Measurement of Intraocular Pressure by Telemetry in Conscious, Unrestrained Rabbits

C. R. Schnell,* C. Debon,† and C. L. Percicot‡

**Purpose.** The aim of this study was to develop a system for the continuous recording of the intraocular pressure (IOP) in rabbits maintained in their normal environment. A telemetric system originally designed for the measurement of cardiovascular parameters in unrestrained, conscious laboratory animals was adapted for this purpose.

**Methods.** Experiments were performed in adult albino female rabbits. The transmitter was placed under the skin in the neck region. Its catheter was tunneled subcutaneously to the superior conjunctival sac and inserted into the midvitreous. Correlation with the IOP in the anterior chamber was performed by pneumatonography measurement and by manometric pressure perfusion in the implanted rabbits. Transitory increase in IOP was induced by a rapid intravenous injection of 20 ml/kg of 5% glucose. Timolol maleate (0.5%) or saline was administered (50 μl) in the instrumented eye before the intravenous glucose injection.

**Results.** The intraocular catheter remained patent and was well tolerated for at least 2 months. A constant circadian rhythm of IOP was recorded as previously reported. Intraocular pressure measurements were highly correlated to pneumatonographic and to manometric measurements, indicating the accuracy and reliability of the recording system. A significant inhibition of the IOP increases following the intravenous injection of glucose was induced by 0.5% timolol treatment when compared to saline instillation.

**Conclusions.** The continuous recording of IOP by our telemetric method represents a breakthrough for studying the effect of various pharmacologic agents in conscious, unrestrained rabbits under physiological conditions that have not been possible with previously described methods. Invest Ophthalmol Vis Sci. 1996; 37:958–965.

An animal model for glaucoma research requires accurate and reproducible measurements of intraocular pressure (IOP). Tonometer devices for the estimation of IOP are applanation or indentation types. The applanation pneumatonometer (PTG) is the usual method applied in pharmacology and eye research. The main disadvantages of this method are that the animals must be restrained and human intervention is required. Therefore, the circadian rhythm of IOP reported in the literature always is measured in conscious and restrained rabbits. Moreover, a local anesthesia must be topically administered before IOP readings by PTG are obtained. This may interfere with the activity or absorption pattern of the new drug in experiment.

Radiotelemetry provides an alternative means of obtaining measurements from freely moving conscious animals. The scientific literature documents the use of implanted devices to measure IOP by telemetry in laboratory animals as early as the late 1960s. However, only recently has the evolution of electronics, packaging, and sensor and battery technology made it feasible to mass produce reliable, miniaturized, implantable devices. In our study, we used a fully implantable miniaturized radiotelemetry system developed for measuring blood pressure (BP), heart rate (HR), and motor activity in small animals such as rats, guinea pigs, and marmosets. We have adapted this system for the continuous recording of IOP in freely moving, unstressed rabbits in their normal environment. We describe here the method of implantation and different manometric and application tonometry calibration studies performed to validate this new approach of recording IOP. Data obtained on basal diurnal rhythms of IOP in freely moving rabbits will be presented. The accuracy of this system was evaluated.
on a transitory ocular hypertension model induced by 5% glucose bolus injection. In addition, we adapted this telemetry system for the continuous recording of mean arterial pressure (MAP), HR, and motor activity in freely moving, unstressed rabbits in their home cages.

**MATERIALS AND METHODS**

**Animals**

The female New Zealand albino rabbits used in this study, weighing between 2.5 and 4 kg, were supplied by Ciba Animal Farm (Basel, Switzerland) and were housed under standard conditions (hygrometry and temperature-controlled room, 12-hour light–12-hour dark cycle). Animals were identified by a tattoo in the ear. Before the beginning of the study, all animals were examined (full ophthalmologic and physical examination). Only animals without pathologic findings were included in this study. All experimental procedures complied with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

**Telemetry System**

The telemetry system (Data Sciences, St. Paul, MN) used for this study consists of four basic components as described previously: (1) an implantable transmitter (AM unit, model TA11PA-C40) with a fluid-filled catheter (0.7 mm wide, 15 cm long) connected to the sensor in the body of the transmitter, which continuously senses and transmits information from within the animal. To maintain chronic patency, the distal 1 cm of the catheter is coated with an antithrombogenic film. The sensor is connected to an electronic amplifier, and the amplified signal is used to modulate radio frequency waves that radiate from the animal; (2) the receiver located within the cage, which detects the signal from the transmitter and converts it to a form readable by computer; (3) the pressure reference module, which measures atmospheric pressure to allow for the telemetered absolute pressure (because the transmitter is precalibrated relative to a vacuum by the manufacturer) to be converted to a gauge pressure; and (4) a computer-based data acquisition system for collection, analysis, and storage of data. The implant transducer body is cylindrical (length = 2.5 cm, depth = 1.2 cm), weighs 9 g, and has a displacement of 4.5 ml. Motor activity is measured using the variability of the received signal strength as a result of changes in distance and orientation relative to the receiving antennae. One digital pulse is generated each time the signal level changes by more than a predetermined amount. Before implantation of the transmitters, calibration was verified to be accurate within 1 to 3 mm Hg (initial offset relative to zero pressure). Battery life of the transmitter is 12 months on average, depending on the frequency of use, because the units incorporate a magnetically activated on–off switch to save power.

**Implantation of Transmitters for Measurements of Intraocular Pressure**

The pressure transmitters were implanted subcutaneously under aseptic conditions, and anesthesia composed of 35 mg/kg ketamine (Imalgene 1000; Rhone Merieux, Lyons, France) and 15 mg/kg xylazine (Rompun 2%; Bayer, Leverkusen, Germany) administered intramuscularly. One hour before surgery, animals received one intramuscular injection of 35 mg of diclofenac (Voltaren 75 mg; Ciba, Basel, Switzerland) and 50 µl of diclofenac Na (Voltaren Eye-drops; Ciba Vision Ophthalmics, Bö lasc, Switzerland) instilled into the conjunctival sac of the eye to be implanted. The rabbits were shaved in the scalp region and placed on a warmed sterile operative field (Aquamatic K module; American Medical System, Cincinnati, OH) maintained at a temperature of 38°C. A small scalp incision was made, and one subcutaneous air pocket was prepared in the neck region. The telemetry transmitter was then inserted into this air pocket, and its pressure catheter was tunneled subcutaneously to an exit site in the superior conjunctival sac of the implanted eye. The sensor catheter was then inserted in the midvitreous of the eye, 3 to 4 mm behind the corneoscleral junction. It was secured at the site of entry with tissue adhesive (Vetbond; 3M Company, St. Paul, MN). The transmitter body was sutured in the neck pocket with polyester thread 4-0 (Dagrofil; Braun, Neuhausen, Germany), and the skin incision was sutured with absorbable thread 4-0 (Vicryl; Ethicon, Nordersted, Germany). Immediately after surgery, the animals were administered 1 subconjunctival injection of 5 mg methylprednisolone (Solu–Medrol 40 mg; Upjohn, Paris, France) and 1 intramuscular injection of 25 mg enrofloxacin (Baytril 2.5%; Bayer). For the next 3 to 4 days, they received 1 ml of enrofloxacin (Baytril 2.5%; Bayer) in the drinking water. The animals were allowed to recover for 5 days before the start of data collection. During this recovery period, daily ocular examination of the anterior eye segment (slit lamp; Zeiss, Oberkochen, Germany), and direct ophthalmoscopy in semidark conditions without pupil dilatation were performed. We found no sign of anterior inflammation, retina detachment, or fundus disturbance 2 days after implantation. Subsequently, a weekly ocular examination was performed. After 3 weeks, the wound was healed totally, and there was no sign of irritation at the subcutaneous neck pocket site. Animals implanted with a radiotelemetry transmitter did not show a different behavioral pattern when compared with unimplanted animals. Pulse amplitude, measured as a index of the patency of the intraocular catheter, remained unchanged for at least
as long as 2 months. The intraocular catheter was well tolerated during this time, and 80% of the implantations we have performed to date were successful. The failures were related mainly to the catheter of the transmitter, which did not remain in the vitreous of the implanted eye.

Implantation of Transmitters for Mean Arterial Pressure, Heart Rate, and Motor Activity Measurements

In several rabbits (n = 3), an additional telemetry transmitter was implanted to monitor cardiovascular parameters while the animals were freely moving. Pressure transmitters were implanted under aseptic conditions and the same anesthesia described previously. The sensor catheter was placed in the femoral artery caudal to the iliac bifurcation. It was secured at the site of entry into the vessel with tissue adhesive (Vet-bond; 3M Company) and a 5 x 10 mm cellulose patch. The body of the transmitter was implanted intraperitoneally and was sutured to the inner abdominal wall. The same postsurgical care was provided as previously described. After 3 weeks, the wound was healed totally. Pulse amplitude, measured as a index of the patency of the intraarterial catheter, remained unchanged for 2 months, the longest period measured so far.

Measurement of Intraocular Pressure or Mean Arterial Pressure, Heart Rate, and Motor Activity

It was not possible to record simultaneously IOP and cardiovascular parameters from the same animal because all transmitters and receivers operate on the same carrier frequency. Therefore, all data with IOP and MAP and HR measurements were performed in the same animal but at different time points. However, motor activity was recorded simultaneously with IOP or MAP and HR. Because the transmitters are designed to operate at very low power over a short distance (20 to 25 cm), the home cages (0.9 X 0.6 X 0.7 m) were fitted with three receivers (model RLA 3000; Data Sciences) to record parameters adequately while the rabbits were freely moving. The receivers were connected to a multiplexer (RMX10; Data Sciences), and the digital signal was transferred to a computer-based data acquisition system. Each animal was measured in cyclic runs of 5 minutes for 11 seconds with a sampling rate of 250 Hz. Mean values for IOP or MAP, HR (derived from a beat-to-beat analysis of the pulsative waveforms), and motor activity were then computed and stored. Hourly averages were determined using the interval averaging routine on the Dataquest Analysis Software (Dataquest IV, version 2.0; Data Sciences). Blood pressure and IOP are expressed in millimeters of mercury, HR in beats per minute, and motor activity in number of movements (units) per 5 minutes.

Measurements of Normal Diurnal Rhythms in Conscious Unrestrained Rabbits

Intraocular pressure (n = 9) or, alternatively, MAP, HR, and motor activity (n = 3) was continuously measured telemetrically in individual animals for 24 hours for several days to determine the diurnal variations in these parameters. A mean 24-hour profile was calculated for each animal.

Intraocular pressure (n = 7) was recorded telemetrically in the same group of animals for 24 hours for 8 consecutive days. Two animals of the initial group could not be included in this study because their transmitters failed within the week of observation.

Correlation Between Manometric and Telemetric Intraocular Pressure Measurements

The experiments were performed in a separate group of rabbits under anesthesia composed of 35 mg/kg ketamine (Imalgene 1000; Rhone Merieux) and 15 mg/kg xylazine (Rompun-2%; Bayer) administered intramuscularly. The sensor catheter of the telemetry transmitter was inserted in the vitreous of the eye 3 to 4 mm behind the corneoscleral junction. It was secured at the site of entry with tissue adhesive (Vet-bond; 3M Company). The anterior chamber of the implanted eye was cannulated with a 27-gauge needle, 0.5 mm from the limbus, and connected by polyethylene tubing and an open stopcock to a BBS (Alcon Laboratories, Forth Worth, TX) column graduated in mm Hg. The correlation between calibrated manometric invasive perfusion pressure (20 to 45 mm Hg) applied in the anterior chamber of the eye and the corresponding telemetric IOP measurements performed in the vitreous were analyzed.

Comparison of Telemetry and Pneumotonography Measurements of Intraocular Pressure

To compare values obtained by telemetry with those obtained by a calibrated planaplan pneumonometer (Alcon Laboratories), IOP measurements were performed simultaneously in the implanted eye of conscious restrained rabbits by telemetry and PTG. Rabbits were transferred to a quiet room with lighting conditions identical to those in the animal house and were placed in individual restraining boxes (Ifa-Credo, L’Arbresle, France). The rabbits were left quiet during 30 minutes before the beginning of the measurements. Topical anesthesia before each set of IOP measurements was obtained with 50 μl of 0.4% oxybuprocain (Novesiner, Ciba Vision Ophthalmics) instilled into the eye.

Application of Telemetry for Measurement of Topically Applied Drug Effects in a Rabbit Ocular Hypertension Model

The suitability of this telemetry system for the evaluation of the IOP-lowering effects of drugs in a transitory
Telemetry in Conscious Rabbits

The rabbits were left undisturbed for 30 minutes before the beginning of telemetric baseline IOP measurements. One measurement was recorded every 15 seconds for 5 seconds with a sampling rate of 250 Hz. Timolol maleate 0.5% or unpreserved saline solution (Unilarm, Ciba Vision Ophthalmics) was instilled (50 μl) into the conjunctival sac of the implanted eye, and the IOP was recorded for the next 60 minutes. A sterile 5% glucose solution (20 ml/kg body weight) was then injected rapidly into the marginal vein of the ear through a 23-gauge needle. Intraocular pressure was recorded for the next 40 minutes. All studies with timolol were performed using a crossover protocol. A washout period of 5 days was allowed between experiments. Delta values were obtained from initial values corresponding to the calculated mean of a 5-minutes preinjection period.

Drugs

The beta blocker Timoptol (timolol maleate) was supplied by Merck Sharp & Dohme–Chibret (Paris, France).

Statistics

For statistical analysis, the average values of data collected over the time period of interest were calculated for individual animals. For comparisons within groups, statistical analysis was performed on averaged data using two-tailed Student’s t-test for paired data or unpaired test for unpaired data. Areas under or above the curves (AUCs; delta mm Hg × minute) recorded until the IOP recovered were determined by using the trapezoidal rule method. Linear regression analysis (least-squares method) was used to compare telemetered measurements to conventional manometric or pneumatonographic values. Values given in text.
RESULTS

Measurements of Normal Diurnal Rhythms in Conscious Unrestrained Rabbits

A consistent diurnal rhythm of IOP was observed (Fig. 1). Values were significantly lower ($P < 0.005$) during the day. The lowest values of IOP was measured at approximately midday (IOP, 12.6 ± 0.5 mm Hg, $n = 9$). Intraocular pressure rose rapidly during the afternoon (before the lights were turned off at 6 PM) and reached a peak at 8 PM (IOP, 17.6 ± 1.3 mm Hg, $n = 9$). It remained at approximately the same level until 5 to 6 AM (IOP, 17 ± 1 mm Hg, $n = 9$) and fell rapidly after 8 to 9 AM.

Mean arterial pressure showed no significant diurnal rhythm (mean daytime value, 74 ± 2 mm Hg; mean night time value, 73 ± 1 mm Hg, $n = 3$). Moreover, there was no significant diurnal rhythm for motor activity (Fig. 1), except a clear peak at approximately 6 AM corresponding to the time point when the lights were turned on in the animal room. However, we observed a clear diurnal rhythm in HR (Fig. 1) that correlates with the IOP rhythm. A remarkable consistency in the pattern of diurnal variation of IOP ($n = 7$) was observed for each of the 8 days of the experiment (Fig. 2).

Although it was not possible to record simultaneously IOP and BP in the same animal, the pulsatile IOP waveform we obtained in a conscious rabbit (Fig. 3a) was similar to the arterial pressure waveform (Fig. 3b). Pulse amplitude was 1.5 mm Hg and remained stable for up to 2 months.

Correlation Between Manometric and Telemetric Intraocular Pressure Measurements

The change in output for a given change in pressure was measured in 14 rabbits using a open stopcock manometer and a telemetric device. We found that the measured pressure by telemetry was highly correlated to the pressure measured on the manometer ($r = 0.98$, $n = 150$, 14 rabbits, 8 to 12 readings per rabbit; Fig. 4). The slope of the regression line was 0.928 ± 0.02 $\delta$ mm Hg manometer/$\delta$ mm Hg telemetry ($P < 0.0001$) indicating the ability of this system to measure IOP accurately in the vitreous of the rabbit eye. The intercept of the regression line was $+2.8 \pm 0.52$ mm Hg and was significantly different from zero ($P = 0.005$). Two different transmitters were used for this study (initial offset relative to zero pressure = $+2.3 \pm 0.3$ mm Hg).

Comparison of Telemetry and Pneumatonography Measurements of Intraocular Pressure

We found that the measured pressure by telemetry was highly correlated to the measured pressure by PTG ($r$...
Telemetry in Conscious Rabbits

Pressure measured via pneumatonometer
(mmHg)

Pressure measured via telemetry
(mmHg)

FIGURE 5. Correlation between intraocular pressure (IOP) measured by pneumatonometry and pressure measured by telemetry ($r = 0.96$, $n = 38$).

Application of Telemetry for Measurement of Topically Applied Drug Effects in a Rabbit Ocular Hypertension Model

Intraocular pressure was significantly increased ($P < 0.001$) after a single intravenous bolus injection of 20 ml/kg of glucose 5% (Fig. 6). The peak effect developed after 2 minutes ($+16.6 \pm 2$ mm Hg), and it slowly returned to initial values after 40 to 50 minutes. A topical administration of timolol maleate at a dose of 0.5% induced no significant changes in baseline IOP for the first hour after application. However, a subsequent intravenous bolus injection of 20 ml/kg of glucose 5% induced only a reduced increase in IOP ($+11 \pm 1$ mm Hg at peak, $n = 3$) for a period of 5 minutes, followed by a decrease toward initial values after 15 minutes. Drug efficacy based on AUC (delta mm Hg × minute) was estimated to be 79.1% ± 2.7% ($P < 0.01$). There was no significant difference in AUC between two repeated glucose injections performed on different days.

DISCUSSION

We have adapted a telemetry system for the continuous measurement of IOP in freely moving rabbits maintained in their home cages. Advantages of the telemetry system for measuring IOP (or, alternatively, MAP and HR) and motor activity can be summarized as follows: Measurement is continuous, restraint and human intervention as well as local anesthesia of the eye are not necessary, and animals can stay in their normal environment during collection of data.

The telemetry method allowed the measurement of the diurnal rhythm of IOP in conscious, unrestrained rabbits as previously reported in pigmented rabbits. Intraocular pressure was lower during the day and showed day–night profiles opposite to those of humans, which is consistent with their opposite wake–sleep cycles. There is still some controversy concerning the diurnal–circadian rhythm of IOP recorded in humans, depending on the experimental design and measurement methods used.

In the rabbit, IOP was decreased at midday and reached a peak at 8 PM. The difference in IOP between mean peak and mean trough measurements was 5 mm Hg, which constituted a fluctuation of nearly 40%. These observations are in accordance with previous studies in which IOP was measured by PTG in light–dark entrained rabbits or rats. However, values for IOP obtained with telemetry were lower than data reported previously in the literature using PTG in trained, restrained rabbits. This may indicate that the animals were less stressed during the telemetry procedure performed in their normal environment than with other methods of measuring IOP performed in a novel environment. Moreover, it has been reported that rabbits are very sensitive to various sensory stimuli (auditory, visual, tactile, olfactory, thermal), which could induce a transient rise in IOP with an

FIGURE 6. Mean changes in intraocular pressure (IOP) during transitory ocular hypertension induced by 20 ml/kg 5% glucose intravenous injection in restrained albino rabbits ($n = 5$) after instillation of saline (thick line) or 0.5% timolol maleate (thin line).
amplitude as great as 5 to 10 mm Hg\textsuperscript{21,22} for several hours.

The telemetry method also allowed the measurement of the diurnal rhythm of MAP, HR, and motor activity in conscious, unrestrained rabbits. We observed a similar diurnal rhythm in HR as in IOP. Heart rate was lower during the day and reached a peak at 8 PM. Mean arterial pressure and motor activity showed no diurnal rhythm. These observations are in accordance with previous studies\textsuperscript{1,2,23,24} performed in conscious rabbits. However, values for HR measured by telemetry were \textasciitilde 20\% lower compared to the data reported previously using conventional methods in conscious rabbits. Again, this may indicate that the animals were less stressed during the telemetry procedure performed in their normal environment. Moreover, in an experiment performed in our laboratories using telemetry, we could observe a dramatic increase in MAP and HR (+30 mm Hg, +80 bpm, respectively) immediately after a trained rabbit was placed in a restraining box. Baseline values were collected before the experiment when the animal was kept in its home cage. Heart rate decreased toward control values after 40 minutes (+30 bpm), whereas MAP remained elevated (+28 mm Hg) for the entire period of restraint. When the animal was returned to its cage, both parameters returned rapidly to initial values after approximately 10 minutes. This shows again how sensitive this animals are to changes in their environment, even if they were previously trained.

In both validation studies performed with the pressure transmitter (TA11PA-C40) we used in the rabbit, our IOP measurements obtained by telemetry were highly correlated to pneumatonographic and manometric measurements. In both studies, the slope of regressions (0.926 and 0.928, respectively) were almost equal to 1 (i.e., line of identity), demonstrating an excellent concordance between methods. The differences we observed (refer to intercepts of regression analysis) between the telemetered and pneumatonographic (+1.28 \pm 0.69 mm Hg) or manometric (+2.8 \pm 0.52 mm Hg) pressure readings in vivo were consistent with the small initial offsets of the telemetric devices used (+1.1 \pm 0.3 mm Hg and +2.3 \pm 0.3 mm Hg, respectively). Similar findings have been reported in previous studies\textsuperscript{5,25,26} to perform the calibration of the same pressure transmitter. This indicates the accuracy and reliability of this recording system for measuring IOP pressure, even in a range from 5 to 45 mm Hg. Moreover, it demonstrates the possibility of accurately measuring IOP in the midvitreous of the eye. The synchronous globe pulsations (1.5 mm Hg) caused by systolic and diastolic pressure pulsations (25 mm Hg) we observed in the rabbits were similar to those reported in normotensive humans\textsuperscript{27,28} and reflects the sensitivity of this system. The high sampling rate (250 Hz for 11 seconds) we used during our measurements allowed us to follow the IOP continuously for several arterial cycles (~40) and to calculate a real mean value for this period, whereas PTG measurements represent only punctual pressure readings.

Topical administration of 0.5\% timolol maleate in ocularly normotensive, telemetered, conscious, restrained albino rabbits had no significant ocular hypotensive effect during the first hour of instillation. Similar observations have been reported.\textsuperscript{29-31} However, a significant inhibition of the IOP increase after the intravenous injection of 5\% glucose was evidenced 1 hour after timolol 0.5\% administration compared to saline instillation. This observation is in accordance with previous studies in which IOP was measured by PTG.\textsuperscript{7} Moreover, using telemetry, we were able to follow the complete profile of the IOP increase after glucose injection and to demonstrate that the maximum increase occurs 2 minutes after bolus injection. Such an effect could not be detected with intermittent PTG measurements. The ability of telemetry to record IOP continuously during the experiment, without interfering with the animal, certainly increases the sensitivity of this artificial elevated IOP model for testing IOP-lowering drugs.

In summary, we have shown that IOP, MAP, HR, and motor activity can be accurately and reliably measured by telemetry in conscious rabbits freely moving in their home cages. With this system, marked diurnal variations in IOP and HR are observed. The pattern of these diurnal rhythms is remarkably constant under defined conditions. This technique can be used to study the effects of drug treatment on the parameters over short or extended periods of time in conscious rabbits under physiological conditions that have not been possible with previously described methods.

Key Words
activity, blood pressure, chronic monitoring, diurnal, heart rate

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References


