Brain natriuretic peptide: identification of a second natriuretic peptide in human aqueous humour

Joel Salzmann, Daniel Flitcroft, Catey Bunce, David Gordon, Richard Wormald, Clive Migdal

Abstract

**Aims/background**—To measure aqueous humour levels of brain natriuretic peptide (BNP) and atrial natriuretic peptide (ANP) in humans. To compare peptide levels in glaucomatous and control eyes to test the hypothesis that these peptides are increased in glaucoma. BNP and ANP are cyclic endopeptides whose principal biological effects are natriuresis and vasodilatation. Experimental glaucoma in animal models results in elevated aqueous ANP. Intravenous ANP administration in both animals and humans causes lowering of intraocular pressure (IOP). There are equivocal data to support a role for ANP in IOP regulation in human eyes. There are as yet no published data on BNP in human aqueous humour.

**Method**—This was a case-control study. Cases were primary open angle, pseudoexfoliation, and mixed mechanism glaucoma eyes undergoing trabeculectomy. Controls were cataract extraction eyes. There were 47 trabeculectomy eyes (46 patients) matched for age, sex, race, systemic medications, and type of anaesthetic, 100–200 µl of aqueous humour were aspirated by paracentesis as the first step in the surgical procedure. Peptide levels were later measured by radioimmunoassay.

**Results**—The presence of BNP and ANP in human aqueous humour was confirmed. BNP was present in higher concentrations than ANP. BNP levels tended to be greater in control eyes—glaucoma median = 0.961, r control group = 0.894). ANP and BNP were log linearly related in both groups (r glaucoma group = 0.961, r control group = 0.894).

**Conclusion**—This is the first report of BNP and ANP in human aqueous humour. Peptide levels did not differ significantly between glaucoma and cataract extraction eyes. A linear relation between log BNP and ANP was found. Further studies are required to clarify the role of these peptides in aqueous humour production and IOP regulation.

The natriuretic peptides are a class of cyclic peptides which play an important role in sodium homeostasis. Three peptides in this class have been identified to date—atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), and C peptide. These compounds have been extensively studied in hypertensive and cardiac failure where their diagnostic and therapeutic potentials are considerable. Analogies between hypertension (systemic volume overload) and glaucoma (raised intraocular pressure (IOP)) have led to the investigation of ANP as an IOP reducing agent.

The role of ANP in the regulation of IOP and aqueous humour production is ill understood. Experimental data in animals suggest that intraocular ANP increases as IOP elevates. Conversely, intravenous, intracameral, or intravitreal ANP administration results in decreased IOP. Furthermore, natriuretic peptide receptors have been identified on the ciliary epithelium and corneal endothelium. Pilot studies in humans have suggested an IOP lowering effect of both ANP administration and inhibition of ANP degradation by the non-specific neutral endopeptidase inhibitor candoxatril. BNP, for its part, plays an important role in tandem with ANP in systemic volume regulation, but there have been no reports to date on BNP in human aqueous humour. Ophthalmic natriuretic peptide data derive from animal research and there is a paucity of human data. We therefore measured BNP and ANP levels in peroperative aqueous humour, and compared eyes with chronic glaucoma with age matched normals undergoing cataract extraction.

**Methods**

**SUBJECTS**

A case-control study was set up and ethical approval obtained from the local hospital ethics committee. “Cases” were eyes with primary open angle, pseudoexfoliative or chronic narrow angle glaucoma with progressive field loss or inadequate IOP control. Normal tension, inflammatory, and rubeotic glaucoma cases were excluded. Cataract extraction eyes were used as controls.

Exclusion criteria included use of topical steroids, a history of ocular inflammation,
ASSAY METHODOLOGY
Aqueous humour was immediately transferred to an Eppendorf tube containing 50 µl of wetting agent 0.1% Triton X in phosphate buffer 0.1 M and frozen at −70°C to await radioimmunoassay (RIA), the methodology of which was adapted from plasma natriuretic peptide assays. Samples were later thawed on ice and a 50 µl aliquot assayed for total protein using the Biuret method on a Roche Cobas Mira autoanalyser with Sigma Total protein reagent. Results were expressed in pg/ml. Aqueous humour total protein dosage was performed so that BNP and ANP levels could also be expressed as a fraction of total protein (pg peptide/mg protein). A measured volume of each aqueous humour sample was then transferred to a 4.5 ml polypolypropylene tube on ice and 500 µl 1 M acetic acid/25 mM hydrochloric acid in deionised water was added to each tube. The tubes were vortex mixed and centrifuged at 2000 g at 4°C for 60 minutes. Each supernatant was decanted and dried in a Savant SC200 SpeedVac drier. RIA was performed using Peninsula kits (product numbers RIK 8798 (alpha-ANP 1–28) and RIK 9086 (BNP 32 human), Peninsula Laboratories, Belmont, CA, USA). The assays were performed according to manufacturers’ instructions with one modification; 50 µl instead of 100 µl of rabbit anti-ANP/BNP antibody were used per tube as this increased assay sensitivity.

Investigators performing the peptide radioimmunoassays were masked to the case-control status of the samples.

Results
Aqueous taps were performed on 118 eyes; 12 samples had inadequate volumes and were discarded; 106 samples were assayed; 100 eyes were then matched into pairs. Eight samples (six glaucoma, two controls) were from four patients undergoing bilateral consecutive surgery in separate episodes. Following strict application of exclusion criteria 47 eye pairs remained for analysis. In the glaucoma group three eyes (6.5%) had pseudoxfoliative glaucoma, three eyes (6.5%) had chronic narrow angle glaucoma, and 41 eyes (87%) had primary open angle glaucoma. Glaucoma treatment regimens are listed in Table 1.

Table 1 shows aqueous peptides and aqueous protein levels. Results suggest that ANP tended to be slightly greater in glaucomatous
eyes (median 3 pg/ml) than in control eyes (median 0 pg/ml), but that aqueous BNP tended to be slightly greater in control eyes. This was true of both absolute and relative measures (pg aqueous peptide/ml aqueous humour and pg aqueous peptide/mg total aqueous protein) but these differences were not statistically significant as assessed by the Wilcoxon signed rank test.

The distribution of paired case-control data for BNP and ANP is shown in Figure 1. The scatter was greatest for BNP (range 0–3526 pg/ml in the glaucoma group).

There was no discernible correlation between glaucoma and control peptide levels. Figure 2 scatter plots reveal little association between total aqueous protein and aqueous peptides in both glaucoma and control groups. This indicates that high BNP and ANP levels were not associated with a breakdown of the blood-aqueous barrier and consequently increased aqueous protein levels.

A strong correlation was found between log BNP and ANP levels in both glaucoma and cataract eyes. Figure 3 illustrates this relation as revealed by the linearity of a scatterplot of log BNP against ANP concentrations. The correlation coefficients were 0.894 in the control group and 0.94 in the glaucoma group.

Discussion

These results suggest a linear association between ANP and log BNP (Fig 3). In the absence of cross reactivity between BNP and ANP antibodies, confirmed by the assay manufacturers, this finding suggests a link in the physiological role of these peptides. However, although closely correlated in terms of concentration, the peptides may be involved in different functions, the nature of which requires further investigation.

These results do not support the hypothesis that BNP and ANP in glaucomatous aqueous differ significantly from controls. This would suggest that these peptides do not play a primary role in IOP regulation. In the 42/47 glaucoma eyes on glaucoma drugs data interpretation is limited by the impact of treatment. For ethical reasons there was no washout period built into the study. This may have influenced results, since aqueous inflow inhibition such as occurs with timolol17 will increase aqueous protein concentration. No subgroup analysis was performed since this was not specified at the outset.

Ocular anaesthesia itself may influence aqueous peptide levels. Induction agents in adult general anaesthesia (GA) habitually cause a transient IOP reduction.18–20 In local anaesthesia (LA), particularly peribulbar blocks,21–23 IOP increases. The ocular hypertensive effect is more marked in eyes with glaucoma.24 BNP and ANP levels in our study were not significantly different in LA or GA.

Table 3 Aqueous natriuretic peptides and proteins in the study population, by case-control status

<table>
<thead>
<tr>
<th></th>
<th>Glaucoma case (47 eyes)</th>
<th>Matched controls (47 eyes)</th>
<th>Wilcoxon SRT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median</td>
<td>IQ range</td>
<td>Range</td>
</tr>
<tr>
<td>ANP pg/ml</td>
<td>3.00</td>
<td>(0, 6.00)</td>
<td>(0–68.50)</td>
</tr>
<tr>
<td>pg/mg protein</td>
<td>4.38</td>
<td>(0, 12.50)</td>
<td>(0–266.70)</td>
</tr>
<tr>
<td>BNP pg/ml</td>
<td>56.50</td>
<td>(0, 90.00)</td>
<td>(0–3526.50)</td>
</tr>
<tr>
<td>pg/mg protein</td>
<td>114.00</td>
<td>(0, 195.00)</td>
<td>(0–11755.00)</td>
</tr>
<tr>
<td>Mean mg/ml</td>
<td>0.57</td>
<td>0.42</td>
<td>0–3.00</td>
</tr>
</tbody>
</table>

IQ = interquartile; SRT = signed rank test.
pg/mg protein = picograms of peptide per milligram of aqueous protein.
subgroups (Table 4). The confounding effects of anaesthesia could be avoided by performing topical anaesthesia slit lamp paracentesis, a procedure not ethically acceptable in the context of our study.

These results show a marked scatter of aqueous humour natriuretic peptide levels with a small number of outliers showing very high levels. The patients from whom these samples were taken did not appear to fit into any particular clinical subgroup, and the isolated cluster of high values remains unclear. ANP results differ from those in the animal study by Fernandez-Durango et al in which glaucoma was induced in rabbits by posterior chamber chymotrypsin injection. Aqueous ANP increased over 20 times while the mean IOP doubled. There was no sign of inflammation. The authors advanced two possible mechanisms for the ANP elevation: "volume distension" of the globe or a physiological response of the eye to high IOP. The experiment, however, simulates acute IOP elevation in humans and is a poor model for chronic glaucoma. Furthermore, since neuropeptide receptor concentrations vary between species, extrapolating from animal model results can only be tentative.

Aqueous humour protein values were similar to those previously published. The lack of association between aqueous humour proteins and peptides indicates that breakdown or abnormal permeability of the blood-aqueous barrier is an unlikely explanation of the high levels of peptides in some eyes. Nathanson compared aqueous humour and plasma peptide levels and found a very low aqueous to plasma ratio. He suggested that the blood-aqueous barrier "may largely exclude atriopeptins from passing into the aqueous humour but also that "atriopeptins are probably not actively secreted into the aqueous from uveal tissues". Recent evidence from the rabbit eye model provides evidence to the contrary: peptide receptor mRNA for ANP, BNP, and natriuretic peptide C obtained by polymerase chain reaction cDNA amplification showed a differential distribution of mRNAs in various ocular tissues; in addition, mRNA for the natriuretic peptides themselves were also differentially distributed in the eye. This suggests that intraocular natriuretic peptides are—at least partly—locally synthesised. Furthermore, BNP and ANP receptors have been demonstrated in human neural retina and retinal pigment epithelium and natriuretic peptide C receptors identified on the corneal endothelium, further suggesting a role of these peptides in the eye.

In conclusion, this is the first study to measure BNP in the human eye and to document ANP and BNP levels in human aqueous humour. The results do not support the hypothesis that aqueous levels of BNP or ANP are increased in glaucoma although in this respect study power was adversely affected by the wide range of peptide values. The relative contribution to aqueous humour production of locally synthesised and plasmatic natriuretic peptides, and their role in aqueous and IOP regulation remains to be clarified. Current data suggest that the natriuretic peptides and their degradative inhibitors such as candesartan may have a role in the treatment of some forms of glaucoma.

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The authors have no proprietary interest in the compounds described in this study.


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