Timolol Reduces IOP in Normal NZW Rabbits During the Dark Only

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The role of beta-adrenergic receptors in mediating the circadian rhythm of intraocular pressure was examined by determining the effect on intraocular pressure of timolol applied topically at different times during the circadian cycle to New Zealand White rabbits entrained to alternating 12 hr periods of light and dark. Timolol lowered intraocular pressure when applied during the dark but not during the light phase of the circadian cycle. Timolol did not lower intraocular pressure when applied during the dark to animals after cervical ganglionectomy. These results are consistent with the idea that beta-adrenergic receptors participate in mediating the circadian rhythm of intraocular pressure, and suggest that endogenous agonists released by adrenergic nerves in the eye during the dark stimulate ocular beta-adrenergic receptors to increase intraocular pressure in rabbits. Invest Ophthalmol Vis Sci 31:715-721, 1990

There is a circadian rhythm of intraocular pressure (IOP) in rabbits; IOP is low during the light phase and high during the dark phase. A circadian rhythm of aqueous flow in phase with the rhythm of IOP makes a major contribution to the rhythm of IOP. Adrenergic input to the eye is required for complete expression of the rhythm of IOP; cervical ganglionectomy (CGX) or decentralization (DX) decrease the dark phase increase of IOP. The experiments reported here were designed to determine whether beta-adrenergic receptors participate in mediating the rhythm of IOP. To do this the effect on IOP of topical application of timolol, a nonselective beta-adrenergic antagonist which decreases aqueous flow, was examined at different times during the circadian cycle in New Zealand White (NZW) rabbits.

Materials and Methods

Male NZW rabbits were used in all experiments. The animals weighed 3 lbs when purchased, and were entrained to a lighting schedule of alternating 12 hr periods of light and dark (12L:12D) for at least 3 weeks prior to use as previously described. Lights ON is defined as 00:00 circadian time (CT).

All studies with timolol were performed using a crossover protocol. One half of each group was treated with a topical application of 50 μl timolol in water to both eyes; 50 μl water was applied to both eyes of the other half of the group. No less than 5 days later animals previously treated with water were treated with timolol and those previously treated with timolol were treated with water. At least 7 days of "washout" were allowed between experiments.

IOP was recorded before (at different times, see figures) and 1/2, 1, 2, 4 and 6 hr after application of timolol or water.

Group 1 (n = 11) was treated with 0.001, 0.01, 0.1, and 1.0% timolol (0.5, 5.0, 50 and 500 μg, free base) at 12:00 CT then with 1.0% timolol at 04:00 and 09:00, and with 0.01% timolol at 15:00 CT.

Group 2 (n = 11) was treated with 0.001, 0.01, 0.1 and 1.0% timolol at 15:00 CT.

Bilateral CGX was performed as described previously. CGX was confirmed by the method of Thompson and Mensher using hydroxyamphetamine (Paredrine, 1%, Smith, Kline & French Laboratories, Philadelphia, PA).

IOP was recorded using a Digilab 30D or Micro One pneumotonometer (Bio-Rad Ophthalmic Division, Cambridge, MA) calibrated in rabbits by the closed stopcock technique. A drop of proparacaine HCl (0.5%) diluted 1 to 10 into balanced salt solution was applied topically before each measurement of IOP. IOP was recorded during the dark by the light of a Bright Lab Jr. red light. IOP was recorded for every animal at 3 hr intervals over at least one 24 hr circadian cycle to confirm that each animal had a normal (or, in the case of CGX animals, a dampened) circadian rhythm of IOP.

All studies with timolol were performed using a crossover protocol. One half of each group was treated with a topical application of 50 μl timolol in water to both eyes; 50 μl water was applied to both eyes of the other half of the group. No less than 5 days later animals previously treated with water were treated with timolol and those previously treated with timolol were treated with water. At least 7 days of "washout" were allowed between experiments.

IOP was recorded before (at different times, see figures) and 1/2, 1, 2, 4 and 6 hr after application of timolol or water.

Group 1 (n = 11) was treated with 0.001, 0.01, 0.1 and 1.0% timolol (0.5, 5.0, 50 and 500 μg, free base) at 12:00 CT then with 1.0% timolol at 04:00 and 09:00, and with 0.01% timolol at 15:00 CT.

Group 2 (n = 11) was treated with 0.001, 0.01, 0.1 and 1.0% timolol at 15:00 CT.

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Group 3 (n = 10): IOP was recorded in these animals over three consecutive 24 hr periods every 3 hr beginning 1.5 hr after lights ON (01:30 CT) prior to and at least 18 days after bilateral CGX. Data from the three pre- and three post-CGX 24 hr cycles were meaned for each animal to produce pre- and post-CGX summary IOP cycles for each animal. The data from all animals were meaned to produce pre- and post-CGX summary IOP cycles for the whole population of CGX animals.

Group 4A (at least 15 weeks after bilateral CGX) was treated with 1.0% timolol at 12:00 (n = 9), 04:00 (n = 9) and 15:00 CT (n = 8).

Group 4B (7 animals from group 4A plus four additional animals at least 20 days after bilateral CGX, n = 11) was treated with 1.0% timolol at 09:00.

Statistical significance was determined using the student t-test for paired samples. All data are presented in Figures as the mean ± SEM where n is the number of animals.

Timolol maleate was generously provided by Merck, Sharp & Dohme Research Laboratories (West Point, PA).

All experimental procedures adhered to the ARVO Resolution on the Use of Animals in Research.

Results

A dose-response relationship for the effect of bilateral topical instillation of timolol on IOP was established at 12:00 and 15:00 CT (Figs. 1, 2). Timolol showed a small, but statistically significant, reduction of IOP at 0.1 and 1.0% when applied at 12:00 CT. Timolol reduced IOP at lower doses when applied at 15:00 than when applied at 12:00 CT; 0.001 and 0.01% timolol, in addition to 0.1 and 1.0%, reduced IOP when applied at 15:00 CT. The reduction of IOP observed after application of 0.01% timolol at 15:00 CT was confirmed by repeating the experiment with another group of animals (data not shown). On the other hand, topical installation of 0.1% (data not shown) and 1.0% timolol at 04:00 and 09:00 CT did not lower IOP during the light phase (Fig. 3); the decrease in IOP 4 hr after 1.0% timolol treatment at 09:00 (Fig. 3B) comes during the dark phase.

Data presented in Figure 4 show that bilateral CGX, like unilateral CGX,3 results in a decrease in IOP during the dark phase, but has little effect during the light. Although timolol reduced IOP when applied to normal animals at 12:00 and 15:00 CT, 1.0% timolol was without effect on IOP when applied at the same times to rabbits after bilateral CGX (Fig. 5C, D). As was the case in normal animals, timolol had no effect on IOP when applied to CGX animals at 04:00 or 09:00 CT (Fig. 5A, B). The statistically significant reductions in IOP observed in these animals 2.0, 1.0 and 1.0 hr after instillation of timolol at 09:00, 12:00 and 15:00 CT, respectively, are probably too small to be meaningful.

Discussion

Timolol is the drug most often used in the United States to reduce IOP in glaucoma patients.21 The marked, prolonged decrease in IOP produced by topical application of this drug results from reduced aqueous flow.11,12 Timolol has also been shown to reduce IOP and flow in monkeys13-15 and in cats,16-19 but in rabbits its effect on IOP is less clear. Radius et al22 found that topical application of 0.03 to 0.5% timolol produced a decrease in IOP in normal albino rabbits. Nathanson23 and Sugrue et al24 showed that 1% timolol decreased IOP in NZW and in albino rabbits, respectively. Vareilles et al25 observed small decreases in IOP after topical application of 0.01-1.0% timolol in normal albino rabbits, but the effect was not consistent. On the other hand, Bartels et al26 showed no effect of 0.5 or 4.0% topical timolol on IOP in urethane anesthetized albino rabbits. Boas et al27 showed that 0.5% timolol had no effect on IOP in female albino rabbits. Woodward et al28,29 showed no effect of timolol at concentrations up to 1.0% in normal cross-bred NZW-Dutch belted rabbits. However, it has been possible to demonstrate that timolol is capable of reducing IOP in rabbits with artificially elevated IOP. Vareilles et al30 showed that 0.5 and 1.5% topical timolol reduced IOP in chymotrypsintreated rabbit eyes and in water-loaded rabbits. Bonomi et al30 showed that 0.25 to 1% timolol reduced IOP in NZW rabbits with elevated IOP induced by betamethasone treatment or by infusion with 5% glucose. Vareilles et al showed that 0.01 to 1.0% timolol reduced IOP in buphthalmic rabbits.31 Liu et al32 showed that topical timolol is more effective at reducing IOP in water loaded pigmented than in water loaded NZW rabbits, and Sugrue et al34 showed that topical application of 0.01 to 0.1% timolol reduced IOP in chymotrypsin-treated albino rabbit eyes. The results reported here show that timolol reduces IOP during the dark in rabbits entrained to 12L:12D but does not reduce IOP during the light in these rabbits. Whether these results can explain the apparently contradictory results reported by other authors is not clear.

It has been suggested that adrenergic stimulation increases the rate of aqueous formation and that timolol reduces flow by blocking stimulation of adrenergic receptors in the ciliary processes.33,34 Therefore, one interpretation of the data presented here is that neurotransmitters released during the dark by the...
Figs. 1, 2. Effect on IOP of 0.001% (A), 0.01% (B), 0.1% (C) and 1.0% (D) timolol applied (arrow) at 12:00 (Fig. 1) or 15:00 CT (Fig. 2). All experiments used a cross-over protocol. Half of the animals were treated with timolol and the other half with water; no less than 5 days later animals previously treated with timolol were treated with water and those previously treated with water were treated with timolol. Data are expressed as the mean ± SEM, n = 11; filled symbols indicate a statistically significant difference in IOP when animals were treated with timolol (O) or with water (A), P < 0.05. Solid lines connect data points from control eyes; cross-hatches along the abscissa indicate darkness.
This interpretation is also supported by the observation that timolol does not reduce IOP in rabbits after CGX (Fig. 5). Although Wentworth and Brubaker showed that timolol reduces aqueous flow in both eyes of patients with unilateral Horner’s Syndrome, CGX markedly reduced the ability of timolol to reduce IOP in cats and in water-loaded pigmented rabbits. Rowland et al have shown that the concentration of aqueous humor cyclic AMP increases in rabbits during the dark phase of the circadian cycle. Although it is not clear that this increase represents increased synthesis of cyclic AMP by the ciliary processes, it is consistent with the idea that beta-adrenergic receptors and adenylate cyclase in rabbit ciliary processes are stimulated during the dark. That timolol does not reduce IOP during the light (Fig. 3) suggests that blockade of beta-adrenergic receptors has no effect on the rate of aqueous formation during the light, and therefore, that endogenous adrenergic agonists may be released during the dark only. However, the small magnitude (2–3 mm Hg) of the reduction of IOP produced by timolol in the dark relative to the range of the circadian rhythm of IOP (10–11 mm Hg in the animals used in these experiments) suggests that mechanisms other than those mediated by beta-adrenergic receptors play important roles in mediating the dark phase increase of IOP and that beta-adrenergic receptors play a relatively minor role. Rowland et al have reached a similar conclusion based on an unpublished study with timolol in entrained rabbits. Furthermore, Bausher et al showed that inhibitory alpha2-adrenergic receptors of rabbit ciliary processes bind catecholamines better than the beta-adrenergic re-

adrenergic nerves in ciliary processes increase aqueous humor formation, and therefore IOP, in rabbits. Timolol has been shown to lower IOP in humans, monkeys and cats by reducing the rate of aqueous formation. Although this has not yet been demonstrated in rabbits, a reasonable expectation is that timolol reduces IOP in rabbits by the same mechanism. Therefore, the observation that timolol reduces IOP during the dark (Figs. 1, 2) suggests that timolol interacts with ocular beta-adrenergic receptors in the ciliary processes, blocks stimulation of these receptors (and therefore adenylate cyclase) by endogenous agonists released by adrenergic nerves during the dark, and reduces aqueous formation, and therefore IOP. The suggestion that timolol blocks stimulation of beta-adrenergic receptors is supported by the observation that timolol applied at 15:00 CT (Fig. 2) is effective at lowering IOP at concentrations as low as those shown by Woodward et al to block the reduction of IOP produced by 0.01% isoproterenol. 

Fig. 3. Effect on IOP of 1.0% timolol applied at 04:00 (A) and 09:00 CT (B). Data are expressed as the mean ± SEM, n = 10; filled symbols indicate a statistically significant difference in IOP when animals were treated with timolol (○) or water (△), P < 0.05. Solid lines connect data points from control eyes.

Fig. 4. Effect of bilateral CGX on the circadian rhythm of IOP. Data are expressed as the mean IOP ± SEM, n = 10 before (○) and after (●) CGX; * indicates when IOP after CGX is significantly different (P < 0.025) from IOP before CGX. The horizontal line represents the mean daily IOP (mean of IOP at all eight times) in all animals prior to CGX (23.9 ± 0.8).
ceptors of the same tissue and argued therefore that α2-adrenergic receptor mediated inhibition of β-adrenergic receptor mediated stimulation of adenylate cyclase may limit the importance of β-adrenergic receptors in regulating aqueous flow. Although the suggestion that catecholamines increase the rate of aqueous formation by interacting with β-adrenergic receptors in the ciliary processes is supported by data in humans,34,38-42 this appears at odds with the idea advanced by Sears that increased cyclic AMP in the ciliary processes leads to decreased aqueous formation43-46 and data which support this idea.47-53

Another equally plausible explanation of the data presented here is that timolol appears more effective at reducing IOP in the dark than in the light simply because a small fractional decrease of elevated dark phase IOP produces a statistically significant absolute decrease of IOP; whereas, the same fractional decrease of the lower IOP obtained in the light phase or in animals after CGX does not. If this interpretation is correct, then all agents capable of reducing IOP in rabbits would be expected to be more effective during the dark than during the light phase of the circadian cycle. Although the interpretation of the data presented here is not yet clear, it is clear that IOP in normal NZW rabbits is reduced by timolol during the dark but not during the light.

Humans have diurnal rhythms of IOP (for reviews of early studies see refs. 54-56) and aqueous flow34,41,42,54,57-62 which are approximately 180° out of phase with these rhythms in rabbits. The temporal change in sensitivity to timolol reported here is analogous to that observed in humans by Topper and Brubaker,34 who showed that aqueous flow was reduced by timolol during the day (when flow is high) but not during the night (when flow is low). They also showed that epinephrine increased flow more at night than during the day. These results led to their suggesting that the rate of aqueous formation is increased by circulating epinephrine and that timolol reduces flow.
by blocking β-adrenergic receptors. Similar results have been obtained with the combination of isoproterenol and theophylline in humans with Horner’s Syndrome and with terbutaline in normal humans. The results presented here suggest the possibility that aqueous humor dynamics in rabbits during the dark, rather than during the light, may be more similar to aqueous humor dynamics in humans during the day. Therefore, normal rabbits may be a better model for studying aqueous humor dynamics than has been generally appreciated if they are entrained to 12L:12D and used during the dark.

Key words: timolol, intraocular pressure, circadian rhythm, beta-adrenergic receptor, rabbit

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