
Characteristics and pharmacologic utility of an intraocular pressure (IOP) model in unanesthetized rabbits. R. J. SeidehameL and K. W. DunGAN.

A laboratory model was designed to assess the effects of drugs on intraocular pressure (IOP) in rabbits. Its utility for evaluating the antiglaucoma potential of drugs was tested by employing l-epinephrine, an adrenergic agent known to be effective in glaucoma. When l-epinephrine was applied topically to the eye, it caused a concentration-dependent reduction of normal IOP and marked antagonism of elevated IOP induced by water load. These effects were of long duration. Antagonism of IOP elevation was earlier in onset than reduction of normal IOP, suggesting different underlying mechanisms. Similarities between these results and those reported for glaucomatous man establish the utility of this IOP model in the evaluation of the antiglaucoma potential of adrenergic-like compounds.

The search for improved medical therapy in glaucoma has prompted development of numerous methods for laboratory evaluation of drug effects on intraocular pressure (IOP). Investigations in our laboratory utilizing some of these methods allowed recognition of particular features which, when incorporated into a single animal model may provide a more meaningful evaluation of a drug's antiglaucoma potential. Features of the model include the following: (1) animals are unanesthetized and thus normally responsive to drugs and stimuli; (2) normal and elevated IOP's are provided sequentially in the same eye; (3) reproducible IOP measurements are made by electronic tonometry; (4) testing can be of sufficient duration to observe the time-course of drug action; (5) appropriate controls are available for post-drug IOP comparisons. The pharmacologic utility of this model for detecting and evaluating potential antiglaucoma agents, particularly of the adrenergic type, was examined employing l-epinephrine, an adrenergic agent with known clinical efficacy in open-angle glaucoma.

Methods. Female New Zealand-White rabbits, 1.8 to 2.5 kilograms body weight, were deprived of food for 18 hours and maintained in restrainer boxes during experimentation. IOP was measured indirectly from the corneal surface, without local anesthesia, using a Mackay-Marg Model No. 12 electronic tonometer. The tip of the tonometer probe was moistened with wetting solution (Barnes-Hind) to avoid corneal abrasion. Pupil diameter was measured to the nearest 0.5 mm. under constant illumination with a clear, straight-edge ruler.

IOP elevation was accomplished in unanesthetized rabbits using a modification of a procedure previously reported.1-2 Rabbits were administered 60 ml per kilogram of tap water rapidly via gavage following measurement of normal or baseline IOP. IOP was measured 10, 20, and 30 minutes thereafter to determine maximal increase (elevated IOP) during this time. The procedure was repeated in the same animals 2, 4, and 6
Fig. 1. Normal IOP and water load-induced IOP elevations versus time obtained from a single rabbit before (PRE) and 2, 4, and 6 hours after topical application of saline to both eyes. The figure illustrates the protocol used in subsequent experiments.

Fig. 2. Mean IOP and pupil diameters obtained from right (R) and left (L) eyes of rabbits before (PRE) and 2, 4, and 6 hours after topical application of saline to both eyes. Normal IOP (bases of the bars) and maximal water load-induced IOP elevations (tops of the bars) are shown. Vertical lines are standard errors of mean values obtained from six rabbits.

hours after topical application of either drug or saline to the eye according to the protocol depicted in Fig. 1.

Saline solutions of Z-epinephrine d-bitartarate were prepared, without adjustment of pH or tonicity, immediately prior to use. Per cent concentrations refer to the salt form. Solutions (drug or saline) were applied topically to the eye in a volume of 100 μl (approximately 2 drops) and the eye lids held closed momentarily. Drug and saline treatment were randomized between right and left eyes, except in the experiments with saline treatment alone.

Results. Fig. 1 illustrates the protocol used in these experiments and shows typical data obtained from a single rabbit in which both eyes were treated with saline. Thus, after water load IOP increased similarly in right and left eyes, reached maximal elevation within 30 minutes, and returned to pretreatment levels before the next water load. Fig. 2 shows mean values for normal IOP, maximal IOP induced by water load, and pupil diameter obtained from six rabbits administered saline topically to both eyes. IOP was elevated markedly in both eyes from normal (pre-water load) levels of about 22 mm. Hg to hypertensive levels of 29 to 35 mm. Hg after each water load. IOP elevation tended to increase with consecutive water loads. Pupil diameter was unaffected by the procedures or treatment.

Topical application of a 2.0 per cent solution of l-epinephrine to one eye of rabbits (saline in contralateral eyes) elicited marked reductions in both normal IOP and IOP response to water load and produced significant mydriasis (Fig. 3). As shown in Fig. 3, l-epinephrine markedly affected elevated IOP and pupil size at the 2-hour treatment interval, whereas normal IOP was not affected until later time intervals. The drug's effect on both normal and elevated IOP remained near maximal six hours after topical application, while pupil size had returned to control levels at this time. Thus, the action of l-epinephrine on IOP was of greater duration than was the effect on pupil size.

Dose-response relationships for the effects of l-epinephrine on normal and elevated IOP are presented in Fig. 4. Quantitatively larger reductions and earlier times of onset of drug action occurred in elevated than in normal IOP. Maximal
drug effects developed 4 to 6 hours after treatment in both normal and elevated IOP states. Pupil diameter was unaffected by concentrations of L-epinephrine less than 2.0 per cent.

**Discussion.** Ideally, laboratory evaluation of antiglaucoma drugs should be conducted in glaucomatous animals. However, the availability of naturally occurring glaucomatous animals is limited and the etiology and pathology of open-angle glaucoma is not well enough understood to permit experimental replication of the disease. Alternatively, animals with “ocular hypertension” generated by varying procedures are used for drug testing in many laboratories.

The model presented herein provides for the determination of a drug's effects on both elevated and normal IOP relative to efficacy, time course of action, and specificity of IOP versus pupillary effects. The pharmacologic utility of this model was demonstrated with L-epinephrine which effectively lowered IOP in normotensive eyes but was most effective in hypertensive eyes. The time-course of action of L-epinephrine on IOP was similar to both that reported from other experiments employing normal IOP in rabbits and that found in normal and glaucomatous man.

The fact that maximal effects of L-epinephrine on normal and elevated IOP consistently occurred at different time intervals after drug treatment suggests that different mechanisms may be involved in the hypotensive versus the antihypertensive actions of this drug. Both effects could be important for the drug’s antiglaucoma action in man, although the antihypertensive action seems most applicable. The pupillary effects of L-epinephrine also differed markedly in time course from IOP effects, again suggesting that these two responses may be mediated by independent mechanisms.

The method of elevating IOP in rabbits via intragastric water load has been utilized previously for drug evaluation in experiments of short duration employing anesthetized rabbits only. The procedure, as described herein, in conscious rabbits exhibited the following desirable characteristics: (1) neither the aqueous fluid production nor the outflow components of the eye were damaged, leaving these sites available for drug action; (2) there was no eye irritation to affect baseline IOP or pupil size; (3) IOP elevation was reproducible and thus suitable for unlimited drug duration studies; (4) similar responses were reliably ob-
tained in both eyes of the same animal; and (5) IOP elevation was susceptible to inhibition by L-epinephrine, a drug effective against human glaucoma. Furthermore, the elevated IOP induced in human glaucomatous patients as a result of the diagnostic "water-drinking test" suggests a possible analogy between glaucomatous human eyes and normal rabbit eyes in response to water load. It should be noted, however, that the animal model as presented measures prophylactic drug action rather than ability to lower a sustained, elevated IOP as it exists in glaucomatous eyes. Hopefully these actions are, in effect, analogous.

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REFERENCES


