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Measurement of intraocular pressure (IOP) in chickens using a rebound tonometer: Quantitative evaluation of variance due to position inaccuracies

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Abstract

Intraocular pressure (IOP), an important risk factor for glaucoma, is a continuous trait determined by a complex set of genetic and environmental factors that are largely unknown. Genetic studies in laboratory animals may facilitate the identification of genes that affect IOP. We examined the use of the rebound tonometer for measuring IOP in non-anaesthetised birds, along with the device's robustness to alignment errors. Calibration curves were obtained by measuring the IOP of cannulated chicken eyes with the rebound tonometer over a range of pressures. To simulate different types of alignment errors that might be expected with measurement of IOP in alert chickens, for some calibrations the tonometer was positioned (1) at various distances from the cornea, (2) laterally displaced from the visual axis, or (3) angled away from the visual axis. In vivo measurements were taken on three-week-old alert chickens from a layer line, a broiler line, and a layer-broiler ''advanced intercross line'' (AIL) designed to facilitate QTL mapping. The rebound tonometer showed excellent linearity ($R^2 = 0.95–0.99$) during calibration, as well as robustness to variation in the probe-to-cornea distance over the range 3–5 mm and to lateral displacement over the range 0–2 mm. However, the tonometer appeared less robust to off-axis misalignment over the range 0–20° ($P < 0.05$). Also, the slope of calibration curves sometimes differed between eyes ($P < 0.001$), presumably reflecting differences in ocular structure. The IOP measured in non-anaesthetised three-week-old AIL chickens was 17.51 ± 0.13 mmHg (mean ± S.E.; $N = 105$ birds). IOP was significantly associated with corneal thickness ($P < 0.05$) and body weight ($P < 0.001$) in a regression model. Replicate measurements were necessary in order to gauge IOP accurately in individual birds; a series of seven tonometry sessions over a 12-h period during the light phase of the light/dark cycle permitted IOP to be measured with a 95% CI of ±0.7 mmHg. IOP did not differ significantly between the broiler and layer chicken lines which served as the progenitor lines for the AIL. In conclusion, the rebound tonometer permits rapid estimation of IOP in chickens and is well tolerated. The small alignment errors that are expected when taking measurements in non-anaesthetised animals are unlikely to affect accuracy. Since high IOP is a major risk factor for glaucoma, identifying QTL controlling IOP may offer future health benefits. However, our preliminary findings highlight several obstacles to mapping such QTL using the chicken advanced intercross line evaluated here.

Keywords: intraocular pressure; chicken; tonometry; quantitative trait loci; linkage analysis

1. Introduction

Ocular hypertension is a major risk factor for primary open-angle glaucoma (Le et al., 2003). Both genetic and environmental factors are implicated in controlling IOP (Viswanathan et al., 2004; Duggal et al., 2005). Mapping genes controlling quantitative traits in human populations is challenging, however, linkage analysis in model organisms may be beneficial in overcoming these disadvantages (Flint et al., 2005). Inbred strain surveys have been carried out for IOP in both mice and zebrafish (John et al., 1997; Savinova

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et al., 2001; Link et al., 2004). In each of these latter studies, IOP was measured by directly cannulating the eyes of anaesthetised animals.

Accurate assessment of IOP is essential for genetic studies. We evaluated the methods available for measuring IOP in the chicken, a popular model organism for genetic and developmental biology research. As described below, we found that general anaesthesia in chickens produced a dramatic reduction in IOP within minutes, ruling out the use of direct cannulation. Previous studies in the chicken (Table 1) have measured IOP using the Tonopen tonometer in the anaesthetised eyes of conscious birds (Nickla et al., 1998; Papastergiou et al., 1998; Schmid et al., 1999, 2000, 2003). In a preliminary experiment, we compared the Tonopen to the more recently introduced ''rebound tonometer'', and found the rebound tonometer to be the more reliable and better tolerated of the two (see below). Therefore, we carried out a series of trials to investigate how robust the rebound tonometer was to the various types of positional errors (e.g. off-axis measurements) likely to be encountered when measuring IOP in gently restrained, alert birds.

2. Materials and methods

All experiments were carried out in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Visual Research. Birds were housed in large, temperature controlled, transparent Plexiglas brooders under a 12-h light/dark cycle (with lights on at 07:00 h). The light intensity of the brooders was 250–300 lux at chick eye level. Food and water were provided ad libitum.

The tonometer used in this study was an ICare rebound tonometer (Tiolat Ltd, Helsinki, Finland) (Fernandes et al., 2005; Garcia-Resua et al., 2006). This instrument projects a small, light probe towards the cornea, and uses the probe's rebound kinetics to calculate the IOP (Kontiola et al., 2001). The tonometer requires six tonometry measurements to be taken, at which point it displays an IOP value corresponding to a weighted average of the six readings rounded to the nearest integer. For clarity, we refer to these weighted average IOP values as single readings.

2.1. Calibration of the rebound tonometer and assessment of alignment errors

Calibration experiments were performed on enucleated, cannulated eyes from three-week-old chickens. Immediately after enucleation, eyes were mounted on a stand and cannulated at the limbus with a 28-gauge needle which passed into the anterior chamber. A trace of cyanoacrylate adhesive was used at the point of cannulation to prevent leakage of aqueous humour. The cannulated eye was mounted with its visual axis horizontal, to mimic the natural primary position. A short length of polypropylene tubing linked the cannulation needle to a three-way connector, which was attached to a burette containing Balanced Salt Solution (Aqsia, Bausch and Lomb, U.K.), a pressure transducer with a full-scale reading 0–2.5 psi (Omega PX800-002GV, Omega Corp., USA), and a custom-manufactured screw-gauge syringe. Pressure in the system could be varied with the screw-gauge or by altering the level of fluid in the burette. With the cannulated eye and screw-gauge syringe isolated from the system, the pressure transducer was calibrated by recording its output voltage as a function of fluid height in the burette (pressure in mmBSS was converted to mmHg using a conversion factor of 0.0735, under the assumption that mmBSS = mmH_2O). Then the burette was isolated, and pressure in the eye was varied using the screw-gauge. IOP was increased from approximately zero up to 40 mmHg and then back to zero, in steps of ~4 mmHg. The IOP was measured at each step using the rebound tonometer and recorded alongside the IOP measured by the pressure transducer.

Three types of ''tonometer position variation'' (i.e. changes in the position of the tonometer relative to the eye) were considered likely to occur when measuring alert birds (Fig. 1). Thus, in order to test the effect of small changes in the position of the tonometer relative to the eye, calibration curves were obtained: (i) with the tonometer probe at varying distances (3, 4 or 5 mm) from the cornea, (ii) with the tonometer displaced laterally from the centre of the cornea (0, 1 or 2 mm displacement) and (iii) with the tonometer probe directed towards the centre of the cornea but at different angles to the visual axis (on axis or either 10 or 20° off-axis). In each case, rebound tonometry measurements were taken as the IOP was increased and decreased in a stepwise manner as described above. Freshly prepared eyes were mounted after 2–3 different tonometer positions had been examined, or if the cannulation site developed a leak. In total, calibrations were carried out for 13 different tonometer position configurations in a total of five eyes, as detailed in Table 2. The mean ± S.E. regression coefficient in calibration experiments was 2.80 ± 0.06 (Table 2), hence we used the equation: true IOP = $2.80 \times$ [rebound tonometer reading] to convert rebound tonometry readings to true IOP in mmHg.

Table 1
Intraocular pressure in chickens in prior studies and the present study. IOP values are presented as mean ± S.E. (but note that the S.E. was not reported in all studies). IOP measured during the night is shown in square brackets

Study	Breed of chicken	Age (post hatch), weeks	Tonometer	IOP, mmHg
Nickla et al. (1998)	White Leghorn (USA)	2 weeks	Tonopen	22.3 [12.3]
Papastergiou et al. (1998)	White Leghorn (USA)	1 week	Tonopen	20.8 ± 1.1 [16.8 ± 0.7]
Schmid et al. (2000, 2003)	Rhode Island Red/White (Australia)	1–4 weeks	Tonopen	13 – 27
	White Leghorn/New Hampshire	1–6 weeks	Tonopen	18.0 ± 5.1
Present study	White Leghorn (UK)/Broiler intercross	3 weeks	Rebound	17.5 ± 0.1
	White Leghorn (UK)	3 weeks	Rebound	16.3 ± 0.2
	Broiler	3 weeks	Rebound	16.1 ± 0.2