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Functional Expression of Components of the Natriuretic Peptide System in Human Ocular Nonpigmented Ciliary Epithelial Cells

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The expression of the natriuretic peptide system in the human ocular ciliary epithelium (CE) and in cultured nonpigmented (NPE) ciliary epithelial cells was examined. By RT-PCR and DNA sequencing, we demonstrated that the CE and NPE cells express mRNA for (i) ANP; (ii) BNP; (iii) NPR-A, NPR-B, and NPR-C receptors; and (iv) the neutral endopeptidase 24.11. Radioimmunoassay results indicate that BNP is secreted by cultured NPE cells at much higher levels than ANP. NPR-A and NPR-B receptors elicited a cGMP response to ANP, BNP, and CNP, in a rank order of potency (CNP ⩾ ANP ≥ BNP), indicative that the NPR-B receptor is predominant in NPE cells. A71915, an inhibitor of NPR-A activity, attenuated (65–75%) cGMP response to ANP and BNP, but not to CNP. C-ANP$_{4-23}$ elicited an inhibitory effect (30–37%) on basal levels of cAMP in NPE cells and on forskolin NPE-treated cells, indicative that the NPR-C receptor is functional in these cells. PMA induced, in NPE cells, a long-term down-regulation (75–85%) of NPR-C receptor mRNA, but not of NPR-A or NPR-B receptor mRNA, suggesting a differential regulation of NPR-C receptor mRNA via activation of PKC. Collectively, our data provide molecular evidence that all the components of the natriuretic peptide system with the exception of CNP are coexpressed in the ocular NPE ciliary epithelial cells, where they may function as local autocrine/paracrine modulators to influence eye pressure. © 1999 Academic Press

The natriuretic peptide system is comprised of three structurally related peptides: atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP) and C-type natriuretic peptide (CNP), and at least three distinct subtype natriuretic peptide receptors (NPR-A, NPR-B and NPR-C) (1). There is also evidence that several proteases are involved in the metabolic clearance and specific degradation of natriuretic peptides. Among these enzymes figured: i) the neutral endopeptidase (NEP) 24.11, a zinc-containing metalloendopeptidase that cleaves at the Cys^{7}-Phe^{8} bond of ANP (2), and ii) the insulin-degrading enzyme (IDE), a thiol-metalloendopeptidase that cleaves ANP at the Ser^{25}-Phe^{26} bond and exhibits a substrate and cleavage specificity comparable to that originally observed with insulin (3, 4).

There is now a significant amount of information supporting the view that natriuretic peptides are involved in the regulation of important physiological functions including blood pressure and fluid homeostasis (5). The actions of natriuretic peptides are mediated by their selective interaction with the their cognate receptors, leading to the generation of second messengers cyclic AMP (cAMP), cyclic GMP (cGMP), or the modulation of ion channels (6). There is physiological and medical interest in the role of the natriuretic peptide system in hypertension (1), and it has also been suggested that a similar system may be involved in the regulation of intraocular pressure in the mammalian eye (7). A pathological elevation in the intraocular pressure is usually associated with the development of glaucoma (8).

Previous studies have documented the expression of natriuretic peptides and natriuretic receptors in the rat and rabbit eyes (9); and the hypotensive effects of ANP on experimental animals (10) or in humans with an elevated intraocular pressure (11) has been described. The site of aqueous humor production in the mammalian eye is the ciliary epithelium (CE), and recent studies have shown that the nonpigmented (NPE) ciliary epithelial cells of the human CE express regulatory peptides and receptors with known hypo-

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Abbreviations used: NPE, ocular nonpigmented ciliary epithelial cells; ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide; (CNP), C-type natriuretic peptide; NPR-A, -B and -C, natriuretic peptide receptors; PMA, phorbol-12-myristate 13-acetate; PKC, protein kinase C; RT-PCR, reverse transcription-polymerase chain reaction.

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tensive/hypertensive activities in the cardiovascular system (12–14). It has been suggested that the regulation of intraocular pressure might be under neuroendocrine control through peptides synthesized and released by the CE and targeting the peptide producing ciliary epithelial cells by an autocrine mechanism and/or cells at the outflow system (trabecular meshwork cells) by a paracrine mechanism (7).

In this report, we present experimental evidence that all the components of the natriuretic peptide system with the exception of CNP are coexpressed at the site of secretion of aqueous humor, in the NPE ciliary epithelial cells of the human eye.

MATERIALS AND METHODS

Source of human eye tissue and aqueous humor. Eyes were obtained from cadavers within 24 h after enucleation through the National Disease Research Interchange (Philadelphia, PA). Under a dissecting microscope the ciliary processes containing the ciliary epithelium (CE) were microdissected and stored in liquid nitrogen until further analysis. Aqueous humor fluid samples were obtained from normal donor patients (50 to 75 years old) through the Yale Eye Center (kindly provided by Dr. Marc Weitzman). Investigations with the human subjects was approved by the Human Subjects Committee of Yale University and, as far as it applies, followed the tenets of the Declaration of Helsinki.

Cell culture and drug treatment. A human ciliary epithelial cell line, ODM-2, established from the NPE cell layer of a 2-year old donor was used in this work (15). This cell line was maintained in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal calf serum (FCS) in humidified 5% CO_2–air at 37°C. ODM-2 cells were grown to semiconfluency in 25-cm^2 Primaria tissue culture flasks (Becton–Dickinson, Franklin Lakes, NJ), and then treated with the single addition of phorbol-12-myristate 13-acetate (PMA) at 50 nM for 6, 24, and 48 h. When treated with natriuretic peptides, cells were incubated in DMEM in the absence of serum and containing 1 μM concentrations of ANP, BNP or CNP for the times indicated in the figure legends. A71915 was a gift from Abbott Laboratories and used at concentrations indicated in the figure legends. Forskolin was used at 1 μM, KCl at 75 mM and veratridine at 10 μM. All drugs, with the exception of A71915 (Abbott Laboratories, Abbott Park, IL) were purchased from Sigma, Co. (St. Louis, MO).

Cyclic GMP assay. ODM-2 cells were cultured on 24 multiwell plates and grown overnight in DMEM with 10% FCS at a density of 10^5 cells/well. Cells were washed twice with DMEM without serum, and then incubated for 5 min with DMEM containing 1 mM isobutylmethylxanthine (IBMX), in the presence or absence of A71915. Immediately after, 1 μM ANP, BNP or CNP were added separately to individual wells and further incubated for 5 min at 37°C. The supernatant was collected and discarded, and cells harvested in 0.5 ml of 6% (v/v) trichloroacetic acid. Cells were pelleted after centrifugation at 2500*g* at 4°C for 15 min and the supernatant collected and extracted five times with 2 vol of water-saturated ether, separating and discarding in each case the organic phase. After the last extraction, sample was lyophilized, dissolved in sodium acetate buffer and used directly in the radioimmunoassay following the commercial instructions for the cGMP [^{125}I]RIA kit (NEX-133) purchased from New England Nuclear. The determinations were carried out in triplicate.

Cyclic AMP assay. ODM-2 cells were cultured on 24 multiwell plates and grown overnight in DMEM with 10% FCS at a density of 10^5 cells/well. Cells were preincubated for 5 min with DMEM containing 1 mM theophiline. Immediately after, 0.1 μM of C-ANP_{4-24} was added for 10 min in the presence or absence of 1 μM forskolin and incubated at 37°C. Cells were harvested in 250 μl of 6% (v/v) trichloroacetic acid, pelleted for 5 min at 2500*g* and the supernatant collected and saved. An additional 250 μl of water was added to the pellet of cells, resuspended, and pelleted again. Supernatants were combined and extracted five times with 2 vol of water-saturated ether, separating and discarding the organic phase. After the last extraction, sample was lyophilized, dissolved in sodium acetate buffer and used directly in the radioimmunoassay following the commercial instructions for the cAMP [^{125}I]RIA kit (BT-300) purchased from Biomedical Technologies Inc (Stoughton, MA). The determinations were carried out in triplicate.

Radioimmunoassay for ANP and BNP peptides. Commercial radioimmunoassay systems were used to measure the concentration of ANP and BNP peptides in human aqueous humor, and in cultured NPE cells. Briefly, aqueous samples (100–200 μl) were collected from human eye donors and kindly provided by Dr. Marc Weitzman at the Yale Eye Clinic. Human NPE cells (ODM-2 cell line) were cultured on petri dishes and grown up to 80% semiconfluent in DMEM containing 10% FCS, and further incubated in DMEM serum-free for 2 h, in the presence or absence of 75 mM KCl, or 1 μM veratridine. The supernatants and cells in each experiment were collected separately and analyzed by radioimmunoassay for ANP and BNP peptides. The determinations were carried out in triplicate, following the instructions indicated in the kits for ANP (RIK 8798), and BNP (RIK 9086) respectively from Peninsula Laboratories, Inc. (Belmont, CA).

Reverse transcription-polymerase chain reaction (RT-PCR) assays. Oligonucleotides sets of primers used in PCR amplification for sequencing and polymerase chain reaction (PCR) were synthesized in the DNA Synthesis Facility at Yale University. Set of primers were selected based on published cDNA nucleotide sequences and with the aid of a Primer-select program of DNASTAR (DNASTAR Inc. Madison, Wisconsin) as shown in Table I. The PCR method of Saiki *et al.* (16) was used to anneal primers to cDNA synthesized *in vitro* from total RNA extracted from intact ciliary processes of human eyes, and the human NPE cell line ODM-2, using a RT-PCR kit (Stratagene, La Jolla, CA) as previously described (12). Each PCR cycle consisted of a denaturation step at 94°C for 1 min, 1 min of annealing at the optimal temperature and 1 min of polymerization at 72°C. This cycle was repeated 30 cycles for each of the set of primers used in this work. The final polymerization step was extended an additional 5 min.

Semiquantitative RT-PCR. Optimization of RT-PCRs for semiquantitative analysis was carried out as previously described (13). Values of the level of expression estimated by semiquantitative analysis are represented as means of triplicate experiments ± standard errors of the means, and are plotted as percent of control. Asterisks indicate statistical significance calculated using Student's *t* test ($p < 0.05$).

RESULTS

Expression of atrial (ANP) and brain (BNP) natriuretic peptides, natriuretic peptide receptors subtypes NPR-A, NPR-B, and NPR-C, and neutral neuropeptidase 24.11 in the human ciliary epithelium and nonpigmented ciliary epithelial cells. Reverse transcriptase (RT), followed by polymerase chain reaction (PCR) and nucleotide sequencing, was used to determine ANP, BNP and CNP mRNA in the human CE and in a human NPE cell line (ODM-2) derived from the ocular ciliary epithelium (15). Set of pairs of oligonucleotide primers were selected (see Table I), based on the human cDNA sequences for ANP, BNP and CNP respectively. The primers were annealed to DNA templates synthesized *in vitro* from total RNA extracted from the CE and from cultured ODM-2 cells. The predicted DNA

TABLE I

Oligonucleotide Primer Pairs

Gene	Forward/Reverse	Location	PCR prod (bp)	Annealing temp (°C)	GenBank Acc. #
Atrial natriuretic peptide (ANP)	5′>CAGTGAGCCGAATGAAGAAG<3′ 5′>GGGCTCCAATCCTGTCCA<3′	(880 to 896) (1095 to 1112)	233	64	X01471
Brain natriuretic peptide (BNP)	5′>GGGCGCTCCTGCTCCTGCTCTTC<3′ 5′>ACACCTGTGGGACGGGGGCTCTC<3′	(523 to 545) (955 to 933)	202	70	M31776
C-type natriuretic peptide (CNP)	5′>GCCTGCGCCCTGCTGCTCA<3′ 5′>GCGCGTTGGGGTGCTCTTG<3′	(331 to 349) (1021 to 1039)	265	68	E03596
Neutral neuroendopeptidase (NEP)	5′>AGCAGCCTCAGCCGAACCTACAAG<3′ 5′>AGAATGCCGGCTGGGAAGACTATC<3′	(1276 to 1299) (1814 to 1791)	539	56	Y00811
Natriuretic peptide receptor (NPR-A)	5′>CTTGGGGAGAGGGGGAGTAGCAC<3′ 5′>GGGGGTCGGGGGAGCAGGTATTGT<3′	(3197 to 3219) (3687 to 3664)	491	62.5	X15357
Natriuretic peptide receptor (NPR-B)	5′>AACGGGCGCATTGTGTATATCTGCGGC<3′ 5′>TTATCACAGGATGGGTCGTCCAAGTCA<3′	(1308 to 1334) (1999 to 1973)	692	55	L13436
Natriuretic peptide receptor (NPR-C)	5′>GAAGGTATCGCCGGGCAGGTGTCC<3′ 5′>TCTTCCCGTAATTCCCGATGTTTT<3′	(1246 to 1269) (1646 to 1623)	401	57.4	X52282
β-Actin	5′>TGCGCAGAAAACAAGATGAGATT<3′ 5′>TGGGGGACAAAAAGGGGGAAGG<3′	(1219 to 1241) (1656 to 1635)	438	60	X00351

fragments for precursors of ANP (233-bp) and BNP (202-bp) were amplified and resolved on agarose gels (Fig. 1). However, no amplification was obtained for CNP (data not shown). The PCR products amplified for ANP and BNP were purified, their nucleotide sequence determined, and verified to share 100% homology with their respective cDNA sequences.

To determine the expression of natriuretic peptide receptors, NPR-A, NPR-B or NPR-C, we also applied RT-PCR by annealing pair of oligonucleotide primers selected for the human nucleotide receptors cDNAs (Table I), to DNA synthesized from RNA prepared from the CE and ODM-2 cells. The expected DNA fragments amplified for NPR-A (491-bp), NPR-B (692-bp) and

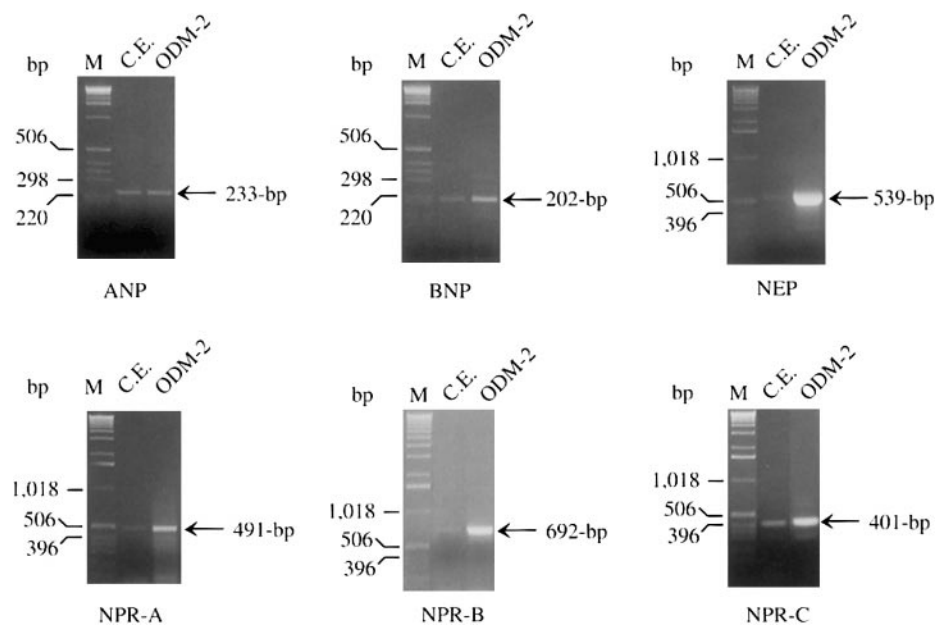

FIG. 1. RT-PCR analysis of atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), neutral endopeptidase 24.11 (NEP), and natriuretic peptide receptors (NPR-A, NPR-B, and NPR-C) in the human ciliary epithelium (C.E.) and in the human NPE ciliary epithelial cell line, ODM-2. Total RNA isolated from ciliary processes of a human eye donor and cultured ODM-2 cells were subjected to RT-PCR analysis using oligonucleotide primer pairs that amplifies regions corresponding the natriuretic peptides, their cognate receptors and NEP. DNA products amplified were resolved in 1% agarose gel, and visualized with ethidium bromide. The gel-purified PCR DNA fragments of the expected size were sequenced directly and verified to share 100% homology with the corresponded cDNAs. The size of the predicted DNA product is indicated at the right of each gel, and of a 1-kb DNA standard marker ladder at left.

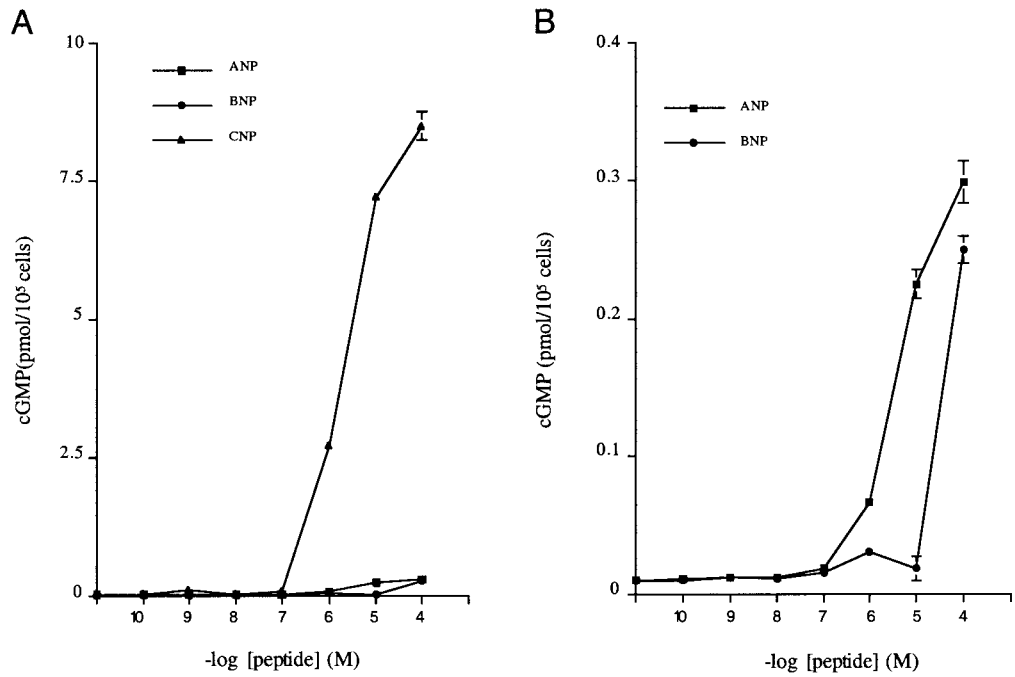

FIG. 2. Stimulation of intracellular cGMP production by natriuretic peptides ANP (■), BNP (●), and CNP (▲) on cultured human nonpigmented ciliary epithelial ODM-2 cells. (A) Cells were preincubated with 1 mM isobutylmethylxanthine (IBMX) for 5 min and then incubated in the presence of the above natriuretic peptides at various concentrations (from 10^{-10} to 10^{-4} M) for 5 min. Intracellular cGMP, expressed as pmol/10^5 cells, was determined using a radioimmunoassay kit from New England Nuclear, as described under Materials and Methods. Values are means SE of three independent experiments. (B) ANP and BNP curves in A are shown now here to a higher scale to distinguish the higher potency of ANP to stimulate cGMP versus BNP.

NPR-C (401-bp) were resolved on agarose gels (Fig. 1), purified and sequenced. The nucleotide sequences of these fragments indicated that they were 100% identical to the nucleotide sequences of the respective human NPR-A, NPR-B and NPR-C receptors.

Finally, we explored by RT-PCR the expression of the neutral endopeptidase 24.11 (NEP) in human CE and cultured ODM-2 cells. A predicted DNA product (539-bp) was amplified by RT-PCR (Fig. 1) using a pair of oligonucleotide primers for NEP (Table I). The nucleotide sequence of this DNA product shared 100% homology with the nucleotide sequence of the human NEP cDNA.

These results indicated that the with the exception of CNP mRNA, transcripts for all the other known components of the natriuretic peptide system are coexpressed in the human CE and human NPE-derived cell line.

The natriuretic peptide NPR-A, NPR-B, and NPR-C receptors expressed on the NPE-derived cell line, ODM-2, are functional. Previous studies have demonstrated that NPR-A and NPR-B receptors are linked to guanylate cyclase activation, whereas the NPR-C receptor is linked to adenylate cyclase inhibition or to activation of phospholipase C (6). To determine whether the three natriuretic receptors coexpressed on cultured ODM-2 cells were functional, we assessed their responsiveness to ANP, BNP and CNP treatments to stimulate guanylate cyclase. Cultured ODM-2 cells were subjected to treatment with increasing concentrations of ANP, BNP or CNP (from 10^{-10} to 10^{-4} M) for 5 min and the accumulated intracellular cGMP was measured using a cGMP ^{125}I-RIA kit (New England Nuclear) as described under Materials and Methods.

Figure 2 shows these results. At the maximal concentrations (0.1 mM), CNP elicited a 28-fold increase on intracellular cGMP (8.5 $\pm$ 0.25 pmol/10^5 cells), when compared to ANP (0.3 $\pm$ 0.01 pmol/10^5 cells) or a 34-fold increase when compared to the response elicited by BNP (0.25 $\pm$ 0.01 pmol/10^5 cells) respectively (Fig. 2A). Although the accumulation of cGMP induced by ANP and BNP was small compared to CNP, the potency of ANP was clearly distinguishable from that of BNP when plotted to a higher scale (Fig. 2B). These results suggested that natriuretic peptides stimulate guanylate cyclase/cGMP accumulation in NPE cells in a rank order of potency, CNP $\gg$ ANP $\geq$ BNP, that is selective for NPR-B receptors (1).

To further verify the ligand selectivity of CNP for NPR-B receptors in NPE cells, we tested A71915, an analogue of ANP that inhibits NPR-A activity (18). A71915 in a concentration-dependent manner (1 and 10 μM), was able to inhibit up to 75% of the stimulation on cGMP induced by ANP (0.1 mM) and 65% of that induced by BNP (0.1 mM). However, it had not effect

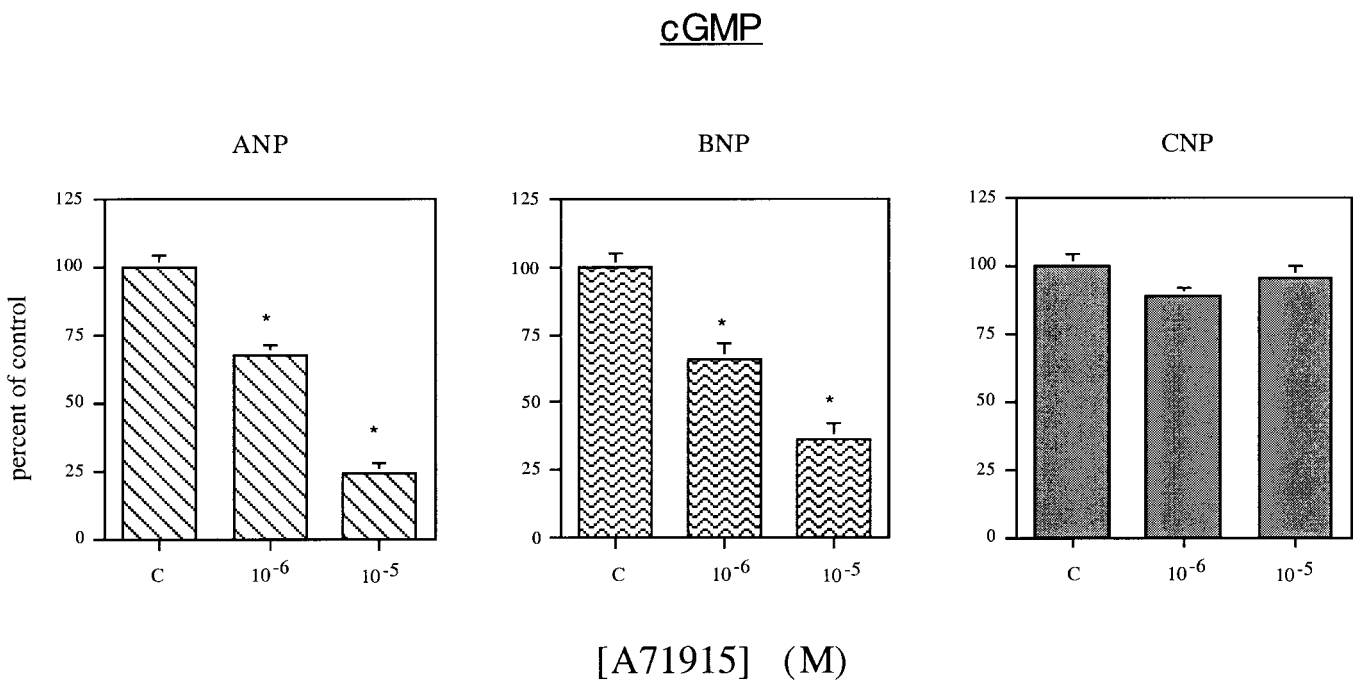

FIG. 3. Inhibitory effect of A71915 on cGMP-mediated stimulation by ANP and BNP. Cultured ODM-2 cells were preincubated with 1 mM IBMX in the presence or absence of various concentrations of A71915 (10^{-6} M and 10^{-5} M), followed by the addition of 1 μM concentration of ANP, BNP or CNP for up to 5 min. Intracellular cGMP levels were then determined as indicated in Fig. 2. Values represent means of triplicate experiments ± standard errors of the means, and they are plotted as percent of control (C). Asterisks indicate statistical significance calculated using Student's *t* test ($p < 0.05$). Notice the lack of inhibitory effect of A71915 on CNP-mediated stimulation of cGMP.

on cGMP accumulation induced by CNP (0.1 mM) (Fig. 3). Thus, these results indicate that ANP and BNP mediate their actions via NPR-A receptors on NPE cells, and CNP mediated its action through NPR-B receptors.

The natriuretic peptide NPR-C receptor is functionally coupled to the inhibition of cyclic AMP in the NPE-derived cell line ODM-2, and its expression is down-regulated by phorbol ester. Previous studies have shown that the activation of NPR-C receptors mediate the inhibition of intracellular cAMP through inhibitory guanine nucleotide-regulating protein (6, 17). To demonstrate that NPR-C receptors in cultured ODM-2 cells are functional we used C-ANP$_{4-23}$, a ring-deleted peptide of ANP, that specifically binds to NPR-C receptors (6). On untreated cells, C-ANP$_{4-23}$ (10^{-7} M) inhibited the basal level of cAMP (0.72 ± 0.08 pmol/10^5 cells) by approximately 37% (0.45 ± 0.06 pmol/10^5 cells), and on forskolin (1 μM) ODM-2-treated cells (25.8 ± 1.50 pmol/10^5 cells) by approximately 30% (18.2 ± 2.6 pmol/10^5 cells).

Recent studies have shown that the direct activation of protein kinase C by phorbol ester, down-regulated NPR-B and NPR-C receptors in a differentially manner (18). To assess whether this inhibitory effect could be observed at the mRNA level, we measured the expression of NPR-A, NPR-B and NPR-C receptor mRNA by semiquantitative RT-PCR after treatment with phorbol-12-myristate 13-acetate (PMA) for various times. The results are shown in Fig. 4. At 24 h of treatment with 50 nM PMA the expression of NPR-C receptor mRNA was down regulated approximately 75%; and 84% at 48 h. In contrast the expression of NPR-A or NPR-B receptor transcripts were unaffected.

Content of ANP and BNP in the human aqueous humor and in the conditioned medium of cultured ODM-2 cells: Effect of secretagogues on secretion. We used radioimmunoassay to determine the concentration of ANP and BNP in the aqueous humor of human eyes and in cultured ODM-2 cells respectively as described under Materials and Methods. In the human aqueous humor, the concentration of ANP was 33.9 ± 9.01 pg/ml (n = 14 samples), and of BNP of 209.8 ± 60 pg/ml. In cultured ODM-2 cells, we estimated the concentration of ANP and BNP accumulated intracellularly and in the culture medium (serum-free) during a 2-h growth period, in the absence and presence of secretagogues. We found that ANP is mostly accumulated intracellularly (15 ± 1.2 pg/10^5 cells), and accumulates in the medium in very low level (less than 1 pg/10^5 cells). KCl (75 mM) or veratridine (10 μM), commonly used to induce secretion, had no effect on the release of ANP in culture. In contrast, BNP was readily detectable intracellularly (65 ± 9.21 pg/10^5 cells) and

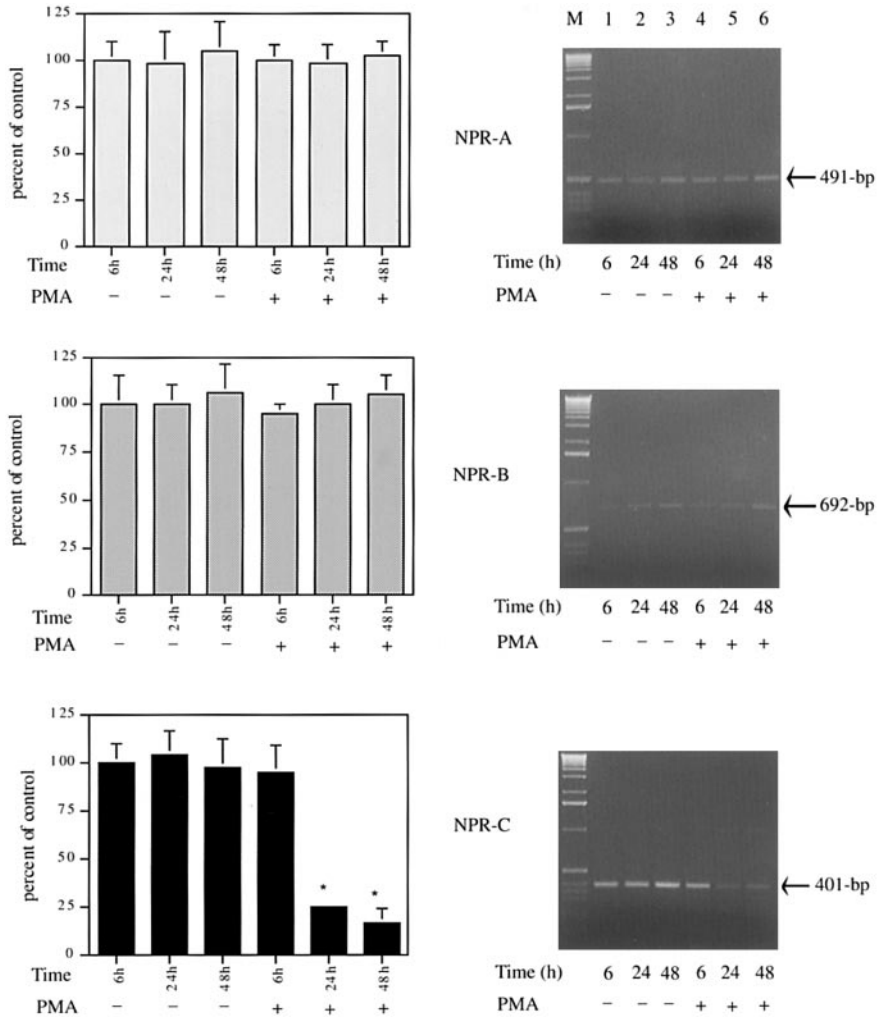

FIG. 4. Semiquantitative RT-PCR analysis of the differential regulation of expression of NPR-A, NPR-B and NPR-C receptors by phorbol ester on cultured NPE cells. Cultured cells grown to semiconfluency were incubated at 37°C in culture medium (DMEM) containing 2% heat-inactivated serum in the absence (−) presence (+) of 50 nM phorbol-12-myristate 13-acetate (PMA), for 6, 24, and 48 h. At the end of each treatment, expression of NPR-A, NPR-B, and NPR-C receptor was determined by semiquantitative RT-PCR as previously described (13). Data, at left, are means ± standard errors (bars) values of three independent experiments, plotted as percentages of control (100%). Asterisks indicate statistical significance calculated using Student's *t* test ($p < 0.05$). At right are representative RT-PCR profiles for each of the natriuretic peptide receptors in the absence (−) or presence (+) of PMA at various times, with the DNA product (in bp) resolved on 1% agarose gel and stained with ethidium bromide.

in the culture medium (71.25 ± 10.5 pg/10^5 cells). Furthermore BNP release into the culture medium was enhanced in about 15–30% in the presence of 75 mM KCl or 10 μM veratridine. These results indicate that both BNP and ANP are detected intracellularly in the NPE-derived cell line ODM-2, and that BNP was detected at higher levels (6-fold) than ANP in the human aqueous humor and in the conditioned medium (70- to 80-fold) of cultured ODM-2 cells.

DISCUSSION

The major finding in this work is the molecular evidence that all the components ascribed to the natriuretic peptide system, with the exception of CNP, are coexpressed in the ocular human nonpigmented ciliary epithelial cells (Fig. 1). Several observations provide evidence which strongly suggests that NPR-A, NPR-B and NPR-C receptors are functional in NPE cells, and can be differentially regulated. In the case of NPR-A receptors, A71915 was able to block the cGMP activity of the receptor. NPR-B receptors were selectively activated by CNP in a more potent manner than ANP or BNP, and when activated by C-$ANP_{4-23,}$ elicited an inhibition of cAMP accumulation in cultured ODM-2 cells. These observations are consistent with the signal transduction pathways elicited by NPR-A and NPR-B receptors through the production of cGMP, and the inhibition of adenylyl cyclase activity by NPR-C receptors (6).

Previous studies have documented the hypotensive effect of systemic or intracameral injections of ANP on intraocular pressure in rabbits, primates and humans (10, 19–21), however the precise source of natriuretic peptides in aqueous humor has remained largely unknown. Previous studies have documented mRNA expression for the three natriuretic peptides and three natriuretic peptide receptors in the rat and rabbit ciliary processes (9), and natriuretic peptides have been detected by radioimmunoassays in aqueous humor of the rat, rabbit and human eyes (22–24). The findings presented in this work indicate that at least in the human eye, the three natriuretic peptide receptors (NPR-A, NPR-B, NPR-C) and two natriuretic peptides (ANP and BNP) are all coexpressed in the same cell, the NPE cells of the ciliary epithelium. These cells are considered to display most of the secretory activity within the ocular ciliary epithelium in aqueous humor production. The present work also supports that the NPE cells contribute to the natriuretic peptide levels of ANP and BNP found in the human aqueous humor.

Potential target cells for the natriuretic peptides released by the ciliary NPE cells in the aqueous humor are (i) the NPE cells themselves; (ii) the adjacent ocular pigment ciliary epithelial cells (PE); (iii) the vascular endothelial cells in the stroma of ciliary processes; and (iv) the trabecular meshwork cells. Small peptides such as natriuretic peptides, after their synthesis in NPE cells, could pass to the PE cells through the numerous gap junctions channels localized at the apical membranes of both cells. Peptides transferred into PE cells could be further secreted into the stroma of the ciliary processes where blood vessels are present. Finally, natriuretic peptides released by the ciliary NPE cells could target natriuretic peptide receptors on the trabecular meshwork cells (25), at the site of drainage of the aqueous humor, through the mediation of carrier proteins such as α2-macroglobulin which exhibits proteinase inhibitor activity (26). This scenario would imply that by a paracrine/endocrine mechanism the rate of outflow could be also under control by the ciliary epithelial cells. This hypothesis is supported by recent evidence that NPE cells synthesize and secrete regulatory peptides, and hormones other than natriuretic peptides, also with hypertensive/hypotensive biological activities in the cardiovascular system (7, 12).

Several neutral endopeptidases have been identified to be involved in the clearance of natriuretic peptides, one of which is the endopeptidase 24.11 (NEP) (2), and another is the insulin-degrading enzyme (IDE) (3). Recently, a cDNA encoding IDE has been isolated from a subtracted ciliary body library (26). Thus, both endopeptidases, NEP and IDE, with affinity to degrade natriuretic peptides are expressed by the human ciliary epithelium and cultured NPE cells. These enzymes could control the level of natriuretic peptides in the aqueous humor. There is now compelling evidence which indicates that the selective inhibition of the NEP enzyme could be used as a potential target in the treatment of cardiovascular hypertension (27). Preliminary studies have indicated that this approach could be taken to control eye pressure in humans with glaucoma (28). In rabbits, with experimentally induced glaucoma, a reduction in atrial natriuretic peptide receptors in the ciliary processes and a concomitant elevation of ANP in the aqueous humor has been observed (29). Thus, the natriuretic peptide system in the human ocular NPE ciliary epithelium may be part of the complex mechanism of regulation of eye pressure.

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