Neurotoxicity Research, 2005, VOL. 8(3,4). pp. 227-233

F.P. Graham Publishing Co.

Degree of Damage Compensation by Various Pacap Treatments in Monosodium Glutamate-induced Retinal Degeneration

NORBERT BABAI[a], TAMÁS ATLASZ[a], ANDREA TAMÁS[b], DÓRA REGLODI[b,c,*], GÁBOR TÓTH[d], PÉTER KISS[b] and RÓBERT GÁBRIEL[a,e]

[a]Department of General Zoology and Neurobiology, Pécs University; [b]Department of Anatomy, Pécs University Medical Faculty; [c]Neurohumoral Regulations Research Group of the Hungarian Academy of Sciences; [d]Department of Medical Chemistry, University of Szeged; [e]Adaptational Biology Research Group of the Hungarian Academy of Sciences, Hungary. dora.reglodi@aok.pte.hu

(Received 04 May 2005; In final form 20 June 2005)

Pituitary adenylate cyclase activating polypeptide (PACAP) has been shown to be neuroprotective in retinal ischemia and monosodium L-glutamate (MSG)-induced retinal degeneration. Here we describe how different MSG treatments (1x and 3x application) cause retinal damage and finally lead to the destruction of the entire inner retina and how PACAP attenuates this effect. Newborn rats from both sexes were injected subcutaneously with 2 mg/g bodyweight MSG on postnatal days 1, 5 and 9. The left eye was left intact while we injected 5 µl PACAP38 solution (100 pmol) into the vitreous of the right eye with a Hamilton syringe at the time of (i) the first, (ii) the first two or (iii) all three MSG injections. Histological analysis has shown that the above described MSG treatment caused the entire inner plexiform layer (IPL) to degenerate, and the inner nuclear (INL) and ganglion cell layers (GCL) seemed fused. One time PACAP38 treatment at the first MSG application did not change the degenerative capacity of MSG. However, if animals received PACAP38 into the vitreous of the eye at the first 2 or all 3 times, a substantial protective effect could be observed. The IPL remained well discernible, the INL retained 2-3 cell rows and the number of cells in the GCL was substantially higher than in the MSG-treated retinas, and was not significantly different from that observed in the control tissue. We conclude that (i) 2 or 3 times PACAP treatment attenuates retinal degeneration; (ii) one PACAP treatment does not provide protection against repeated excitotoxic insults, and (iii) repeated application of PACAP under these experimental conditions may lead to a primed state in which further neurotoxic insults are ineffective.

Keywords: PACAP; Glutamate; Retina; Degeneration; Excitotoxicity; Neurotoxicity; Neuroprotection

INTRODUCTION

There are many types of retinal degeneration models described in the literature. They can be grouped into two main categories: one is with a known genetic background (review by Paicone *et al.*, 2003), the other is induced by metabolic or traumatic events. These induced retinal degenerations, among others, may be caused by aging, glaucoma, ischemic damage, autoimmune processes, diabetes, toxic agents and exposure to extremely strong light (Sucher *et al.*, 1997; Osborne *et al.*, 1999; Vidal-Sanz *et al.*, 2000; Villani *et al.*, 2001).

Pathological activation of glutamate receptors is thought to play a key role in neuronal damage in many neurological diseases (Sucher *et al.*, 1997; Smythies, 1999; Pedersen and Schmidt, 2000; Danysz and Parsons, 2002). In the eye, several pathological conditions such as ischemia and some types of glaucoma can be mimicked by experimentally elevating extracellular glutamate concentrations or applying its analogues (Romano *et al.*, 1995; Vidal-Sanz *et al.*, 2000; Osborne *et al.*, 2004). One such agent is monosodium L-glutamate (MSG) which can be administered in the form of subcutaneous (s.c.) injection, and finally leads to the destruction of the entire inner retina (van Rijn *et al.*, 1986; Chambille and Serviere, 1993). This treatment leads to depolarization of inner retinal cells and causes Ca^{2+} influx into the neurons (Toriu *et al.*, 2000).

Several agents have been shown to alleviate degenerative changes caused by elevation of extracellular glutamate levels in the retina under experimental

*Corresponding author. Tel.: +36 72 536001 ext. 5398; FAX: +36 72 536 393; E-mail: dora.reglodi@aok.pte.hu
ISSN 1029 8428 print/ ISSN 1476-3524 online.

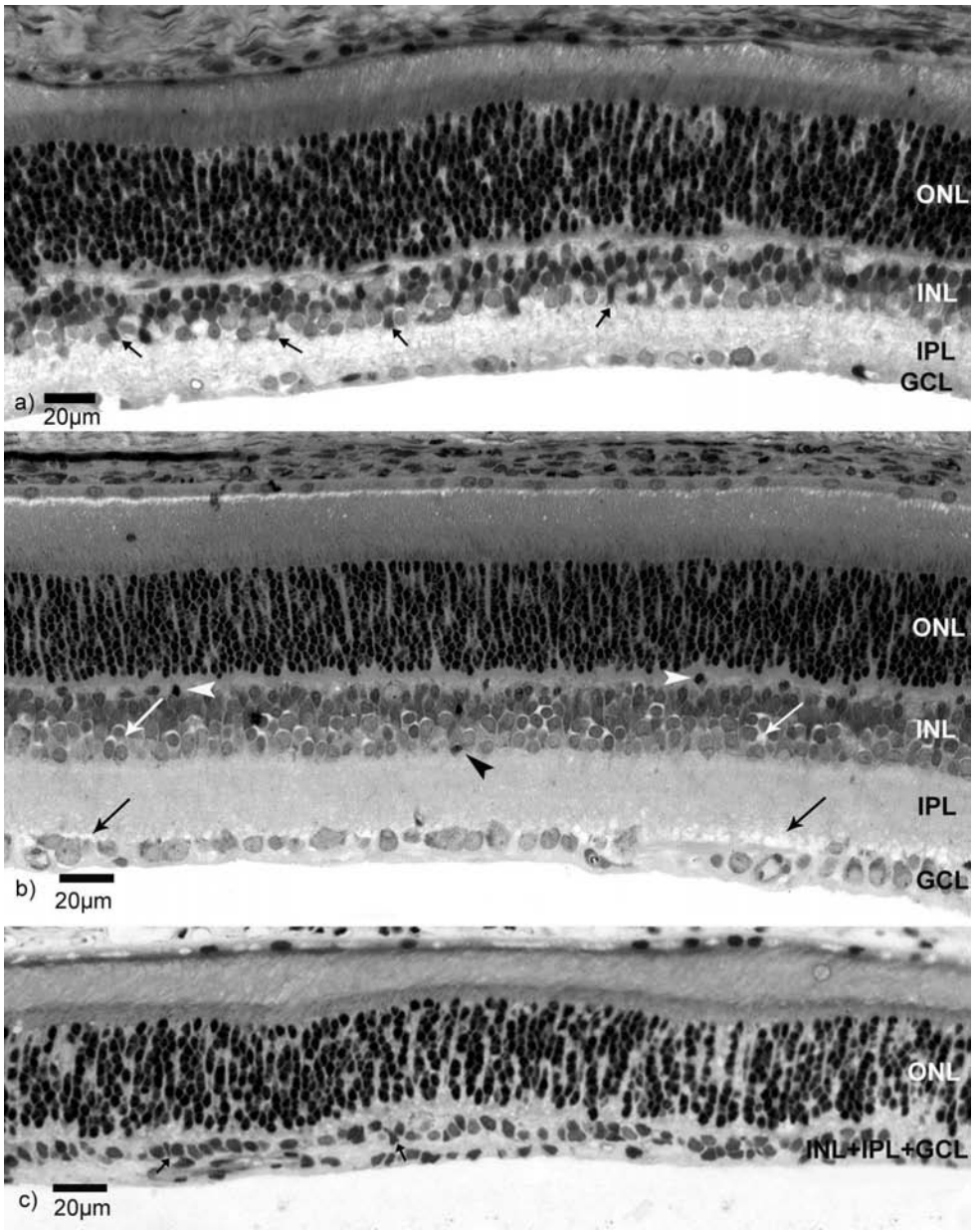

FIGURE 1 MSG-induced retinal degeneration in rats. ONL: outer nuclear layer; INL: inner nuclear layer; IPL: inner plexiform layer; GCL: ganglion cell layer. **a**. Control section. Arrows: Müller cells. **b**. 1x MSG treatment. Arrows: swollen, degenerating structures; arrowheads: picnotic nuclei. **c**. 3x MSG treatment. In several places the INL and GCL fused, the IPL is barely discernible. Arrows: Müller cells.

conditions, such as NMDA-antagonists, Ca^{2+}-channel blockers and fenamates (Sucher *et al.*, 1997; Chen *et al.*, 1998; Toriu *et al.*, 2000; Sun *et al.*, 2001). Trophic factors are also effective in retinal pathologies (Vidal-Sanz *et al.*, 2000). A member of the vasoactive intestinal peptide (VIP)/glucagon/secretin peptide family, pituitary adenylate cyclase activating polypeptide (PACAP), has recently been referred to as a trophic factor for its important roles during nervous development (Waschek, 2002; Somogyvari-Vigh and Reglodi, 2004). The neuroprotective effects of PACAP against various toxic agents *in vitro* and in different models of neuronal pathologies *in vivo* have been demonstrated (review by Somogyvari-Vigh and Reglodi, 2004). Its protective effects have also been shown in retinal hypoxia (Rabl *et al.*, 2002) and in retinal degeneration induced by toxic agents including glutamate both *in vitro* (Shoge *et al.*, 1999; Silveira *et al.*, 2002) and *in vivo* (Tamas *et al.*, 2004). Also, it promotes ganglion cell survival after optic nerve transection (Seki *et al.*, 2003). These effects are mediated through PACAP receptors which are found on inner retinal neurons, mostly in amacrine and ganglion cells (Seki *et al.*, 1997). The mechanism that leads to PACAP-induced neuroprotection is unidentified as yet.

In the present study we describe how different MSG

treatments cause retinal damage and finally lead to the destruction of the entire inner retina. Evidence is provided that with suitable treatment regime a model system for inner retinal degeneration can be generated in which neuroprotective agents might be tested effectively. We also show that PACAP, applied simultaneously with MSG, can reduce the damage of the retinal structure. We conclude that repeated application of PACAP under these experimental conditions may lead to a primed state in which further neurotoxic insults are ineffective.

MATERIALS AND METHODS

The experimental animals derived from the local colony of Wistar rats. They were housed in individual cages, fed and watered *ad libitum*, under light/dark cycles of 12/12 h. The NIH and local animal care committee guidelines were carefully followed throughout the entire procedure.

Newborn rats from both sexes (n=20) were injected s.c. with 2 mg/g b.w. MSG on postnatal days (PD) 1, 5 and 9. PACAP38 (100 pmol in 5 µl saline solution) was injected into the vitreous of the right eye with a Hamilton syringe at the time of (i) the first, (ii) the first two or (iii) all three MSG injections. According to previous observations, injection of physiological saline does not lead to any morphological change compared to normal eyes (Gabriel *et al.*, 1993; Tamas *et al.*, 2004). Therefore, the left intact eye served as a control MSG-treated eye. The dose of PACAP was based on previous observations where this dose was proven to be effective in optic nerve transection and MSG toxicity (Seki *et al.*, 2003; Tamas *et al.*, 2004).

At 3 weeks of age, rats were killed with an overdose of anesthetic (pentobarbital, Nembutal, Sanofi-Phylaxia, Hungary), the eyes were immediately dissected in ice-cold phosphate buffered saline and fixed in 4% paraformaldehyde dissolved in 0.1 M phosphate buffer. Tissues were embedded in Durcupan ACM resin, cut at 2 µm and stained with toluidine blue. The sections were then mounted in Depex medium and examined in a Nikon Eclipse 80i microscope. Photographs were taken with a digital CCD camera using the Spot program. Files were then further processed with Adobe Photoshop 7.0 program. Measurements were taken from the digital photographs with the NIH Image 1.55 program. Samples for measurements derived from at least six tissue blocks prepared from at least three animals (n=2-5 measurements from one tissue block). The following parameters were measured: (i) cross-section of the retina from the outer limiting membrane to the inner limiting membrane; (ii) the width of the inner plexiform layer (IPL) from the bottom of perikarya of the last cell row in the inner nuclear layer (INL) to the top of perikarya in the ganglion cell layer (GCL); the number of cells/100 µm section length in the GCL. Statistical comparisons were made using the ANOVA test followed by Neuman-Keul's *post hoc* analysis.

RESULTS

Effect of Repeated s.c. Application of MSG in the Rat Retina

Repeated application of MSG causes progressively more severe alterations in retinal morphology. In control preparations all the layers characteristic for the mammalian retina are well visible. Under the pigment epithelium, several rows of photoreceptors are present, followed by the thin outer synaptic layer. The INL usually consists of 4-5 rows of cells, followed by a thick inner synaptic layer. Finally, cells in the GCL layer are frequent but not side by side (FIG. 1a). One time MSG treatment at PD 1 does not cause much damage to the retina. The layers are similar in size to that of the control (FIG. 2), although signs of degenerative processes (swollen cells and nervous profiles, small holes in the tissue, some pyknotic cell nuclei; FIG. 1b) could be seen. The otherwise characteristic Müller glial cells identified on the basis of their dark rectangular nuclei are difficult to find in these preparations.

Retinal tissue from animals treated 3x with MSG show severe degeneration compared to the controls. Much of the IPL disappears and the INL and GCL are intermingled (FIG. 1c). As a consequence, the total thickness of the retina is significantly reduced (FIG.

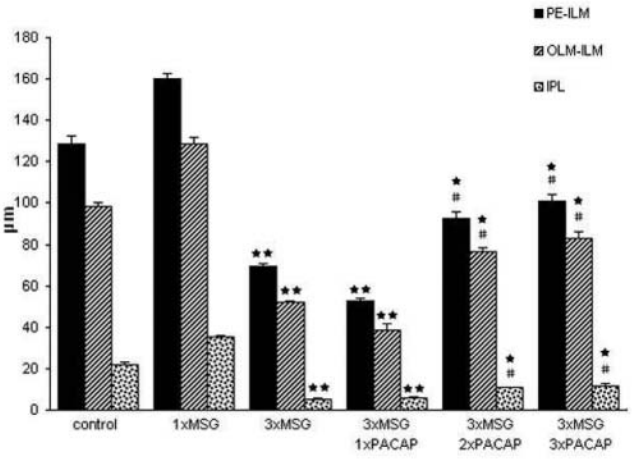

FIGURE 2 Comparison of retinal layers in control, MSG treated and MSG+PACAP-treated retinas. PE: pigment epithelium, OLM: outer limiting membrane, ILM: inner limiting membrane. *P <0.05, **P <0.01 *vs* control, #P <0.05 *vs* 3x MSG-treated group.

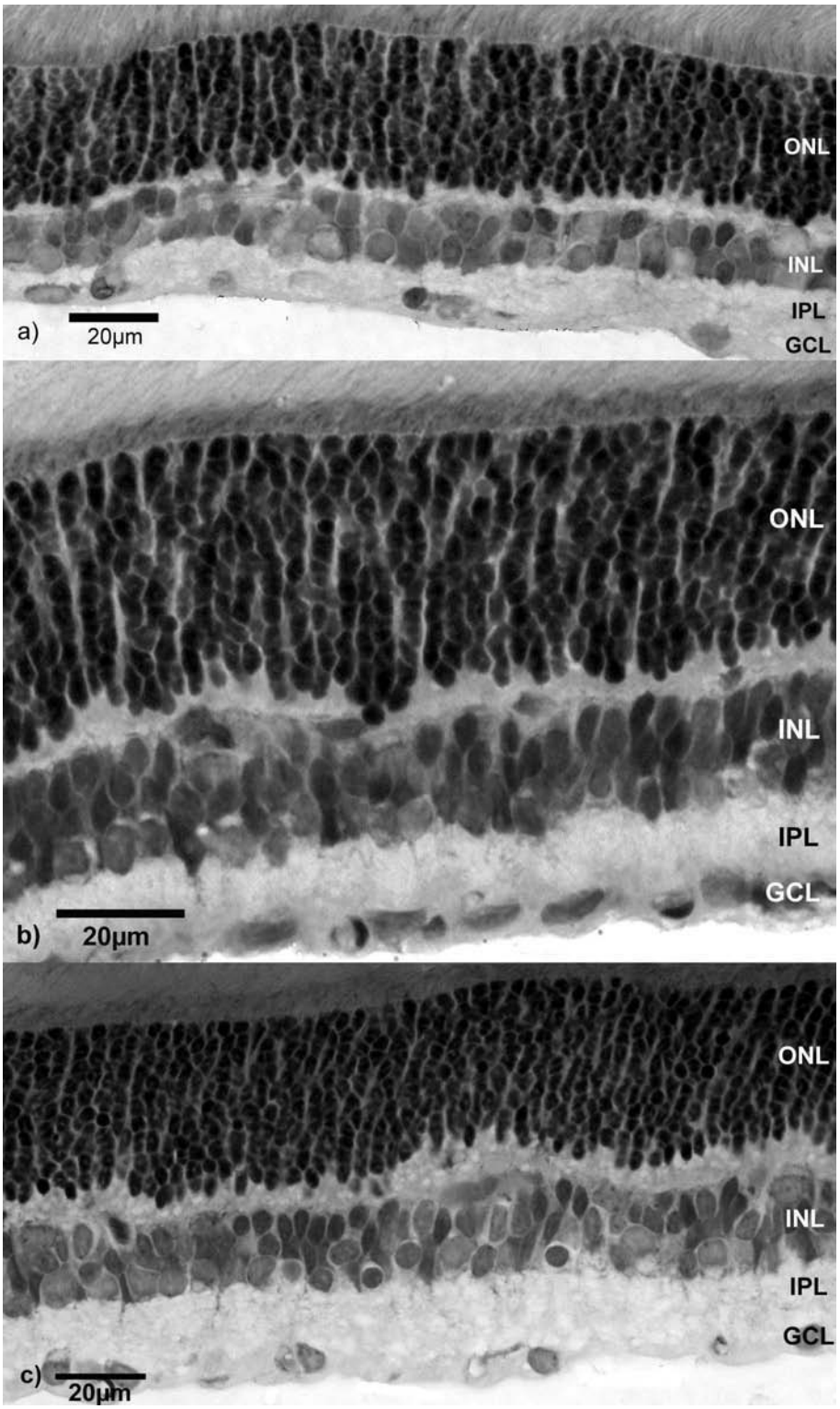

FIGURE 3 Alleviation of 3x MSG treatment-induced degeneration with PACAP. Retinal layers as in figure 1. **a**. 1x PACAP treatment at the first MSG application does not alter the degenerative capacity of MSG. **b**. 2x PACAP treatment at the first two MSG application. The IPL remains well visible, the INL and GCL all clearly separated at all places. **c**. Substantial but not full protection can be achieved if all MSG injections are followed by PACAP application.

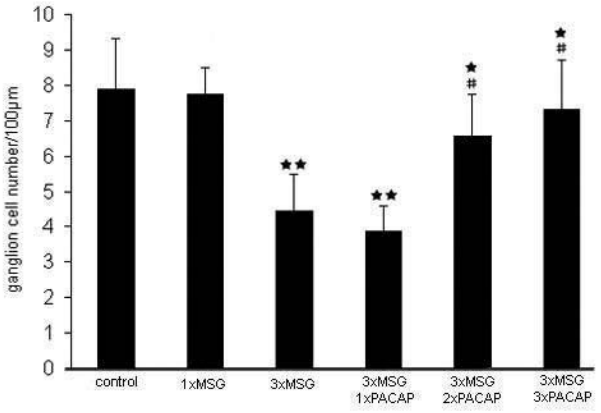

FIGURE 4 The number of cells/100 µm GCL length in control and differently treated retinas. *P <0.05, **P <0.01 *vs* control, #P <0.05 *vs* 3x MSG-treated group.

2). Only the photoreceptor layer seems unchanged. The entire retinal thickness is basically not much larger than that of the photoreceptor layer (FIG. 2).

Simultaneous Application of MSG (s.c.) and PACAP (Intraocular injection)

Analysis has shown that the above described MSG treatment caused the entire IPL to degenerate, and the INL and GCL seemed fused (FIG. 1c). In this series of experiments we used 3x MSG application, while PACAP treatment varied. If only one time PACAP treatment was made at the first MSG application, it did not change the degenerative capacity of MSG (FIG. 2). The retinal layers remained fused, although in some preparations more than two cell layers could be discerned in the inner retina (FIG. 3a). However, if animals received PACAP into the vitreous of the eye at the first 2 (FIG. 3b) or all 3 times (FIG. 3c) during MSG application, a substantial protective effect could be observed. The IPL remained well discernible (FIG. 2, 3c, d, 4), the INL was prominently present and retained 2-4 cell rows (FIG. 3c, d). The number of cells in the GCL was not significantly less than that of the untreated retina (FIG. 4).

DISCUSSION

The vertebrate retina uses glutamate as neurotransmitter in the so-called through-pathway (that is, the photoreceptor, bipolar and ganglion cells, connected with synapses in this order). Inner retinal cells, with the exception of ON bipolars, bear functional ionotropic glutamate receptors (Sucher *et al.*, 1997; Thoreson and Witkovsky, 1999). Therefore, these cells are all potential targets of MSG toxicity. On the other hand, nearly the same set of neurons bear PACAP receptors (Seki *et al.*, 2000). This explains why these cells could be able to survive the excitotoxic injury if PACAP is present in high concentration at the same time of the insult. The IPL remained slightly reduced after combined MSG and PACAP treatment in our experiments. This may be explained if we suppose that the OFF bipolar cells possess Ca^{2+}-permeable non-NMDA type ionotropic glutamate receptors but do not bear PACAP receptors, thus they are susceptible to damage. Also, some of the ganglion cells possess numerous NMDA receptors, so PACAP-induced pathways may not provide full protection. A further less susceptible population of inner retinal cells is that of the calcium-buffer protein containing neuron types. These include the AII amacrine cells which contain parvalbumin (Wässle *et al.*, 1993), the calbindin-positive dopaminergic cells (Hamano *et al.*, 1990), the cholinergic starburst amacrine cells (Gabriel and Witkovsky, 1998) and several types of medium and small-sized ganglion cells (Hamano *et al.*, 1990; Wässle *et al.*, 1993; Gabriel and Witkovsky, 1998).

The presence and distribution of PACAP, along with its cAMP-increasing and vascular effects have been described in the retina and ocular tissues of various species (Nilsson, 1994; Onali and Olianas, 1994; Wang *et al.*, 1995; Samuelsson-Almen and Nilsson, 1999; Izumi *et al.*, 2000; Koves *et al.*, 2000; Seki *et al.*, 2000; Jozsa *et al.*, 2001; Yoshitomi *et al.*, 2002). PACAP is also present in the embryonic retinas, and according to recent studies, it may play a role during the development of the retina (Olianas *et al.*, 1997; Bagnoli *et al.*, 2003; Mathieu *et al.*, 2004). PACAP receptors have also been demonstrated in the retina: the selective PAC1 receptor is predominant in the retina, and is present in amacrine and Muller cells in the inner nuclear layer and in the ganglion cells (Nilsson *et al.*, 1994; Seki *et al.*, 1997; Kubrusly *et al.*, 2005). *In vitro* studies on the neuroprotective effects of PACAP against glutamate-induced toxicity have demonstrated that PACAP treatment leads to a significant elevation in cAMP levels and MAP kinase activity, and the observed protective effect along with the MAP kinase activation are reduced in the presence of the selective protein kinase A antagonist, H89 (Morio *et al.*, 1996; Shoge *et al.*, 1999; Frechilla *et al.*, 2001; Silveira *et al.*, 2002). The predominant occurrence of the specific PAC1 receptor in the retina and the *in vitro* studies on the protective effects of PACAP suggest that PACAP counteracts the glutamate-induced toxicity via the cAMP/PKA/MAP kinase pathway by binding to PAC1 receptors. In addition, effects via the common VIP/PACAP receptors (VPAC1 and VPAC2) cannot be excluded, since these receptors are also present in the retina (Nilsson *et al.*, 1994). Although the cAMP increasing effects of VIP is

much less pronounced in various tissues, including the retina (Olianas *et al.*, 1997; Shoge *et al.*, 1999), protective effects of VIP in retinal ischemia/reperfusion injury and *in vitro* glutamate-toxicity have been demonstrated (Tuncel *et al.*, 1996; Shoge *et al.*, 1998).

We found that after the second PACAP injection, no further neuroprotection could be achieved with subsequent injection of PACAP. It is possible that repeated injection of PACAP leads to a primed state, which provides a long-lasting protection. In connection with this observation we currently test if there is a particularly sensitive period for MSG induced degeneration in retina development. Similar findings have been reported in cerebellar granular cells. Exposure of granule cells to PACAP during only 1 hour induced a maximum survival of cultured cells, and longer incubations did not further increase cell survival (Vaudry *et al.*, 1998). This indicates that acute treatment with PACAP induces immediate activation of a specific biochemical pathway which promotes a long-lasting survival (Vaudry *et al.*, 1998). Also, in a rat model of focal cerebral ischemia, a 7-day-pretreatment with PACAP resulted in no further reduction of the infarct size compared to a single preischemic bolus injection (Reglodi *et al.*, 2002).

Glutamate-induced toxicity is known to play a role in several retinal pathologies, such as glutamate released in retinal ischemia, initiating a cascade leading to retinal cell death (Sucher *et al.*, 1997; Osborne *et al.*, 1999). Also, elevated levels of glutamate cause further damage in glaucoma patients (Sucher *et al.*, 1997). The damaging effect of excitotoxicity by elevated glutamate has also been shown in optic neuritis, central artery occlusion, optic nerve trauma and AIDS (Sucher *et al.*, 1997). Therefore, our present results could have further clinical implications in reducing glutamate-induced excitotoxicity in several ophthalmic diseases.

Acknowledgements

This work was supported by OTKA T046589 and the Hungarian Academy of Sciences.

References

Bagnoli P, M Dal Monte and G Casini (2003) Expression of neuropeptides and their receptors in the developing retina of mammals. *Histol. Histopathol.* **18**, 1219-1242.

Chambille I and J Serviere (1993) Neurotoxic effect of neonatal injections of monosodium L-glutamate (L-MSG) in the retinal ganglion cell layer of the golden hamster: anatomical and functional consequences on the circadian system. *J. Comp. Neurol.* **338**, 67-82.

Chen Q, JW Olney, PD Lukasiewicz, T Almli and C Romano (1998) Fenamates protect against ischemic and excitotoxic injury in chick embryo retina. *Neurosci. Lett.* **242**, 163-166.

Danysz W and CG Parsons (2002) Neuroprotective potential of ionotropic glutamate receptor antagonists. *Neurotoxicity Res.* **4**, 119-126.

Frechilla D, A Garcia-Osta, S Palacios, E Cenarruzabeitia and J Del Rio (2001) BDNF mediates the neuroprotective effect of PACAP38 on rat cortical neurons. *Neuroreport* **12**, 919-923.

Gábriel R and P Witkovsky (1998) Cholinergic, but not the rod pathway-related glycinergic (AII), amacrine cells contain calretinin in the rat retina. *Neurosci. Lett.* **247**, 179-182.

Gábriel R, BS Zhu and C Stranicky (1993) Synaptic contacts of serotonin-like immunoreactive and 5,7-dihydroxytryptamine accumulating neurons in the anuran retina. *Neuroscience* **54**, 1103-1114.

Hamano K, H Kiyama, PC Emson, R Manabe, M Nakauchi and M Tohyama (1990) Localization of two calcium-binding proteins, calbindin (28 kDa) and parvalbumin (12 kDa) in the vertebrate retina. *J. Comp. Neurol.* **302**, 417-424.

Izumi S, T Seki, S Shioda, CJ Zhou, A Arimura and R Koide (2000) Ultrastructural localization of PACAP immunoreactivity in the rat retina. *Ann. NY Acad. Sci.* **921**, 317-320.

Jozsa R, A Somogyvari-Vigh, D Reglodi, T Hollosy and A Arimura (2001) Distribution and daily variations of PACAP in the chicken brain. *Peptides* **22**, 1371-1377.

Koves K, O Kantor, V Vereczki, M Kausz, A Nemeskeri, K Fogel, A Kiss, TJ Gorcs, G Szeiffert and A Atrimura (2000) PACAP and VIP in the photoneuroendocrine system. From the retina to the pituitary gland. *Ann. NY Acad. Sci.* **921**, 321-326.

Kubrusly RC, MC da Cunha, RA Reis, H Soares, AL Ventura, E Kurtenbach, MC de Mello and FC de Mello (2005) Expression of functional receptors and transmitter enzymes in cultured Muller cells. *Brain Res.* **1038**, 141-149.

Mathieu M, M Ciarlo, N Trucco, F Griffero, G Damonte, A Salis and M Vallarino (2004) Pituitary adenylate cyclase activating polypeptide in the brain, spinal cord and sensory organs of the zebrafish, *Danio rerio*, during development. *Dev. Brain Res.* **151**, 169-185.

Morio H, I Tatsuno, A Hirai, Y Tamura and Y Saitoh (1996) Pituitary adenylate cyclase activating polypeptide protects rat-cultured cortical neurons from glutamate-induced cytotoxicity. *Brain Res.* **741**, 82-88.

Nilsson SF (1994) PACAP-27 and PACAP-38: vascular effects in the eye and some other tissues in the rabbit. *Eur. J. Pharmacol.* **253**, 17-25.

Nilsson SF, P De Neef, P Robberecht and J Christophe (1994) Characterization of ocular receptors for pituitary adenylate cyclase activating polypeptide (PACAP) and their coupling to adenylate cyclase. *Exp. Eye Res.* **58**, 459-467.

Olianas MC, A Ingianni, V Sogos and P Onali (1997) Expression of pituitary adenylate cyclase activating polypeptide (PACAP) receptors and PACAP in human fetal retina. *J. Neurochem.* **69**, 1213-1218.

Onali P and MC Olianas (1994) PACAP is a potent and highly effective stimulator of adenylyl cyclase activity in the retinas of different mammalian species. *Brain Res.* **641**, 132-134.

Osborne NN, M Ugarte, M Chao, G Chidlow, JH Bae, JP Wood and MS Nash (1999) Neuroprotection in relation to retinal ischemia and relevance to glaucoma. *Surv. Ophtalmol.* **43** Suppl. 1, S102-S128.

Osborne NN, RJ Casson, JP Wood, G Chidlow, M Graham and J Melena (2004) Retinal ischemia: mechanisms of damage and potential therapeutic strategies. *Prog. Retin. Eye Res.* **23**, 91-

147.

Paicone LR, MJ Szego, S Ikeda, PM Nishima and RR McInnes (2003) Progress toward understandig the genetic and biochemical mechanisms of inherited photoreceptor degenerations. *Ann. Rev. Neurosci.* **26**, 657-700.

Pedersen V and WJ Schmidt (2000) The neuroprotective properties of glutamate antagonists and antiglutamatergic drugs. *Neurotoxicity Res.* **2**, 179-204.

Rabl K, D Reglodi, T Banvolgyi, A Somogyvari-Vigh, I Lengvari, R Gabriel and A Arimura (2002) PACAP inhibits anoxia-induced changes in physiological responses in horizontal cells in the turtle retina. *Regul. Pept.* **109**, 71-74.

Reglodi D, A Tamas, A Somogyvari-Vigh, Z Szanto, E Kertes, L Lenard, A Arimura and I Lengvari (2002) Effects of pretreatment with PACAP on the infarct size and functional outcome in rat permanent focal cerebral ischemia. *Peptides* **23**, 2227-2234.

Romano C, MT Price and JW Olney (1995) Delayed excitotoxic neurodegeneration induced by excitatory amino acid agonists in isolated retina. *J. Neurochem.* **65**, 59-67.

Samuelsson-Almen M and SF Nilsson (1999) Pituitary adenylate cyclase activating polypeptide- and VIP-induced activation of adenylate cyclase in the porcine non-pigmented ciliary epithelium: effects of antagonists. *J. Ocul. Pharmacol. Ther.* **15**, 389-400.

Seki T, S Shioda, D Ogino, Y Nakai, A Arimura and R Koide (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, S Shioda, S Izumi, A Arimura and R Koide (2000) Electron microscopic observation of pituitary adenylate cyclase activating polypeptide (PACAP)-containing neurons in the rat retina. *Peptides* **21**, 109-113.

Seki T, S Izumi, S Shioda and A Arimura (2003) Pituitary adenylate cyclase activating polypeptide (PACAP) protects against ganglion cell death after cutting of optic nerve in the rat retina. *Regul. Pept.* **115**, 55.

Shoge K, HK Mishima, T Saitoh, K Ishihara, Y Tamura, H Shiomi and M Sasa (1998) Protective effects of vasoactive intestinal peptide against delayed glutamate neurotoxicity in cultured retina. *Brain Res.* **809**, 127-136.

Shoge K, HK Mishima, T Saitoh, K Ishihara, Y Tamura, H Shiomi, and M Sasa (1999) Attenuation by PACAP of glutamate-induced neurotoxicity in cultured retinal neurons. *Brain Res.* **839**, 66-73.

Silveira MS, MR Costa, M Bozza and R Linden (2002) Pituitary adenylate cyclase activating polypeptide prevents induced cell death in retinal tissue through activation of cyclic AMP-dependent protein kinase. *J. Biol. Chem.* **277**, 16075-16080.

Smythies J (1999) The neurotoxicity of glutamate, domapine, iron and reactive oxygen species: functional interrelationships in health and disease: a review - discussion. *Neurotoxicity Res.* **1**, 27-39.

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

Sucher NJ, SA Lipton and EB Dreyer (1997) Molecular basis of glutamate toxicity in retinal ganglion cells. *Vision Res.* **37**, 3483-3493.

Sun Q, VEC Ooi, and SO Chan (2001) *N*-methyl-D-aspartate-induced excitotoxicity in adult rat retina is antagonized by single systemic injection of MK-801. *Exp. Brain Res.* **138**, 37-45.

Tamas A, R Gabriel, B Racz, V Denes, P Kiss, A Lubics, I Lengvari and D Reglodi (2004) Effects of pituitary adenylate cyclase activating polypeptide in retinal degeneration induced by monosodium- glutamate. *Neurosci. Lett.* **372**, 110-113.

Thoreson WB and P Witkovsky (1999) Glutamate receptors and circuits in the vertebrate retina. *Prog. Retinal Res.* **18**, 765-810.

Toriu N, A Akaibe, H Yasuyoshi, S Zhang, S Kashii, Y Honda, M Shimazawa and H Hara (2000) Lomerizine, a Ca^{2+} channel blocker, reduces glutamate-induced neurotoxicity and ischemia/reperfusion damage in rat retina. *Exp. Eye Res.* **70**, 475-484.

Tuncel N, H Basmak, K Uzuner, M Tuncel, G Altiokka, V Zaimoglu, A Ozer and F Gurer (1996) Protection of rat retina from ischemia-reperfusion injury by vasoactive intestinal peptide (VIP): the effect of VIP on lipid peroxidation and antioxidant enzyme activity of retina and choroid. *Ann. NY Acad. Sci.* **805**, 489-498.

van Rijn CM, E Marani and WJ Rietveld (1986) The neurotoxic effect of monosodium glutamate (MSG) on the retinal ganglion cells of the albino rat. *Histol. Histopathol.* **1**, 291-295.

Vaudry D, BJ Gonzalez, M Basille, Y Anouar, A Fournier and H Vaudry (1998) Pituitary adenylate cyclase activating polypeptide stimulates both c-fos gene expression and cell survival in rat cerebellar granule neurons through activation of the protein kinase A pathway. *Neuroscience* **84**, 801-812.

Vidal-Sanz M, M Lafuente, P Sobrado-Calvo, I Selles-Navarro, E Rodriguez, S Mayor-Torroglosa and MP Villegas-Perez (2000) Death and neuroprotection of retinal ganglion cells after different types of injury. *Neurotoxicity Res.* **2**, 215-227.

Villani L, A Beraudi, A Guiliani and A Poli (2001) MPTP-induced apoptosis in the retina of goldfish. *Neurotoxicity Res.* **3**, 255-257.

Wang YZ, P Alm and R Hakanson (1995) Distribution and effects of pituitary adenylate cyclase activating peptide in the rabbit eye. *Neuroscience* **69**, 297-308.

Waschek JA (2002) Multiple actions of pituitary adenylyl cyclase activating peptide in nervous system development and regeneration. *Dev. Neurosci.* **24**, 14-23.

Wassle H, U Grunert and J Rohrenbeck (1993) Immunocytochemical staining of AII amacrine cells in the rat retina with antibodies against parvalbumin. *J. Comp. Neurol.* **322**, 407-420.

Yoshitomi T, K Yamaji, H Ishikawa and Y Ohnishi (2002) Effect of pituitary adenylate cyclase activating peptide on isolated rabbit iris sphincter and dilator muscles. *Invest. Ophtalmol. Vis. Sci.* **43**, 780-783.