# Angiotensin, Vasopressin, and Atrial Natriuretic Peptide in the Rat Eye (43776)

DONALD E. PALM,[1] LANNY C. KEIL, AND WALTER B. SEVERS[2]

*Department of Pharmacology, College of Medicine, Pennsylvania State University, Hershey, Pennsylvania 17033 and (LCK) NASA, Ames Research Center, Moffett Field, California 94035*

*Abstract.* **Angiotensin (Ang), vasopressin (VP) and atrial natriuretic peptide (ANP) were assayed in the anterior uvea and retina from eyes of decapitated rats and rats perfused through the heart with phosphate buffered saline to remove peptides from blood and ocular fluids. All peptides were detected, and ANP was the most abundant. Uveal content of each peptide was greater than the retina. Perfusion did not affect ANP or VP content, but Ang was eliminated. Washout may explain the lack of immunohistochemical localization in the eye for Ang, but not VP. Also, washout does not account for available immunohistochemical data describing the localization of ANP in ocular tissue.** [P.S.E.B.M. 1994, Vol 206]

Angiotensin II (Ang II) (1, 2), vasopressin (VP) (3, 4), and atrial natriuretic peptide (ANP) (5, 6) may all affect intraocular pressure as well as ocular fluid dynamics. *A priori,* this could be anticipated because each peptide affects cardiovascular function and the movement of sodium and water. Evidence that links Ang, VP, and ANP with ocular tissue is based, variably, on research employing immunohistochemistry, autoradiography or membrane preparations to detect "receptors," and assay of freshly dissected tissue. However, information is incomplete. None of the peptides and their receptors has been fully localized by histological and autoradiographic analyses and quantified by regional assays.

ANP (5) and VP (3) have been measured in the eye by sensitive radioimmunoassays (RIA). Components of an endogenous renin-angiotensin system in the eye have also been described (7). Ang has been measured by RIA in vitreous humor and retina of freshly dissected kitten eyes (8). Specific "receptors" have been reported for all three peptides (3, 9, 10). Immunohistochemical studies to localize the effector peptides at the cellular level have been sparse. We are unaware of localization data for Ang or VP. ANP has been localized in the retina (11), but not in uveal regions, and a brain-specific natriuretic peptide has been found in uveal structures but not the retina (12).

The present study had two objectives. The first was to measure the content of each of the three peptides in the uvea and retina of fresh rat eyes by sensitive RIAs. Secondly, we wanted to evaluate whether peptides in fresh ocular tissue were labile. For this purpose we assayed eyes from rats that had been perfused *in situ* at physiological pressures by the transcardiac route with a large volume of phosphate-buffered saline. This perfusion would have been expected to remove peptides from blood and ocular fluids. Assessment of "washout" should identify how blood or ocular fluids affect peptide assays of fresh tissue and reveal the likelihood of peptide loss during tissue processing protocols for immunohistochemistry. Knowledge of peptide lability may help to resolve discordant findings in the literature. For example, RIAs of ANP revealed a higher content in the anterior eye compared with the retina (5), whereas immunohistochemical studies detected and localized ANP in the retina but not in the anterior eye (11). Peptide lability may explain this apparent discrepancy.

## Materials and Methods

Adult male Sprague-Dawley rats (300–500 g) were used after at least 2 hr of adaptation to the lighting

[1] Current address: Department of Neurosurgery, Brown University School of Medicine, Providence, RI 02911.
[2] To whom requests for reprints should be addressed at Department of Pharmacology, Hershey Medical Center—C7714B, Hershey, PA 17033.

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conditions of the laboratory. Some rats were sacrificed by decapitation and the eyes were immediately enucleated. Other rats were anesthetized (pentobarbital sodium, 50 mg/kg ip) and perfused (16 g ball needle, 80–85 Hz) by the transcardiac route with 100 ml phosphate buffered, 0.85% saline (PBS, 50 m*M*, pH 7.4). The abdominal aorta was clamped and the right atrium was lanced during the perfusion. The efficacy of perfusion could be directly verified by the loss of color (blood) from the nonpigmented rat eyes. These rats were decapitated after perfusion and the eyes enucleated. It should be noted that the time delay for tissue harvesting after decapitation was the same for both the conscious rats and those that were anesthetized and perfused.

The anterior uvea (ciliary tissue and iris) and retina were dissected with the aid of a stereomicroscope on Parafilm® kept on ice. Tissues were blotted, weighed, and placed in microcentrifuge tubes containing 0.75 ml of 0.1 *N* reagent grade hydrochloric acid with 150 μg phenylmethanesulfonyl fluoride (PMSF) and 50 μl Triton X per deciliter. Tissues from both eyes of two rats were pooled in each tube.

The tubes were placed in ice and the tissue was completely disrupted by ultrasound (Tekmar Instruments) and centrifuged at 10,000*g* for 30 min. An aliquot of 600 μl of supernatant was transferred to another microcentrifuge tube. Tubes were frozen in dry ice–acetone, lyophilized, and stored at −20°C until RIAs were performed. The small tissue mass precluded HPLC confirmation of peptide identity; therefore, the peptides measured in the RIAs are referred to with the notation, "IR", for "immunoreactive."

Lyophilized samples were shipped in dry ice to the NASA facility for assay. They were reconstituted in buffer and assayed at two dilutions according to published radioimmunoassays for angiotensin II (13) and vasopressin (14) using antibodies described therein. A commercial kit (Peninsula Labs Inc., Belmont, CA) was used for ANP assays. Synthetic ile$^5$-angiotensin II, rat 1–28 atrial natriuretic peptide and arg$^8$-vasopressin were used as reference standards. Briefly, displacement analysis at 50% binding with vasopressin antibody showed less than 0.01% cross-reactivity with vasotocin, oxytocin, ANPs or angiotensins. The angiotensin II antibody had less than 0.02% cross-reactivity with neurotensin, substance P, vasotocin, angiotensin I, saralasin, synthetic renin substrate, or purified dog renin. There was 70% cross-reactivity with angiotensin III. Intra-assay variability for angiotensin and vasopressin was less than 5%, and 10% for ANP. Interassay variability was less than 10% for angiotensin and vasopressin, and 20% for ANP.

Recoveries of peptides were estimated by adding synthetic peptides to tubes with retinal or anterior uveal tissue just before sonication and processing. The average recoveries for anterior uvea and retina were, respectively, 88% and 61% for VP; 110% and 115% for Ang; and 58% and 78% for ANP.

Data were expressed as pg of IR-peptide per mg wet weight, uncorrected for recovery, and compared by two-way analysis of variance. The post-hoc Newman-Keul test was used to evaluate significant ($P < 0.05$) $F$ ratios. Comparisons of two means was made by Student's $t$ or Mann-Whitney $U$ tests when appropriate.

## Results

The data for the three IR-peptides are shown in Table I. All were detectable in both the anterior uvea and retina obtained from eyes of decapitated rats. The anterior uvea contained more of each IR-peptide than the retina ($P < 0.05$). IR-ANP was the most abundant peptide. IR-Ang and IR-VP levels were clearly low, but detectable under the conditions described. Anesthesia and transcardiac perfusion with PBS did not alter ($P > 0.05$) the content of IR-ANP or IR-VP in either eye tissue. However, perfusion reduced IR-Ang to undetectable levels in every case in both anterior uvea and retina ($P < 0.05$, U test).

## Discussion

Once thought to be exclusively circulating hormones, Ang, VP, and ANP have been detected in other tissues where they may be endogenously produced (15–17). The eye is one such tissue.

The presence of renin, renin substrate, converting enzyme and high-affinity binding sites in ocular tissue suggests that the eye produces Ang (1, 2, 7, 10). However, to our knowledge, the peptide has not been assayed in ocular tissue when Ang from the blood and ocular fluids was excluded. Our data show the presence of a low amount of IR-Ang in the anterior eye and

**Table I.** Immunoreactive Atrial Natriuretic Peptide (ANP), Vasopressin (VP), and Angiotensin (Ang) in Anterior Uvea and Retina of Rat Eyes[a]

| | Anterior uvea | Retina |
|---|---|---|
| IR-ANP | | |
| Decapitated | 10.7 ± 1.4 | 3.47 ± 0.42 |
| Perfused | 10.2 ± 1.5 | 4.37 ± 0.71 |
| IR-VP | | |
| Decapitated | 2.7 ± 1.1 | 0.25 ± 0.03 |
| Perfused | 3.3 ± 0.9 | 0.22 ± 0.03 |
| IR-Ang | | |
| Decapitated | 2.4 ± 1.0 | 0.08 ± 0.05 |
| Perfused | N.D. | N.D. |

[a] Data are shown as the mean ± SEM in pg/mg wet weight. $n$ = 12/cell for IR-ANP and IR-VP, and 8 for IR-Ang. N.D. = not detectable. Values in the anterior uvea were always higher than corresponding retina values (ANOVA, $P < 0.01$ for IR-ANP and IR-VP, $P < 0.05$ for IR-Ang, $t$ test). There was no effect of perfusion ($P > 0.05$) for IR-ANP or IR-VP. IR-Ang was depleted by perfusion in the anterior uvea and retina ($P < 0.05$, Mann-Whitney $U$ test).

the retina. However, Ang was completely eliminated from the eye after anesthesia and perfusion with transcardiac PBS. An anesthetic effect appears unlikely, because barbituate anesthesia in rats would increase plasma angiotensin II concentration (18). Therefore, the depletion indicates that little, if any, Ang is stored in the eye. The source of this Ang may be blood or ocular fluid. Alternately, the low levels of Ang may be endogenously produced by the local renin-angiotensin system (7).

Another implication of the lability of IR-Ang in the eye is that many immunohistochemical protocols use perfusions before harvesting tissue. Often, PBS is perfused to remove blood, followed by a fixation perfusion. Such perfusions may explain the lack of reports on histological localization of Ang in eyes. We have been unable to localize Ang in rat eyes using an immunohistochemical protocol that localized ANP in the rat retina (11 and unpublished observation). That protocol does, however, easily localize Ang in kidneys from the same rats.

The present data on ANP content in the eye, including a content higher in uvea versus retina, independently confirm a study by Stone and Glembotski (5). In addition, our data indicate that ANP in uvea and retina is not labile during tissue processing protocols for immunohistochemistry. Limited data describe the immunohistochemical localization of ANP in the eye. In perfusion-fixed rat eyes, ANP was highly localized to the inner and outer plexiform (synaptic) layers of the retina (11). None was found in the anterior eye (11), although the antibody used in that study binds ANP in rat uveal tissue prepared for RIA as described herein. Thus, the inability to localize ANP in rat uvea, although it is clearly present, is not due to "washout" of the peptide. A spatial orientation or storage form of ANP inaccessible to antibody applied to tissue sections offers a potential explanation. In immersion-fixed porcine eyes, brain natriuretic peptide (BNP) has recently been immunohistochemically localized in nerve fibers of anterior ocular tissue, but not in the retina (12). Natriuretic peptides may be in ocular structures protected by the blood-ocular barrier (11, 12). Thus, it remains unexplained why an antibody raised against rat 1–28 ANP (a circulating form) detects the peptide in the retina (11), whereas an antibody specifically recognizing BNP does not stain neural retinal areas (12). It is possible that multiple natriuretic peptides exist in both anterior and retinal portions of the eye.

VP has been measured by RIA in acid extracts of freshly enucleated rabbit eye, with a higher content in uvea versus retina (3). The present experiments confirm these data for rat eyes. In addition, they show that VP is not washed out by perfusion protocols. To our knowledge, VP has not been immunochemically localized in ocular structures. As with our experience with Ang, the antibody for VP RIAs described herein failed to localize the peptide in the eye when tested with a standard immunohistochemical protocol (11 and unpublished observation) that easily localizes VP in the supraoptic nuclei. Although VP is clearly present in the eye, it is not labile. It may be stored after accumulation from blood or cerebrospinal fluid or, possibly, produced locally.

In summary, Ang, VP, and ANP are present in the rat eye. Uveal structures contain more of each peptide than the retina. Only Ang is labile after perfusion of the eye. This distinction could account for the lack of reports describing histochemical localization of Ang, but not of VP, which is not washed out of the eye. ANP is also not washed out of the eye by perfusion, but positive reports of histochemical localization of ANP and BNP in the eye require further resolution with available RIA data.

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