Hydroxyamphetamine Increases Intraocular Pressure in Rabbits

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Objective: To determine the effect of norepinephrine (NE) released from endogenous ocular stores on intraocular pressure (IOP) and aqueous flow in rabbits.

Methods: The IOP was measured with a pneumotonometer, the aqueous flow with a scanning fluorophotometer, and the aqueous NE by methylation with catechol-O-methyltransferase in the presence of S-adenosyl-L-[methyl-3H]methionine.

Results: Hydroxyamphetamine increased IOP in a dose-dependent fashion. Surgical removal of the superior cervical sympathetic ganglion eliminated the increase in IOP and pupil diameter; preganglionic section of the cervical sympathetic trunk did not. Hydroxyamphetamine increased the concentration of NE in the aqueous. Increased IOP was not accompanied by increased aqueous flow and was eliminated by blockade of α₁-adrenergic receptors but not β- or α₂-adrenergic receptors.

Conclusions: Increased IOP after hydroxyamphetamine application is consistent with earlier suggestions that the nocturnal circadian increase in IOP in rabbits is mediated in part by NE released from ocular sympathetic nerves. However, failure of hydroxyamphetamine to increase aqueous flow and of β-adrenergic blockade to blunt the increase in IOP does not support our suggestion that the nocturnal increase in IOP results in part from NE stimulation of ciliary process β-adrenergic receptors and increased aqueous flow.

Clinical Relevance: In addition to increasing pupil diameter, hydroxyamphetamine increases IOP.


Rabbits have circadian rhythms of intraocular pressure (IOP) and aqueous flow; both are increased at night.1-6 There is also a nocturnal increase in the aqueous norepinephrine (NE) concentration, which is probably also circadian.7,8 This suggests that there is a circadian increase in sympathetic input to the rabbit eye during the night and that increased sympathetic input may be responsible for the nocturnal increases in IOP and aqueous flow. This idea is supported by the observations that the nocturnal increases in IOP and aqueous flow are partially blunted by superior cervical ganglionectomy (CGX) or preganglionic section of the cervical sympathetic trunk (decentralization [DX])6,9-11 and that the nocturnal increase in aqueous NE is abolished by CGX or DX.7,8 Furthermore, Gallar and Liu12 have shown that low frequency preganglionic stimulation of the cervical sympathetic trunk increased IOP in rabbits, and Liu et al13 showed that intravenous injection of low doses of NE (10 and 100 ng) increased IOP. Because of the difficulties associated with interpreting IOP changes after systemic delivery of drugs, we decided to attempt to circumvent this problem by studying the effects on IOP and aqueous flow of hydroxyamphetamine, a drug known to cause reversible release of NE from sympathetic nerve endings in the anterior segment.14 We reasoned that NE released from sympathetic nerve endings by hydroxyamphetamine is more likely to act at physiologically relevant targets than is NE applied topically or delivered systematically. It is known that hydroxyamphetamine increases pupil diameter in rabbits15 and humans,16 and it is an important clinical tool for diagnosis of Horner syndrome.17,18 Increased IOP after topical instillation of hydroxyamphetamine has been previously described19 in patients with open angle glaucoma but is thought not to compromise the clinical utility of this drug for diagnosis of Horner syndrome. We report herein that hydroxyamphetamine also increases IOP in rabbits.
SUBJECTS AND METHODS

Rabbits for all studies were used in accordance with the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research. Male New Zealand White rabbits weighing 2 to 21⁄2 kg were housed in rooms with lighting schedules of alternating 12-hour periods of light and dark (12L:12D) for at least 2 weeks before being used.1,9 Lights on is defined as 00:00 circadian time (CT), and, therefore, lights off is at 12:00 CT. Unilateral CGX or DX was done and evaluated as previously described10,11; after surgery, rabbits were housed in 12L:12D for at least 2 weeks before being used. The IOP was measured using either a Digilab 30D or Micro One tonometer (Bio-Rad Ophthalmic Division, Cambridge, Mass) or a 30 Classic pneumotonometer (Mentor O & O, Inc, Norwell, Mass), previously calibrated in rabbits. Drugs were dissolved in Hanks balanced salt solution and delivered by unilateral topical instillation; the same volume of balanced salt solution was instilled in contralateral eyes. Hydroxyamphetamine was also delivered by intravitreal injection; contralateral eyes were injected with the same volume of balanced salt solution. A drop of 0.5% proparacaine hydrochloride (Alcaine; Alcon, Inc, Humacao, Puerto Rico) diluted to 0.05% with balanced salt solution. A drop of 0.5% proparacaine hydrochloride (Alcaine; Alcon, Inc, Humacao, Puerto Rico) diluted to 0.05% with balanced salt solution was applied to each eye immediately before applanation. The IOP was measured during darkness by the light of a dim red bulb (Delta 1, Dallas, Tex).1,9,20 Pupil diameter was estimated by comparing rabbit pupils with semicircles of known diameter on a clinical examination card. Drugs were applied topically to the cornea in a volume of 50 µL. Intravitreal injection was done as previously described31. 0.5% proparacaine hydrochloride was applied topically, and then 10 µL of 1% hydroxyamphetamine was injected through the sclera and into the vitreous with a 50-µL syringe (Hamilton, Reno, Nev) fitted with a 30-gauge needle. Aqueous flow was measured by the corneal depot method using a scanning fluorophotometer (Fluorotron Master; Coherent Medical Division, Palo Alto, Calif) as previously described.22 Fifty microliters of 0.3% hydroxyamphetamine was applied at 02:00 and 04:00 CT; corneal and anterior chamber fluorescence was measured every hour for 3 hours beginning at 02:30 CT. Aqueous NE was measured as previously described using catecholamine assay kits (Amersham Intl, Buckingham, England). Catecholamines in aqueous samples were converted to [3H]-O-methylated derivatives by treating aliquots of aqueous with catechol-O-methyltransferase and S-adenosyl-L-[methyl-3H]methionine; the [3H]-O-methylated derivatives were separated by thin-layer chromatography on silica-gel plates (Analtech Inc, Newark, Del); and spots corresponding to NE were scraped off the plates and counted in a liquid scintillation counter (RACKBETA, LKB; Wallac, Gaithersburg, Md).

Data are given as mean±SE.

RESULTS

Topically instilled hydroxyamphetamine increased IOP and pupil diameter in rabbits; however, the dose dependence of the 2 effects of hydroxyamphetamine differed. Pupil diameter was increased by 1% hydroxyamphetamine only, whereas IOP was increased by lower doses as well (Figure 1). Superior cervical ganglionectomy eliminated increased IOP and pupil diameter after hydroxyamphetamine instillation (Figure 2), but DX did not (Figure 3). Pupil diameter at 01:00 CT (before hydroxyamphetamine treatment) was 5.1±0.3 and 6.2±0.2 mm in CGX and contralateral eyes and 5.1±0.2 and 5.7±0.2 mm in DX and contralateral eyes, respectively. Decentralization slightly increased the response of pupil diameter to hydroxyamphetamine; the change in pupil dilation in DX eyes was significantly different from that in contralateral eyes at 02:30, 03:00, and 04:00 CT (P<.05, t test). Decentralization decreases NE release and thus prejunctional stores of NE may accumulate in these eyes, resulting in a greater response to hydroxyamphetamine. Intravitreal injection of hydroxyamphetamine also increased IOP, although the time course of increased IOP differed from that observed after topical instillation (Figure 4).

Aqueous NE measured in rabbits killed 30 minutes after unilateral instillation of 1% hydroxyamphetamine at 03:15 CT was 2.38±0.29 and 0.93±0.20 ng/mL of aqueous from treated and contralateral eyes, respectively (n=7;
P<.01, t test for paired data). As previously reported, the concentrations of aqueous epinephrine and dopamine were barely detectable (below the assay limits) and were not increased by hydroxyamphetamine.

The aqueous flow rate was measured after unilateral topical instillation of 0.3% hydroxyamphetamine at 02:00 and 04:00 CT; this protocol maintained elevated IOP for about 3 hours. Aqueous flow from 02:30 to 05:30 CT was 2.79±0.14 and 2.86±0.22 µL/min in treated and contralateral eyes, respectively (n=8).

Rabbits were treated with adrenergic antagonists 30 minutes before 0.1% hydroxyamphetamine instillation to determine which adrenergic receptor(s) mediates the effects of hydroxyamphetamine on IOP. Pretreatment with 1% timolol, a β-adrenergic antagonist, or 0.3% rauwolsine, an α2-adrenergic antagonist, did not block increased IOP after hydroxyamphetamine instillation; pretreatment with 0.3% bunazosin, an α1-adrenergic antagonist, completely eliminated the increase in IOP during the day (Figure 5). These experiments were also done at night. In rabbits, ocular sympathetic tone, IOP, and the aqueous flow rate are higher at night.1,7,8 In contrast, epinephrine secretion from the adrenal medulla and the aqueous flow rate decrease at night in humans, and timolol reduces the aqueous flow rate only during the day.23,24 Adrenergic antagonists were applied bilaterally at 13:30 CT and hydroxyamphetamine unilaterally at 14:00 CT. The same results were obtained with the antagonists at night, although hydroxyamphetamine increased IOP less at night, when IOP and sympathetic tone are higher. Neither timolol nor rauwolsine blocked increased IOP after hydroxyamphetamine treatment (data not shown), but bunazosin eliminated the increase. The difference between IOP at 30 minutes after unilateral 0.1% hydroxyamphetamine instillation in treated eyes and that in contralateral eyes in rabbits pretreated with bunazosin was −1.3±0.7 (n=6, P=.15), whereas in the same rabbits on a day when they were not pretreated with bunazosin the difference was 2.2±0.5 (n=6; P<.01, t test for paired data).

The IOP measured at 04:00 and 13:00 CT in contralateral eyes (before application of hydroxyamphetamine or adrenergic antagonists) was 18.2±0.1 and 24.1±0.9 mm Hg, respectively (n=5). The IOP in these rabbits is at its daily minimum at about 04:30 CT and its maximum at about 14:30.10 Hydroxyamphetamine did not change pupil diameter in these experiments, nor did timolol or rauwolsine. Bunazosin decreased pupil diameter at night but not during the day. Pupil diameter in contralateral eyes (treated with bunazosin but not with hydroxyamphetamine) relative to pupil diameter in the same eyes before bunazosin instillation was −1.3±0.2 mm at 14:30 CT, 1 hour after bunazosin instillation (P<.005, t test for paired data).
The increase in IOP after hydroxyamphetamine treatment is mediated by NE released from ocular sympathetic nerve endings. The effects of CGX and DX on the IOP response and our observation of increased aqueous NE after topical application of hydroxyamphetamine are consistent with this. Kronfeld et al\textsuperscript{19} confirmed by gonioscopy that hydroxyamphetamine did not increase IOP in their patients by acute angle closure. Angle closure is also unlikely in our study because pupil diameter was unaffected by hydroxyamphetamine, except at the highest dose: 1%.

Topical instillation of sympathomimetic agents is known to produce an initial increase in IOP; the \( \alpha_2 \)-adrenergic agonist brimonidine has been shown to produce an initial increase in IOP, followed by decreased IOP.\textsuperscript{25,26} The initial increase was eliminated by surgical section of the rectus muscles or by intracameral infusion of the drug, prompting us to surmise that increased IOP is unrelated to interaction of brimonidine with intraocular receptor sites or to changes in aqueous dynamics.\textsuperscript{25,27} Intravitreal injection of hydroxyamphetamine increased IOP; therefore, its effect on IOP is likely to result from its action at local intraocular sites and reflects changes of 1 or more parameters of aqueous dynamics.

Sympathetic tone increases at night in rabbits,\textsuperscript{7,8} and the nocturnal increases in IOP and the aqueous flow rate require sympathetic input.\textsuperscript{6,7,9-11} Therefore, we anticipated that hydroxyamphetamine-mediated NE release would increase IOP and aqueous flow. Because timolol decreased IOP and aqueous flow in rabbits at night, but not during the day, and did not decrease IOP at night in sympathectomized rabbits, researchers have argued\textsuperscript{21,28} that part of the nocturnal increase in IOP in this species results from NE stimulation of ciliary process \( \beta \)-adrenergic receptors and increased aqueous flow. The failure of timolol to block increased IOP after hydroxyamphetamine treatment and our observation of unchanged aqueous flow after hydroxyamphetamine application do not support this idea. Because stimulation of \( \alpha_2 \)-adrenergic receptors decreases IOP by reducing aqueous flow,\textsuperscript{29-32} failure of rauwolscine to block increased IOP after hydroxyamphetamine treatment is not surprising.

Noradrenaline increases outflow facility in rabbits,\textsuperscript{33-35} and therefore hydroxyamphetamine-induced NE release would be expected to increase outflow facility and decrease IOP.

How can we explain the increase in IOP after hydroxyamphetamine treatment? Bunazosin reduced IOP in rabbits during the day and night, and sympathectomy eliminated its effect on IOP.\textsuperscript{22,36} Decreased IOP after bunazosin instillation was not accompanied by de-
creased aqueous flow.\textsuperscript{22,37} Bunazosin blocked the initial increase in IOP after topical application of NE in sympathectomized rabbits,\textsuperscript{38} and we show here that bunazosin eliminated increased IOP after hydroxyamphetamine application. Prazosin hydrochloride, another \(\alpha_1\)-adrenergic antagonist, blocked the circadian increase in IOP observed from 10:00 to 14:00 CT, but had no effect on the increase in aqueous flow during the same time.\textsuperscript{38} More recently, Zhan et al\textsuperscript{39} have shown that decreased IOP after topical application of bunazosin in rabbits resulted predominantly from increased uveoscleral outflow. These observations suggest that hydroxyamphetamine-induced NE release increased IOP by decreasing uveoscleral outflow. However, the uveoscleral outflow pathway plays only a minor role in aqueous flow in the rabbit—less than 10\% of the total outflow.\textsuperscript{40} This makes it unlikely that a significant increase in IOP could result from decreased uveoscleral outflow in this species. In summary, hydroxyamphetamine increased IOP in rabbits by stimulating \(\alpha_1\)-adrenergic receptors; the mechanism for increased IOP remains to be identified.

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