Converting Enzyme Inhibition Normalizes QT Interval in Spontaneously Hypertensive Rats

Christophe Baillard, Pascale Mansier, Pierre Vladimir Ennezat, Laurence Mangin, Claire Medigue, Bernard Swynghedauw, Brigitte Chevalier

Abstract—We quantified the repolarization time (so-called QT interval) in a rat, an animal species that does not show a well-characterized T wave on surface ECG. We used spontaneously hypertensive rats (SHR) and converting enzyme inhibition to demonstrate a reversible increase in QT interval in pressure-overloaded hearts in the absence of ischemia. An implanted telemetry system recording ECG data in freely moving rats was used to automatically calculate the RR interval. The QT duration was manually determined by use of a calibrated gauge, and a time-frequency domain analysis was used to evaluate heart rate variability. Left ventricular mass was sequentially assessed by echocardiography. Before treatment, 12-month-old SHR had higher left ventricular mass, QT and RR intervals, and unchanged heart rate variability compared with age-matched Wistar rats. A 2-month converting enzyme inhibition treatment with trandolapril reduces systolic blood pressure, left ventricular mass, and QT interval. The RR interval and heart rate variability remains unchanged. There is a positive correlation between the QT interval and left ventricular mass. The SHR is suitable for longitudinal studies on the QT interval. Thus, the detection of the QT interval reflects the phenotypic changes that occur during mechanical overload and, on the basis of these criteria, allows an in vivo determination of the adaptational process. (Hypertension. 2000;36:350-354.)

Key Words: hypertrophy ■ hypertension, arterial ■ electrocardiography ■ converting enzyme inhibition ■ QT interval

Several ECG indexes have been proposed for identifying patients at risk of sudden death, including an increased QT-interval dispersion.1–3 Clinical trials had demonstrated that this index is specifically altered in chronic cardiac hypertrophy and/or during myocardial ischemia and is reversible under therapy. Nevertheless, the meaning of the QT-interval dispersion is a complex issue that includes at least 2 different phenomena, namely, a lengthening of the average duration of the action potential (AP) and myocardial heterogeneity; indeed, in clinical conditions, the QT interval is both prolonged and dispersed, because hypertensive cardiopathy usually associates cardiac hypertrophy with myocardial fibrosis.4–7

There is a need for models of pure mechanical overload without any ischemia, a condition that is nearly impossible to observe in clinical situations, and experimental animal models would help to resolve this issue. Among different animal species, the rat may be an ideal model because it never suffers from atherosclerosis. In addition, there exists a well-documented rat strain, the spontaneously hypertensive rat (SHR), which complies with the above criteria and has been extensively used for pharmacological research and particularly in experimental testing of most of the available antihypertensive drugs.

The QT interval represents repolarization time. It is not easy to measure in humans, despite a well-characterized T wave. In rats, the situation is still more difficult because the T wave is not clearly separated from the QRS complex.8,9 Both pharmacological research and pathophysiology require a method to analyze repolarization time without anesthesia in this animal species.

The goal of the present study was then to present a method suitable for ambulatory detection of repolarization time (otherwise known as the QT interval) in the rat. The necessary validation of such a method has been principally obtained by demonstrating that the duration of the QT interval is increased in a pure model of pressure overload, the SHR, and that this augmentation is reversible under converting enzyme inhibition (CEI) and parallels the echocardiographically detected left ventricular (LV) hypertrophy.

Methods

Animal Model and Experimental Protocol

The laboratory used for the present study complies with the requirements of the French Ministry of Agriculture and has been authorized to experiment on living animals according to executive order No. 887-848 of October 19, 1987. The rats were provided the same animal facilities (Iffa-Credo Farm, Lyon, France) and housed with a...
diurnal light cycle. Early in the treatment, rats were 12 months old and drank tap water (control groups were Wistar [WST] control [WST-C] and SHR control [SHR-C]; n=10 in each group) or trandolapril (1 mg/kg per day given in tap water) for 2 months (treated SHR group [SHR-T]; n=10). Rats were 14 months old at the end of the study period. Such middle-aged rats roughly correspond to patients aged 50 to 60 years, which is the age at which essential hypertension usually begins. Systolic blood pressure measurements were made twice monthly by using the tail-cuff method. Two SHR-C died during the study period. CEI has been selected because of its efficiency in reducing cardiac hypertrophy. A daily dose of 1 mg/kg per day trandolapril has previously demonstrated its efficiency in reducing cardiac hypertrophy in SHR.5,10

Echocardiographic Measurements
Rats were slightly anesthetized with Na\textsuperscript+ pentobarbital (20 mg/kg IP). Echocardiograms were performed with animals in the left decubitus position after the thorax was shaved. A Vingmed echocardiographic unit (model CFM 750) equipped with a 9-MHz transducer was used to obtain short-axis, 2D, guided M-mode recordings of the LV at the papillary muscle level. With the leading edge method, LV dimensions at end diastole and end systole were directly measured from the M-mode recordings, as were interventricular septal (IVS) and posterior wall (PW) thickness. The IVS and PW thicknesses were measured at the time of maximum diastolic dimension. End-systolic dimension was assessed at the time of maximum anterior motion of the PW. The average of 3 cardiac cycles was calculated. LV mass was determined by using the standard cubic function formula: LV mass (mg) = 1.055 \times [(end-diastolic dimension + PW and IVS thickness)\textsuperscript{3} - end-diastolic dimension\textsuperscript{3}] + 1.055 being the myocardial specific gravity.11 For each animal, the LV mass was normalized for body weight. Echocardiograms were performed by the same investigator, who was blinded to the study conditions, at the beginning (12-month-old rats) and at end (14-month-old rats) of the study period to assess the modification of cardiac measurements.

ECG Monitoring and Measurement of Heart Rate Variability
Three ECG recordings were monitored at 3 kHz by use of telemetry in conscious rats as previously reported.3 Briefly, with the rats anesthetized (Na\textsuperscript+ pentobarbital, 25 mg/kg IP), an emitter (ETA-F20, Data Sciences) was subcutaneously implanted in the abdomen. Two leads were placed in the direction of the forelimbs to obtain a derivation similar to lead II in humans. Because the QT duration depends on the derivation, the rats were always tested by the same person. The recordings were made 72 hours after anesthesia, while the rat was freely moving.

For each rat, 3 periods of 3 minutes were recorded to determine the RR interval and heart rate variability (HRV) with the use of Axotape software. Accurate R-wave detection was achieved by level crossing (Dasip, DSP Development). HRV was evaluated by using an instant time-frequency domain method of analysis, the pseudo-smoothed Wigner-Ville transform.12 The analysis was based on the discrete Wigner distribution, which broke the initial time function signal down to a function of time and frequency. Time-frequency mapping gives beat-to-beat estimations and is particularly appropriate for a nonstationary time series. High resolution is achieved by independent time and frequency smoothing with the use of a 16-bit moving window for the time and 128 events for the frequency (LaryC software developed at INRIA, under Sildex environment, TNI). Such a method provided instant spectra every 4 seconds by using a moving window. The spectral powers were calculated for each window and averaged for all the windows for a given recording. The spectral power of the low-frequency component was defined as the total area between 0.04 and 0.50 Hz, and that of the high-frequency component was defined as the total area between 0.6 and 1.4 Hz. The results were expressed both in absolute (ms\textsuperscript{2}) and in frequency component was defined as the total area between 0.6 and 0.50 Hz, and that of the high-

QT-Interval (Repolarization Time) Detection
On the same recordings, the ECG signal was analyzed by using software that recognized the shape of the tracing and that stopped automatically after each R-wave detection and amplified the last QRST complex in another window (Sildex). Using a gauge calibrated in milliseconds, the operator manually evaluated the QT duration as the time elapsed between the onset of the Q wave and the end of the complex. As already described, in small rodents, in contrast to humans, the T wave is not well characterized and is a shoulder of the QRS complex. Consequently, ventricular repolarization was complete when the ECG signal returned to the isoelectric line. The time interval between 2 consecutive R deflections was then automatically calculated and recorded with the QT interval. For each 3-minute ECG recording, 100 measurements were made. Thus, the mean value for RR and QT intervals represented the average of 300 measurements (3 ECGs per rat). As for clinical studies, the QT interval corrected for heart rate (QTc) was also evaluated by use of the Bazett equation: QTc\textsuperscript{2} = QT (in seconds)/RR (in seconds).13 All QT measurements were made by the same investigator who was blinded to the study conditions.

To assess the reliability of the method, several preliminary experiments were performed (1) to assess the physiological relationship between cardiac cycle and the QT duration, (2) to confirm the ability to detect change in QT interval with amiodarone, a drug known to lengthen the repolarization time, and (3) to measure the intraobserver variability. Heart rate is a major determinant of the QT interval, and QT shortens when the heart rate accelerates. An accurate method of QT measurement has to confirm such well-documented physiologic relationships. Thus, 63 ECG recordings were made in seven 3-month-old conscious rats; to obtain different ranges of heart rate, several pharmacological interventions were performed. Recordings were made at rest and before and after each pharmacological injection; these injections were separated by 24-hour intervals. We used a nonlinear regression analysis. A wide range of RR intervals was recorded. Maximal and minimal values of RR intervals were 240 and 120 ms, respectively. The formula was QT = 7.048 + 0.304RR, and the correlation coefficient was r\textsuperscript{2} = 0.574. The effect of amiodarone (30 mg/kg IP) on QT duration was measured in 3-month-old rats (n=7); amiodarone led to a QT-interval lengthening (54 ± 4 versus 65 ± 5 ms, P<0.05). Finally, the Bland and Altman14 method was used to compare QT measurements in 27 recordings. Two random measurements were made for each recording by an observer blinded to the study conditions. The bias method (mean difference between the 2 measurements) was used. The bias represents the systematic error between the measurements. Mean difference ± 2 SD is known as “the limits of agreement.”14 QT measurements ranged between 39.4 and 76.5 ms. The bias was 1.1 ± 4.3%; 95% CI (mean difference ± 2 SD) was 9.7 to 7.5.

Blood and Urine Samples: Anatomic Data
The day before the animals were euthanized for study, urine was collected in a metabolic cage during 24 hours to assess diuresis, ion concentration, and ammonium and ketosis. Creatinine plasma concentration rise and were measured.

Blood samples were collected before death to measure plasma ion concentration, including sodium, potassium, calcium, and magnesium. Urea and creatinine plasma concentration were also measured.

At the end of the study, the rats were euthanized by use of an intraperitoneal pentobarbital overdose. The hearts were removed, and the atria and ventricles were separated. The ventricles were dried and weighed on an analytical scale.

Statistical Analysis
Results were expressed as mean ± SEM. Statistical significance was set at 5%. The statistical processing was performed by use of StatView. A simple regression analysis was used to study the relationship between RR and QT duration and both electrophysio-
TABLE 1. Anatomic Data

<table>
<thead>
<tr>
<th>Parameter</th>
<th>WST-C (n=10)</th>
<th>SHR-C (n=8)</th>
<th>SHR-T (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>640±20</td>
<td>480±19*</td>
<td>437±13</td>
</tr>
<tr>
<td>LV mass, mg</td>
<td>905±21</td>
<td>1508±67*</td>
<td>1105±43‡</td>
</tr>
<tr>
<td>RV weight, mg</td>
<td>233±7</td>
<td>281±14†</td>
<td>253±8</td>
</tr>
<tr>
<td>LV/RV weight ratio</td>
<td>3.9±0.1</td>
<td>5.4±0.02*</td>
<td>4.4±0.2</td>
</tr>
</tbody>
</table>

Values are mean±SEM. RV indicates right ventricle (ventricular). WST-C vs SHR-C assesses the strain effect; SHR-C vs SHR-T assesses the effect of treatment.

$^*P<0.001$ and $^†P<0.01$ vs WST-C; $^‡P<0.001$ vs SHR-C.

Results

Effect of Strain

As expected, the systolic blood pressure of SHR was higher (228±1 mm Hg) than that of the control group (136±2 mm Hg) during the 2-month period (Figure 1). No difference in plasma and urine contents was observed in the 2 strains (data not shown). The SHR groups, as usual, were slightly hypotrophic, with a 27% reduction in the body weight compared with age-matched WST (Table 1). Echocardiography shows that LV mass, LV mass/body weight ratio, PW, and IVS thickness (+89%, +260%, +95%, and +75%, respectively; $P<0.001$) increase progressively with time. Both the LV mass and the LV mass/body weight ratio were higher in SHR-C than in WST-C at the end of the study period (+166% and +221%, respectively; $P<0.001$). The LV/right ventricle weight ratio was higher in SHR-C (Table 1). Anatomic findings correlated with echocardiographic measurements.

Both the 12- and 14-month-old SHR have a slightly slower heart rate compared with controls; the total spectral power was unchanged, but the high-frequency component was significantly enhanced in the hypertensive group. SHR have an increased duration of both the QT interval (52±2 versus 70±1 ms for WST and SHR, respectively; $P<0.01$) and QTc interval (119±4 versus 154±2 ms for WST and SHR, respectively; $P<0.01$).

Effects of Treatment

CEI reduced the systolic blood pressure in SHR (Figure 1) but did not modify plasma and urine contents (data not shown). The treatment significantly reduced the LV mass and mass indexes by −60% and −65%, respectively ($P<0.01$) and reduced the PW and IVS thickness by −66% and −65%, respectively ($P<0.01$) (Table 2). The LV mass, LV mass/body weight (by −66% and −80%, respectively; $P<0.001$), and LV/right ventricle weight ratio were also diminished after treatment (Table 1).

Trandolapril did not significantly modify the average RR interval, the global spectrum, or the relative high-frequency component (Table 3). Nevertheless, 2 months of treatment with CEI significantly reduced the QT interval (61±2 versus 71±1 ms) in SHR. Because the QT interval reduction occurred at the same time as cardiac hypertrophy, the QT interval also correlates with the LV mass (Figure 2).

Discussion

The main results of the present study are the following: (1) The repolarization time is measurable by telemetry in freely moving rats. (2) The QT is longer in SHR than in control rats, and the QT length correlates with the LV mass. (3) Two months of treatment with CEI reduces in parallel the LV mass and QT length.

QT-Interval Detection in Rats

The reliability of the QT measurement method was assessed as follows: (1) Intraobserver reproducibility evidenced a 95% CI that was 9.7 to 7.5, which matched the interobserver reproducibility of QT measurement in humans. (2) The cardiac cycle correlates with QT duration in rats ($r^2=0.57$). (3) In the present study, the value of the QTc-interval duration was in the same range as the AP duration value.

The QT-interval duration found in the present study agrees with recent studies in which the QT interval in rats was measured with ECG in standard limb leads.

At a cellular level, ventricular repolarization is prolonged in cardiac hypertrophy in every animal species, including SHR. In hypertrophic cardiomyopathy in humans, the QT interval, which represents both the dispersion and the lengthening of the AP duration, is also prolonged and correlates with the LV mass as assessed by 2D echocardiography. In the present study, hypertensive cardiopathy in SHR is associated with an increased QT duration and cardiac hypertrophy, and LV mass is correlated with QT duration. In addition, sequential echocardiographic and ECG measurements made during the study period clearly showed that the 2 events were closely linked to each other.

Effect of CEI

At a cellular level, Thollon et al showed that the electrophysiological changes developed with cardiac hypertrophy in infarcted rat hearts were considerably attenuated by CEI. In humans, it was shown by Gonzalez-Juanatey et al that CEI simultaneously reverses cardiac hypertrophy and QT length-
en. Experimental data have shown that CEI reverses LV hypertrophy by reducing the load and by a direct trophic effect on cardiac myocyte proliferation, through its lowering effect on angiotensin II and its inhibition of bradykinin degradation. Further studies using other antihypertensive therapies may clarify this issue; nevertheless, there is much available evidence showing that the 2 components are in fact degradation. Further studies using other antihypertensive therapies may clarify this issue; nevertheless, there is much available evidence showing that the 2 components are in fact closely linked and that the intramyocardial renin-angiotensin system is an important determinant of the phenotypic changes that occur during pressure overload.

**QT Lengthening and AP Duration**

The most consistent electrical abnormality that has been described in association with cardiac hypertrophy is extending AP duration. In rats, among the different K currents, the transient outward current, a major repolarizing current, is the major determinant of AP duration. In experimental cardiac hypertrophy, a number of electrophysiological abnormalities have been reported, including myocardial areas of both short and long AP duration. Such a heterogeneous repolarization occurs mainly in fibrotic areas, which are commonly associated with pressure overload. Cell death could also contribute to the trophic balance of the heart. In SHR, hypertrophy appeared to be an earlier alteration that developed at the same time as arterial hypertension, whereas apoptosis developed later and is associated with hyperactivity of the local angiotensin-converting enzyme. CEI is able to reduce both apoptosis and cardiac angiotensin-converting enzyme activity.

**Conclusion**

Several arguments demonstrate the necessity for QT measurement reliability in rats. The present study shows that the QT interval is longer in SHR than in WST control rats and that QT length correlates with LV mass. Two months of treatment with CEI reduces both the LV mass and QT length. Thus, the QT measurement offers the possibility of assessing its potential role in arrhythmias. With all the resources currently focused on this topic, it is well worth developing and using such a tool. The SHR is a pharmacological model that is useful in the study of the effects of antihypertensive therapy on repolarization and arrhythmias.

**Acknowledgments**

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**TABLE 2. Echocardiographic Data**

<table>
<thead>
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<th>Measurements</th>
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<th>After Treatment</th>
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<tbody>
<tr>
<td></td>
<td>WST (n=10)</td>
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</tr>
<tr>
<td>BW, g</td>
<td>607±31</td>
<td>440±12</td>
</tr>
<tr>
<td>LVM, mg</td>
<td>866±22</td>
<td>1379±29</td>
</tr>
<tr>
<td>LVM/BW ratio, mg/g</td>
<td>1.45±0.1</td>
<td>3.16±0.1</td>
</tr>
<tr>
<td>IVS, mm</td>
<td>1.62±0.1</td>
<td>2.15±0.1</td>
</tr>
<tr>
<td>PWT, mm</td>
<td>1.61±0.1</td>
<td>1.99±0.1</td>
</tr>
</tbody>
</table>

Values are mean±SEM. BW indicates body weight; LVM, LV mass; and PWT, posterior wall thickness. WST-C vs SHR-C assesses the strain effect. SHR-C vs SHR-T assesses the effect of treatment.

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**TABLE 3. ECG Data, RR Interval, HRV, and QT Interval**

<table>
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<tr>
<td></td>
<td>WST (n=10)</td>
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</tr>
<tr>
<td>RR interval, ms</td>
<td>195±6</td>
<td>206±4*</td>
</tr>
<tr>
<td>QT interval, ms</td>
<td>52±2</td>
<td>70±1†</td>
</tr>
<tr>
<td>QTc, ms</td>
<td>119±4</td>
<td>154±2†</td>
</tr>
</tbody>
</table>

Values are mean±SEM. QTc indicates QT/RR (n=5); SP, spectral power; HF, high frequency; LF, low frequency; and ND, not determined. WST vs SHR assesses strain effects. C vs T assesses the effect of treatment.

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**TABLE 4. ECG Data, RR Interval, HRV, and QT Interval**

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**TABLE 5. ECG Data, RR Interval, HRV, and QT Interval**

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Figure 2. QT interval in rats. Correlation is shown between QT interval (in milliseconds) and LV mass (LVM, in milligrams).

Groups were as follows: WST-C, n=10; SHR-C, n=8; and SHR-T, n=10 (r²=0.723, P<0.001).

References

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