
Catecholamines in Human Aqueous Humor

Graham E. Trope* and Alan G. Rumley†

Aqueous humor catecholamine levels were measured in 14 patients admitted to hospital for cataract or glaucoma surgery. Norepinephrine was detected in all patients. Epinephrine was detected in one patient who had received a preoperative retrobulbar injection of epinephrine. Dopamine was not detected in any patients. The highest level of norepinephrine was detected in cataract patients with normal intraocular pressures (mean = 7.18 nmol/l). Invest Ophthalmol Vis Sci 26:399-401, 1985

It is presently believed by some workers that aqueous humor does not contain catecholamines. It has been suggested that the mechanisms of inactivation of released catecholamines are so efficient that it is not possible to detect norepinephrine in aqueous humor.1-3 Recently sensitive radioenzymatic techniques have been developed, which allow for the detection of catecholamines present in minute concentration in various fluids. Catecholamines have been reported to exist in plasma,4 and we have detected them in as little as 10-20 µl of tears.

The main purpose of this study was to confirm the presence of catecholamines in human aqueous humor and to extend the study to determine catecholamine levels in patients with glaucoma.

Materials and Methods. Fourteen aqueous humor samples from 14 patients were studied. Thirteen patients were admitted for routine eye surgery under general anaesthetic, one patient was admitted for surgery under local anesthetic. Aqueous humor samples were obtained from three groups of patients.

Group 1: This group consisted of six patients admitted for unicocular intracapsular lens extraction (cases 1-6) under general anaesthetic. None received preoperative sympathetic eyedrops. All underwent a limbal paracentesis with removal of aqueous humor on the operating table before the anterior chamber was entered with surgical instruments. Once aqueous humor had been withdrawn into a tuberculin syringe, sympathetic drops were applied to the eye to dilate the pupil for further surgery.

Group 2: This group consisted of four patients newly diagnosed as having open-angle glaucoma (OAG). These patients were involved in a study to determine the role of primary trabeculectomy in patients with this disease3 (cases 7-10). None of these patients had ever received topical or systemic antiglaucoma therapy. Three of the patients had primary open-angle glaucoma (POAG). One had pigmentary glaucoma (case 8). Three of the patients underwent trabeculectomy under general anesthesia (cases 7-9). One patient, however, had a local anesthetic. In this case a retrobulbar injection of 1% lignocaine plus 1:100,000 adrenaline was used (case 10). Samples of aqueous humor were removed through a limbal puncture under the superficial trabeculectomy flap.
before the anterior chamber was entered with surgical instruments.

**Group 3:** This group consisted of four patients with OAG not controlled on antiglaucoma medical therapy (cases 11–14). All four patients had aqueous humor samples removed under general anesthesia by paracentesis during trabeculectomy as described in group 2. One patient had pigmentary glaucoma (case 12), one had POAG (case 11), and two patients had POAG associated with dysthyroid eye disease (cases 13, 14).

Aqueous humor was removed from the anterior chamber of all patients using a 25-gauge needle and collected into a tuberculin syringe. The amount of aqueous humor removed varied from patient to patient according to how much the surgeon was prepared to withdraw (between 10–100 μl were studied). The aqueous humor was removed and transferred into preweighed tubes containing 0.1 M HCl. The tubes then were reweighed and deep frozen within 2 hr of collection at −20°C. The volume of aqueous humor removed was determined by subtracting the initial weight of the tube from the final weight (assuming the SG of the contents to be 1).

Aqueous humor catecholamines levels were studied using the technique of Da Prada and Zurcher⁴ as modified by Ball et al.⁶ In this assay 100 μl of diluted sample was assayed in duplicate with a simultaneous assay (also in duplicate) of sample containing added internal standard (166.7 pg of epinephrine, norepinephrine, and dopamine) to enable correction of results to be made to compensate for losses of catecholamine during the assay procedure. These samples were then added to an incubation mixture containing crude catechol-o-methyl transferase (20 μl), L-(methyl-3H) methionine (3.6 μCi) (Radiochemical Centre; Amersham, Bucks, England), tris buffer (1 mmol/l), magnesium chloride (75 mmol/l) and benzylxamine (a dopa decarboxylase inhibitor: 0.5 mmol/l). Total incubation volume was 150 μl at pH 8.4. Incubation was for 1 hr at 37°C.

The tritiated 3-methoxy derivatives were extracted into 1 ml diethyl ether in the presence of tetra phenyl boron and excess “cold” 3-methoxy derivatives and then back extracted into 50 μl HCl (0.1 mol/l). This 50 μl extraction was applied directly to a thin layer chromatography plate and after development for 1 hr in a chloroform:ethanol:ethyamine (80:15:10) solvent, the three distinct spots of the methylated derivative were scraped off the plates for liquid scintillation counting.

Epinephrine and norepinephrine derivatives were eluted into 1 ml ammonium hydroxide (0.05 mol/l) and oxidized to vanillin with 4% sodium periodate (50 μl) and after 5 min, were acidified with 1 ml acetic acid (0.1 mol/l) and extracted into 8 ml of toluene containing 0.6% butyl-PBD (scintillant). Dopamine derivative was eluted into 0.5 ml ammonium hydroxide (0.05 mol/l) acetylated with acetic anhydride (25 μl) for 30 min at room temperature, acidified with glacial acetic aid (100 μl) and extracted into toluene containing scintillant as before.

There is very little cross interference in this assay. Da Prada and Zurcher measured no interference by dopamine in epinephrine and norepinephrine measurement, less than 0.1% interference by norepinephrine in epinephrine measurement and 1.8% interference by epinephrine in norepinephrine measurement.⁴

The detection limits were set, in the standard way for this type of assay, as the concentration equivalent to twice the blank value. The limits therefore varied from assay to assay, but concentrations below the detection limit for each assay were reported as “not detected.”

Informed consent was obtained from patients prior to undertaking the study. Statistical analysis of results was performed using the Student’s t-test and the Mann Whitney test.

**Results.** All 14 patients had detectable levels of norepinephrine in their aqueous humor. Epinephrine and dopamine were not detected in significant quantities in the aqueous humor of any patients who had undergone general anesthesia. Blank levels for these catecholamines were, however, fairly high, and this may explain why we were unable to detect them in most patients. Epinephrine was, however, detected in one sample. This sample came from the patient who had undergone a retrobulbar injection containing epinephrine, approximately ½ hr before the aqueous humor had been removed (sample 10). The epinephrine level was 5.8 nmol/L in this case.

Table 1 contains details of group 1 patients. The mean norepinephrine level for this group of patients was 7.18 (±4.43) nmol/L. Table 2 contains details of the four patients in group 2. Mean norepinephrine levels in this group was 3.44 (±0.97) nmol/L. Table 3 contains details of the four patients in group 3.

<table>
<thead>
<tr>
<th>Sample/case number</th>
<th>Age</th>
<th>Surgical procedure</th>
<th>Norepinephrine level (nmol/L)</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>79</td>
<td>ICLE</td>
<td>7.3</td>
</tr>
<tr>
<td>2</td>
<td>76</td>
<td>ICLE</td>
<td>12.9</td>
</tr>
<tr>
<td>3</td>
<td>81</td>
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<td>3.57</td>
</tr>
<tr>
<td>4</td>
<td>71</td>
<td>ICLE</td>
<td>2.18</td>
</tr>
<tr>
<td>5</td>
<td>60</td>
<td>ICLE</td>
<td>5.10</td>
</tr>
<tr>
<td>6</td>
<td>76</td>
<td>ICLE</td>
<td>12.0</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td>7.18 (±4.43)</td>
</tr>
</tbody>
</table>

ICLE = intracapsular lens extraction.

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**Table 1. Details of Group 1 patients with cataract and normal intraocular pressures**
Table 2. Details of group 2 patients who had glaucoma but no previous antiglaucoma medication

<table>
<thead>
<tr>
<th>Sample/case number</th>
<th>Age</th>
<th>Surgical procedure</th>
<th>Norepinephrine level (nmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>75</td>
<td>Trabeculectomy</td>
<td>3.4</td>
</tr>
<tr>
<td>8</td>
<td>41</td>
<td>Trabeculectomy</td>
<td>3.3</td>
</tr>
<tr>
<td>9</td>
<td>84</td>
<td>Trabeculectomy</td>
<td>4.7</td>
</tr>
<tr>
<td>10</td>
<td>75</td>
<td>Trabeculectomy (under local)</td>
<td>2.35</td>
</tr>
</tbody>
</table>

Mean norepinephrine levels in this group was 2.53 (±0.63) nmol/L.

Statistical analysis of the above data indicated significant differences in mean norepinephrine levels between group 1 and groups 2 and 3 combined ($P < 0.0422$), and between groups 1 and 3 ($P < 0.0311$). Nonparametric tests (Mann Whitney test) revealed significant differences between group 1, and groups 2 and 3 combined ($Z = 2.068, P < 0.0468$).

Discussion. The purpose of this study was twofold. Firstly, we wished to determine if catecholamines exist in aqueous humor. Despite some evidence to the contrary, this study confirms a recent report that human aqueous humor contains significant quantities of norepinephrine. The mechanisms of catecholamine inactivation therefore are not so efficient as to prevent detection of norepinephrine in aqueous humor as previously suggested.

The origin of human aqueous norepinephrine is uncertain. It has been reported that total iridectomy decreases primate aqueous humor norepinephrine to about 17%, indicating that a small quantity of aqueous humor norepinephrine is derived from non-iris sources. Circulating norepinephrine generally is derived from incomplete re-uptake and metabolism of this neurotransmitter following its release into the synaptic clefts. The ciliary processes are known to be innervated by sympathetic fibers. The possibility therefore exists that aqueous humor norepinephrine is similarly derived in part from overflow of the neurotransmitter from the ciliary processes.

The second purpose of this study was to try to determine whether patients with glaucoma have significantly different aqueous humor catecholamine levels compared with patients with normal intraocular pressures. This study indicates that norepinephrine levels may be lower in patients with OAG compared with patients with cataracts and normal intraocular pressures. The lowest mean levels of norepinephrine were found in patients on therapy for this disease. In view of the fact that group 1 contains two patients with high norepinephrine levels (cases 2 and 6), there is significantly more variability in the norepinephrine levels of this group than in the glaucoma groups. The possibility, therefore, exists that the statistical differences between group 1 and the other groups is artifactual. Larger numbers of samples should be studied before a definite conclusion regarding aqueous norepinephrine levels in glaucoma compared with nonglaucomatous eyes can be made.

Key words: catecholamines, aqueous humor, norepinephrine, epinephrine, dopamine, glaucoma intraocular pressure

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From the Ophthalmology Department,* University of Toronto, Toronto, Canada, and the Department of Pathological Biochemistry,† Western Infirmary, Glasgow, Scotland. Submitted for publication: April 19, 1984. Reprint requests: Dr. G. Trope, Toronto General Hospital, Eaton Building 5-303, 200 Elizabeth Street, Toronto, Canada M5G 1L7.

References