Effects of Systemic Desmopressin on Aqueous Humor Dynamics in Rabbits

Iro Wallace, Jay Moolchandani, Theodore Krupin, Allan Wulc, and Richard A. Stone

Intravenous desmopressin, a synthetic antidiuretic hormone, resulted in a dose-dependent increase in intraocular pressure (IOP) in rabbits. IOP was increased 3.6 ± 0.8 mm Hg 6 hr following injection of desmopressin 200 mUnits/kg with the increase lasting over 10 hr. IOP returned to baseline 24 hr after the injection. Systemic blood pressure, plasma osmolarity and arterial blood gases were not altered by desmopressin. The increased IOP was not associated with alterations in measured outflow facility or episcleral venous pressure. Five hours after desmopressin injection, calculated aqueous humor flow was increased approximately 57%. Aqueous humor ascorbate measurements for calculation of flow to diffusion ratios and anterior chamber fluorophotometry also were consistent with an increased rate of aqueous humor formation as the mechanism for the IOP elevation. Desmopressin administration did not increase aqueous humor protein or aqueous humor cyclic AMP concentration. Systemic pretreatment with indomethacin only partially blocked the IOP increase. Systemic pretreatment with demeclocycline completely blocked the desmopressin-induced increase in IOP. Invest Ophthalmol Vis Sci 29:406-410, 1988

Vasopressin or antidiuretic hormone is a nine amino acid peptide with a broad range of biological activities, including effects on the eye. Topical administration of vasopressin to the human eye lowers intraocular pressure (IOP) probably because of its vasoconstriction action. In contrast, an intravenous infusion of vasopressin elevates IOP in rabbits. In the isolated iris-ciliary body preparation, vasopressin increases the short circuit current across the tissue, suggesting that vasopressin may affect active sodium transport across the ciliary epithelium.

Aqueous humor dynamic studies are difficult to evaluate following intravenous vasopressin. Vasopressin-mediated vasoconstriction elevates systemic blood pressure as well as causing ocular vasoconstriction and secondary effects on IOP. Also, because of rapid hydrolysis by serum peptidases, the plasma half-life of vasopressin is only 5 to 10 min. Vasopressin must be given by continuous infusion to elevate IOP. To overcome some of these problems, we have studied the effect of intravenous desmopressin (1-deamino-8-D-arginine vasopressin), a synthetic analog of vasopressin, on aqueous humor dynamics in rabbits. Desmopressin is a highly specific antidiuretic agent exhibiting an antidiuretic to vasopressor activity ratio of 2000:1 (vasopressin = 0.9:1). In addition, desmopressin is not degraded by serum peptidases and has a 6 to 20 hr plasma half-life.

Materials and Methods

Awake pigmented rabbits weighing 2.5 to 4 kg were restrained in cloth wrappers or in specially designed plexiglass boxes. Animals were not fasted prior to the experiment; however, water was withheld during the 10 hr duration of the experiments. After topical 0.5% proparacaine HCl anesthesia, IOP was measured with a pneumotonometer (Digilab Model 30R, Cambridge, MA) calibrated manometrically (closed stopcock technique) against rabbit eyes. After obtaining stable baseline IOP measurements, desmopressin acetate (Armour Pharmaceutical Company, Kankakee, IL) was dissolved in filtered normal saline (osmolarity 295–305 mosmols/l, pH 7.0) and was injected into a marginal ear vein at doses between 12.5 and 1000 mUnits/kg. IOP was measured hourly during the first 8 hr and at 10 and 24 hr following injection.

Aqueous humor outflow facility was determined by tonography using an electronic Schiotz tonometer connected to a graph recorder. Baseline tonograms were performed on nine awake rabbits prior to and 5 hr after intravenous desmopressin (200 mUnits/kg). Episcleral venous pressure was measured with an episcleral venomanometer in four rabbits before and at
various times after intravenous desmopressin (200 mUnits/kg). Aqueous humor flow was investigated in a group of eight rabbits which received unilateral intravitreal injections of 0.02 ml of 10% fluorescein-labeled dextran (molecular weight, 67000). Three days later, baseline anterior chamber fluorophotometry was performed over a 3 hr interval to determine the concentration of aqueous humor fluorescein. Desmopressin 200 mUnits/kg was injected intravenously and fluorophotometry repeated 1, 3 and 6 hr later. The fluorophotometer was calibrated with a fluorescein glass standard and the instrument was linear between fluorescein concentrations of $10^{-5}$ and $10^{-9}$ mg/ml. To study the effects of desmopressin on systemic blood pressure and blood chemistry, a femoral arterial catheter was placed in restrained awake animals using locally injected 1.0% lidocaine HCl anesthesia. Systemic blood pressure was measured directly by connecting the cannula to a pressure transducer and a polygraph. Arterial blood samples, obtained from the same cannula, were analyzed for pO$_2$, pCO$_2$ and blood pH with a Corning (Medfield, MA) blood gas analyzer, and plasma osmolarity was measured using a Precision Instruments (Sudbury, MA) Osmette S automatic osmometer.

Aqueous humor samples were obtained on awake animals by posterior and anterior chamber paracentesis after topical 0.5% proparacaine anesthesia. Baseline chamber taps were done in one eye and intravenous desmopressin (200 mUnits/kg) was administered. IOP was remeasured either 1 or 5 hr later on the fellow eye and anterior chamber taps were then performed on this fellow eye. Aqueous humor samples were immediately placed in 4.0% metaphosphoric acid and titrated with dichlorophenol-indophenol for estimation of ascorbate concentration. The Kinsey-Palm formula was used to calculate the ascorbate ratio of the flow coefficient ($k_{fa}$) to the diffusion coefficient ($k_{ppa}$). In addition, anterior chamber aqueous humor protein concentration was measured using Biuret and Folin phenol reagents (Total Protein Kit No. 690, Sigma Chemical Co., St. Louis, MO).

In another group of 20 rabbits, anterior chamber aqueous humor samples were analysed for cyclic adenosine monophosphate (cAMP). Aqueous humor samples were precipitated immediately in cold 10% trichloroacetic acid, the samples were acetylated, and cAMP concentrations determined by radioimmunoassay (DuPont Specialty Diagnostics, Boston, MA). A baseline aqueous humor sample was obtained from one eye. Desmopressin (200 mUnits/kg) was given intravenously and aqueous humor was sampled from the anterior chamber of the fellow eye either 1 or 5 hr later.

Two additional groups of rabbits were pretreated either with oral demeclocycline hydrochloride (Sigma Chemical Co., 20 mg/kg) 2 hr before intravenous desmopressin (200 mUnits/kg) or with intraperitoneal indomethacin (Sigma Chemical Co., 10 mg/kg) 1 hr before intravenous desmopressin (200 mUnits/kg). IOP was measured prior to pretreatment, just prior to injection of desmopressin and then hourly for 8 hr.

Statistical analyses were performed using a t-test on paired differences for results within each experimental group and a t-test on unpaired differences for intergroup analysis. The IOPs of the two eyes of each rabbit were averaged at each measurement interval with the average used as one observation. Drug effects are reported as mean ± SEM, and $P$ values less than 0.05 were considered significant. The study conformed to the ARVO Resolution on the Use of Animals in Research.

Results

Intravenous desmopressin elevated IOP; both the magnitude and the duration of the increase show a dose-dependent response. IOP was significantly increased following a desmopressin 25 mUnits/kg injection with a peak increase in IOP of $+2.9 ± 0.4$ mm Hg and a duration of 5 hr. Higher doses of desmopressin resulted in a greater increase in IOP (Fig. 1) which remained elevated for a longer time (Fig. 2). The elevated IOP returned to baseline levels by 24 hr after intravenous injection. The maximum response was observed with desmopressin doses of 200 mUnits/kg; the higher doses gave essentially the same IOP response.

Baseline mean arterial blood pressure (106 ± 4 mm Hg) was not significantly altered 4 hr (107 ± 2 mm Hg).
Table 2. Effects of desmopressin (200 mU/kg I.V.) on aqueous humor chemistries

<table>
<thead>
<tr>
<th>Time after injection</th>
<th>Cyclic-AMP (mean ± SEM nM)</th>
<th>Protein (mean ± SEM mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline eye</td>
<td>15.2 ± 2.0</td>
<td>66 ± 10</td>
</tr>
<tr>
<td>Fellow eye</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 hr</td>
<td>12.5 ± 2.0</td>
<td></td>
</tr>
<tr>
<td>5 hr</td>
<td>10.2 ± 1.0*</td>
<td>52.5 ± 3*</td>
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</tbody>
</table>

* Significant difference from baseline, paired t-test, P < 0.05.

Table 1. Effects of desmopressin (200 mU/Kg I.V.) on aqueous humor dynamics

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>5 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Outflow facility (μl/min/mm Hg)</td>
<td>0.20 ± 0.03</td>
<td>0.22 ± 0.03</td>
</tr>
<tr>
<td>Episcleral Venous pressure (mm Hg)</td>
<td>8.3 ± 1.0</td>
<td>7.2 ± 2</td>
</tr>
<tr>
<td>IOP (mm Hg)</td>
<td>16.7 ± 0.8</td>
<td>19.2 ± 0.8*</td>
</tr>
<tr>
<td>Calculated aqueous flow† (μl/min)</td>
<td>1.68</td>
<td>2.64*</td>
</tr>
</tbody>
</table>

* Significant change from baseline value, paired t-test, P < 0.01.
† Goldmann equation where flow = (IOP-episcleral venous pressure) out-flow facility.

Hg), 5 hr (107 ± 2 mm Hg), or 6 hr (103 ± 2 mm Hg) following intravenous administration of 200 mU/kg desmopressin (P > 0.5; n = 6). Baseline arterial blood pH (7.40 ± 0.01) was unchanged at 4 hr (7.43 ± 0.02) and 6 hr (7.44 ± 0.03) after the same desmopressin dose. Plasma osmolarity of 285.5 ± 2.1 mOsm/l at baseline was 291.8 ± 1.4 mOsm/l at 4 hr and 289.0 ± 1.7 mOsm/l at 6 hr; both statistically unchanged (P > 0.1; n = 6).

The baseline Kinsey-Palm flow to diffusion ratio, Kf/dpa, was calculated for ascorbate.9 The baseline ratio of 2.12 ± 0.23 (ascorbate anterior chamber 29.1 ± 3.4 mg%, posterior chamber 42.8 ± 4.2 mg%) increased 1 hr after injection of desmopressin (200 mUnits/kg) to 3.14 ± 0.47 (ascorbate anterior chamber 29.9 ± 3.1 mg%, posterior chamber 39.4 ± 2.2 mg%) (P < 0.05; n = 9). The baseline ascorbate ratio (2.95 ± 0.32) (ascorbate anterior chamber 29.1 ± 2.7 mg%, posterior chamber 41.5 ± 2.7 mg%) in the eyes of animals studied 5 hr after desmopressin also was significantly increased to 4.07 ± 0.38 (ascorbate anterior chamber 28.5 ± 2.1 mg%, posterior chamber 35.5 ± 3.0 mg%) (P < 0.05; n = 8).

The mean fluorescein concentration (10^-4 mg/ml) in the anterior chamber in eight rabbits 3 days after intravitreal injection of fluorescein-labeled dextran was 8.98 ± 0.35. Repeat baseline concentration 3 hr later, prior to intravenous desmopressin 200 mUnits/kg, was 9.00 ± 0.38 (P > 0.6). Anterior chamber fluorescein concentration was significantly (P < 0.001) decreased 1 hr (7.46 ± 0.39), 3 hr (7.48 ± 0.32) and 6 hr (7.34 ± 0.33) following intravenous administration of desmopressin.

One hour after desmopressin administration, aqueous humor cAMP showed a small reduction which was not statistically significant (Table 2). However, 5 hr after administration of desmopressin, aqueous humor cAMP was significantly reduced compared to baseline. Anterior chamber aqueous humor protein concentration was also significantly reduced 5 hr after desmopressin administration (Table 2).

Pretreatment with oral demeclocycline blocked the desmopressin-induced rise in IOP (Table 3). Oral demeclocycline alone in five control animals had no significant effect on IOP during a 6 hr interval following administration of the tetracycline. Pretreatment with intraperitoneal indomethacin partially blocked the desmopressin-induced increase in IOP (Table 3). It was already established that intraperitoneal indomethacin alone does not significantly alter IOP.10

Discussion

Like vasopressin,3 the synthetic antidiuretic hormone desmopressin elevates IOP in rabbits. Because
Table 3. Effect of systemic pretreatment with demeclocycline or indomethacin on the desmopressin-induced increase in intraocular pressure*

<table>
<thead>
<tr>
<th></th>
<th>Baseline IOP (mm Hg ± SEM)</th>
<th>Change in IOP (mm Hg ± SEM)</th>
<th>No. animals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 hr</td>
<td>3 hr</td>
</tr>
<tr>
<td>Desmopressin</td>
<td>14.8 ± 1.0</td>
<td>+1.4 ± 0.7</td>
<td>+2.4 ± 0.8</td>
</tr>
<tr>
<td>Demeclocycline pretreatment (20 mg/kg PO)</td>
<td>16.8 ± 1.2</td>
<td>+0.1 ± 0.2†</td>
<td>+0.3 ± 0.5†</td>
</tr>
<tr>
<td>Indomethacin pretreatment (10 mg/kg IP)</td>
<td>16.5 ± 0.3</td>
<td>+0.5 ± 0.4</td>
<td>+1.4 ± 0.8</td>
</tr>
</tbody>
</table>

* Animals were pretreated with oral demeclocycline hydrochloride 2 hr or intraperitoneal indomethacin 1 hr before 200 mUnits/kg of intravenous desmopressin.
† Significant difference compared to desmopressin alone, student t-test, \( P < 0.05 \).

of longer plasma half-life and possibly because of more selective antidiuretic activity, desmopressin’s effect on IOP is more prolonged than that of vasopressin. Systemic desmopressin (200 mUnits/Kg) does not significantly alter blood pressure or plasma osmolarity. The lack of a change in plasma osmolarity may relate to the desmopressin dose and to the state of animal hydration during the experiment. In addition, an increase in plasma osmolarity would lower and not increase IOP. The desmopressin-induced increase in IOP is independent of alterations in outflow facility and episcleral venous pressure. Aqueous humor production is increased as calculated from the tonographic data. Also, measured alterations in the Kinsey-Palm formula for ascorbate and the reduced anterior chamber fluorescein-labeled dextran concentrations are suggestive of an increased entry of water into the eye following the administration of desmopressin.

In the kidney, vasopressin stimulates adenylate cyclase and increases urinary cyclic AMP.11 We are unable to document a parallel elevation of aqueous humor cyclic AMP during the interval when IOP is elevated by desmopressin. The reduction in aqueous humor cyclic AMP levels at 5 hr are consistent with increased aqueous humor production. In the isolated iris-ciliary body preparation vasopressin similarly does not elevate cyclic AMP.12 While increased levels of intracellular cyclic AMP may not be reflected in the aqueous humor, these data suggest that adenylate cyclase does not mediate the IOP response to antidiuretic hormone in rabbits.

Demeclocycline, a tetracycline antibiotic, antagonizes the antidiuretic effect of vasopressin in the kidney; it inhibits both the vasopressin-induced rise in cAMP and the cAMP effects on urine flow.13,14 In the eye, demeclocycline also blocks the vasopressin-induced rise of IOP, despite the lack of evidence linking cAMP to vasopressin’s effect on the eye. We have no ready explanation to reconcile this difference between the two organs.

Antidiuretic hormone stimulates renal production of prostaglandins, PGE-1, PGE-2 and PGE-2α. Prostaglandin release acts as a negative feedback to decrease water absorption in the renal collecting tubule system.15,16 In the eye, prostaglandins have complex effects that may vary between species. In the rabbit, they generally elevate IOP with an associated increase in aqueous humor protein levels.10 Following intravenous desmopressin, aqueous humor protein levels actually fall, probably reflecting dilution of aqueous constituents by increased aqueous humor production. Pretreatment with indomethacin, a prostaglandin synthetase inhibitor, partially blocks the desmopressin-induced increase in IOP. Prostaglandin production would therefore appear to contribute to the IOP response following intravenous desmopressin and does not antagonize it as in the kidney.

The present study confirms prior work indicating an effect of vasopressin on aqueous secretion, but also indicates that the mechanism by which this hormone acts on the eye differs from that in the kidney. The interaction of vasopressin/desmopressin with intracellular messenger systems and prostaglandins in the ciliary epithelium remain to be more precisely defined.

Key words: desmopressin, antidiuretic hormone, intraocular pressure, aqueous humor cyclic AMP, rabbit

References