Current Eye Research, 30:189–194, 2005

ISSN: 0271-3863 print / 1460-2202 online
DOI: 10.1080/02713680490908715

Taylor & Francis
Taylor & Francis Group

VIP Immunoreacty in Human Aqueous Humor

Shay-Whey M. Koh,
Allan R. Rutzen,
Timothy J. Coll,
Ramzi K. Hemady,
and Eve J. Higginbotham
Department of Ophthalmology,
University of Maryland,
Baltimore, Baltimore,
Maryland, USA

ABSTRACT *Purpose*: To demonstrate the presence of vasoactive intestinal peptide (VIP)-immunoreactive molecule in the human aqueous humor collected from eyes undergoing either cataract or glaucoma surgeries and to identify the masses of molecules responsible for the VIP immunoreactivity. *Methods*: Aqueous humor specimens were collected by parencentesis from nine cataract patients and also from eight patients undergoing glaucoma surgery following the creation of the limbal based conjunctival flap, partial dissection of the scleral flap, and application of mitomycin-C. The aqueous humor specimens were analyzed by radioimmunoassay to determine the level of VIP immunoreactivity. Specimens from 10 other cataract patients were pooled and analyzed for VIP immunoreactivity by Western blot analysis. *Results*: Levels of VIP immunoreactivity in aqueous humor of cataract and glaucoma patients were significantly different and were 610 ± 160 and 260 ± 64 pg VIP/ml, respectively ($p = 0.03$), while there was no correlation between the donor age and the level of VIP immunoreactivity. VIP immunoreactivity was detected as a single molecule with a molecular weight of 9000. *Conclusions*: The disease status and the treatments of the eye that led to surgery and procedures applied to the eye immediately before aqueous humor collection, but not the age of the patients, affected the level of VIP immunoreactivity in the aqueous humor. The relationship between the 9000 Da VIP-immunoreactive molecule and the authentic (3326 Da) VIP remains to be studied.

KEYWORDS aqueous humor; corneal endothelial cells; immunoblotting; neuropeptides; trophic factors

INTRODUCTION

The presence of a neuroendocrine peptidergic system that is capable of synthesizing, processing, and releasing neuropeptides in the human ciliary epithelium has very recently been postulated on the basis of analysis of the cDNA libraries of this tissue.[1] Expression of neuropeptides and neuropeptide-processing enzymes in the ciliary epithelium and the presence of these molecules in the aqueous humor have also supported this postulate.[2,3] On the other hand, the human and bovine corneal endothelium have been shown to express the mRNA and protein of a neuropeptide, vasoactive intestinal peptide (VIP),[4] suggesting that the neural-crest–derived corneal endothelium[5–8] may also have

Received 13 September 2002
Accepted 1 July 2004

Correspondence: Shay-Whey M. Koh, Ph.D., Rm. 500C MSTF, 10 S. Pine Street, Baltimore, MD 21201, USA. Tel: 410-706-0775; Fax: 410-706-7057; E-mail: skoh@umaryland.edu

neuroendocrine properties. Because both the ciliary epithelium and the corneal endothelium are constantly bathed in the aqueous humor *in vivo*, it is not surprising to find in the aqueous humor immunoreactivity of neuropeptides including calcitonin-gene–related peptide,[9] neurotensin,[2] pituitary adenylate cyclase activating peptide,[10] and VIP.[4,11,12]

Neuropeptides that are released into the aqueous humor may exert important physiological functions through autocrine and/or paracrine mechanisms in tissues that are constantly bathed in the aqueous humor. Recently, we have found that VIP promotes the survival of corneal endothelium under acute oxidative stress in H_2O_2-treated human and bovine corneoscleral explants,[4] an effect that may be associated with VIP-stimulated intracellular cAMP production in the corneal endothelium.[13] Whereas VIP has been shown by Nilsson and colleagues,[14,15] to increase the aqueous humor outflow in animals, probably via modulation of the trabecular meshwork, we and others have previously reported that VIP stimulates cAMP production and proliferation of trabecular meshwork cells in cell culture and in laser-treated trabecular meshwork in corneoscleral explant cultures.[16–18] VIP stimulates intracellular cAMP production in nonpigmented ciliary epithelium (a tissue bathed in the aqueous humor), but not in the pigmented ciliary epithelium (which is shielded by the nonpigmented epithelium and not bathed in the aqueous humor).[19–21] Furthermore, VIP immunoreactivity has been shown to contribute to the immunosuppressive property of the aqueous humor[11] that may be responsible, at least in part, for the maintenance of an immune-privileged environment in the anterior chamber (reviewed in Ref. 22). The diverse functions of VIP in tissues that are bathed in the aqueous humor *in vivo* reflect the broad spectrum of biological activities of this neuropeptide. In addition to being a growth and differentiation promoting factor of cells cultured from neural and non-neural tissues,[18,23–26] VIP is also a modulator of specialized functions of differentiated cells. For example, VIP stimulates fluid secretion in intestines,[27,28] prolactin secretion in the pituitary gland,[29] enzyme secretion in the pancreas,[30] macromolecule secretion at the apical membrane of the retinal pigment epithelium,[31] glycoconjugate secretion by the conjunctival goblet cells,[32] and protein secretion by lacrimal gland acinar cells.[33,34] VIP exerts protective effects on a variety of neuronal cells including the retinal ganglion cells through mechanisms that include stimulation of the release of glia-derived trophic factors.[35–38] The immunomodulatory functions of VIP include that of inhibition of T-lymphocyte proliferation and interleukin-2 production[39] and downregulation of T-lymphocytes by inhibiting the costimulatory function of activated macrophages.[40,41] Furthermore, VIP suppresses the IFN-γ-producing and the delayed-type hypersensitivity-causing abilities of lymph-node cells collected from immunized animals.[11]

The nonpigmented epithelium[42,43] and the trabecular meshwork[44] are responsible for the aqueous humor formation and outflow, respectively, while both tissues are responsive to VIP modulation (above). It has recently been reported that the VIP gene in human trabecular meshwork is upregulated by increased intraocular pressure *in vitro*.[45] In this study, we determined by radioimmunoassay (RIA) the level of VIP immunoreactivity in the aqueous humor collected during cataract and glaucoma surgeries and identified for the first time the molecule responsible for the VIP immunoreactivity in the aqueous humor.

MATERIALS AND METHODS

Sample Collection

Aqueous humor samples were collected from patients undergoing either cataract or glaucoma surgery. At the first step of the cataract surgery, a paracentesis was performed, and an aqueous humor specimen ranging from 50 to 150 μl was drawn using a cannula. During glaucoma surgery, the aqueous humor was removed at the time of the parencentesis following the creation of the limbal-based conjunctival flap, partial dissection of the scleral flap, and application of mitomycin-C. Aqueous humor specimens were chilled on ice and quickly frozen at -70°C Aqueous humor samples of 250 μl were drawn from each of the enucleated donor human eyes (with less than 36 h postmortem time) obtained from the Central Florida Eye Bank (Tampa, FL, USA), by inserting a hypodermic needle (with an attached syringe) through the center of the cornea and then were frozen on dry ice.

RIA for VIP in Human Aqueous Humor

VIP immunoreactivity in the samples was assayed using an RIA kit (Research & Diagnostic Antibodies; Berkley, CA, USA). From each of the aqueous humor samples, duplicate 20 μl samples were incubated with

the anti-VIP rabbit serum (100 μl) at 4°C for 20 h. This was followed by addition of ^{125}I-VIP and the secondary antibody (goat anti-rabbit immunoglobulin serum). The immune complex was spun down after centrifugation at 1500 $\times$ *g* for 20 min, and its radioactivity was measured using a gamma counter. The level of VIP immunoreactivity was calculated from the standard curve generated at the time of assay.

Western Blot Analysis

Ten aqueous humor samples from cataract patients with a volume of 50–150 μl were pooled for a total of 0.8 ml. The pooled specimen was lyophilized and reconstituted in 75 μl of a sample buffer containing 0.9 M Tris-HCl, pH 8.45, 24% glycerol, 8% SDS, 0.015% Coomassie Blue G, and 0.005% phenol red (LC 1676; Novex, San Diego, CA, USA) plus 2.5% β-mercaptoethanol for SDS-polyacrylamide gel electrophoresis (PAGE) using preformed Tricine/10–20% polyacrylamide gels (Novex). A sample of 15 μl was placed in each lane of the gel. The eletrophoresed proteins were electrophoretically transferred to a polyvinylidene difluoride membrane for Western blot analysis using an ECL kit with horseradish peroxidase–linked anti-rabbit IgG secondary antibody (Amersham Pharmacia; Piscataway, NJ, USA). An anti-VIP rabbit serum (ICN Biochemicals, Aurora, OH, USA) was used at 1:1250 dilution in 1% bovine serum albumin/phosphate-buffered saline (BSA/PBS). To block the VIP-specific reactivity of the anti-VIP antibody, synthetic VIP (MW = 3326; Sigma, St. Louis, MO, USA) at 1.7×10^{-7} M was used to preincubate the antibody at 4°C for 20 h prior to Western blotting. In control experiments, preimmune (normal) rabbit serum was used in place of the anti-VIP rabbit serum in the Western blot analysis of aqueous humor from donor human eyes.

Statistical Analysis

The significance level of the difference between the two groups was analyzed by Student's t-test.

RESULTS

VIP Immunoreactivity in Human Aqueous Humor

VIP immunoreactivity was present in human aqueous humor of all samples. As shown in Figure 1, the level of VIP immunoreactivity present in the aqueous humor ranged from 100 to 1350 pg VIP/ml. The average concentrations of VIP immunoreactivity in the aqueous humor from nine patients undergoing cataract surgery and eight patients undergoing glaucoma surgery were 607 $\pm$ 156 pg VIP/ml and 260 $\pm$ 64 pg VIP/ml, respectively. The difference between the samples from patients undergoing surgery for cataract and those for glaucoma was significant ($p = 0.03$). There was no correlation between VIP-immunoreactivity level and donor age in samples from patients going through either cataract or glaucoma surgery. The average concentration (mean in Figure 1) of VIP immunoreactivity of all 17 samples from either cataract or glaucoma patients was 445 $\pm$ 95 pg VIP/ml. Among the five samples demonstrating concentrations of VIP immunoreactivity that were higher than 445 pg VIP/ml, four of them (80%) were from cataract patients. One out of eight (13%) aqueous humor samples from glaucoma patients demonstrated above-average level of VIP immunoreactivity, whereas four out of nine (44%) of that from cataract patients did.

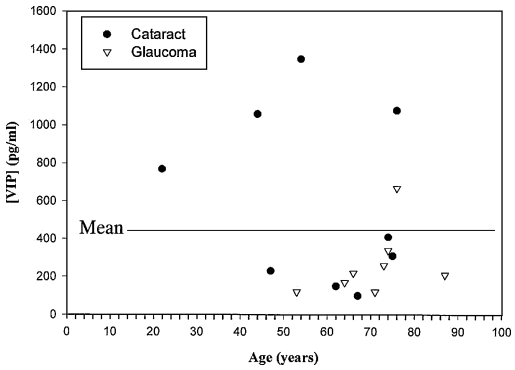

FIGURE 1 **Radioimmunoassay (RIA) for VIP immunoreactivity in the aqueous humor of eyes undergoing cataract and glaucoma surgeries. Aqueous humor samples were collected from patients undergoing cataract (•) and glaucoma (▽) surgeries. VIP immunoreactivity in each of these samples was measured in duplicate. The line indicates the mean level calculated from all samples.**

A 9000-Da Molecule Responsible for the VIP Immunoreactivity Detected in Human Aqueous Humor

Western blot analysis showed that human aqueous humor contained a VIP-immunoreactive molecule with a molecular weight of 9000 (Figure 2, lane 1). The immunoreactivity of the 9000 Da molecule was blocked by preincubation of the anti-VIP antibody with synthetic VIP (Figure 2, lane 2). The 9000 Da

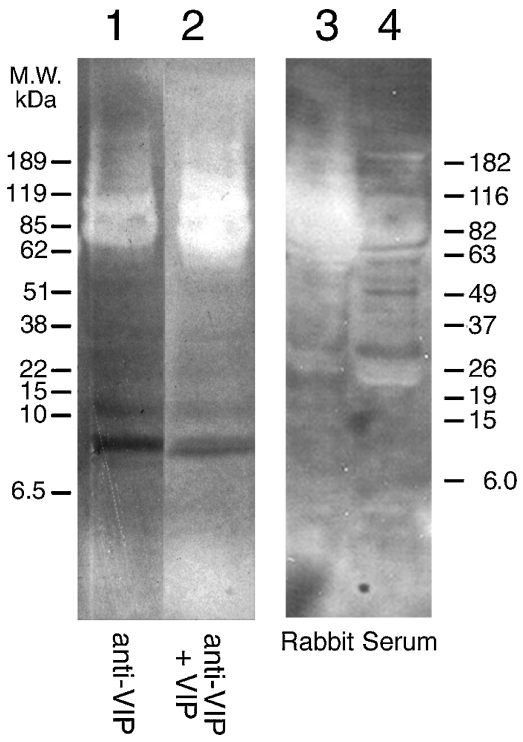

FIGURE 2 **Anti–VIP-immunoreactive molecule in human aqueous humor. Western blot analysis of pooled human aqueous humor samples from 10 cataract patients showing a 9000-Da molecule reacted with the anti-VIP rabbit serum (lane 1). The reaction was diminished by preincubating the anti-VIP rabbit serum with synthetic VIP (lane 2). Preimmune rabbit serum did not react with the 9000-Da molecule in the analysis of aqueous humor from eyes of two donors (lanes 3 [age 73] and lane 4 [age 67]). Each lane contained 160 μl (lanes 1 and 2), 250 μl (lane 3), and 125 μl (lane 4) aqueous humor sample (lyophilized and reconstituted).**

VIP-immunoreactive molecule was absent from blots using preimmune rabbit serum instead of the anti-VIP rabbit serum in the analysis of aqueous humor collected from donor human eyes (Figure 2, lanes 3 and 4).

DISCUSSION

VIP is a 3326-Da, 28-amino-acid neuropeptide.[46] VIP immunoreactivity was found at concentrations of approximately 200 pM VIP (610 ± 160 pg VIP/ml) and 78 pM VIP (260 ± 64 pg VIP/ml) in samples collected from patients going through surgery for cataract and that for glaucoma, respectively. Using the same method of detection, we have previously found that the concentration of VIP immunoreactivity in the aqueous humor of enucleated bovine eyes is 40 pM.[4] Troger *et al.* detected VIP immunoreactivity by RIA in cataract patients and patients with proliferative vitreoretinopathy (PVR) and found concentrations of 0.34 pM VIP in cataract patients and 4.5 pM VIP in patients with PVR.[12] Taylor *et al.* found that VIP immunoreactivity in normal rabbit aqueous humor was 12 nM VIP using an indirect ELISA.[11] The levels of VIP immunoreactivity are not directly comparable between these reports because of differences in species, specimen preparation, and methods, including differences in antibodies. Nevertheless, the current study showed that the averaged level of VIP immunoreactivity in the aqueous humor from glaucoma patients was lower than that from the cataract patients. Although the presence of uncontrollable high intraocular pressure was the reason for glaucoma surgery, the aqueous humor collected from these patients may reflect this abnormality. It has been shown that increased intraocular pressure suppresses the stimulatory effect of VIP on the outflow in an animal model[14,15] and upregulates the VIP gene expression in trabecular meshwork cells in an organ culture system.[45] However, caution must be taken in the interpretation of the current data because eyes with glaucoma and cataract have gone through different therapies before their respective surgeries. In addition, the aqueous humor samples from the cataract eyes were taken before any other procedures were performed, while those from the glaucomatous eyes were from eyes that have been traumatized (see "Materials and methods").

Using Western blot analysis, we detected only one molecule of 9000 Da that was responsible for VIP immunoreactivity in human aqueous humor. No VIP immunoreactivity was detectable with a molecular weight of 3326 corresponding to the known MW of the mature 28-amino-acid VIP. It is possible that the 3326-Da VIP immunoreactivity was present, but our methods may not have been able to detect it. Repeated Western blot analysis of the synthetic 3326-Da VIP was not successful, likely due to improper transfer of this small molecule from the gel to the membrane. The biosynthetic precursor of VIP is the prepro-VIP (MW = 20,000).[47,48] We have previously identified a 20,000-Da VIP-immunoreactive molecule in the human corneal endothelium. Although the nonpigmented ciliary epithelium secretes neuropeptide processing enzymes,[1–3] the presence of these enzymes has been detected in the aqueous humor.[1,2]

Using synthetic peptides corresponding to five prepro-VIP–derived peptides in chromatography, Nilsson and Fahrenkrug have identified in the VIP-producing tumors as well as in the plasma of patients bearing these tumors prepro-VIP 22-79, 111-122, 80-108, 111-122, and 150-170.[49] Although their study was not designed to detect a 9000-Da prepro-VIP–derived

peptide, it demonstrated that either the prepro-VIP itself or its derived peptides was secreted by these tumor cells. Whether the mature VIP (preproVIP 125-152) is secreted by these or any other cells has not been determined. It is probable that the VIP-immunoreactive molecule was released as a 20,000-Da molecule into the aqueous humor and was then processed by the neuropeptide processing enzymes in the aqueous humor to form the 9000-Da VIP-immunoreactive molecule. How the 9000 Da VIP-immunoreactive molecule was cleaved from the prepro-VIP in the aqueous humor is not known at the present time. Whether the 9000-Da VIP-immunoreactive molecule is biologically active remains to be investigated. Most of the biologically active peptides including the mature VIP are amidated at its carboxy-terminal in a reaction catalyzed only by the peptidylglycine α amidating monooxygenase (PAM), whereas PAM is synthesized and secreted by the ciliary epithelium.[2] Furthermore, PAM is an ascorbic acid–dependent enzyme.[50] Ascorbic acid, on the other hand, is concentrated in the human aqueous humor, in which the concentration of ascorbic acid is 14-fold that in the plasma.[51]

In conclusion, we determined the level of VIP immunoreactivity in the human aqueous humor collected during cataract and glaucoma surgeries, and the current study was the first to have demonstrated that only one molecule was responsible for the VIP immunoreactivity in the human aqueous humor.

ACKNOWLEDGMENTS

The current study was supported in part by National Institutes of Health grant RO1EY-11607 (S-W.M.K.), Pangborn Award from the University of Maryland, Baltimore (S-W.M.K.), and Research to Prevent Blindness, Inc., New York, NY (S-W.M.K., T.J.C., R.K.H., A.R.R., E.J.H.).

REFERENCES

[1] Escribano J, Coca-Prados M. Bioinformatics and reanalysis of subtracted expressed sequence tags from the human ciliary body: Identification of novel biological functions. *Mol Vis.* 2002;8:315–332.

[2] Ortego J, Escribano J, Crabb J, Coca-Prados M. Identification of neuropeptide and neuropeptide-processing enzymes in aqueous humor confers neuroendocrine features to the human ocular ciliary epithelium. *J Neurochem.* 1996;66:787–796.

[3] Ortego J, Coca-Prados M**.** Molecular characterization and differential gene induction of the neuroendocrine-specific genes neurotensin, neurotensin receptor, PC1, PC2, and 7B2 in the human ocular ciliary epithelium. *J Neurochem*. 1997;69:1829–1839.

[4] Koh S-WM, Waschek JAW. Corneal endothelial cell survival in organ cultures under acute oxidative stress: Effect of VIP. *Invest Ophthalmol Vis Sci.* 2000;41:4085–4092.

[5] Johnston MC. A radioautographic study of the migration and fate of cranial neural crest cells in the chick embryo. *Anat Rec.* 1966;156:143–155.

[6] Johnston MC, Noden DM, Hazelton RD, Coulombre JL, Coulombre AJ. Origins of avian ocular and periocular tissues. *Exp Eye Res.* 1979;29:27–43.

[7] LeLievre CS, LeDouarin NM. Mesenchymal derivatives of the neural crest: Analysis of chimeric quail and chick embryos. *J Embryo Exp Morphol.* 1975;34:125–154.

[8] Noden DM. The control of avian cephalic neural crest cytodifferentiation. I. Skeletal and connective tissues. *Dev Biol.* 1978;67:296–312.

[9] Troger J, Kremser B, Stockl T, Kralinger M, Schmid E, Kunze C, Kieselbach GF. Elevated levels of calcitonin gene-related peptide in aqueous humor of patients with proliferative vitreoretinopathy. *Graefes Arch Clin Exp Ophalmol.* 2000;38:237–242.

[10] Wang ZY, Alm P, Hakanson R. Distribution and effects of pituitary adenylate cyclase-activating peptide in the rabbit eye. *Neuroscience*. 1995;69:297–308.

[11] Taylor AW, Streilein JW, Cousins SW. Immunoreactive vasoactive intestinal peptide contributes to the immunosuppressive activity of normal aqueous humor. *J Immunol.* 1994;153:1080–1086.

[12] Troger J, Kieselbach G, Gottinger W, Metzler R, Kahler C, Saria A. Different concentrations of vasoactive intestinal peptide in aqueous humor of patients with proliferative vitreoretinopathy and cataract patients. *Ger J Ophthalmol.* 1994;3;245–247.

[13] Koh S-WM, Yue BYJT. VIP stimulation of cAMP production in cultures of rabbit corneal endothelial cells. *Cornea.* 2002;21:270–274.

[14] Nilsson SF, Sperber GO, Bill A. Effects of vasoactive intestinal polypeptide (VIP) on intraocular pressure, facility of outflow and formation of aqueous humor in the monkey. *Exp Eye Res.* 1986;43:849–857.

[15] Maepea O, Nilsson SF. Suppression of VIP-and terbutaline stimulated aqueous humor flow by increased intraocular pressure in the cynomolgus monkey. *Curr Eye Res.* 1991;10:703–712.

[16] Koh S-WM, Yue BYJT. Effects of agonists on the intracellular cyclic AMP concentration in monkey trabecular meshwork cells. *Curr Eye Res.* 1988;7:75–80.

[17] Busch MJ, Kobayashi K, Hoyng PF, Mittag TW. Adenylyl cyclase in human and bovine trabecular meshwork. *Invest Ophthalmol Vis Sci.* 1993;34:3028–3034.

[18] Koh S-WM, Yeh T-H, Morris SM, Leffler M, Higginbotham EJ, Brenneman DE, Yue BY. Vasoactive intestinal peptide stimulation of human trabecular meshwork cell growth. *Invest Ophthalmol Vis Sci.* 1997;38:2781–2789.

[19] Mittag TW, Tormay A, Podos SM.Vasoactive intestinal peptide and intraocular pressure: Adenylate cyclase activation and binding sites for vasoactive intestinal peptide in membranes of ocular ciliary processes. *J Pharmacol Exp Ther.* 1987;241:230–235.

[20] Crook RB, Lui GM, Alvarado JA, Fauss DJ, Polansky JR. High affinity vasoactive intestinal peptide receptors on fetal human nonpigmented ciliary epithelial cells. *Curr Eye Res.* 1994:12:272–279.

[21] Samuelsson-Almen M, Nilsson SF. 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.* 1999;15:389–400.

[22] Taylor AW. Neuroimmunomodulation and immune privilege: The role of neuropeptides in ocular immunosuppression. *Neuroimmunomodulation.* 2002;10:189–198.

[23] Koh S-WM, Kane GJ. VIP stimulates proliferation and differentiation of the cultured retinal pigment epithelium with disparate potencies. *Cell Biol Int Rep.* 1992;16:175–183.

[24] Pincus DW, DiCicco-Bloom EM, Black IB. Vasoactive intestinal peptide regulates mitosis, differentiation and survival of cultured sympathetic neuroblasts. *Nature*. 1990;343:564–567.

[25] Gozes I, Brenneman DE. Neuropeptides as growth and differentiation factors in general and VIP in particular. *J Mol Neurosci.* 1993;4:1–9.
[26] Pankhaniya R, Jabrane-Ferrat N, Gaufo GO, Sreedharan SP, Dazin P, Kaye J, Goetzl EJ. Vasoactive intestinal peptide enhancement of antigen-induced differentiation of a cultured line of mouse thymocytes. *FASEB J.* 1998;12:119–127.
[27] Arcuni JC, Stoner MC, Kellum JM. Vasoactive intestinal peptide is a neuropeptide mediator of the secretary response to serotonin in rat. *J Surg Res.* 2000;91:118–122.
[28] Sjoqvist A, Fihn BM. Transcellular fluid secretion induced by cholera toxin and vasoactive intestinal polypeptide in the small intestine of the rat. *Acta Physiol Scand.* 1993;148:393–401.
[29] Rodriguez de Fonseca F, Wenger T, Navarro M, Murphy LL. Effects of delta9-THC on VIP-induced prolactin secretion in anterior pituitary cultures: Evidence for the presence of functional cannabinoid CB1 receptors in pituitary cells. *Brain Res.* 1999;84:114–122.
[30] Ito T, Hou W, Katsuno T, Igarashi H, Pradhan TK, Mantey SA, Coy DH, Jensen RT. Rat and guinea pig pancreatic acini possess both VIP(1) and VIP(2) receptors, which mediate enzyme secretion. *Am J Physiol Gastrointest Liver Physiol.* 2000;278:G64–74.
[31] Koh S-WM. VIP stimulation of polarized macromolecule secretion in cultured chick embryonic retinal pigment epithelium. *Exp Cell Res.* 1991;197:1–7.
[32] Rios JD, Zoukhri D, Rawe IM, Hodges RR, Zieske JD, Dartt DA. Immunolocalization of muscarinic and VIP receptor subtypes and their role in stimulating goblet cell secretion. *Invest Ophthalmol Vis Sci.* 1999;40:1102–1111.
[33] Adeghate EA, Singh J, Howarth FC, Burrows. S. Control of porcine lacrimal gland secretion by non-cholinergic, non-adrenergic nerves: Effects of electrical field stimulation, VIP and NPY. *Brain Res.* 1997;758:127–135.
[34] Sanghi S, Kumar R, Walton S, Laurie GW. Quantitation of rat lacrimal secretion: A novel sandwich ELISA with high sensitivity. *Exp Eye Res.* 2000;70:651–658.
[35] Brenneman DE, Nicol T, Warren D, Bowers LM. Vasoactive intestinal peptide: A neurotrophic releasing agent and an astroglia mitogen. *J Neurosci Res.* 1990;25:386–394.
[36] Gozes I, Bardea A, Reshef A, Zamostiano R, Zhukovsky S, Rubinraut S, Fridkin M, Brenneman DE. Neuroprotective strategy for Alzheimer disease: Intranasal administration of a fatty neuropeptide. *Proc Natl Acad Sci USA*. 1996;93:427–432.
[37] Brenneman DE, Westbrook G, Fitzgerald SP, Ennist DL, Elkins KL, Ruff MR, Pert CB. Neuronal cell killing by the envelope protein of HIV and its prevention by vasoactive intestinal peptide. *Nature.* 1988;335:639–642.
[38] Shoge K, Mishima HK, Saitoh T, Ishihara K, Tamura Y, Shiomi H, Sasa M. Protective effects of vasoactive intestinal peptide against delayed glutamate neurotoxicity in cultured retina. *Brain Res.* 1998;809:127–136.
[39] Ganea D. Regulatory effects of vasoactive intestinal peptide on cytokine production in central and peripheral lymphoid organs. *Adv Neuroimmunol.* 1996;6:61–674.
[40] Delgado M, Sun W, Leceta J, Ganea. D. VIP and PACAP differenentially regulate the costimulatory activity of resting and activated macrophages through the expression of B7.1 and B7.2 expression. *J Immunol.* 1999;163:4213–4223.
[41] Delgado M, Pozo D, Martinez C, Leceta J, Calvo JR, Ganea D, Gomariz RP. Vasoactive intestinal peptide and pituitary adenylate cyclase-activating polypeptide inhibit endotoxin-induced TNFalpha production by macrophages: In vitro and in vivo. *J Immunol.* 1999;162:2358–2367.
[42] Chen S, Sears M. A low conductance chloride channel in the basolateral membranes of the non-pigmented ciliary epithelium of the rabbit eye. *Curr Eye Res.* 1997;16:710–718.
[43] Fujita H, Kondo K, Sears M. A new function of the non-pigmented epithelium of ciliary processes in the formation of aqueous humor. *Klin Monatsbl Augenheilkd.* 1984;185:28–34.
[44] Lutjen-Drecoll E, Gabelt BT, Tian B, Kaufman PL. Outflow of aqueous humor. *J Glaucoma.* 2001;10(5 Suppl 1):S42–44.
[45] Vittitow JL, Borrás T. Genes expressed in the human trabecular meshwork during pressure-induced homeostatic response. *J Cell Physiol.* 2004;2001:126–137.
[46] Carlquist M, McDonald TJ, Go VL, Bataille D, Johansson C, Mutt V. Isolation and amino acid composition of human vasoactive intestinal polypeptide (VIP). *Horm Metab Res*. 1982;14:28–29.
[47] Obata K, Ito N, Okamoto H, Yanaihara C, Yanaihara N, Suzuki T. Identification and processing of biosynthetic precursors to vasoactive intestinal polypeptide in human neuroblastoma cells. *FEBS Lett.* 1981;136:123–126.
[48] Ito N, Obata K, Yanaihara N, Okamoto H. Human preprovasoactive intestinal polypeptide contains a novel PHI-27-like peptide, PHM-27. *Nature.* 1983;340:547–549.
[49] Ronnov-Jensen D, Gether U, Fahrenkrug J. PreproVIP-derived peptides in tissue and plasma from patients with VIP-producing tumors. *Eur J Clin Invest.* 1991;21:154–160.
[50] Kolhekar AS, Mains RE, Eipper BA. Peptidylglycine alpha-amidating monooxygenase: An ascorbate-requiring enzyme. *Methods Enzymol.* 1997;279:35–43.
[51] Taylor A, Jacques PF, Nowell T, Perrone G, Blumberg J, Handelman G, Jozwiak B, Nadler D. Vitamin C in human and guinea pig aqueous, lens and plasma in relation to intake. *Curr Eye Res.* 1997; 9:857–864.