Goldmann Applanation Tonometry in the Conscious Rat

Bruce E. Cohan and David F. Bohr

PURPOSE. To determine whether the Goldmann applanation tonometer can be modified to measure intraocular pressure (IOP) in the conscious rat.

METHODS. In anesthetized rats Goldmann tonometers were tested that had reduced biprism angles in the applanating tips and reduced weights in the tonometer body from those used in humans and species with similar size eyes. Tonometers with tips with biprism angles of 48° and an applied weight of 25 mg per Goldmann scale division (2 g full scale) were calibrated for the rat against manometrically measured IOP. Tonometers, thus modified, were then used in conscious, unsedated rats.

RESULTS. In conscious rats the measured mean Goldmann value was 15.5 ± 0.6 mm Hg (confidence interval = 14.1, 16.6 mm Hg). This was the plateau level reached after the repeated applanations (approximately 10) required to eliminate an artifactual decline in initial Goldmann readings, which was larger than that in humans.

CONCLUSIONS. The Goldmann applanation tonometer was modified to measure IOP in the conscious, unsedated rat. This instrument, the standard for measuring this key physiological parameter in the human eye, can now be applied to the laboratory rat. This may advance the use of this important animal as a model in IOP and glaucoma research. (Invest Ophthalmol Vis Sci. 2001;42:340–342)

The animal model, one of the most important tools in biomedical research, was introduced to studies of glaucoma by Gaasterland and Kupfer1 and Quigley and Holeman,2 who convincingly demonstrated that the condition resembling ocular hypertension in humans could be experimentally produced in the monkey. The most productive laboratory investigations of the effects of elevated intraocular pressure (IOP) in the past quarter century have been conducted with this model. Recently, in part because of the expense of acquiring and maintaining monkeys in sizable numbers and the ease and familiarity of work with the laboratory rat, this animal has begun to share the role of model for IOP and glaucoma studies. The anatomy of the aqueous humor outflow pathway in this rodent eye is similar to the primate,3 unlike rabbit, dog, and cat eyes, which therefore are rarely used in glaucoma-related studies. Morrison’s group4,5 and Sharma’s group6 have described methods for producing ocular hypertension in the rat.

Noninvasive IOP measurement is accomplished by tonometry, and two tonometers, that were designed for the human eye and cannot be modified for the rat eye, have been used to detect qualitative IOP elevation in this animal. The pneumotonometer, which lacks calibration studies in the rat, has been used in these animals under general anesthesia.7 The Tono-Pen, which has been subjected to calibration studies in the rat,8,9 has been applied in the conscious animal,4,10 but with difficulty.11

A third tonometric method is Goldmann applanation tonometry.12 It depends on the Imbert-Fick hypothesis, which holds that when a flat surface is pressed against a closed sphere with a given internal pressure, an equilibrium will be attained when the force exerted against the spherical surface is balanced by the internal pressure of the sphere exerted over the area of contact. Ideally, the sphere applanated by the flat surface is thin, perfectly elastic, and perfectly flexible, and the only force acting against it is the pressure of the applanating surface. In any case the applanated area and the subsequently displaced volume must be small in relation to the total area and volume of the sphere.

The Goldmann tonometer has a transparent plastic applanating tip in the shape of a truncated cone through which corneal contact is observed with the slit-lamp biomicroscope. The tip contains a biprism (two prisms touching at their apices), which produces optical doubling of the image of the flattened surface and separates the two components by a fixed amount, dependent on the apex angles of the prisms. The tonometer tip is connected by a lever arm to the tonometer body, which contains a variable weight. Force is applied to the cornea through the tip until the diameter of the applanated area reaches that fixed separation.

Goldmann empirically tested eyes while measuring IOP with a manometer. He found that for the human eye biprism angles of 60° gave the appropriate diameter of the area of corneal applanation, relating the force of a variable weight applied to it (100 mg per scale division of his instrument; 8 g full scale) to the manometrically measured IOP. He reported that this diameter in the human eye (and in monkey) is 3.06 mm and in rabbit, dog, and cat 4 mm.

MATERIALS AND METHODS

Modification of the Goldmann Applanation Tonometer

Because the eyes of rats are naturally protuberant, they are readily accessible to the applanating tip of the Goldmann tonometer without reduction of its external dimensions and without having to touch the eye lids. In anesthetized rats a range of reductions of the biprism angles of the Goldmann tonometer tip were tested empirically in calibration experiments. The results of these tests determined the values of the angles of the biprism in the tonometer tip (48°; Fig. 1) and of the weight applied by the instrument to the cornea (25 mg per scale division; 2 g full scale). The resulting changes yielded a smaller area, approximately 2 mm in diameter, of corneal applanation than in the larger eyes of the species in Goldmann’s experiments.

Calibration of the Modified Goldmann Applanation Tonometer

The experiments adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and the guidelines of the

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Application of the Goldmann Applanation Tonometer in Conscious Rats

The modified tonometer was used in conscious, unseated rats \((n = 10)\) that were wrapped in a small towel and held gently on a small platform at the slit-lamp biomicroscope, with one person holding the animal and another making the applanation readings. Brown Norway rats are especially docile and require no training or frequent handling before an applanation session. Goldmann tonometry was performed as it is in humans, with topical anesthesia obtained with an eye drop of proparacaine hydrochloride 0.5% and the edge of the applanated area accentuated by staining the tears with a minute drop of sodium fluorescein 2% dye. A series of applanation readings of both eyes was obtained in 194 animal-sessions. Readings were generally made at intervals of from 15 to 45 seconds and in a few sessions at 1- or at 2-minute intervals, over an 8-minute test period for each eye, the right eye first. Tonometer tip-corneal contact time was usually 10 to 15 seconds.

RESULTS

Calibration

The results, compared by linear regression analysis of the Goldmann readings with the manometer measurements, obtained both in random and step fashion, showed good agreement across the range of IOP tested (Fig. 2). And one Goldmann scale unit on the tonometer dial corresponded to 1.1 mm Hg IOP.

Applanation Tonometry in the Conscious Rat

Because the means of the Goldmann readings in the conscious rats did not differ significantly by ANOVA between eyes of an animal, among animals, or at different times of the day, all readings of all test sessions of the 10 animals were pooled. It was found that repeated applanations resulted in a decline in Goldmann readings to a plateau or steady state level. A nonlinear model was applied to this pooled data to determine the number of tonometer contacts \((\pm SE)\) required to reach the asymptote: "mathematical" stability was reached at 11.3 \(\pm 1.2\) (95% confidence interval \([9.3, 13.3]\) applanations). The means of Goldmann readings were close to each other at 9, 10, 11, 12, and 13 applanations, and their confidence intervals were narrow (Table 1). At 11.3 applanations, the interpolated mean Goldmann reading in this series of conscious, unseated brown Norway rats was 15.5 \(\pm 0.6\) (95% CI \([14.1, 16.6]\) mm Hg), the same mean that Goldmann reported for the normal human eye.12 The decline was from a mean initial reading of 23.5 \(\pm 0.4\) (95% CI \([22.7, 24.4]\) mm Hg).

Decline in Goldmann Readings with Repeated Applanations

That in the rat the decline in Goldmann readings with repeated applanations was not the result of a response of the conscious

<table>
<thead>
<tr>
<th>No. of Applanations</th>
<th>Expected Goldmann Reading*</th>
<th>Confidence Interval†</th>
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<tr>
<td>9</td>
<td>16.6 (\pm 0.5)</td>
<td>15.6, 17.7</td>
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<tr>
<td>10</td>
<td>15.6 (\pm 0.6)</td>
<td>14.5, 16.8</td>
</tr>
<tr>
<td>11</td>
<td>15.7 (\pm 0.6)</td>
<td>14.5, 16.8</td>
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<tr>
<td>12</td>
<td>15.3 (\pm 0.6)</td>
<td>14.1, 16.4</td>
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<tr>
<td>13</td>
<td>15.7 (\pm 0.6)</td>
<td>14.4, 17.0</td>
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* Mean \(\pm SE\).
† 95%.
animal to the measurement process was suggested by its presence in both the first-measured right eye and the left eye. Also, it is not due to a reflex because it persisted after both retrobulbar anesthesia and under general anesthesia (with lower initial and plateau levels). When this decline in the rat was first observed, the impulse was to attribute it to the expression of aqueous humor during applanation. Experiments to test this possible explanation were subsequently performed on rats under general anesthesia with a cannula in the anterior chamber to measure IOP manometrically; the pressure regulator was not used, and IOP was allowed to reach a steady state, generally approximately 12 mm Hg. It was noteworthy then that with repeated applanations, the manometrically measured IOP baseline remained unchanged (Fig. 3). This showed that the decline in Goldmann tonometer readings could not be attributed to expression of fluid from the eye during applanation and confirmed the validity for the rat eye of the Imbert-Fick requirement that displaced volume is small in relation to its total area and volume. Further, when initial Goldmann readings were compared simultaneously with manometrically measured IOP, the Goldmann readings were higher and with repeated applanations declined to match the manometrically measured level. This showed that the higher initial readings were artifactual.

**DISCUSSION**

The phenomenon in the rat of a decline in Goldmann tonometer readings with repeated applanations has a close parallel in the human eye in which a similar, usually smaller, decline has long been recognized and clinically either ignored or taken into account by repeating applanations until the readings stabilize and discard ing initial readings. Initially observed by Goldmann, this effect was characterized by Moses and by Bechrakis, whose name is sometimes attached to it. The phenomenon has been most studied by Krakau's group, but even now its mechanism(s) remains obscure.

We speculated that in the rat this decline phenomenon is of corneal origin but could not demonstrate, in images of projected patterns, corneal flattening after the 8-minute test sessions of multiple applanations, perhaps because our corneal topography was not sufficiently sensitive. It still seems plausible that repeated applanations may subtly alter the central rat cornea, making it approach more closely a perfect membrane. Goldmann did describe in human eyes a decline in readings when the tonometer tip was allowed to remain in contact with the cornea, which he attributed to creep or "flowing" of the tissue. To try to reduce the decline phenomenon in the rat by using tonometer tips with smaller biprism angles would require reducing further the weight applied to the cornea, which is beyond the practical limit of the instrument (Pfister R, personal communication, 2000).

The rat is by a factor of 10 the most widely used experimental animal in biomedical research, and its physiology and pathology have been extensively studied. Inbred rat strains provide models of many diseases, among them systemic arterial hypertension, heart failure, diabetes, and obesity. A reliable method for measurement of IOP in the conscious, unsedated rat is now available using the world standard instrument, the Goldmann applanation tonometer, modified as we have described. The availability of this fundamental measurement of this key physiological parameter in this important experimental animal opens new avenues for IOP and glaucoma research and the possibility of development of an inbred rat strain with spontaneous elevated IOP (ocular hypertension) by transgenic and inbreeding strategies.

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**References**


