Autonomic Nervous System Influences on QT Interval in Normal Subjects

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OBJECTIVES We sought to determine whether the relationship between heart rate (HR) and QT interval (QT) differs as HR increases in response to exercise, atropine and isoproterenol.

BACKGROUND Autonomic nervous system influences on repolarization are poorly understood and may complicate the interpretation of QT measurements.

METHODS Twenty-five normal subjects sequentially underwent graded-intensity bicycle exercise, atropine injection and isoproterenol infusion. Serial 12-lead electrocardiograms were recorded at steady state during each condition and analyzed using interactive computer software. The HR-QT data were modeled linearly and the slopes (quantifying QT adaptation to HR) as well as the QT intervals at 100 beats/min for each intervention were compared by repeated-measures analysis of variance.

RESULTS As HR increased, QT was longer for isoproterenol in comparison to exercise or atropine, which were similar. The HR-QT slope (ms/beats/min) was less steep for isoproterenol (−0.83 ± 0.53) than for atropine (−1.45 ± 0.21) or exercise (−1.37 ± 0.23) (p < 0.0001). In comparison to men, women had more negative HR-QT slopes during all interventions. At 100 beats/min, the QT was 364 ms during isoproterenol, which was significantly longer than that during exercise (330 ms) or atropine (339 ms) (p < 0.0001). Isoproterenol produced a dose-dependent increase in U-wave amplitude that was not observed during exercise or atropine.

CONCLUSIONS In comparison to exercise and atropine, isoproterenol is associated with much less QT shortening for a given increase in HR and, therefore, greater absolute QT intervals. Our findings demonstrate that autonomic conditions directly affect the ventricular myocardium of healthy subjects, causing differences in QT that are independent of HR. (J Am Coll Cardiol 2002;39:1820–6) © 2002 by the American College of Cardiology Foundation

It has been known since Bazett’s initial investigations in the 1920s that the QT interval (QT) varies with resting heart rate (HR) (1). More recently, studies of atrial pacing have provided direct evidence that changes in HR alone affect the QT (2,3). However, the relationship between HR and QT during nonresting autonomic states is not fully understood. Autonomic conditions affect the sinus node as well as the ventricular myocardium (4). Sinus node effects cause a change in HR, which in turn influences the QT. However, autonomic changes also have direct actions on the ventricular myocardium and, therefore, may also have an impact on the duration of cardiac repolarization (5–8).

Although evaluation of autonomic influences on the QT may aid in understanding arrhythmia vulnerability, there also may be important diagnostic implications. It has been suggested that maneuvers such as isoproterenol infusion may "uncover" QT prolongation that may not be evident at rest (9–12); however, normative nonresting QT behavior must be clarified before such findings can be interpreted clinically. The aim of this study is to evaluate the effect of autonomic state on QT in normal subjects.

METHODS

Study population. Healthy subjects were recruited and screened by history, physical examination and 12-lead electrocardiogram (ECG). Subjects did not use tobacco, alcohol, illicit drugs, caffeine or any medications during the study period. The Institutional Review Board approved the protocol and informed consent was obtained.

Study protocol. On day 1, subjects underwent a bicycle exercise with the ECG limb-leads placed in the standard configuration for exercise electrocardiography. The workload was increased gradually. Electrocardiograms were performed at rest in the sitting position and once a steady HR was achieved at each workload (2 to 5 min). On a separate day, fasting subjects returned to the clinical research center between the hours of 9:00 AM and 2:00 PM. Standard ECG electrodes were applied and a peripheral intravenous line was placed. Baseline data were recorded during a period of quiet rest in supine position and at 60° tilt. While supine, subjects received graded infusions of phenylephrine, isoproterenol, esmolol, atropine and combined atropine/esmolol (double autonomic blockade). A rest period followed each stage allowing for metabolism of the study drug (four half-lives) and return of vital signs to baseline. This report addresses only the interventions causing an increase in HR: exercise, isoproterenol infusion and atropine injection.

Atropine was administered as 0.005 mg/kg boluses. Once
a constant HR was achieved at each dose level (average of 2 min), repeated doses of 0.005 mg/kg were given until no further change in HR was observed. Isoproterenol was initiated at a continuous infusion rate of 0.5 μg/min. After a constant HR was achieved at each dose level (average of 3 min), the infusion rate was increased in 0.5 μg/min increments to a maximum infusion rate of either 5.0 μg/min or until HR exceeded 110 beats/min (a HR similar to that typically achieved during atropine). At each dose level of isoproterenol and atropine, multiple ECGs were recorded after a constant HR was achieved.

Electrocardiography. Electrocardiograms of 10 s duration were recorded with a Marquette Electronics MAC-12 (Milwaukee, Wisconsin) acquisition unit using standard filters and a 250 Hz sampling frequency. Electrocardiograms were transferred to a computer workstation and analyzed using the QT-Guard program (v.1.3, GE-Marquette, Milwaukee, Wisconsin). The QT–Guard computed a “median beat” for each ECG lead. A tangent to the maximal terminal slope of the T-wave was derived by the least-squares technique and then extrapolated to the isoelectric line where the end of T-wave was identified for each lead (13,14). The QT for each lead was defined as the time between the common QRS onset and the individual lead T-wave offset. Leads with low T-wave amplitude, excessive noise or undetermined T-wave morphology were excluded by the QT–Guard program (15).

Each lead of each ECG was overread by a cardiologist (A. R. M.) for accuracy in determining the T-wave offset. Of the interventions studied, isoproterenol typically produced a substantial increase in U-wave amplitude. In general, QT measurement using the least-squares method of the QT–Guard is least affected by U-waves because this method uses the maximal terminal slope of the T-wave and determines the intersection of that slope with the isoelectric line. In cases where the QT–Guard could not distinguish the T-wave and U-wave, the overreading cardiologist would designate that lead invalid. The QT–Guard incorrectly marked the T-wave offset in 64 of 3,415 ECG leads during isoproterenol, 0 of 3,303 leads during atropine and 1 of 3,844 leads during exercise. In each case, the terminal slope of a large U-wave was mistaken for that of the T-wave; these leads were excluded from analysis. Erroneous measurement of the U-wave most frequently occurred in leads V3 to V4, accounting for 50 of the 65 manually excluded leads.

The QT on a given ECG was computed as the mean QT of all valid leads. Electrocardiograms with <2 valid leads were excluded. Given the limitations in accurate measurement of the QT at high HRs, the analysis was restricted to HRs <140 beats/min. Bazett’s formula (QTc = QT/RR1/2) was used to calculate all reported QTc intervals, which were classified as prolonged at >440 ms for males and 450 ms for females.

Statistical methods. For each subject, the HR and QT values from all ECGs obtained during a dose-level of medication (or a work-level of exercise) were averaged (mean of 2.3 ECGs per stage). These mean values of HR and QT from each stage were subsequently used in the construction of all models. For each condition (exercise, isoproterenol and atropine), QT was plotted as a function of HR and fitted using linear regression analysis for each subject. Heart rate was chosen instead of RR-interval because it had previously been shown to have a more linear relationship with QT (16). The HR–QT slopes, quantifying QT adaptation to HR, were determined. For each condition, the mean HR–QT slope was determined by averaging the individual HR–QT slopes. The QT at 100 beats/min (a HR consistently attained during each condition) was determined for each subject by solving the linear regression equation during each condition for HR = 100 beats/min. The HR–QT slopes and QT intervals at 100 beats/min were compared during different conditions by repeated measures analysis of variance (each subject was studied under all conditions). Gender comparisons were made by including gender as a factor. Different conditions were compared by pairwise contrasts of least-squares means from the analysis of variance; the two genders were compared similarly. SAS (version 8, Cary, North Carolina) was used for all analyses and results are reported as means with standard deviations.

RESULTS

Subject characteristics. The study population consisted of 12 male and 13 female subjects of mean age 30 ± 8.4 years (range 22 to 60 years). The mean baseline HR was 66 ± 8 beats/min and the mean resting QT was 387 ± 24 ms (QTc 403 ± 17 ms). The peak workload obtained during exercise was 110 ± 50 W, the total dose of atropine was 255 ± 26 μg/kg and the peak infusion rate of isoproterenol was 2.85 ± 1.05 μg/min.

HR–QT interval relationship: evaluation of a single subject. For each subject, overall QT was evaluated as a function of HR. Figure 1 shows the relationship between HR and QT for a single subject. The QT was similar at baseline for all three interventions studied. However, as HR increased, less QT shortening was observed during isoproterenol than during atropine or exercise. This difference was reflected in the attenuated HR–QT slope observed during isoproterenol as compared to atropine and exercise. At a given HR, the QT appeared longer and the terminal downslope of the T-wave was less steep during isoproterenol as compared to exercise or atropine (Fig. 2).
Autonomic effects on the QT interval. For the entire sample of subjects, the maximal HRs, HR-QT slopes, and QT intervals at 100 beats/min for each intervention are provided in Table 1. At higher HRs, QT intervals were longer for isoproterenol than for exercise or atropine, which were similar. The HR-QT slope was significantly less steep for isoproterenol (0.83 ± 0.53 ms/beats/min) than for atropine (1.45 ± 0.21 ms/beats/min) or exercise (1.37 ± 0.23 ms/beats/min) (p < 0.0001). At a HR of 100 beats/min, the QT was 364 ms during isoproterenol—significantly longer than during exercise or atropine, which were 330 ms and 339 ms, respectively (p < 0.0001) (Fig. 3). During isoproterenol, 23 of 25 subjects had a prolonged QTc at 100 beats/min. Although maximal HR differed among the interventions studied, exclusion of all ECGs with HRs ≥110 beats/min (the maximal HR ob-

**Figure 1.** The heart rate (HR)-QT interval (QT) relationship in a single subject during exercise, atropine and isoproterenol. At rest QT was similar during each condition. However, as HR increased, isoproterenol (circle) was associated with less shortening of the QT than exercise (square) or atropine (triangle). As a result, the slope describing the relationship between HR and QT is much less steep for isoproterenol than for exercise or atropine. This individual demonstrated a transient increase in QT as HR increased from 85 to 95 beats/min. Twelve of our 25 subjects demonstrated transient increases (>50 ms) in QT as HR increased in response to isoproterenol. However, such events were rare, representing only 22 of 3,415 consecutive electrocardiograms during isoproterenol. None of our subjects demonstrated this type of increase in QT in response to atropine or exercise.

**Figure 2.** Effects of exercise, atropine and isoproterenol on the electrocardiogram (ECG) complex at 100 beats/min. As heart rate increases in response to each experimental intervention, less decrement in the QT interval was observed during isoproterenol as compared to exercise or atropine. The changes in the ECG complex of lead V2 are shown for one typical subject at 100 beats/min.

<table>
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<tr>
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<th>Women (n = 13)</th>
<th>Men (n = 12)</th>
<th>All Subjects (n = 25)</th>
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<tr>
<td>Max HR exercise (beats/min)</td>
<td>134 ± 4</td>
<td>130 ± 7</td>
<td>132 ± 6</td>
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<td>Max HR atropine (beats/min)</td>
<td>113 ± 8</td>
<td>104 ± 15</td>
<td>109 ± 12</td>
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<tr>
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<td>114 ± 6</td>
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<td>113 ± 7</td>
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<td>HR-QT slope exercise (ms/beats/min)</td>
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<td>HR-QT slope atropine* (ms/beats/min)</td>
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<td>−1.36 ± 0.23</td>
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<td>HR-QT Slope Isoproterenol (ms/beats/min)</td>
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<tr>
<td>QT at 100 beats/min Isoproterenol (ms)</td>
<td>362 ± 15.2</td>
<td>365 ± 30.0</td>
<td>364 ± 21.2</td>
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Significance level for differences between women and men: *p < 0.05. HR = heart rate; QT = QT interval.

**Table 1. HR and QT Interval Responses to Exercise, Atropine and Isoproterenol**
The QT interval (QT) at 100 beats/min during exercise (EX), atropine (AT) and isoproterenol (ISO). At a heart rate of 100 beats/min, the QT during isoproterenol was significantly (p < 0.001) longer than the QT during either exercise or atropine. The difference in QT between exercise and atropine was also statistically significant (p < 0.005).

Figure 3. The QT interval (QT) at 100 beats/min during exercise (EX), atropine (AT) and isoproterenol (ISO). At a heart rate of 100 beats/min, the QT during isoproterenol was significantly (p < 0.001) longer than the QT during either exercise or atropine. The difference in QT between exercise and atropine was also statistically significant (p < 0.005).

Comparison between men and women. At baseline, women had significantly longer resting QTc intervals than men (411 ± 14 ms vs. 394 ± 16 ms, p < 0.01). As HR increased, regardless of the intervention, women shortened their QT more than men (Table 1), minimizing QT differences at higher HRs. Compared with men, the HR-QT slope for women was 13% steeper for atropine (p < 0.05), 11% steeper for exercise (p = 0.12) and 40% steeper for isoproterenol (p = 0.20). There were no significant differences between genders in QT at 100 beats/min during any of the interventions studied.

DISCUSSION

The QT interval is strongly influenced by autonomic conditions. Our data demonstrate that beta-adrenergic stimulation with isoproterenol is associated with significantly less QT shortening than either exercise or atropine, both states associated with substantial vagal inhibition. For each 10-beats/min increase in HR, the QT shortens by approximately 8 ms during isoproterenol infusion. In contrast, the same increment in HR during atropine or exercise would be associated with a nearly twofold greater decrease in QT (14.5 ms and 13.7 ms, respectively).

The autonomic interventions studied influence the QT in two ways: 1) directly, through effects of the intervention on the ventricular myocardium; and 2) indirectly, via the associated change in HR and the accompanying effects of HR on the QT. In and of itself HR is a major determinant

Figure 4. Effect of isoproterenol on the U-wave. In a representative subject, isoproterenol infusion produced a dose-dependent increase in U-wave amplitude in all subjects studied. The U-wave in this subject was present at baseline, but visibly increased in amplitude during low dose isoproterenol. As the infusion rate increased, the U-wave (tracked by arrows in the figure) merged with the T-wave forming a single T-wave complex. At higher doses of isoproterenol, the T-wave is characterized by a prolonged, flattened terminal downslope during isoproterenol. In contrast, these U-wave changes were not observed during exercise (not shown) or atropine infusion. The end of T-wave is indicated by a tick mark for each electrocardiogram complex.
of the QT. The QT has been shown to shorten predictably as HR increases in response to pacing (17), a state in which autonomic conditions remain relatively constant over a wide range of HRs. In several studies, however, direct effects of autonomically active agents on the QT have been demonstrated when atrial pacing was used to control HR (2,4,18). For example, atropine shortens the QT and ventricular effective refractory period during pacing at a fixed HR. Our study similarly shows that autonomic interventions produce changes in the QT that are independent of HR. If HR were the only determinant of QT, then identical QT intervals would have been observed at any given HR. Instead, the QT at 100 beats/min was greatest with isoproterenol: 25 ms longer than with atropine and 34 ms longer than with exercise.

**Autonomic effects on the HR-QT interval relationship.** We found that the QT shortens similarly in response to exercise and atropine. Exercise is a complex physiologic state involving vagal withdrawal, activation of the sympathetic nervous system and an increase in serum catecholamine levels (19,20). During exercise, we found that progressive QT shortening occurs in a linear fashion as HR increases. Other investigators have found similar reductions in the QT during exercise, with HR-QT slopes ranging from −1.3 to −1.7 ms/beats/min (16,21,22). Because multiple autonomic changes are occurring simultaneously during exercise, it is impossible to determine the relative contributions of changes in the sympathetic and parasympathetic nervous systems to the observed degree of QT shortening.

Atropine provides a more direct model of vagal inhibition. Our HR-QT relationship during atropine was similar to previous reports (21,23). The striking similarity in HR-QT behavior during exercise and atropine may reflect the importance of vagal inhibition (with or without concomitant sympathetic excitation) in facilitating QT shortening. In comparison to exercise and atropine, the QT shortened less as HR increased during beta-adrenergic stimulation with isoproterenol. In contrast to the other states investigated, isoproterenol infusion is not associated with significant vagal withdrawal. Interestingly, previous studies have been inconsistent with regard to the effect of isoproterenol on QT (21,23), likely reflecting differences in QT measurement methodology, treatment of U-waves and duration of adrenergic exposure (24,25), all of which could produce important effects on the measurement of QT duration.

Studies using various cardiac myocyte preparations have reported both increased (5,6,26,27) and decreased (6,26,28) action potential duration with isoproterenol. The inconsistent findings reflect important differences in the cell types studied, degree of cellular maturation and methodological variations. Using microelectrode techniques, Litovsky and Antzelevitch found that isoproterenol produced greater action potential shortening in the canine subepicardium than in the subendocardium (29). Because the inscription of the T-wave on the ECG is a reflection of instantaneous transmural repolarization gradients, changes in the relative timing of repolarization across the thickness of the myocardium would be expected to affect the appearance and duration of the T-wave.

**Effects of isoproterenol on the U-wave.** In this study, beta-adrenergic stimulation with isoproterenol was associated with unique effects on the U-wave. As demonstrated in Figure 4, isoproterenol caused a noticeable increase in the amplitude of the U-wave, an observation previously reported by Biberman et al. (24). At higher doses, the U-wave typically merged with the T-wave to produce a single, broad T-wave complex. This phenomenon was not observed during atropine or exercise. When the U-wave is fully incorporated within the T-wave, the electrical gradients that previously inscribed the U-wave may be included in measurement of the QT. Thus, increased size of the U-wave followed by T-U-wave merging is one possible explanation for the greater QT at a given HR during isoproterenol in comparison to atropine or exercise.

Given that the QT is used as an index of repolarization time, these data highlight the importance of extending our understanding of the U-wave. Is the U-wave part of ventricular repolarization or does it represent some other electrophysiologic process such as ventricular afterdepolarizations (30–32)? If the U-wave is part of ventricular repolarization, then should the QT-U interval be the correct measure of repolarization time? Our data demonstrate that isoproterenol causes an increase in U-wave amplitude that is not observed during exercise or atropine. We suspect that the U-wave differences observed during isoproterenol reflect either a greater degree of beta-adrenergic stimulation or less complete vagal withdrawal as compared with exercise or atropine.

Animal studies have demonstrated that beta-adrenergic stimulation increases both U-waves on the surface electrocardiogram and delayed afterdepolarizations on monophasic action potential recordings (31). Shimuzu and Antzelevitch demonstrated that isoproterenol, through differential effects on action potential duration in the endocardium and epicardium, alters T-wave morphology on a simulated surface ECG (33). Such transmural heterogeneity on action potential duration could account for the complex T-wave and U-wave morphologic changes observed with isoproterenol infusion in our study. The molecular mechanism for these effects on action potential is not fully understood; however, beta-adrenergic stimulation is known to increase cell membrane calcium conductance and intracellular calcium levels in cardiac myocytes (34,35). These effects may increase both action potential duration and the amplitude of afterdepolarizations (27). Further investigation is required to understand the cellular electrophysiologic processes responsible for the generating the U-wave. Given the effects of isoproterenol on T and U waves, the isoproterenol model may be useful in future studies aimed at better defining the physiologic meaning of the U-wave.
Gender differences in HR-QT behavior. Women demonstrated a greater extent of QT shortening for all three interventions. At baseline, women had longer QTc intervals than men. During all three interventions, the HR-QT slope tended to be steeper for women as compared to men, consistent with other studies (22). The gender difference in HR-QT slopes was statistically significant in the case of atropine, and there were strong trends in the same direction for isoproterenol and exercise. The mechanism of greater exercise-associated QT shortening in women remains unclear. However, our data suggest that this phenomenon is present during three diverse autonomic states.

Implications for clinical interpretation of QT intervals. QT prolongation during isoproterenol has been reported in patients with the long QT syndrome (36,37). In fact, several groups have proposed that isoproterenol may be useful to “unmask” a long QT in suspected patients without manifest QT prolongation on their resting ECG (36,38,39). Although isoproterenol infusion has been proposed as a potential screening tool (11), data from studies of healthy volunteers have raised the concern that isoproterenol induced QTc prolongation can occur in healthy individuals as well (40,41). The present data substantiate this concern, demonstrating that the QT shortens less during isoproterenol as compared with other interventions that increase HR in normal individuals. Isoproterenol provocation would only be a useful diagnostic test for evaluation of repolarization time if this normal response were systematically compared to that of patients with specific forms of the long QT syndrome.

Methodologic issues in QT interval measurement. The methods used in measuring QT are critically important to ensure accuracy and reproducibility. The standard Marquette MAC-12 QT measurement algorithm, a first-derivative threshold method, was not used because it was extremely sensitive to U-waves (42) (personal communication, P. Elko, Global Products Manager, GE-Marquette, Milwaukee, Wisconsin, 2000). This method frequently measured the end of the U-wave rather than T-wave in leads with large U-waves. We used individual-lead QT measurement using the least-squares method (13,15), which more consistently distinguished between T-wave and U-wave. The potential for U-wave inclusion demonstrates the importance of verifying automated measurements of QT, particularly when prominent U-waves are present as is the case with isoproterenol.

Conclusions. In contrast to conditions under exercise and with atropine, isoproterenol is associated with much less QT shortening as HR increases. In addition, isoproterenol is associated with complex morphological changes of the U-wave, which may contribute to prolongation of the QT at high HRs. Our findings demonstrate that autonomic conditions directly affect the ventricular myocardium causing differences in QT that are independent of HR and may confound the clinical assessment of cardiac repolarization time.

REFERENCES