The Influence of Reserpine and Propranolol upon the IOP Response to Vanadate in the Rabbit

Nicholas A. Delamere and Richard N. Williams

The authors examined the influence of intravenously administered sodium ortho-vanadate upon the intraocular pressure (IOP) of the albino rabbit. Vanadate was administered by intravenous injection and the IOP was measured by applanation tonometry. Vanadate (2 mg/kg) caused a marked reduction of IOP which lasted for several hours. Pretreatment with systemic reserpine 24 hr prior to vanadate administration markedly diminished ocular hypertensive response to vanadate. Similarly, systemic treatment with propranolol prevented the IOP-lowering effect of vanadate. In addition, propranolol administered during the course of the vanadate-induced hypertensive response caused the IOP to return to a level close to the control value. The IOP-lowering effect of vanadate appeared to be unrelated to cardiovascular changes; vanadate was observed to have no significant influence upon the blood pressure of anesthetized animals even though the IOP was markedly reduced. On the basis of these experiments, the authors suggest that adrenergic mechanisms contribute to the IOP-lowering effect of vanadate. Invest Ophthalmol Vis Sci 26:1442–1445, 1985

Sodium ortho-vanadate, applied topically to the eye, has been shown to lower intraocular pressure both in rabbits and nonhuman primates. The vanadate ion is a potent inhibitor of Na,K-ATPase, and it has been suggested that it could inhibit the activity of the sodium pump in the ciliary body and thereby compromise aqueous humor formation. A reduced aqueous humor flow rate following vanadate treatment has, in fact, been demonstrated experimentally by Podos et al. However, direct evidence is lacking to support the concept that transport enzyme inhibition does actually underlie the reduced aqueous flow rate following vanadate application.

A typical pharmacologic response to vanadate in vivo is an alteration of vascular tone, suggestive of an influence upon sympathetic nervous activity (see Erdmann et al for review). This aspect of vanadate action led us to examine the possibility of adrenergic involvement in the IOP response to vanadate. In order to avoid the recognized uncertainty of penetration of topically applied vanadate, we determined the intraocular pressure response to systemically administered vanadate in the albino rabbit. The response to vanadate was measured in control groups of animals and also in animals pretreated with reserpine or propranolol.

Materials and Methods. Chemicals: Sodium orthovanadate was purchased from Fisher Chemical Company (Fair Lawn, NJ). All other drugs were obtained from Sigma Chemical Company (St. Louis, MO). The sodium ortho-vanadate was dissolved in sterile isotonic saline and the pH was adjusted to 7.4 with 0.1 M hydrochloric acid. All the other compounds were dissolved in sterile isotonic saline.

Measurement of IOP: The IOPs of conscious albino rabbits were measured using an Alcon Digilab applanation tonometer (Alcon; Fort Worth, TX). Prior to measurement of the IOP, the animals were gently restrained by wrapping in a cloth towel, and the cornea was anesthetized with 0.5% proparacain. Measurements of IOP were undertaken several times during a 24-hr period before each experiment to ensure that the animals became accustomed to handling. The IOP was measured in each rabbit prior to and after the administration of each drug.

Administration of drugs: All compounds were administered by intravenous injection into the marginal ear vein. Reserpine was administered (1.0 mg/kg) 24 hr before the intravenous injection of sodium orthovanadate. This dose of reserpine has been used previously in rabbits. Propranolol (1.0 mg/kg) was administered to a separate group of animals, 2 hr prior to the vanadate. The reserpine-induced depletion of catecholamines is slow in onset, and at this dose, is essentially complete within 24 hr. The blockade of beta receptors by propranolol is, on the other hand, a rapid phenomenon, the actual rate being dependent upon the bioavailability of the drug following intravenous injection. The pretreatment dosage regimens for reserpine and propranolol were therefore chosen to optimize the pharmacologic activity of these drugs.

Anesthesia and measurement of blood pressure: To determine whether the IOP effect of sodium orthovanadate could be attributed to a systemic hypotensive response, we monitored the arterial blood pressure in
the presence of this compound. The animals were anesthetized with urethane (1.7 mg/kg intravenously) and the femoral artery was cannulated and then connected to a manometrically calibrated pressure transducer (Bell and Howell; Pasadena, CA). The blood pressure was monitored for 1 hr prior to and several hours after the intravenous injection of sodium ortho-vanadate.

It should be noted that all the experiments in this study conformed to the ARVO Resolution on the Use of Animals in Research.

**Results.** Systemic administration of 2 mg/kg sodium ortho-vanadate resulted in a prompt reduction of intraocular pressure (Fig. 1). The change in intraocular pressure was maximal after 90 min; at this time, the IOP was 13.5 ± 0.4 mmHg, compared to a control value (determined prior to treatment) of 17.4 ± 0.2 mmHg (mean ± SEM, n = 4).

In a separate series of experiments, rabbits were treated with 1 mg/kg reserpine in order to deplete catecholamines. We determined that reserpine treatment had no significant influence upon the IOP; the control IOP was 19.8 ± 0.2 mmHg, while that measured 4 hr post-treatment was 19.7 ± 0.1 mmHg and at 24 hr was 19.1 ± 0.4 mmHg (mean ± SEM, n = 4). After a 24-hr time period, the reserpine pretreated animals were injected with vanadate (2 mg/kg intravenously) and the IOP response measured. It was observed that pretreatment with reserpine markedly diminished the IOP fall normally induced by vanadate (Fig. 2).

We examined whether the vanadate-induced intraocular pressure decrease could be influenced by propranolol. It has been established previously that propranolol does not alter the IOP in the rabbit. In these experiments, the IOP measured 60 min after propranolol administration (1.0 mg/kg intravenously) was 18.6 ± 0.6 mmHg (mean ± SEM, n = 4) compared to a control (pretreatment) IOP of 19.0 ± 0.3 mmHg. Propranolol treated animals were given vanadate (2 mg/kg intravenously) after 60 min. It was observed that propranolol almost completely abrogated the IOP-lowering effect of vanadate (Fig. 3). Using a separate group of animals, we demonstrated that the IOP reduction caused by vanadate could be rapidly reversed when propranolol was administered at the time of the maximal response to vanadate (Fig. 4).

To establish whether the change of IOP induced by vanadate might be related to a reduction of systemic blood pressure, we performed separate experiments to measure the blood pressure by manometric techniques in anesthetized animals. It was observed that vanadate in doses as high as 2.0 mg/kg did not cause a reduction...
Fig. 4. Reversal of IOP-lowering effect of vanadate by propranolol. Vanadate (2 mg/kg) was administered by intravenous injection, and propranolol (1 mg/kg) was administered 60 min later. Each value is the mean ± SEM for six rabbits.

Discussion. In this study, we have demonstrated that intravenous administration of sodium ortho-vanadate in the albino rabbit lowers the IOP for several hours. This IOP-lowering effect of vanadate could be inhibited by reserpine and, also, propranolol. Furthermore, the IOP reduction following vanadate treatment appeared to be unrelated to changes of blood pressure.

Fig. 5. The influence of sodium ortho-vanadate upon the blood pressure and intraocular pressure of the rabbit. Blood pressure was recorded following cannulation of the femoral artery in the anesthetized animal, and IOP was measured simultaneously by application tonometry. Vanadate was administered intravenously (2 mg/kg) as indicated. Vanadate lowered the intraocular pressure but had no effect on blood pressure. Each value is the mean ± SEM for six rabbits. The dotted line represents the mean control IOP.

It has been shown that vanadate also lowers the IOP following topical administration to monkeys and rabbits.1,2 Investigators have speculated that because vanadate has effects upon ATPase function, its IOP-lowering effect might be related to inhibition of active transport by the iris-ciliary body.4 However, Mittag et al9 have recently presented a convincing argument against this hypothesis; they determined that the concentration of vanadate accumulating in ocular tissues following topical administration was not sufficient to cause a significant effect upon iris-ciliary body Na,K-ATPase. The present study provides direct new evidence to support the concept that mechanisms other than Na,K-ATPase inhibition might contribute to the IOP-lowering effect of vanadate.

The finding that both reserpine and propranolol block the IOP-lowering effect of vanadate raises the possibility that the action of vanadate upon the IOP is connected with an influence on sympathetic neuronal activity. Reserpine is a powerful catecholamine depleting agent, and pretreatment with reserpine has previously been shown to cause effective sympathetic denervation.7 In our study, propranolol inhibited and reversed the IOP-lowering effect of vanadate. Since propranolol specifically blocks post-synaptic beta receptors, this finding suggests a major contribution of an adrenergic component to the vanadate effect.

Although we have demonstrated that vanadate does not diminish systemic blood pressure, it is conceivable that vanadate could exert a significant influence upon localized blood flow. In fact, Morgan et al10 have provided experimental evidence to suggest that topical application of vanadate alters blood flow in the rabbit eye. It remains to be determined whether a vanadate-induced alteration of ocular blood flow might contribute to the observed vanadate-induced reduction of IOP in the rabbit.

Key words: vanadate, intraocular pressure, rabbit, propranolol, reserpine

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References

**Stereochronometry: Quantitative Measurement of Optic Disc Cup Changes**

Takenori Takamoro and Bernard Schwartz

The authors have developed an objective, quantitative method for detecting optic disc cup changes that occur with time. Called stereochronometry, this new technique uses a stereoplotter to measure changes recorded in the combination of two single photographs taken of the same optic disc at different times. The reproducibility and sensitivity of stereochronometric measurements were evaluated using a model eye. Stereochronometric measurements were significantly correlated with calibrated changes of depth and width of the cup of the model eye. Standard deviations for five measurements of cup changes without camera shift range from 2 μm to 18 μm, and errors (deviations of the mean of measurements from calibrated cup changes) ranged from —26 μm to +33 μm. Standard deviations and errors in the measurement of cup depth and width were significantly increased when the camera was shifted by 2.5 mm between photographs to simulate possible changes in photographic conditions. Invest Ophthalmol Vis Sci 26:1445–1449, 1985

Changes in the optic disc cup are an early sign of glaucoma; a quantitative screening method to detect such changes would prove a valuable clinical tool. Schirmer¹ devised a procedure, which Goldmann and Lotmar² called stereochronoscopy, that employs two single photographs of the optic disc taken at different times (a stereochronoscopic pair). This photographic pair, when viewed stereoscopically after a rotation of the photographs to eliminate stereoeffects caused by cup depth, shows cup changes as stereoeffects.³⁴

Stereochronoscopy allows image shifts caused by optic disc cup changes to be detected subjectively, that is, according to the stereo acuity of the observer. Using a stereoplotter, we made objective measurements of the amount of image shift caused by optic disc cup changes. We call this quantitative method for determining optic disc changes stereochronometry.

We evaluated the reproducibility and sensitivity of stereochronometry by measuring changes in a model eye that could simulate alterations in optic disc cup depth and width that occur in time in glaucoma and ocular hypertension.

**Materials and Methods.** Our procedure comprised the following steps: (1) adjustment of a model eye (Fig. 1) to a different cup width and depth for each series of photographs; (2) photography of the model eye with a Donaldson stereoscopic fundus camera to produce simultaneous stereophotographs; (3) calibration of cup depth and width in the model eye; (4) assembly of a stereochronoscopic pair from a series of photographs so that the pair represented a depth change or a width change; (5) stereochronometric measurements of stereochronoscopic pairs using a Kern PG2 stereoplotter (Kern; Aarau, Switzerland); (6) correlations between stereochronometric measurements and cup changes in the model eye; and (7) computations of reproducibility of stereochronometric measurements.

The model eye (Fig. 1) was constructed to simulate changes in the optic cup. We fixed the position of the camera relative to the model eye so that all photographs taken from the same side, left or right, were taken along the same camera optical axis and were, therefore, geometrically identical.

To simulate changes in cup depth we varied the position of the micrometer head over a range of 600 μm, from —300 μm to +300 μm, measured relative to the fixed position of the ring. Two pairs of stereophotographs were taken at each of seven positions along this range, with intervals of 100 μm between each position.

We assembled stereochronoscopic pairs with no camera shift using only the left photograph of a stereopair so that the stereochronoscopic pair had identical geometry. To simulate changes that might occur in photographic conditions (eg, camera angle or distance,