Aqueous Humor Messengers in the Transient Decrease of Intraocular Pressure After Ganglionectomy

John H. K. Liu

Intraocular pressure (IOP) decreases in rabbits 1 day after superior cervical ganglionectomy. It was hypothesized that this IOP decrease was caused by an accumulation of norepinephrine (NE) released from the iris–ciliary body into the aqueous humor during nerve degeneration. Direct measurement of aqueous humor NE concentration, however, was not successful because of the technical difficulty. In the current study, aqueous humor NE after superior cervical ganglionectomy was extracted and quantified using high-performance liquid chromatography–electrochemical detection. Twelve New Zealand albino rabbits were maintained in a daily 12-hr light–12-hr dark environment. Unilateral ganglionectomy was done on these rabbits during the light phase under halothane anesthesia. Twenty-two hours after the procedure, a significant IOP decrease occurred. The IOP was 16.1 ± 0.6 mmHg (mean ± the standard error of the mean) in the operated eye and 20.9 ± 0.6 mmHg in the contralateral eye (P < 0.01). Aqueous humor NE concentration in the operated eye (475 ± 81 pg/ml) was not different from that in the contralateral eye (469 ± 58 pg/ml). However, the concentration of aqueous humor cyclic adenosine monophosphate (cAMP) in the operated eye (29.8 ± 6.8 pmol/ml) was significantly higher (P < 0.05) than that in the contralateral eye (11.7 ± 0.8 pmol/ml). These data indicate that aqueous humor NE per se does not cause the transient IOP decrease after superior cervical ganglionectomy and cAMP-mediated ocular activities may be involved in this change in IOP. Invest Ophthalmol Vis Sci 33:3181–3185, 1992

The transient decrease of intraocular pressure (IOP) in rabbits 1 day after superior cervical ganglionectomy is well documented.1–6 It is termed the ganglionectomy effect.7 It was hypothesized 32 yr ago, that this effect was caused by a massive release of norepinephrine (NE) into the aqueous humor from the iris–ciliary body during the degeneration of the sympathetic nerves.4 This hypothesis was supported indirectly by several experiments.6–9 However, a direct identification of an increase in aqueous humor NE concentration was not successful because the techniques available then were not sensitive enough to detect this neurotransmitter in the aqueous humor.5

Recently, our ability to detect NE has improved significantly through the development of two techniques. The first technique is high-performance liquid chromatography (HPLC) in conjunction with an electrochemical detector. Using this technique, the NE concentration in rabbit aqueous humor was measured successfully in physiologic and several experimental conditions.10,11 The second technique is the radioenzymatic method, which was used to detect aqueous humor NE in humans12 and rabbits.13 Studies with rabbits using both techniques found a surprising result about the relationship between aqueous humor NE and IOP.10,11,13 A high NE concentration in the aqueous humor appeared concomitant with an IOP elevation. This result directly challenged the hypothesis4 that the transient IOP decrease after superior cervical ganglionectomy was caused by an elevation of NE concentration in the aqueous humor. To test the hypothesis, the concentration of aqueous humor NE in rabbits, during the transient IOP decrease, was measured using the HPLC–electrochemical detection method.

The concentration of cyclic adenosine monophosphate (cAMP) in aqueous humor might reflect some biologic activities in the anterior segment.14,15 Measurement of aqueous humor cAMP was included in our study as another indicator of the biochemical changes in ocular tissues during the ganglionectomy effect.

Materials and Methods

Male New Zealand albino rabbits were used in accordance with the ARVO Resolution on the Use of...
Animals in Research. Twelve rabbits (weight range, 2.9-4.0 kg) were purchased from a local supplier. They were housed in a daily 12-hr light–12-hr dark environment. The light period was from midnight to noon. After 3 weeks of maintenance in this light–dark cycle, an IOP elevation between 10 AM and 2 PM was verified. Measurement of IOP using a pneumotonometer was done as previously described.

The NE concentration in the rabbit's aqueous humor varied at different times of the day. It was shown in laboratory rabbits that aqueous humor NE and IOP change in a circadian pattern. The NE concentration and IOP during the light phase are lower than those during the dark phase. Because the values of aqueous humor NE and IOP 2 hr before the onset of dark were documented in our laboratory, superior cervical ganglionectomy and sampling of aqueous humor for NE determination were done near this time.

On the day of ganglionectomy, bilateral IOPs and pupil sizes were measured at 10 AM. Pupil size was measured using a transparent ruler in constant illumination. Between 10:45-11:15 AM, two rabbits were anesthetized with halothane using a Quantiflex VMC anesthesia machine (Matrix Medical, Orchard Park, NY). Halothane was chosen because it has a fast induction and recovery compared with commonly used anesthetic agents given by injection. Extirpation of the superior cervical ganglion was done between 11 AM and noon. The whole surgical procedure, under aseptic conditions, took less than 45 min per rabbit. Before our current study, it was established that halothane anesthesia at 10 AM for 1 hr would not change the circadian IOP pattern measured the next day. It was assumed that this halothane anesthesia would have a minimal effect on the circadian change of NE concentration in the aqueous humor. After removal of the anesthesia mask, the rabbit was fully awake in 20 min. Ten operations were done on 10 animals' right sides, and two were done on two animals' left sides in 6 days.

Postoperatively, the rabbits were allowed to recuperate in the same light–dark cycle. On the second day, pupil sizes from both eyes were measured at 21 hr (8-9 AM) and 22 hr (9-10 AM) postoperatively. The IOPs in both eyes were measured at 22 hr postoperatively. These time points were reported to be near the peak of IOP and pupillary responses. To avoid the interference of changes in aqueous humor dynamics, no IOP measurement was made before 22 hr postoperatively. The rabbits then were killed immediately, using an overdose of pentobarbital (Nembutal; Abbott, North Chicago, IL) given through the ear vein. Aqueous humor was collected between 9 AM and 10 AM by paracentesis, divided into one 150-μl and one 50-μl sample, and then frozen at −70°C.

The NE in the 150-μl aqueous humor sample was extracted and quantified by HPLC-electrochemical detector using the protocol described previously. The sensitivity of this assay for aqueous humor NE is approximately 100 pg/ml. The reagents used for the assay were obtained from Waters (Milford, MA) and ESA (Bedford, MA). Concentrations of cAMP in the 50-μl aqueous humor samples from all 24 eyes were determined in one batch using an assay kit purchased from Advanced Magnetics (Cambridge, MA).

The paired student t-test was used to analyze the circadian IOP elevation by comparing IOP values of the same eye at 10 AM and 2 PM. A paired student t-test also was used to compare IOP, pupil size, NE concentration, and cAMP concentration between the two eyes. A difference of \( P < 0.05 \) was regarded as statistically significant.

### Results

All data are summarized in Table 1. All 12 rabbits had an IOP elevation between 10 AM and 2 PM in

| Time                         | Measurement     | Operated eye | Contralateral eye | \( P \) value
|------------------------------|-----------------|--------------|-------------------|--------------
| Baseline at 10 AM            | IOP (mmHg)      | 19.9 ± 0.5\* | 20.0 ± 0.5        | >0.05        |
| Baseline at 2 PM             | IOP (mmHg)      | 26.9 ± 1.2   | 26.6 ± 1.2        | >0.05        |
| Preoperative at 10 AM        | IOP (mmHg)      | 19.5 ± 0.4   | 19.5 ± 0.3        | >0.05        |
| Postoperative 21 hr          | Pupil size (mm) | 6.7 ± 0.1    | 6.7 ± 0.2         | >0.05        |
| Postoperative 22 hr          | Pupil size (mm) | 6.8 ± 0.2    | 6.8 ± 0.1         | >0.05        |
|                             | IOP (mmHg)      | 16.1 ± 0.6   | 20.9 ± 0.6        | <0.01        |
|                             | NE (pg/ml)      | 475 ± 81     | 469 ± 58          | >0.05        |
|                             | cAMP (pmol/ml)  | 29.8 ± 6.8   | 11.7 ± 0.8        | <0.05        |

*Rabbits (n = 12) were housed in a 12 hr/12 hr light-dark cycle. The onset of dark was at noon. Extirpation of the ganglion was done between 11 AM and noon.

\* Mean ± standard error of the mean.

\*n = 10.

NE, norepinephrine. cAMP, cyclic adenosine monophosphate.

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Table 1. IOP, pupil size, and biochemical components in aqueous humor after unilateral superior cervical ganglionectomy*
both eyes. In the eye that underwent the ganglionectomy on its side (defined as the operated eye), the mean IOP elevation was 7.0 ± 0.9 mmHg (from 19.9 ± 0.5 to 26.9 ± 1.2 mmHg). In the contralateral eyes, the IOP elevation was 6.6 ± 0.9 mmHg (from 20.0 ± 0.5 to 26.6 ± 1.2 mmHg).

Every superior cervical ganglionectomy was successful (verified by vasodilation of the ear artery after the extirpation of the ganglion and by histologic examination of the ganglion). On postoperative day 2, the operated eyes showed hyperemia on the iris and conjunctiva. The pupillary sizes of the two eyes were not different at 21 hr and 22 hr after the operation and were not different from the preoperative values. Twenty-two hours after the ganglionectomy, the IOP was 16.1 ± 0.6 mmHg in the operated eye and 20.9 ± 0.6 mmHg in the contralateral eye, a significant decrease in IOP (P < 0.01). Aqueous humor NE concentration was 475 ± 81 pg/ml in the operated eye and 469 ± 58 pg/ml in the contralateral eye. No statistically significant difference was found (P > 0.05). However, the concentration of aqueous humor cAMP in the operated eye (29.8 ± 6.8 pmol/ml) was significantly higher (P < 0.05) than that in the contralateral eye (11.7 ± 0.8 pmol/ml).

## Discussion

Many physiologic factors are believed to be involved in the regulation of aqueous humor dynamics. Neural impulses, changes in cardiovascular parameters, and fluctuations of circulating and local hormones are among these factors. The significance of each factor in different species may vary. In rabbits, the neural influence on aqueous humor dynamics is significant.18 After an interruption of sympathetic neural inputs in rabbits, changes in aqueous humor dynamics occur. The interruption usually is done in two ways: superior cervical ganglionectomy and decentralization by sectioning the preganglionic nerve trunk.

These two surgical procedures, ganglionectomy and decentralization, cause profound effects on IOP. Weeks after unilateral ganglionectomy or decentralization, IOPs measured during the light phase in the rabbits' two eyes are usually not different.3 When IOP measurement is taken during the dark phase weeks after the operation, both procedures eliminate the circadian IOP elevation.10,11,20 Decentralization does not cause any acute effect on IOP during the light phase.3 However, a dramatic IOP decrease occurs 20 hr after the ganglionectomy, and it may last for 20 hr (with a peak appearing at 24 hr postoperatively).10,21

Extensive studies were done on the mechanisms of this ganglionectomy effect in the years of 1955–1970.22 Most studies showed an increase of outflow facility during the transient IOP decrease,4,6,21,23 although earlier studies did not detect such a change.1,2 The formation of aqueous humor22 and the episcleral venous pressure4 were reported to be similar between the two eyes. The iris–ciliary body seems to be essential for the ganglionectomy effect because iridectomy eliminates the effect.8 The key messenger (which must travel through aqueous humor and work on the outflow apparatus for the ganglionectomy effect to occur) was unclear. It was hypothesized in 1960 that the event was caused by an accumulation of NE in aqueous humor during nerve degeneration. The concentration of NE in aqueous humor was not measurable at that time, and therefore, supporting evidence came from experiments using anatomic and pharmacologic approaches6–8 and from experiments tracing the labeled circulating NE into the aqueous humor.19 Each experimental design was based on various assumptions, and the conclusion required complicated reasoning.

The role of aqueous humor NE in the ganglionectomy effect was questioned once in the literature by two studies that attempted to analyze the NE concentration in aqueous humor using a HPLC–electrochemical detector system.24,25 After injecting untreated aqueous humor into the detecting system, an unusually high concentration of NE (approximately, 7.5 ng/ml) was found in the baseline aqueous humor. Using the standard extraction procedure with alumina and an internal standard, the baseline concentration of aqueous humor NE (sampled 2 hr before the onset of dark) was only 489 ± 65 pg/ml in our laboratory.10 The latter value is reasonably close to the values obtained by the radioenzymatic method.13 Thus, the challenge raised by the two studies24,25 seemed to be overshadowed by the uncertainty of an NE signal in the detecting system caused by the absence of an isolation procedure.

Recently, the hypothetic mechanism in the ganglionectomy effect was challenged again by observations on the circadian rhythm of IOP in rabbits; an increase in aqueous humor NE concentration occurred in parallel with the circadian IOP elevation in the dark.10,13 In addition, after unilateral transsection of the cervical sympathetic trunk, the circadian IOP elevation in the decentralized eye was eliminated, and the concentration of aqueous humor NE was reduced.10

Our first consideration in the current study was the baseline NE concentration in the aqueous humor. Because there was a circadian elevation of IOP in every rabbit used, it was assumed that a similar circadian pattern of NE concentration in aqueous humor existed. We measured NE during the light phase, which
would have a low NE concentration. If the hypothetic role of NE in the ganglionectomy effect were correct, we would expect a significant increase in postoperative aqueous humor NE over the baseline value.

We found that the concentration of aqueous humor NE in the operated eye was close to the concentration in the contralateral eye 22 hr after the procedure. Both values were similar to the baseline NE concentration. It is known that the NE level in the iris-ciliary body diminishes to a very low level near this time. There are two possible explanations for the substantial amount of NE in the aqueous humor of the operated eye. First, the NE may come from a postoperative exclusion of NE from the degenerating nerves, as proposed in the hypothesis. Second, the NE might come from the ocular circulation. In a normal physiologic condition, the circadian change in aqueous humor NE concentration is not related to the NE concentration in the circulation. However, the situation may be different after ganglionectomy. Postoperative vasodilation may facilitate the movement of vascular NE into the aqueous humor, and the resorption of NE into the degenerating nerve terminals has diminished. By combining these two effects, more vascular NE may remain in the aqueous humor. This possibility was supported by the observation that there was a higher concentration of labeled NE (administered systemically) in the aqueous humor of the operated eye than in the contralateral eye.

The NE in the aqueous humor in the operated eye, no matter what its origin, is not able to cause the transient decrease of IOP by itself. To have such an effect, supersensitivity to the baseline aqueous humor NE has to be developed at this time in tissues involved in the ganglionectomy effect, presumably along the outflow pathway. One previous report indicated that a supersensitivity of pupillary reaction to external epinephrine develops near this time. However, excess availability of the external epinephrine in the latter case (caused by lack of resorption) may be solely responsible for the supersensitivity.

There is anatomic evidence of sympathetic innervation along the aqueous outflow pathway in rabbits. Can the transient IOP decrease after ganglionectomy be related to the degenerative release of NE from tissues along the outflow of aqueous humor? The small amount of NE released would be sufficient to activate adrenergic receptors, but not sufficient to elevate the NE concentration in aqueous humor over the baseline value. This modification of the previous hypothesis is less incompatible with our data. However, this modification does not reconcile the role of the iris-ciliary body in the ganglionectomy effect.

An increase of aqueous humor cAMP concentration (22 hr after the ganglionectomy) indicated that the cAMP-mediated activities in the anterior segment were high during the transient IOP decrease. There are many studies showing that an increase in cAMP-mediated ocular activities in rabbits may cause an IOP decrease. Intracameral injection of cAMP, the messenger itself, can cause a reduction in IOP in rabbits. In this regard, aqueous humor cAMP may act either directly on the outflow channel or as an intracellular messenger in triggering cellular responses in the tissues of the outflow channel. Therefore, our study suggests the involvement of cAMP in the ganglionectomy effect, either as an intracellular messenger in relevant ocular tissues or as a messenger in the aqueous humor that affects the outflow of aqueous humor.

In our study, a pupillary effect after the superior cervical ganglionectomy was not observed at 21 hr or 22 hr postoperatively. Mydriasis in conjunction with the ganglionectomy effect has been reported inconsistently in the literature. One laboratory found significant mydriasis for 10–18 hr. Another reported a duration of only 1–2 hr. Others noticed little change in pupil size. It is likely that the pupillary response depends on the light condition and the type of anesthesia used in each study. Because the transient IOP decrease after the ganglionectomy can be separated from the pupillary effect in some cases, the mechanisms causing the ganglionectomy effect is likely to be different from the mechanisms causing the postoperative mydriasis.

Key words: aqueous humor, cyclic AMP, ganglionectomy, intraocular pressure, norepinephrine

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