nal NAT rhythm survives pinealectomy in chicks. Aimoto et al\textsuperscript{12} have demonstrated that melatonin in rabbit iris and ciliary body increases in the dark, and Chiou et al\textsuperscript{13} have shown that intracameral injection of melatonin increases IOP in rabbits. Our results after unilateral CGX or DX suggest that if ocular melatonin mediates the dark phase increase of IOP, its rhythm of production is driven by circadian signals carried to the eye by postganglionic adrenergic nerves.

Key words: intraocular pressure, circadian, adrenergic, decentralization, rabbit

Acknowledgments. The authors would like to thank Marvin L. Sears for his encouragement and Alden Mead for his technical instruction.


References


5. Gregory DS, Aviado DG, and Sears ML: Cerebral ganglionec-


12. Aimoto T, Brooks HR, Chiou GCY, and Lauder JK: N-acetyl-
transferase activity and melatonin level in the eyes of glau-


Parasympathetic Denervation Hypersensitivity of the Iris in Ocular Hypertension

Charles V. Clark and Roy Mapsone

Seventy-eight ocular hypertensive patients and 47 age- and sex-matched control subjects were assessed for parasympa-
thetic denervation hypersensitivity of the iris using topical application of 2.5% methacholine chloride solution. Con-
striction of the pupil in response to methacholine stimula-
tion of the sphincter pupillae was significantly greater in the ocular hypertensive patients than the control group (P < 0.001). The implications are discussed, with particular reference to the association between autonomic neuropathy and the primary glaucomas. Invest Ophthalmol Vis Sci 28:1732–1735, 1987

Adrenoceptor density, determined by radioligand binding, is significantly increased at the postsynaptic receptor site in autonomic denervation hypersensitivity.\textsuperscript{1} This mechanism may be applied to the anterior segment of the eye, where pupillary diameter is di-
rectly controlled by the autonomic nervous system; parasympathetic nerves effect pupillary constriction via the sphincter pupillae, and sympathetic nerves effect pupillary dilation via the dilator pupillae. Pup-
illary diameter therefore represents an external manifestation of relative autonomic activity in the anterior segment of the eye. Methacholine at 2.5% is an effective determinant of parasympathetic denerva-
tion hypersensitivity; topical guttae 2.5% metha-
choline constricts Adie’s tonic pupils, whilst normal pupils may not be affected by concentrations up to 15%.\textsuperscript{2} Methacholine at 2.5% was therefore used in the present study to determine the degree of parasympa-
thetic denervation hypersensitivity in the anterior segment of patients with ocular hypertension.
**Materials and Methods.** After informed consent had been obtained, pupillary responses to guttae 2.5% methacholine chloride were assessed in 78 patients with ocular hypertension (mean age 66.6 ± 8.7 years) and 47 age- and sex-matched control subjects (mean age 67.0 ± 11.1 years). The control group consisted of hospital staff, and patients attending the casualty department who were subsequently determined to have no detectable ocular abnormality. Subjects known to have medical disorders predisposing to autonomic nerve dysfunction, or taking medication with effects on the autonomic nervous system, were excluded from the study. A comprehensive ocular examination was performed on all subjects. In particular, there was no evidence of visual field defects following dynamic and static Goldmann perimetry; dynamic perimetry with I4, I3, I2 isopters; static perimetry with I3 and I2 isopters; blind spot with I3 and I2 isopters. Intraocular pressures, by Goldmann applanation tonometry, were as follows (mean ± SEM): control group 16.6 ± 0.2 mm Hg; ocular hypertensive patients 25.2 ± 0.2 mm Hg. Assessment of pupillary denervation hypersensitivity was performed 4 weeks after initial ocular examination. Eyes were excluded from assessment if there was a history of ocular operations, ocular trauma, or current ophthalmic drug treatment. No subject included in the study—either control or ocular hypertensive—had ever been treated with anti-glaucoma medication.

Pupil diameters were recorded photographically. Subjects' eyes were photographed between 9–11 AM under standard lighting conditions, with measured luminance of 20 apostilbs. The subject was instructed to rest his/her forehead against an ophthalmic headrest, and a scale was positioned against the lower eyelid in the perpendicular plane of the iris. The subject fixated at 6 m, then a photograph at ×3 magnification was taken of both eyes together. One drop of 2.5% methacholine chloride solution was placed in the conjunctival sac of the right eye. Forty-five minutes later, a second photograph was taken in a similar manner. The photographic slides were projected on to a white screen at 5 m, producing a final magnification of ×17. Horizontal pupillary diameters were measured to an accuracy of ±0.5 mm, and corrected to actual values by comparison with relative magnification of the scale; this resulted in measurements of actual pupillary diameters to ±0.03 mm.

2.5% methacholine ratio =
\[
\frac{\text{horiz. pupil diameter 45 min post-test}}{\text{horiz. pupil diameter pre-test}}
\]

The concurrent assessment of an age- and sex-matched control group provided effective standards for comparison. One eye was tested in each subject, and all assessments were performed on a single-masked basis.

Diabetes mellitus is significantly associated with ocular hypertension, and may cause parasympathetic denervation hypersensitivity of the pupil. To determine the possible influence of diabetes on pupil function in ocular hypertension, the diabetic status of each subject was assessed by 75 g oral glucose tolerance test according to the standard criteria of the National Diabetes Data Group. Subjects with known diabetes mellitus were excluded from oral glucose tolerance test.

**Results.** Age-adjusted normal tolerance intervals for 2.5% methacholine ratios in the control group were assessed for dependence on age by regression analysis. The data were fitted according to various mathematical models using the computer program SPSSx on an IBM 4341 computer to obtain a normal distribution on age, thus permitting determination of tolerance intervals. Linear regression analysis was calculated from:

\[
\text{RLM}_{2.5} = \frac{\left(\log_{10} M_{2.5} - (0.01756 - 0.00076 \times \text{age})\right)}{0.05241}
\]

\[
\log_{10} (2.5\% \text{ methacholine ratio})
\]

Log_{10} (2.5% methacholine ratio) was shown to depend significantly on age (r = -0.40; P < 0.001) (Fig. 1).

Pupil responses to 2.5% methacholine chloride in ocular hypertension. Comparisons were made between the results of the control group and patients with ocular hypertension; significance was assessed by student unpaired t-test. Two and one-half percent
methacholine ratios (mean ± SEM) were significantly lower in patients with ocular hypertension (0.87 ± 0.01) than the control group (0.94 ± 0.01) \( (P < 0.001) \). Pupillary constriction resulting from methacholine stimulation of the sphincter pupillae in a patient with ocular hypertension is shown in Figure 2.

**Diabetic status and denervation hypersensitivity:** Diabetes mellitus was present in 17 patients with ocular hypertension (21.8\%) and three control subjects (6.4\%). There were no significant differences \( (P > 0.05) \) in pupil responses to 2.5\% methacholine between diabetic and non-diabetic subjects, in either the ocular hypertensive subjects or the control group.

**Discussion.** Neuropharmacological manipulations are effectively used in the localization of defects in autonomic innervation of the iris musculature. Observations of hypersensitive reactions to topical cholinergic agonists in parasympathetic denervated irides\(^8\) resulted in the use of dilute guttae methacholine as a pathognomonic test of pupillotonia,\(^2\) and subsequently to the definitive treatise on denervation hypersensitivity by Cannon and Rosenbluth in 1949.\(^9\) However, constriction of the pupil per se, in response to methacholine stimulation of the sphincter pupillae, may not be indicative of an increase in postsynaptic receptors; whilst previous descriptions have attributed the manifestations of denervation hypersensitivity to an increase in postsynaptic receptor numbers—not without alteration in receptor affinity\(^1\)—alternative explanations may be equally applicable. Thus although this test is of undisputed value in the clinical assessment of anterior segment denervation hypersensitivity, explanations of the observed effect remain speculative. This study has shown that pupil responses to 2.5\% methacholine are significantly age-dependent in normal subjects, a fact not previously well-recognised, and therefore comparison with an age- and sex-matched control group is essential. Pupillary constriction following administration of topical 2.5\% methacholine was significantly greater in patients with ocular hypertension than the control group \( (P < 0.001) \), supporting the hypothesis of parasympathetic denervation hypersensitivity of the iris in patients with ocular hypertension. The presence of ocular autonomic neuropathy in these patients is not unexpected, as systemic parasympathetic dysfunction—demonsrtated by tests based upon cardiovascular reflexes—is present in 42\% of patients with ocular hypertension.\(^10\) Autonomic neuropathy is not characteristically a localized disorder; it is unlikely that significant autonomic neuropathy in the anterior segment of the eye is restricted to the iris. Autonomic dysfunction may affect the outflow mechanism directly; parasympathetic nerve stimulation has been shown to increase facility of outflow by inducing ciliary muscle contraction on the scleral spur, thereby actively “opening” the trabecular meshwork. In the presence of parasympathetic dysfunction, as in ocular hypertension, one may reasonably assume this mechanism to be impaired.

Effects of the autonomic nervous system are recognized to be of fundamental importance in the determination of intraocular pressure, forming the basis of treatment for primary glaucoma with topical application of autonomic agonists (pilocarpine, adreneline) and antagonists (timolol, guanethidine). In this context, the demonstration of significant parasympathetic neuropathy in the anterior segment of patients with raised intraocular pressure is an interesting observation, the exact relevance of which remains to be determined.

**Key words:** denervation hypersensitivity, ocular hypertension, autonomic neuropathy, primary glaucoma

From the Glaucoma Unit, St. Paul’s Eye Hospital, Old Hall Street, Liverpool, United Kingdom. Supported by a grant from the W. H. Ross Foundation. Submitted for publication: May 7, 1986. Correspondence to: Charles V. Clark, MD, FRCS, Moorfields Eye Hospital, City Road, London EC1V 2PD, United Kingdom.

**References**

We have postulated that abnormal mechanical support of the optic nervehead at the level of the lamina cribrosa could be the precursor of glaucomatous damage. Recent studies have shown deformations of the lamina cribrosa to be among the earliest changes in glaucoma. To evaluate the support of the nervehead, we have developed a noninvasive optical method to measure the optic nervehead compliance, namely, the displacement of the optic nervehead induced by an artificial increase in intraocular pressure. To test the validity of the method, we have compared noninvasive measurements obtained in post-mortem enucleated human eyes with those recorded using an invasive technique. Both methods had a reproducibility better than 6 μm and induced no damage capable of interfering with the results. The displacements measured by both methods were similar, thus indicating that our optical method is capable of measuring bulk motion of the optic nervehead. Our results were identical with those obtained by other authors using a third method. The data obtained also established the normal range of optic nervehead displacements induced by a range of intraocular pressure increments. Invest Ophthal Mol Vis Sci 28:1735–1739, 1987

Histologic studies of post-mortem human eyes with glaucoma have shown that damage to the axonal fibers is located at the optic cribrosa. Maumenee suggested in 1973 that the damage may be caused by a misalignment of holes within the lamellar layers through which the bundles of axons pass. The important role played by the lamina cribrosa was further documented by electron microscopic studies that showed the following: (1) The normal lamina cribrosa is less dense at the superior and inferior poles, suggesting a sensitivity compatible with the location of arcuate scotomas; (2) at an early stage of glaucoma, when the visual field (by Goldmann perimetry) is still intact, the lamellar sheets are abnormally compressed and little or no posterior bowing can be documented; and (3) significant bowing and distortion of the lamina cribrosa are present in later stages of glaucoma.

The above findings, especially those related to early stages of glaucoma, support our 1980 hypothesis. We suggested that an altered elastic strength of the lamina cribrosa caused the susceptibility to nerve fiber damage in eyes that develop glaucoma. The findings of Quigley and associates may be interpreted to indicate either an increased weakness or an increased stiffness of the lamina cribrosa: if the lamellar sheets are weak, they would become stressed, ultimately reaching their nonelastic limit. If this limit is surpassed, permanent deformation would result, expressed as the observed compression of the anterior sheets that increases with the severity and duration of the process. The reversible bowing present in the elastic mode of lamellar stress would not be documented histologically, because the tissues are fixed at zero intraocular pressure (IOP). On the other hand, if the lamellar sheets are too stiff, they cannot stretch and accommodate the strain produced by the pressure gradient. Consequently, they would tear and collapse. This would be observed as a collapse of the sheets.

The potential role that the elastic compliance of the lamina cribrosa may play in the pathogenesis of glaucomatous damage has prompted us to develop a method to measure the compliance noninvasively. We compare herein, in post-mortem human eyes, the results from our noninvasive method with those of an invasive technique that measures elastic compliance directly. Levy and coworkers have developed an invasive method, but we chose to design a new procedure that may be less susceptible to artifacts and would allow an independent comparison with their results. Finally, the values obtained in normal human eyes should provide a baseline to which glaucomatous eyes can be compared.

Materials and Methods. Preparation of the globe: Human eyes were obtained from the Illinois Eye