Intraocular pressure measurement in the conscious rat

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ABSTRACT.

Purpose. **To develop a method for measurement of intraocular pressure in conscious, unsedated rats.**

Methods. **The animal was gently held with a thick fabric mitten, topical anesthetic drops were instilled and the Tono-Pen was applied to the cornea.**

Result. **Measurements in a total of 51 animals did not differ significantly among four strains studied: the overall mean intraocular pressure±standard deviation was 13.0±1.2 mm Hg. Several intraocular pressure tolerance limits were calculated from this conscious rat data to provide a baseline estimate for future studies.**

Conclusions. **This measurement method in conscious rats may contribute to making this widely used laboratory animal available for intraocular pressure research.**

Key words: glaucoma – intraocular pressure – rat – tonometry – Tono-Pen.

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Animal models of intraocular pressure (IOP) research have been essentially limited to the rabbit and monkey. However, the anatomy of the rabbit aqueous outflow system is very different from that of the primate; and availability of the monkey in sizable numbers for research is impractical. Until recently the rat has itself been impractical as a subject for IOP research because of limitations of measuring methods (Ohnesorge et al. 1968; Schulz & van Zwieten 1971; Funk et al. 1985). The Tono-Pen, a tonometer widely used clinically, has been evaluated for use in the rat (Moore et al. 1993; Mermoud et al. 1994) and this instrument (Mermoud et al. 1994; Moore et al. 1995a) and also a pneumotonometer (Shareef et al. 1995) have been used to make noninvasive IOP measurements in anesthetized rats. The present study examines IOP in the conscious, unsedated rat, measured with the Tono-Pen, an approach first mentioned by Moore et al. (1995b) and then outlined by them (1996).

Materials

All experiments were performed using the Tono-Pen XL (TP) (Mentor Ophthalmics, Inc., Norwell, MA., U.S.A.).

Experiments were performed on four rat strains: Sixteen male Sprague-Dawley (Harlan Sprague Dawley, Indianapolis, IN), weight 180–220 grams, 8–12 weeks old; University of Michigan colonies were the sources of the other three strains: two subgroups of male outbred Long Evans hooded, eight in one and ten in the other, weight 300–350 grams, 10 months old; twelve female genetically hypertensive, stroke prone (SHRSP), weight 200–250 grams, 7 months old; five male normotensive Wistar Kyoto (WKY), weight 250–300 grams, 8 months old. All experiments were performed in compliance with the Declaration of Helsinki.

Methods

Previous studies which calibrated the TP in anesthetized rats (Moore et al. 1993; Mermoud et al. 1994) described the problems of applying this instrument, which was designed for the human eye, to the rat. Therefore, we began developing our measuring method in anesthetized animals, and incidentally obtained data on the behavior of this instrument in our hands. We used a method previously described (Mermoud et al. 1994) in each of our four rat strains: the anterior chamber was cannulated and, with an IOP pressure regulating device interposed, connected to a pressure transducer and recorder. IOP was then adjusted to a series of pressure settings, and TP readings were obtained at each of these. Linear regression analyses of these TP readings for each of the strains were very close to the manometric settings over the full range of pressures tested (10–40 mm Hg). Further, there was excellent agreement between data obtained sequentially increasing and sequentially decreasing manometric pressure settings, and when the experimenter was masked and successive manometric pressure settings were random. Thus in our hands the TP measured directly IOP as quantified manometrically.

The experiments in the present study were done in conscious, unsedated rats which were restrained by a modified version of a recently described method (Moore et al. 1996). The animals were gently held on a low laboratory counter top with one hand in a large, fabric mit-

Table 1. Rat cornea and Tono-Pen XL geometry.

Rat Cornea		Tono-Pen XL	
Dimension (method)	Measurement (mm)	Dimension (methods)	Measurement (mm)
Diameter (variable slit)	5.5	Tip diameter (micrometer)	3.16
Radius of curvature (photokeratometer)	2.5	Transducer face diameter (micrometer)	1.26
Thickness (optical pachymeter)	0.21	Tip-transducer face diameter (metallurgical microscope)	flush

Axial length of rat eye measured by A-scan ultrasonography=5.5 mm.

Table 2. Tono-Pen measurements of intraocular pressure (IOP) in conscious rats (mm Hg)*.

Strain	Sprague Dawley (n=16)	Long Evans** (n=18)	Hypertensive (SHRSP) (n=12)	Wistar Kyoto (WKY) (n=5)
Mean IOP	12.8	12.5	13.1	12.7
Standard Deviation	±1.3	±1.2	±1.0	±0.9

* for each animal the two eyes were averaged.
** measurements of both subgroups at first session.

ten, using primarily the weight of the mitten for restraint and with minimal pressure caudal to the shoulders. Immobilization of the head was accomplished with gentle pressure on the shoulders and the top of the head. Topical anesthesia was obtained with a drop of proparacaine (0.5%) which was repeated after two minutes. The animals are usually comfortable and readings can be obtained with infrequent evidence of animal restlessness. TP readings were made in each eye, beginning with the right eye. In the first subgroup of the Long Evans strain IOP readings were repeated on successive days, and in the second subgroup after an interval of 30 days. All measurements were made during the light phase of the light-dark cycle.

The TP, which employs an applanation method for measuring IOP, based on the principle of Mackay and Marg, is hand-held and positioned "by eye." Within its tip its transducer senses the force required for applanation of the cornea. The TP was designed for the human cornea which has a substantial redundancy of the target area of contact; still, consistent readings come after a learning curve. The rat cornea is so small, steeply curved and thin that the target area is a small fraction of that in the human, resulting in an extended learning curve. The dimensions of the rat eye and of the TP were measured because they are relevant to this method of IOP measurement in this species (Table 1).

To arrive at an IOP value in the conscious rat, we used the TP according to its design: as multiple applanations are made, the instrument's microprocessor senses the signals, seeking four signals within 18 seconds provided by the TP's timer which, first, have satisfactory Mackay-Marg force curves, second, are processed through circuits to be converted to IOP and, third, are filtered statistically of aberrant instrument-related values; the TP then provides a statistically processed result of these four accepted values with their coefficient of variation. (Personal communication, 1997. Mr. Morey H. Waltuck, Mentor Ophthalmics, Inc.)

We made applanations until five such averages were obtained, each with a coefficient of variation <5%. We accepted values >7 mm Hg and <21 mm Hg to objectively filter aberrant TP-rat corneal interface related values.

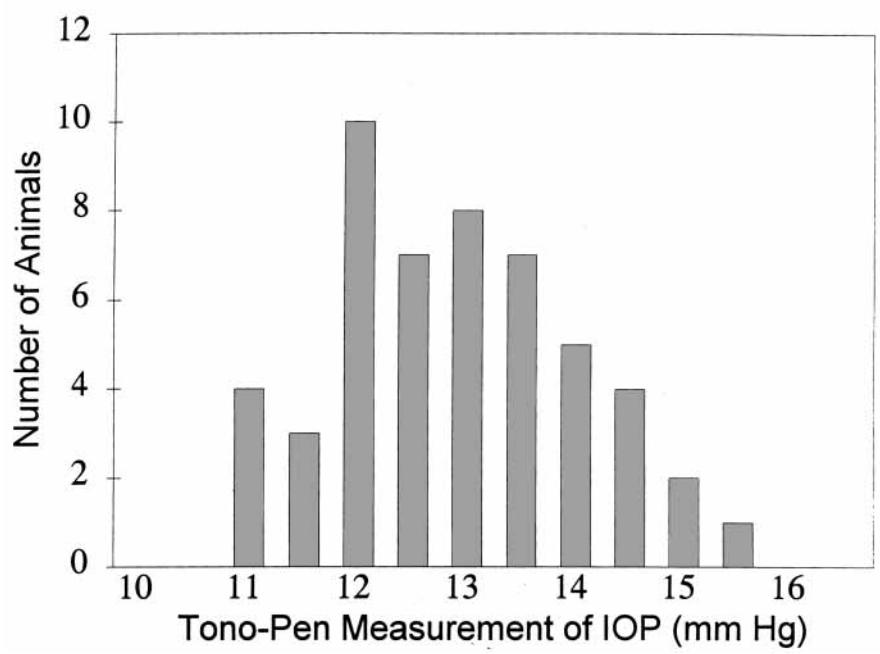

Fig. Histogram of intraocular pressure in 51 conscious rats of the four strains; the readings for the two eyes of each animal were averaged.

Results

The results of TP readings, mean± standard deviation, in the four strains of conscious rats are shown in Table 2. Since by paired t-test there was no significant difference in mean IOP of right and left eyes of each rat strain, or of means of all right eyes (12.9±1.7 mm Hg) versus all left eyes (13.1±1.8 mm Hg), the readings for the two eyes of each animal were averaged.

Since mean IOP of the conscious rats did not differ significantly by ANOVA

Table 3. Tolerance Limits.

Tolerance Limits			IOP (mm Hg)	Coverage* (percent)
Distribution of Eyes (n=102)			10–16	85
			9–17	95
Asymmetry Between Eyes** (n=51)			4	75
			5	90
			6	97
Difference Over Time**	Each eye (right to right and left to left)	One day (n=16)	3	90
			4	97
			5	99
		30 days (n=20)	5	50
			6	70
			7	80
	Average of the two eyes	One day (n=8)	5	50
			6	70
			7	80
		30 days (n=10)	4	87
			5	97
			6	99

* For 95% confidence, assuming an underlying normal distribution.
** Using these absolute values of IOP difference as upper bounds.

among the four strains, IOP of the 51 animals were pooled: mean IOP± standard deviation was 13.0±1.2 mm Hg. A histogram (Fig.) shows the pooled IOP data with the readings for the two eyes of each animal averaged. Of the 102 rat eyes studied, 95 (93%) had IOP between 10 and 16 mm Hg and 100 (99%) between 9 and 17 mm Hg. Considering asymmetry of IOP between eyes of each of the 51 rats, 49 of 51 (96%) pairs of eyes differed by less than 5 mm Hg; 47 (92%) less than 4; 41 (80%) less than 3; 30 (59%) less than 2; and 21 (41%) less than 1.5.

Another way of showing the distribution of IOP is by calculating tolerance limits, assuming an underlying normal distribution of the IOP measurements. Table 3 shows these calculations, with 95% confidence for the following distributions: the 102 rat eyes and the asymmetry between eyes of each of the 51 animals; and difference over time in the two Long Evans subgroups. In these subgroups, first, for each rat the absolute value of IOP difference in each eye (right to right and left to left) was used for the eyes (n=16) in which the measurements were separated by one day, and also for the eyes (n=20) in which the time between measurements was 30 days. Second, for each of these same rats the absolute value of IOP difference of the two eyes averaged was used for the one day (n=8), and also for the 30-day subgroup (n=10).

Discussion

These observations show that IOP can be measured in the conscious, unsedated rat using the TP. Using this instrument according to its design, a mean IOP±standard deviation of 13.0±1.2 mm Hg was obtained in 51 animals of four different strains, with no significant difference between the strains.

In anesthetized rats, previous noninvasive studies of IOP in different strains have produced different means: 13.8 mm Hg with carbon dioxide sedation in Lewis rats (Mermoud et al. 1994); 15.2 mm Hg with isoflurane in brown Norway rats (Moore et al. 1995a); and 12.5 mm Hg with acepromazine maleate-xylazine-ketamine in Wistar rats (Shareef et al. 1995). The well-known and variable effects on IOP of general anesthesia and the possible effect on IOP of different rat strains was considered in these studies. In the TP studies, what is also relevant is the way the instrument was used: in the first (Mermoud et al. 1994) the TP was used according to its design while in the second (Moore et al. 1995a) the data obtained were selected primary readings, that is, not using the TP's filtering of these data nor its statistically processed result. The same difference in method applies when comparing the mean IOP in the initial study in conscious animals – 19.3 mm Hg in brown Norway rats (Moore et al. 1996) – with the present results – 13.0 mm Hg in four rat strains.

In calibrating the TP for the rat both Moore et al. (1993) and Mermoud et al. (1994) reported sporadic low and high values. This is often encountered in biological measurements made with clinical instruments, and we found it to be progressively more frequent from humans to anesthetized rats to conscious rats. We noted in our rats no evidence of a massage effect from repeated applanations.

We tried unsuccessfully to see whether these sporadic values occur in simple tests with latex membrane bubbles with approximately the same radii of curvature as human and rat corneas, connected to the calibration set-up: in each test the TP measurements over the full range of pressure settings were consistent and accurate. We conclude that the small rat corneal diameter and high radius of curvature, possibly combined with the buttress effect of the limbal region, yields a very small contact area for applanation. When the transducer face touches the cornea partially outside this area, the contact which activates it is incomplete and the result is nonphysiologic low readings, that is, less than episcleral venous pressure.

Clusters of high values were encountered in early experiments with a modified plastic rat holder, the result, it seemed apparent, of restricted venous return from the head. Isolated high values due to over-restraint were noted infrequently with the mitten restraint and were immediately reversed upon relaxing the restraint. A second source of sporadic high values occurred with too firm an application of the TP (Whitacre et al. 1991). We suspect that in the rat the exceedingly thin cornea and shallow anterior chamber result in the posterior corneal surface touching the iris-lens diaphragm. An observation which supports this interpretation occurred with anterior chamber cannulation if the needle tip was placed beneath the contact area of the cornea: then even a gentle touch with the TP gave a reading at the top of the scale because of posterior corneal contact with the needle.

We have dealt with sporadic aberrant TP values by using the TP according to its design and then using objective filtering. Using values >7 mm Hg filters the unphysiologic – less than episcleral venous pressure – values below this. We evaluated the validity of filtering values above our upper limit of <21 mm Hg in

30 IOP measurements in a group of male Sprague Dawley rats. We compared IOP means using limits >7 mm Hg and <21 mm Hg with limits >7 mm Hg and <31 mm Hg, clearly well beyond the upper limit of normal in all previous studies of rat IOP. Despite the probable inclusion of sporadic aberrant high values, the difference in means was about 2.0 mm Hg.

The tolerance limits calculated from the data in the present study can guide evaluations of TP measurements of IOP in conscious rats, thus permitting assessment of IOP of a "typical" rat eye or of the two eyes of a "typical" rat. The distribution of IOP of a "typical" rat eye in this study compares well with the normal young human subject population. The IOP asymmetry between eyes of the "typical" rat compares quite well with the 3 mm Hg generally accepted for human eyes tested by Goldmann tonometry and is an indication of the relatively high reliability of TP measurement in conscious rats. Although the tolerance limits of IOP difference over time were calculated from small subgroups, these limits for a "typical" rat eye are appropriate for assessment of experiments in which, after control IOP measurements, interventions, such as pharmacologic or surgical, are made in one eye of an animal. Similarly, the tolerance limits calculation for IOP difference over time in the average of the two eyes of a "typical" rat is applicable to experiments in which systemic interventions or status change may affect IOP of both eyes, such as systemically administered pharmacologic agents, altered metabolic states, models of diseases or the effect of breeding strategies.

The present study in the rat was carried out because investigations using this animal in series of sizable numbers is practical: the laboratory rat is relatively inexpensive to obtain and maintain, and its physiology and pathology are well studied. Further, inbred strains are available which provide models of diabetes, hypertension, obesity and other diseases. Its anterior segment anatomy, especially the aqueous outflow system, is similar to that in the primate (Morrison et al. 1995). The present study was stimulated by the rat paradigm in circulation research: following the introduction, in 1932, of the tail-cuff method of measuring arterial pressure in the conscious, unsedated animal, the rat was propelled into prominence for a broad and still growing array of studies (Bohr & Dominiczak 1991). A reliable method for measuring IOP in the concious rat now makes this animal available for IOP research.

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