Measurement of heart rate and Q-T interval in the conscious mouse

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Mitchell, Gary F., Andreas Jeron, and Gideon Koren. Measurement of heart rate and Q-T interval in the conscious mouse. Am. J. Physiol. 274 (Heart Circ. Physiol. 43): H747–H751, 1998.—Transgenic mouse models provided a powerful tool to evaluate the physiological significance of altered quantitative or characteristics of specific gene products, such as cardiac ion channels. We have developed a system to record and analyze changes in the electrocardiogram in the mouse using an implantable telemetry system. The R-R and Q-T intervals were measured on individual beats and on signal-averaged complexes derived from 1, 2, or 4 s of contiguous data each hour during a 24-h period in three male and three female FVB mice. Duration of averaging had minimal effect on the measured Q-T. The Q-T interval was shown to be related to the square root of the R-R interval, and an appropriate formula for a rate-corrected Q-T interval (Q-Tc) was derived. Ketamine anesthesia was shown to markedly increase duration and variability in R-R, Q-T, and Q-Tc intervals. In conscious animals, variability in Q-T was low across animals and over time, suggesting that this could be a sensitive model for detection of changes in the Q-T interval in transgenic mice with ion channel defects.

THE TRANSGENIC MOUSE has emerged as an important experimental model that may be used to establish the physiological significance of induced genetic alterations that were previously characterized at the cellular or molecular level only. Expression of abnormal quantitative or altered forms of gene products in an intact animal provides a powerful tool to gain insight into the role of specific genes in cardiac growth, development, and function. For example, we recently developed a transgenic mouse model in which we overexpressed a truncated Shaker-like potassium channel, which when coexpressed with the wild-type channel abolished expression of the potassium current in Xenopus oocytes (1). In preliminary studies, we have shown that expression of this transgene in the intact mouse prolongs the Q-T interval measured under anesthetized conditions (8). This may represent an experimental model of the long-Q-T syndrome, which in humans is associated with spontaneous ventricular arrhythmias and sudden cardiac death. Therefore, we sought to develop and herein describe a system for long-term monitoring and evaluation of electrocardiographic intervals in the mouse.

METHODS

Study animals. Studies were performed in male and female FVB mice (Charles River, Wilmington, MA) ranging in age from 3 to 6 mo and in body weight from 27 to 33 g. All studies were performed in accordance with the guidelines of the Harvard Medical Area Standing Committee on Animals.

Data acquisition system. Data were acquired using a commercially available telemetry system, which employs an implantable 3.6 g (0.85 cm × 0.9 cm × 2.3 cm) radio frequency transmitter (TA10ETA-F20; Data Sciences, St. Paul, MN) and a receiver that is placed under the cage of each animal under study. The analog output option for this system was used to produce a high-level, single-channel, bandpass filtered (1–100 Hz), analog electrocardiogram. This analog electrocardiogram signal was digitized at 500 Hz using a 16-bit analog-to-digital converter (model AT-M10–16XE-10; National Instruments, Austin, TX) in a personal computer. With the use of custom software (9), continuous 24-h digital recordings of the electrocardiogram were acquired to the hard drive of the computer and subsequently transferred to CD-ROM for later analysis.

Transmitter implantation. After induction of general anesthesia with an intraperitoneal injection of a mixture of ketamine (50 mg/kg) and xylazine (10 mg/kg), the hair was removed from the back and the left chest wall using a clipper. The operative fields were prepped with an antiseptic solution and draped in a sterile fashion. A 2-cm midline incision was made on the back along the spine, and a subcutaneous pocket was formed by blunt dissection. The transmitter was sutured to the muscle just to the left of the midline with the leads directed caudally. The cathodal lead was looped to the right within the pocket to an area overlying the right scapula and was anchored in place with a permanent suture. A small incision was then made overlying the apical region of the heart. A trochar with an overlying plastic sleeve was tunneled from this incision around the chest wall to the transmitter pocket. The trochar was removed, leaving the sleeve in place. The anodal lead was passed through the sleeve, which was then withdrawn, placing the lead in the subcutaneous tunnel. The tip of the lead was sutured to the chest wall overlying the apex of the heart, and both skin incisions were then closed. Animals were weighed and returned to their cages. Their electrocardiograms were monitored immediately after the implantation procedure. They were kept under warming lights until fully conscious and moving about the cage. The entire implantation procedure took ~30 min.

Data acquisition and analysis. All analyses were performed on recordings that were obtained starting at least 48 h after the implantation procedure. Full-disclosure 24-h recordings were analyzed off-line. Measurements were performed on 1-, 2-, or 4-s screens of data. The R wave of the QRS was detected automatically using a derivative-based QRS detection algorithm. The peak of the QRS was marked on a high-resolution computer monitor and visually confirmed. The first and last complexes on each screen represent potentially incomplete cardiac cycles and were therefore automatically excluded from the analyses. All remaining complexes on the screen were ensemble averaged using the peak of the QRS as a fiducial point. The Q-T interval of the signal-averaged complex was then determined manually by placing cursors on the beginning of the QRS and the end of the T wave. The R-R interval was determined automatically by averaging individual R-R intervals for all complete cardiac cycles on the screen.
Analyses were performed at the beginning of each hour of the 24-h recordings in six animals (3 male, 3 female), giving a total of 144 measurements of Q-T interval. Signal averaging can produce a significant low-pass filtering effect, depending on the number of complexes averaged and the amount of jitter in the fiducial point (6). Therefore, the effects of averaging on Q-T interval were assessed by taking measurements from single beats and from averaged beats derived from 1, 2, or 4 s of contiguous data for each of the 144 measurements. The same observer performed these analyses on different days. In a subsequent analysis, the Q-T intervals of three consecutive, individual beats of unprocessed data were measured and averaged to obtain the “single beat” Q-T interval. Analyses of 4-s screens were repeated on a separate day by the same observer and subsequently by a second observer to assess intra- and interobserver variability, respectively.

The relationship between the Q-T interval and the R-R interval in the mouse was assessed in the same six mice. To obtain physiologically relevant values for heart rate-corrected Q-T interval, Q-Tc, in units of time (rather than time to a power not equal to 1), the observed R-R interval, R-Ro, was first expressed as a unitless multiple of 100 ms, which is the approximate average R-R interval for this strain of mice. This gives a normalized R-R interval, R-R100 = R-Ro/100 ms. Next, the value of the exponent, y, in the relationship Q-Tc = Q-T × R-R100 was assessed, where Q-To is the observed Q-T (in ms); units for Q-Tc are also milliseconds. Taking the natural logarithm of each side of this relationship gave ln(Q-To) = ln(Q-T) + y ln(R-R100). Thus the slope of the linear relationship between the log-transformed Q-T and R-R100 defined the exponent to which the R-R interval ratio should be raised to correct Q-T for heart rate.

Effects of gender. Studies were performed in a total of six male and five female mice. In each animal, the R-R, Q-T, and Q-Tc were evaluated every 10 min during the 24-h recording period, was 99 ± 13 ms, corresponding to a heart rate of 616 ± 77 beats/min. This represents a coefficient of variability of 13% for the R-R interval. The Q-T interval measured from averaged waveforms from the same 4-s screens was 54.9 ± 4.0 ms, giving a coefficient of variability of 7.3%. Thus the lumped variability in Q-T interval across time and animals was about one-half the variability in R-R interval. The Q-T

RESULTS

The implantation and recovery periods were well tolerated by all animals, with no weight loss within the first 9 days after the procedure (Fig. 1). High-quality recordings were obtained throughout the 24-h recording period in each of the animals (Fig. 2). Although there were occasional periods of muscle and motion artifact, these data were easily analyzed using the signal averaging approach (Fig. 2).

The average R-R interval for the mice, calculated from hourly 4-s samples evaluated throughout the 24-h recording period, was 99 ± 13 ms, corresponding to a heart rate of 616 ± 77 beats/min. This represents a coefficient of variability of 13% for the R-R interval. The Q-T interval measured from averaged waveforms from the same 4-s screens was 54.9 ± 4.0 ms, giving a coefficient of variability of 7.3%. Thus the lumped variability in Q-T interval across time and animals was about one-half the variability in R-R interval. The Q-T

Fig. 1. Average body weight for the 6 animals in the 9 days after implantation of the electrocardiogram transmitter. Initial weight was taken immediately after the implantation procedure in each mouse. Values are means ± SD.

Fig. 2. Signal averaging of the electrocardiogram in the conscious mouse. First second of a 4-s data segment (left) and the signal-averaged complex (right) obtained by averaging all complexes from the entire 4-s period are shown. In conscious animals, active periods may be contaminated by muscle artifact and noise (bottom), which obscure electrocardiogram landmarks that are otherwise readily identifiable (top). After averaging, the T wave, which includes a substantial inverted component in this animal, is easily measured and is seen to be slightly shorter during the active period (bottom).
intervals measured on single beats and on averaged waveforms derived from 1, 2, or 4 s of data were compared. Despite averaging a significantly greater number of beats as sample length increased (8 ± 1 vs. 18 ± 2 vs. 39 ± 5 beats for 1, 2, and 4 s of data, respectively, \( P < 0.001 \)), there was only a minimal trend toward an increased value for Q-T interval.

The square root of the R-R interval. As a result, the trend toward an increased value for Q-T interval was explained by a low-pass-filtering effect of waveform averaging (Fig. 2). As a result, all further analyses were performed using a 4-s data window.

When Q-T analyses were repeated by the same observer on a different day, there was no significant difference in measured Q-T interval \([54.9 ± 4.0 \text{ vs. } 54.9 ± 4.8 \text{ ms, } P = \text{ not significant (NS)}]\), and the SD of the difference was only 2.6 ms (4.7% of the mean), which approximates the resolution of the data acquisition system (2 ms). When the measurements were performed by a second observer, there was a slight difference in the mean Q-T interval \([54.9 ± 4.0 \text{ vs. } 53.5 ± 5.8 \text{ ms, } P < 0.001]\), and the SD of the difference was quantitatively higher (4.6 ms).

As expected, the Q-T interval was highly correlated with the R-R interval in these conscious animals \((r = 0.74, P < 0.01)\). The relationship between R-R interval and Q-T interval was evaluated by assessing the slope of the relationship between the natural logarithms of each of these variables (Fig. 3). The R-R interval was first expressed as a unitless ratio of the approximate average R-R interval (100 ms) for this strain of mice. Linear regression of the data revealed a slope of 0.44, indicating that Q-T is approximately proportional to the square root of the R-R interval. As a result, the following formula for correction of Q-T interval for R-R interval was derived: \(Q-T_c = Q-T_o/(R-R_o/100)^{0.42}\).

The values for Q-Tc, obtained with this approximate formula averaged 55.3 ± 2.8 ms and had minimal residual correlation with the R-R interval \((r = -0.16, P = 0.06)\) as a result of using an approximation in the exponent.

Heart rate and Q-T variability during the 24-h period were assessed by analyzing data from the six animals as a repeated measure with 24 levels (each hour of the day). There was significant circadian variability in R-R interval as assessed by repeated-measures analysis of variance (ANOVA; \( P < 0.005 \), Fig. 4). Changes in Q-T interval generally paralleled those in R-R interval when Q-T was rescaled to match the variability in R-R (Fig. 4). The main effect of time of day on Q-T and Q-Tc was not, however, significant by repeated-measures ANOVA, suggesting that Q-T is subject to less circadian variability than R-R interval in these conscious animals in a controlled environment. The relatively constrained variability in Q-Tc across time and animals was clear when plotted along with R-R interval on the same scale (Fig. 5).

We evaluated the effects of gender on intervals in six male and five female animals that were matched for age (male vs. female, 10.2 ± 1.7 vs. 9.1 ± 2.2 wk, \( P = \text{NS} \)) and body weight (31.5 ± 2.8 vs. 31.2 ± 0.8 g, \( P = \text{NS} \)). We found no difference in R-R interval (male vs. female; 88.0 ± 2.3 vs. 90.5 ± 5.5 ms, \( P = \text{NS} \)), Q-T interval (51.2 ± 1.4 vs. 52.1 ± 2.0 ms, \( P = \text{NS} \)), or Q-Tc (54.6 ± 1.0 vs. 54.8 ± 1.4 ms, \( P = \text{NS} \)).

Ketamine anesthesia had a prompt and substantial bradycardic effect on the five mice that were rechallenged with the drug \((P < 0.001, \text{Fig. 6})\). The marked and highly variable increase in R-R interval was associated with prolongation of the Q-T interval \((P < 0.0001, \text{Fig. 6})\), which remained significant even after correcting for heart rate \((P < 0.01, \text{Fig. 6})\). The peak effect on the Q-T interval \((30–40 \text{ min after intraperitoneal injec-}

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**Fig. 4. Variability in R-R and Q-T interval during the day.** Each point represents the mean for the 6 mice studied. Error bars have been omitted for clarity (see Fig. 5 for full range of data). Note that the R-R interval tends to be above the overall mean (100 ms) during the day (6:00 to 18:00), indicating a slower heart rate during the less active period in these nocturnal animals. When the scale for Q-T interval is expanded (by a factor of 4) and offset to match that of R-R interval, Q-T appears to track R-R interval reasonably well.

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**Fig. 3. Relationship between natural log-transformed Q-T intervals and normalized R-R intervals in mice.** Data represent all 144 measurements performed on the 6 animals (intervals measured hourly over the course of 24 h). Linear fit and 95% confidence intervals are shown. See text for details. Q-To, observed Q-T interval. R-R\(_{100}\), R-R interval expressed as a ratio of 100 ms.
tion of the drug) occurred somewhat later than the peak effect on R-R interval (20 min postinjection).

DISCUSSION

Transgenic mouse models offer the opportunity to evaluate the physiological correlates of altered expression of individual structural proteins, enzymes, receptors, hormones, and ion channels. In this study, we have described a method whereby alterations in electrocardiographic phenotype may be evaluated continuously over an indefinite period of time in an intact, conscious, freely moving mouse. We evaluated the relationship between R-R and Q-T intervals, established a formula for the Q-Tc interval, and established reference values for R-R, Q-T, and Q-Tc intervals in conscious, unrestrained FVB mice. The measurement of Q-T interval was highly repeatable by the same experienced observer and was reasonably reproducible when performed by a second observer with limited experience with the analysis system. We found a relatively constrained range of Q-T intervals in the FVB mice, despite moderate spontaneous variability in the heart rate, suggesting that even modest changes in Q-T interval in this model should be detectable with reasonably small numbers of animals. Furthermore, the data acquisition and analysis methods are sensitive to physiological change, as evidenced by our ability to describe the time course of changes in heart rate, Q-T, and Q-Tc intervals in the mice after administration of ketamine.

Normal electrocardiographic intervals for mice. To the best of our knowledge, this represents the first report of electrocardiographic Q-T and Q-Tc intervals in conscious FVB mice. Berul et al. (3) recently reported normal values for intervals in open-chest anesthetized C57BL/6J mice and found substantially longer R-R and Q-T intervals as well as significantly greater coefficients of variability (26 and 27%, respectively; see Ref. 3). Furthermore, they corrected the Q-T interval using the formula of Bazett (2) and found markedly prolonged values for the Q-Tc interval. This marked prolongation of the Q-Tc was largely an artifact of the units of the corrected interval. Their equation corrected Q-T (in ms) by the square root of the R-R interval (in seconds), yielding nonlinear units. However, application of our equation for Q-Tc to their mean values for R-R and Q-T intervals gave a Q-Tc of 79 ms, which remains markedly prolonged compared with our reference values. As we have shown, one component of the increased Q-Tc is attributable to the effects of ketamine anesthesia, which was used in that study. Additional effects of pentobarbital sodium and open thoracotomy with artificial respiration must also be considered. In a subsequent study, these same investigators found substantially shorter Q-T intervals in anesthetized closed-chest animals (4). Application of our equation for Q-Tc to their mean data for R-R (120.0 ± 18.4 ms) and Q-T (59.2 ± 10.9 ms) yields a mean Q-Tc (54.0 ms) comparable to that recorded in our animals. Note that the coefficient of variability for Q-T interval remained relatively high (18%) in that study. The markedly increased variability in R-R and Q-T intervals under anesthesia could obscure small but important differ-
ences between groups of animals with channel defects. Furthermore, groups of animals with channel defects may exhibit a differential response to various anesthetic agents (8).

In contrast, van Acker et al. (10) studied conscious BALB/c mice and reported much shorter values for Q-T interval in their control animals. At comparable heart rates (600–700 beats/min), these investigators reported a Q-T interval of ~25 ms, which is one-half the value that we have found. This large discrepancy is likely related to technical differences in definition of the Q-T interval. As noted in the studies of van Acker et al. (10) and others (7), the electrocardiogram of an adult mouse differs considerably from that of humans, with the most notable difference being the absence of an identifiable S-T segment. Instead, in lead II, there is an early-peaking, upright T wave that is merged with the QRS. In our experience, the T wave also has a broad and often inverted tail (Fig. 2). The basis for this configuration was established by performing surface electrocardiograms in a separate series of five male mice that were 10 wk of age. We found a QRS axis of 72 ± 33° and a T wave axis of −111 ± 54°, giving a QRS-T angle of 183 ± 27°. This QRS axis in the frontal plane is oriented approximately along the axis of our implanted lead configuration. Furthermore, this T wave axis is oriented along the same line, but in the opposite direction. These surface electrocardiogram findings are consistent with the QRS-T morphology that we have observed in our telemetric studies. We routinely include the inverted and/or biphasic portions of the T wave in our measurement of Q-T interval.

Correction of Q-T for heart rate. We evaluated the power of the relationship between Q-T interval and R-R interval and found that this can be reasonably approximated by a square root function of R-R interval. We modified the standard Bazett equation, however, to account for the high resting heart rates that are encountered in the mouse. Our goal was to produce values for Q-Tc (in ms) that fell within the physiological range of Q-T intervals encountered in mice. To do this, the R-R interval in the denominator first was normalized and expressed as a unitless ratio. We chose a normalization factor of 100 ms because this approximates the physiological R-R interval of the mouse and is easy to remember. After controlling for R-R interval, we found that the Q-Tc interval was relatively tightly controlled across animals and time in these normal mice under controlled conditions.

Limitations of our study. There are several important limitations of our approach that should be underscored. Currently available transmitter systems permit recording of a single lead only. Because Q-T interval may vary among leads on a electrocardiogram, standardization of lead placement is important. The lead configuration that we have used for the telemetric studies, which is oriented approximately along the axis of lead II (the negative of) lead AVR, gives a maximally upright QRS and a maximally inverted T wave in most animals studied. This configuration facilitates determination of the Q-T interval despite the recording of only a single lead. The transmitter is relatively large; however, the procedure was well tolerated in our 3- to 6-mo-old animals, with no loss of weight, as has been reported with abdominal implants (7). Higher variability and a potential for bias were found when comparing values for Q-T interval measured by different observers. These factors should be considered when designing studies aimed at detecting small differences in Q-T interval among groups. If analysis of the data is performed by more than one observer, the analyses should be randomly assigned, preferably in a blinded fashion, to avoid a bias.

Summary. Transgenic mouse models provide a powerful tool for evaluating physiological correlates of individual gene products. We have shown that telemetric studies in the conscious animal allow for precise characterization of the electrocardiographic phenotype in mice, free from the effects of acute anesthesia and experimental manipulation. This model should prove valuable in the evaluation of ion channel defects in transgenic animals.

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