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Type B and type C natriuretic peptide receptors modulate intraocular pressure in the rabbit eye

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Abstract

We investigated (1) the in vivo functional significance of the type B (ANP_B) and type C (ANP_C) natriuretic peptide receptors in the rabbit eye by evaluating the effect of intracameral administration of C-type natriuretic peptide (CNP) and C-ANP-(4–23) on intraocular pressure, and (2) the action of CNP on guanylate cyclase activity in the rabbit ciliary process membranes. Atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) were also studied for comparison. We demonstrated that the natriuretic peptides decrease intraocular pressure and stimulate guanylate cyclase activity, CNP being the most potent. The duration of the effect of C-ANP-(4–23) on intraocular pressure reduction was almost 9-fold that of the BNP and 20-fold that of ANP and CNP effect. This ligand increased threefold the immunoreactive natriuretic peptides levels in aqueous humour. Our data demonstrate the presence of functional ANP_A and ANP_B receptors in the rabbit eye and that the ANP_C receptor modulates the concentration of the natriuretic peptides in the aqueous humour.

Keywords: Natriuretic peptide receptor; Intraocular pressure; Ciliary process

1. Introduction

The natriuretic peptides, atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) are involved in the regulation of natriuresis, diuresis and blood flow (De Bold and Flynn, 1983; Sudoh et al., 1988). A third peptide, the C-type natriuretic peptide (CNP), acts mainly as a vasodilator with little natriuretic activity, and is found principally in the central nervous system and endothelial cells (Sudoh et al., 1990; Suga et al., 1992). There are two types of cell-surface natriuretic peptide receptors which generate cyclic guanosine 3′,5′-monophosphate (cGMP), termed ANP_A and ANP_B. The ANP_A receptors show high affinity for ANP and BNP (Schulz et al., 1989). The ANP_B receptors exhibit high affinity for CNP (Koller et al., 1991; Suga et al., 1992) and lower affinity for ANP and BNP. The third subtype of natriuretic peptide receptor, ANP_C, which is known to have its major role in the clearance of the natriuretic peptides from the bloodstream (Maack et al., 1987), has no intrinsic ability to generate cGMP. It binds not only the three natriuretic peptides with approximately equal affinity, but also ring-deleted and truncated linear peptides (Bennet et al., 1991; Maack, 1992; Suga et al., 1992). The 5-aminoacid, ring-deleted ANP analog, {des ($Gln^{18}Ser^{19}Gly^{20}Leu^{21}Gly^{22}$) rANF-(4–23)-$NH_2$}, which has been designated C-ANP-(4–23), has been reported to be specific for the ANP_C receptor (Maack et al., 1987; Bovy et al., 1989), even though it interacts with the ANP_A and ANP_B receptors at high (> 1 mM) concentrations (Konrad et al., 1991).

In the eye, Bianchi et al. (1986) identified, in the rabbit ciliary process, ANP binding sites negatively coupled to adenylate cyclase. Binding sites for ANP, BNP and CNP positively coupled to guanylate cyclase have also been found in the rat ciliary body (Moya et al., 1998), where the ANP_C receptor type is found in higher proportion than the ANP_A and ANP_B ones. Recently, it has been observed that, in the human trabecular meshwork, ciliary muscle cells (Pang et al., 1996) and in human nonpigmented ciliary epithelial cells (Crook and Chang, 1997), both the ANP and CNP peptides stimulate guanylate cyclase activity, for

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which the ANP_B receptor is the primary functional natriuretic peptide receptor. These data predict that natriuretic peptides, acting through ANP_A and ANP_B receptors, may lower intraocular pressure. Indeed, the intraocular administration of ANP (Mittag et al., 1987; Nathanson, 1987; Samuelsson-Almén et al., 1991) and of BNP (Takashima et al., 1996a) decreases intraocular pressure. In addition, Fernández-Durango et al. (1995) reported that messenger RNAs encoding the natriuretic peptides and their receptors are expressed in the eye.

It is not known which natriuretic peptide receptor is the primary functional one in the rabbit ciliary processes. Furthermore, even though it is believed that the ANP_C receptor type plays a role in the clearance of natriuretic peptides from the bloodstream, nothing is known regarding its function in the eye.

The aims of the present study were therefore to assess (1) the effects of intracameral (i.c.) administration of CNP on the intraocular pressure in the rabbit eye in comparison to the effects elicited by ANP and BNP, together with the actions of the three natriuretic peptides on guanylate cyclase activity in the rabbit ciliary processes, and (2) the in vivo functional significance of the ANP_C receptor, by studying the effects of the i.c. administration of C-ANP-(4–23) on intraocular pressure.

2. Material and methods

2.1. Peptides

The synthetic human ANP-(1–28) (ANP), human BNP-32 (BNP), porcine, rat and human CNP-(1–22) (CNP), {des ($Gln^{18}Ser^{19}Gly^{20}Leu^{21}Gly^{22}$) rANF-(4–23)-$NH_2$} {C-ANP-(4–23)}, ^{125}IhBNP-32 (1828 Ci/mmol) and ^{125}I[Tyr^0]CNP (1627 Ci/mmol) peptides were purchased from Peninsula Laboratories, (Merseyside, UK). The ^{125}IhANP peptide (2000 Ci/mmol) was obtained from Amersham International (Buckinghamshire, UK).

2.2. Intraocular pressure measurement

New Zealand albino rabbits of 3.0–3.5 kg body weight were housed and handled according to the European Community Council Directive of 24 November 1986 (86/609/ECC). The rabbits were trained with 12-h light–dark cycles for at least 2 weeks prior to the experiments. Intraocular pressure was measured using a standardised hand-held aplanation tonometer calibrated as described previously (Fernández-Durango et al., 1991). This instrument was calibrated for use on the eyes of anaesthetised rabbits by cannulating the anterior chamber of the eye as described by Callaway et al. (1973). The rabbits were trained for intraocular pressure measurement for several weeks before the beginning of the experiments. For each determination, the intraocular pressure (expressed in mm Hg) was averaged from three successive readings. The mean intraocular pressure of our control rabbit eyes (13.7 $\pm$ 0.9 mm Hg) is within the range (11.1–20.9 mm Hg) quoted by Callaway et al. (1973). A local anaesthetic (0.2% fluorescein–0.4% oxybuprocaine hydrochloride) was administered topically, and the tonometer was applied tangentially to the cornea for 5 s.

2.3. Intracameral injection and aqueous humour protein determination

The animals were sedated prior to the experiments with intramuscular injections of ketamine hydrochloride (5 mg/kg b.w.) and xylacine (5 mg/kg b.w.). The peptides were dissolved in 0.9% sterile saline containing 0.5% bovine serum albumin, and 10 μl (10 μg) of each solution ($n = 6$) was injected through the sclera into the anterior chamber of one eye 3 mm from the limbus, using a microsyringe (Hamilton) and a 27-gauge needle under topical anaesthesia with 0.4% oxybuprocaine. In order to assess the possible effects of i.c. injection per se on intraocular pressure, an additional group of six rabbits received an i.c. injection of 10 μl of vehicle in one eye; the contralateral eye serving as control.

To determine whether the i.c. injection of either CNP or C-ANP-(4–23) might elicit a nonspecific inflammatory response, two groups of four rabbits were injected intracamerally with 10 μl (10 μg) of both peptides. Four hours after the i.c. injection of CNP and 24 h after the i.c. injection of C-ANP-(4–23) (which is the time yielding the near maximum intraocular pressure effect for the two peptides), the animals were anaesthetised by i.v. administration of sodium pentobarbital (30 mg/kg b.w.) and aqueous humour was drawn with a 1.0-ml syringe as previously described (Fernández-Durango et al., 1990). The needle (0.3 mm diameter) was forced through the corneal-sclera junction into the anterior chamber. The aqueous humour samples were centrifuged at $900 \times g$ for 10 min and the supernatant was removed, frozen immediately and stored at -20°C for later measurement of the protein and natriuretic peptide levels. The pellet was resuspended in 45 μl of phosphate-buffered saline and 5 μl trypan blue dye, and the cells present were counted with a slide haemocytometer. The protein levels were measured by the method of Lowry et al. (1951). At the conclusion of the study, the rabbits were killed with an intravenously administered overdose of sodium pentobarbital.

2.4. Guanylate cyclase activity

The eyes of the New Zealand albino rabbits were enucleated immediately after killing with an intravenous overdose of sodium pentobarbital. The ciliary processes were freed from their attachments to the iris at 4°C, under a dissecting microscope. Ciliary process membranes were prepared as previously described (Fernández-Durango et al., 1991). Briefly, ciliary processes were homogenized on

ice in 5 mM Tris–HCl buffer pH 7.4, containing 0.32 M sucrose, 0.5 mM phenylmethylsulfonyl fluoride, 0.2 mM pepstatin, and 0.8 mM aprotinin. The homogenates were centrifuged at 30,000 × *g* for 30 min in the same buffer. The pellets were resuspended in 50 mM Tris–HCl buffer pH 7.4 at a protein concentration of 0.75–1 mg/ml. The protein concentration was estimated by the method of Lowry et al. (1951). Tissue preparations were stored in aliquots at −70°C and used within 1 month.

Natriuretic peptide-activated guanylate cyclase activity was measured as the rate of conversion of GTP to cyclic GMP as previously described by Inagami et al. (1991). For this assay, reaction tubes were prepared containing (in 0.1 ml final volume): Tris–HCl 50 mM, pH 7.4; 50 mM triethanolamine, 2 mM 3-isobutyl-1-methylxanthine, 10 mM theophylline, 3 mM $MnCl_2$, 1 mM GTP, 75 U/ml creatinine phosphokinase, 72 mM phosphocreatinine, 0.1 mM natriuretic peptides and ciliary process membranes (6 μg of protein). The reaction was started by the addition of Mn_2-GTP. The tubes were incubated for 30 min at 37°C, and the reaction was stopped by addition of 2 ml 30 mM EDTA at 80°C, followed by boiling for 3 min. The GMP formed was subsequently measured by specific radioimmunoassay with the Amersham cGMP kit (RA525), using the nonacetylation protocol. The polyclonal antibody used has a sensitivity (ED_{50}) of 64 fmol/tube and less than 0.001% cross-reactivity with cyclic AMP.

2.5. Measurement of immunoreactive ANP, BNP and CNP in aqueous humour and plasma

The aqueous humour and plasma samples were collected in chilled tubes containing protease inhibitors at the

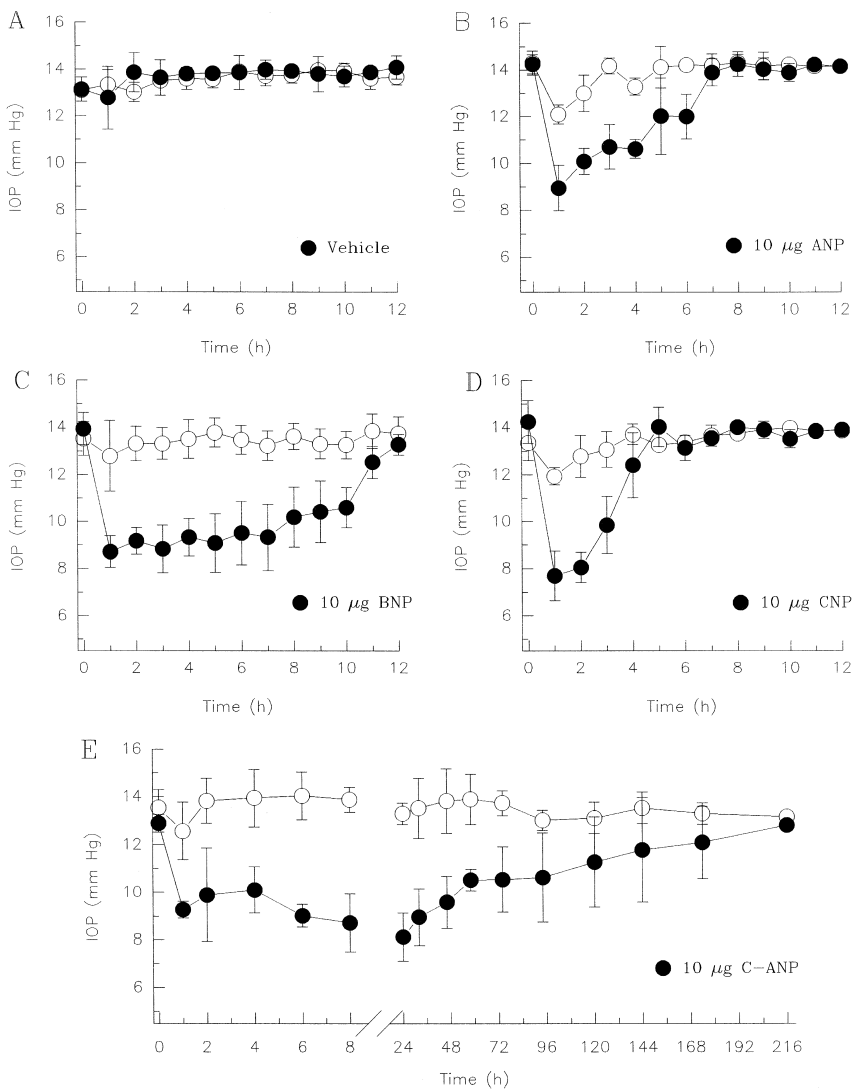

Fig. 1. Intraocular pressure changes after the intracamerular injection of (A) vehicle ($n = 6$), (B) 10 μg ANP ($n = 6$), (C) 10 μg BNP ($n = 6$), (D) 10 μg CNP ($n = 6$) and (E) 10 μg C-ANP-(4–23) ($n = 6$). Each data point represents the mean ± 95% confidence interval. The open circles (O) represent the intraocular pressure values for contralateral uninjected eyes. The ANOVA indicated that all treatments were different ($P < 0.0001$) regarding the maximal intraocular pressure reduction, CNP being the most efficacious ($P < 0.001$, Scheffe's test). The time courses of the intraocular pressure decrease were significantly different for all treatments ($P < 0.001$, Scheffe's test), the duration profiles being: CNP < ANP < BNP ≪ C-ANP.

Table 1
Effect of intracamerular injection of C-ANP and CNP on aqueous humour protein and white cell count

Condition	Aqueous humour	
	Total protein (mg/ml)	White cells (/mm^3)
Control (uninjected) ($n=4$)	0.92 ± 0.2	<1
Vehicle-injected eyes ($n=4$)	0.94 ± 0.1	<1
C-ANP-injected ($n=4$)	1.11 ± 0.3	<1
CNP-injected ($n=4$)	1.02 ± 0.3	<1

Values shown are means ± S.E.M.

following final concentrations: 10^{-5}M EDTA, 10^{-3}M phenylmethylsulfonyl fluoride and 5×10^{-6} M pepstatin. ANP, BNP and CNP extractions were performed as previously described elsewhere for ANP (Fernández-Durango et al., 1990). In brief, ANP, BNP and CNP were extracted using Sep-Pack C-18 cartridges (Waters Associates, Milford, MA, USA). The recoveries of the ANP, BNP and CNP standards added to pooled aqueous humour and plasma were 79.0 ± 3.2, 72.9 ± 2.1 and 75 ± 2.5%, respectively. Immunoreactive ANP was measured by a specific and sensitive radioimmunoassay (RIA) as previously described (Fernández-Durango et al., 1990). The detection limit was 0.75 pg, with a within- and between-assay variability of 10.2% and 14.7%, respectively. The immunoreactive BNP was measured with a RIA, using ^{125}IhBNP-32 and a specific antibody to human BNP-32 obtained from Peninsula Laboratories (Rabbit hBNP-32 antiserum, RAS 9086). The detection limit was 1 pg/tube, and the IC_{50} 24 pg/tube. The within- and between-assay variability was 10.4% and 13.4%, respectively. Cross-reactivity with endothelin, ANP, CNP, BNP-26 (porcine) or BNP-45 (rat) was less than 0.01%. The immunoreactive CNP was measured by RIA, using ^{125}I[Tyr^0]CNP-22 and a specific antibody to human CNP-22 obtained from Peninsula Laboratories (Rabbit CNP-22 antiserum, RAS 9033). The detection limit was 1.5 pg/tube and the IC_{50} 16 pg/tube. The within- and between-assay variability was 9.9% and 16.2%, respectively. Cross-reactivity with endothelin, ANP or BNP was less than 0.01%.

Clear parallelism was observed between various dilutions of aqueous humour extracts, plasma extracts and the standard curve, indicating that BNP and CNP present in aqueous humour are indistinguishable from the peptides used for the preparation of the standard curve (data not shown).

2.6. Data analysis

The intraocular pressure values are expressed as means with 95% confidence intervals. Multifactorial analysis of variance (MANOVA) followed by Hotelling's test was used to examine the statistical significance of differences between the intraocular pressure values of the injected eyes and the uninjected contralateral eyes at all time points. One-way analysis of variance (ANOVA) was applied to test the significance of differences among various treatments regarding the maximal intraocular pressure reduction and the time course of the intraocular pressure decrease. Once the differences between individual means became evident, they were assessed by post-hoc Scheffe's multiple-range test; $P < 0.05$ was taken to indicate statistical significance. The cyclic GMP values are expressed as the means ± standard error of the mean (S.E.M.)

3. Results

The effects of a unilateral i.c. injection of vehicle alone (0.9% sterile saline with 0.5% bovine serum albumin) on the intraocular pressure in a group of six rabbits are shown in Fig. 1A. In the injected eyes there were no significant decreases in intraocular pressure at any time point post-injection as compared to the uninjected contralateral eyes. Fig. 1 shows the effects of unilateral i.c. injection of 10 μg ANF (B), 10 μg BNP (C), 10 μg CNP (D) and 10 μg C-ANP-(4–23) (E). All four experiments showed no significant differences in the intraocular pressure values for the contralateral uninjected eyes and the vehicle-injected eyes. A multifactorial analysis of variance (MANOVA) showed that the treatment effects were different over global curves ($P < 0.001$, Hotelling's test). The ANOVA indicated that the treatment effects were significantly different ($P < 0.0001$) for maximal intraocular pressure reduction, CNP being the most efficacious ($P < 0.001$, Scheffe's test). However, the maximal intraocular pressure reductions produced by ANP, BNP and C-ANP-(4–23) did not

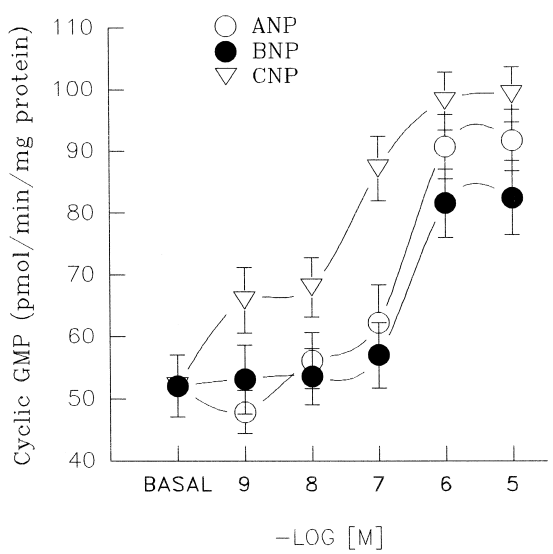

Fig. 2. Concentration-dependent stimulation of particulate guanylate cyclase activity by natriuretic peptides in rabbit ciliary process membranes. Data are presented as the means ± S.E.M. of four independent experiments performed in duplicate.

Table 2
Effect of intracamerular injection of C-ANP on aqueous humour natriuretic peptide levels

	ANP (pM)	BNP (pM)	CNP (pM)
Plasma levels ($n=10$)	3.1 ± 1.1	4.3 ± 1.7	1.5 ± 0.1
Control eyes (uninjected) ($n=6$)	< 0.6	43.0 ± 9.2	10.2 ± 2.8
Vehicle-injected eyes ($n=4$)	< 0.6	46.5 ± 12	13.4 ± 2.4
C-ANP-(4–23)-injected eyes ($n=4$)	17 ± 0.3	128.0 ± 23	37.1 ± 8.4

Values shown are means ± S.E.M.

differ significantly. On the other hand, the time courses of intraocular pressure decrease were significantly different for all four treatments ($P < 0.001$, Scheffe's test), the duration profiles being: CNP < ANP < BNP ≪ C-ANP.

Table 1 shows that there were no changes in the protein concentration and white cell count in the aqueous humour after i.c. injection of either CNP or C-ANP-(4–23). Thus, it appears that the changes in intraocular pressure induced by the injection of either CNP or C-ANP-(4–23) were not related to a nonspecific inflammatory response.

In the rabbit ciliary process membranes, the baseline levels of guanylate cyclase activity were 52.06 ± 5 pmol cGMP/min mg protein ($n = 5$). The three natriuretic peptides activated particulate guanylate cyclase (Fig. 2). The maximal stimulation of the enzyme produced by ANP was achieved with 1 μM (90.72 ± 5 pmol cGMP/min mg protein). The concentration–response curve for ANP was sigmoidal, with an EC_{50} of 118 ± 6 nM. The stimulatory effect of BNP was similar to that of ANP, with an EC_{50} of 124 ± 8 nM and with similar maximal stimulation (82.5 ± 6 pmol cGMP/min mg protein). However, CNP was more potent than ANP and BNP, with an EC_{50} of 24 ± 3 nM. Table 1 shows that the injections of either drug or vehicle did not elicit, at a time when hypotension was present, increases beyond the normal ranges for either the white cell counts or the total protein content.

The concentrations of the natriuretic peptides in plasma and aqueous humour are shown in Table 2. The ANP concentrations in aqueous humour from normal eyes were nondetectable. At 24 h after the injection of 10 μg C-ANP-(4–23), the natriuretic peptide concentrations in aqueous humour had increased to almost three times the baseline values.

4. Discussion

The present study investigated for the first time the role of the ANP_B and ANP_C receptors in the regulation of intraocular pressure. To this purpose, we have examined the effects of i.c. injections of CNP and C-ANP-(4–23), respectively; the effects of ANP and BNP injections were also studied for comparison. Our results demonstrated that the three natriuretic peptides are effective to reduce intraocular pressure. The effects elicited by both ANP and CNP lasted for 4 h, even though CNP decreased the intraocular pressure more intensively than did ANP during the first 3 h. The intraocular pressure reduction induced by BNP was similar to that seen with ANP, but persisted 7 h longer. The intraocular ANP_C blockade with C-ANP-(4–23) produced an intraocular pressure reduction similar to that caused by ANP, and the duration of the reduction was 96 h, a period longer than that achieved with any of the three natriuretic peptides.

On the other hand, we have shown that the guanylate cyclase activity in rabbit ciliary process membranes is stimulated by the three natriuretic peptides, confirming the existence of both biologically active natriuretic receptors, the ANP_A and ANP_B subtypes, in this tissue. The potency profile for the natriuretic peptides concerning the activation of guanylate cyclase was found to be CNP > ANP = BNP (according to the EC_{50} values found). This finding is consistent with the maximal intraocular pressure-reducing effects of the peptides according to Fig. 1. Our data are also consistent with those from a previous study, showing that the intravitreal injection of CNP lowers intraocular pressure in rabbits more effectively than either ANP or BNP (Takashima et al., 1996b).

The dose of natriuretic peptides (10 μg) chosen for injection was based upon estimates that, if the peptide is distributed uniformly within the volume of the aqueous humour (250–300 μl), the final concentration would be about 10^{-5} M, similar to that which caused maximal stimulation of ciliary process guanylate cyclase activity (Fig. 2).

It is noteworthy that, in previous studies, we demonstrated that the K_d of binding of ANP to the rabbit ciliary processes is 30 pM (Fernández-Durango et al., 1991). This K_d value is at least three orders of magnitude lower than that of the EC_{50} of cGMP stimulation (124 ± 8 nM). Similar results have been obtained with other tissues (Koller et al., 1991; Dos Reis et al., 1995). Thus, this appears to be a property of this family of peptide receptors. Different mechanisms have been proposed to explain the phenomenon, such as intramolecular inhibitory domains, existence of essential cofactors, oligomerization of receptors, and interaction with the ANP_C receptor. It has also been reported that, in tissues in which the ANP_C receptor is absent, lower concentrations of ANP appear to be needed to increase cGMP levels (Hamet and Tremblay, 1991). Another possibility raised by Weir et al. (1993) was that one or all of these hormones may be signalling via another second messenger pathway in addition to that of cGMP.

The longer-lasting effect of BNP on intraocular pressure as compared to that elicited by ANP and CNP could be due either to a lower affinity for the ANP_C receptor or to less degradation. The fact that, in the rat ciliary body (Moya et al., 1998), as well as in human foetal nonpigmented ciliary epithelial cells (Crook and Chang, 1997), ANP and BNP have the same affinity for the ANP_C receptors might indicate that BNP could be metabolized

more slowly than ANP and CNP by the neutral endopeptidase 24.11 present in the eye (Sales et al., 1991). Indeed, BNP was hydrolysed by endopeptidase 24.11 at a lower rate than the other three peptides (Kenny et al., 1993).

The fact that the duration of the effect of C-ANP-(4–23) on intraocular pressure reduction was almost 9-fold that of the BNP effect and 20-fold that of the ANP and CNP effects may be due to the blockade of ANP_C receptors, since this compound produced an approximately threefold increase of the immunoreactive ANP, BNP and CNP levels in the aqueous humour. Our results are consistent with the earlier finding that the administration of C-ANP-(4–23) in vivo augments the plasma ANP levels (Maack et al., 1987). The increase of the natriuretic peptide concentrations in the aqueous humour induced by C-ANP-(4–23) was probably secondary to the displacement of natriuretic peptides from the ocular ANP_C receptors into the aqueous humour. This observation is consistent with the view that the ANP_C receptor may function as a hormone buffer system, modulating the ocular natriuretic peptide concentrations. We suggest that the intraocular pressure reduction produced by CNP might be due to the biological actions of the increased concentrations of natriuretic peptides in the aqueous humour, acting through either the ANP_A or the ANP_B receptors. The ANP_C receptor is the prominent natriuretic receptor in the rat ciliary body (Moya et al., 1998) and in human nonpigmented ciliary epithelial cells (Crook and Chang, 1997).

The ocular hypotensive effect of natriuretic peptides does not appear to be related to a nonspecific inflammatory response, since the aqueous humour protein concentrations in C-ANP-(4–23) and/or CNP-injected and vehicle-injected eyes were not significantly different. It has also been reported that ANP and BNP do not seem to be pro-inflammatory substances (Nathanson, 1987; Takashima et al., 1996a). The small contralateral effect observed 1 h after injection of the peptides might have been due to a systemic effect of the peptide released into the circulation from the injected eye.

We chose intracameral administration because drug delivery to the anterior side of the uvea is probably the best way to study the actions of the natriuretic peptides on their receptors. Furthermore, the effects of intravitreal injections of peptides may be influenced by binding of the peptides to the polyanionic glycosaminoglycans of the vitreous humour. Indeed, and in contrast to our results, the intravitreal injection of BNP induces an intraocular pressure reduction of lower intensity than, and similar duration to, that elicited by ANP (Takashima et al., 1996a).

Aqueous humour formation, conventional outflow facility and uveoscleral outflow are the major components determining intraocular pressure. The precise effects of ANP on aqueous humour flow and intraocular pressure are not clear. Several investigators have reported that, in rabbits, ANP decreases either intraocular pressure alone (Sugrue and Viader, 1986; Nathanson, 1987; Mittag et al., 1987) or both parameters (Korenfeld and Becker, 1989). However, it has been reported that, in the *cynomolgus* monkey, intracameral injection of ANP transiently increased the aqueous humour flow, while the uveoscleral outflow tended to increase and the intraocular pressure tended to decrease (Samuelsson-Almén et al., 1991). Similarly, Takashima et al. (1996a) reported that the intravitreal injection of BNP in rabbits reduced intraocular pressure because of an increase in the outflow facility. At least some of these effects are likely to be due to a direct action on the ciliary body, because autoradiographic studies show that natriuretic peptide binding sites are associated with guinea-pig (Mantyh et al., 1986) and rat (Bianchi et al., 1986; Moya et al., 1998) ciliary epithelium. We also have detected ANP_A, ANP_B and ANP_C mRNAs in the rat and rabbit ciliary body (Fernández-Durango et al., 1995). Carré and Civan (1995) presented evidence that ANP modulates ion transport across the rabbit ciliary epithelium. This finding, together with the guanylate cyclase-stimulating effect of natriuretic peptides we have observed in rabbit ciliary processes, suggests a potential action of these peptides on aqueous humour formation.

We now have demonstrated for the first time the presence of immunoreactive BNP as well as of immunoreactive CNP in the rabbit aqueous humour. We have previously reported the existence of biologically active ANP in aqueous humour (García de la Coba et al., 1991). Also, we found that, in rabbits with experimental glaucoma, the ANP receptors are down-regulated (Fernández-Durango et al., 1991), and that the immunoreactive ANP levels in aqueous humour rise as the intraocular pressure increases (Fernández-Durango et al., 1990). Interestingly, the concentrations of both immunoreactive BNP and immunoreactive CNP are higher in aqueous humour than in plasma. However, the opposite occurs with the immunoreactive ANP levels. These results suggest that BNP and CNP may play a more significant role than ANP in the regulation of intraocular pressure. Considering our results on the ocular effect of C-ANP-(4–23), the pharmacological use of ANP_C-specific ligands to reduce the natriuretic peptide clearance and thus enhance their action in the eye could have important implications in the treatment of glaucoma.

In summary, we have demonstrated, in the rabbit eye, (1) the presence of functional ANP_A and ANP_B receptors, since ANP, BNP and CNP all decreased intraocular pressure and stimulated guanylate cyclase activity in ciliary process membranes, CNP being the most potent of the three, and (2) that the ANP_C receptor may function as a clearance receptor since it modulates the concentrations of natriuretic peptides in the aqueous humour.

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