, no such changes were observed in the PACAP-treated group.

Irreversible visual loss is a severe clinical issue commonly caused by glaucoma. Currently, there is no known effective neuroprotective therapy. Brain-derived neurotrophic factor (BDNF) injection into the vitreous body has proven to maintain the number of ganglion cells. PACAP1-38 eye drops therapy has a similar potent preventive effect against retinal ganglion cell death in our glaucoma model. There is a need to develop an effective neuroprotection method which is able to interact with cellular signaling and promotes RGCs survival. The locally produced BDNF might be important in the RGC activation through the TrkB receptor. The two factors are also linked to each other, as PACAP1-38 can induce the expression of BDNF via its specific PAC1 receptor and PACAP's protective effects are partially mediated by BDNF in neuronal cells.

The microbead occlusion model of glaucoma represents an attractive model for determining the impact of PACAP on glaucoma. Our present findings further suggest that PACAP1-38 eye drops could be used in future therapeutic approaches. Taken together, PACAP1-38 eye drops treatment can provide a future accessory strategy designed parallel to the regular glaucoma treatments.

5 Summary of novel findings

I) In the first part of my Ph.D. work, we investigated the retinoprotective effects of PACAP-TAT and VIP-TAT in BCCAO-induced retinopathy. In our histological analysis, we showed that PACAP-TAT and VIP-TAT eye drops significantly inhibited the retinal damage caused by hypoperfusion. PACAP-TAT did not prove to have significantly higher retinoprotective efficacy than untagged PACAP.

II) In the second half of my Ph.D. work, we confirmed the protective role of PACAP eye drops in microbead-induced glaucoma model. Our results also prove that the eye drops treatment of PACAP significantly reduces the elevation of IOP in this glaucoma model.

In summary, we showed that non-invasive eye drops application of PACAP and VIP has protective role in retinal disorders. Our present study also provides evidence, TAT bound VIP has more effective protective role than the VIP. Our previous and present findings further suggest that PACAP and VIP could be used in future therapeutic approaches against different retinal diseases.

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7 Publications

The thesis is based on the following publications (cumulative impact factor (IF): 8.601)

Szabó E., Patkó E., Váczy A., Molitor D., Csutak A., Tóth G., Reglődi D., Atlasz T. (2021). Retinoprotective Effects of PACAP Eye Drops in Microbead-Induced Glaucoma Model in Rats. *Int J Mol Sci* 22, 8825. http://doi.org/10.3390/ijms22168825 (**IF=5.923**)

Atlasz T., Werling D., Song S., **Szabo E**., Vaczy A., Kovari P., Tamas A., Reglodi D., Yu R. (2019). Retinoprotective Effects of TAT-Bound Vasoactive Intestinal Peptide and Pituitary Adenylate Cyclase Activating Polypeptide. *J Mol Neurosci* 68(3), 397–407. http://doi.org/10.1007/s12031-018-1229-5 (**IF=2.678**)

Reglodi D., Atlasz T., Jungling A., **Szabo E**., Kovari P., Manavalan S., Tamas A. (2018). Alternative routes of administration of the neuroprotective pituitary adenylate cyclase activating polypeptide. *Curr Pharm Des* 24(33): 3892–3904. http://doi.org/10.2174/1381612824666181112110934 (**Review; IF=2.412**)

Other publications (cumulative IF: 49.748)

Tekus E., Szenasi NL., **Szabo E**., Heckel Z., Mintal T., Koszegi T., Atlasz T., Gazdag Z., Vaczi M., Wilhelm M. (2022). Well-trained elders have antioxidant responses and equal magnitude of EIMD as young adults. *Int J Environ Res Public Health* 19(15):8889. https://doi.org/10.3390/ijerph19158889 (IF=4.614)

Patko E., **Szabo E**., Toth D., Tornoczki T., Bosnyak I., Vaczy A., Atlasz T., Reglodi D. (2022). Distribution of PACAP and PAC1 receptor in the human eye. *J Mol Neurosci* https://doi.org/10.1007/s12031-022-01985-0 (IF=3.444)

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