Cardiac changes during arousals from non-REM sleep in healthy volunteers

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Cardiac changes during arousals from non-REM sleep in healthy volunteers. Am J Physiol Regul Integr Comp Physiol 292: R1320–R1327, 2007. First published November 16, 2006; doi:10.1152/ajpregu.00642.2006.—Our aim was to evaluate cardiac changes evoked by spontaneous and sound-induced arousals from sleep. Cardiac responses to spontaneous and auditory-induced arousals were recorded during overnight sleep studies in 28 young healthy subjects (14 males, 14 females) during non-rapid eye movement sleep. Computerized analysis was applied to assess beat-to-beat changes in heart rate, atrio-ventricular conductance, and ventricular repolarization from 30 s before to 60 s after the auditory tone. During both types of arousals, the most consistent change was the increase in the heart rate (in 62% of spontaneous and in 89% of sound-induced arousals). This was accompanied by an increase or no change in PR interval and by a decrease or no change in QT interval. The magnitude of all cardiac changes was significantly higher for tone-induced vs. spontaneous arousals (mean ± SD for heart rate: +9 ± 8 vs. +13 ± 9 beats per min; for PR prolongation: 14 ± 16 vs. 24 ± 22 ms; for QT shortening: −12 ± 6 vs. −20 ± 9 ms). The prevalence of transient tachycardia and PR prolongation was also significantly higher for tone-induced vs. spontaneous arousals (tachycardia: 85% vs. 57% of arousals, P < 0.001; PR prolongation: 51% vs. 25% of arousals, P < 0.001). All cardiac responses were short-lasting (10–15 s). We conclude that cardiac pacemaker region, conducting system, and ventricular myocardium may be under independent neural control. Prolongation of atrio-ventricular delay may serve to increase ventricular filling during arousal from sleep. Whether prolonged atrio-ventricular conductance associated with increased sympathetic outflow to the ventricular myocardium contributes to arrhythmogenesis during sudden arousal from sleep remains to be evaluated.

METHODS
Subjects
In total, 14 males and 14 females participated. All subjects were nonsmokers, nonlesnors, took no regular medications, and had no auditory, cardiovascular, respiratory, or sleeping problems. All females were studied in the follicular menstrual phase. The study conformed with the principles outlined in the Declaration of Helsinki and was approved by the Repatriation General Hospital Research and Ethics Committee. All volunteers provided written consent after being fully informed regarding the nature and risks of the study. Subjects attended the laboratory ~2 h before their normal reported bedtime (range 9:30 to 12:30 PM), having abstained from caffeine for at least 8 h.

Data Collection, Experimental Protocol, and Data Analysis
Sleep parameters, including two EEGs (C4-A1, C3-A2), left and right electrooculograms, and submental electromyograms (from the skin area under the chin) were continuously recorded via a Compucare S-series system (Abbotsford, Victoria Australia). In each subject, a ECG signal (lead II) was amplified, band-pass filtered (0.3–30 Hz, Compupedics S series), and recorded using a 1-kHz sample rate.
To elicit sound-induced arousals, auditory tones (0.5 s, 1 kHz, range 55–90 dB) were administered throughout the night via ear-insert headphones. Tones were presented during the expiratory phase of ventilation after at least 5 min of sleep after any period of wakefulness (EEG defined wake lasting >15-s) and at least 2 min of stable sleep without arousal (spontaneous or tone-induced EEG changes lasting 3–15 s). Tone intensity was adjusted in 5-dB increments to achieve as many 3–15 s EEG-defined arousals as practical.

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A single skilled technician, blinded to all but the conventional sleep recordings (EEG, EOG, and EMG) determined sleep stage in 30-s epochs according to standard criteria (25). The same technician identified all 3–15 s EEG defined arousal events, according to the American Sleep Disorders Association’s criteria (1).

ECG Analysis

For each subject, three spontaneous and three tone-induced arousal events recorded during stable stage 2 sleep were selected at random for detailed ECG analysis. Digital ECG recordings from 30 s before to 60 s after the point of onset of EEG-defined arousal event were analyzed using custom-written software in IgorPro (WaveMetrics, Lake Oswego, OR). Our algorithm for ECG wave detection consisted of the following steps (Fig. 1A): a, manual setting of the threshold (above the peak of T-wave but below the peak of the R-wave); b, manual setting of time points for computing the baseline before P-wave (b1) and after T-wave (b2); c, detection of R-wave peak of the first ECG cycle; d, detection of Q-wave peak; e, computing the baseline voltage before P-wave; f, detection of the P-wave peak; g, detection of the P-wave onset (computed as a time when the voltage exceeded 5% of the P-wave amplitude); h, detection of the baseline after T-wave; i, detection of the T-wave peak; k, detection of the T-wave end (computed as a time when the voltage dropped to 5% of the T-wave amplitude); l, shift to the next ECG cycle. On the termination of the computation, the software returned the text values (amplitude and time) for each T-wave, and raw ECG trace with automatically generated markers at P-wave onset, Q-, R- and T-peaks, and T-wave end.

Gender, arousal type, and time-dependent effects on heart rate, PR, and QT intervals were examined using one-way ANOVA for repeated measures using values recorded at 2-s intervals between arousal onset (time zero) and 20-s post-arousal, with arousal type and time as repeated factors within subjects. A 2-s interval was chosen to allow time effects to be examined over the 20-s postarousal period, while keeping the number of replicate measures within limits compatible with ANOVA for repeated measures. Greenhouse-Geisser adjusted P values < 0.05 were considered significant.

In addition to the ANOVA of group data, we used the cumulative sum method (10) to detect statistically significant deviations from baseline within each individual data trace. By examining each ECG trace in this way, the incidence and magnitude of the postarousal changes in ECG parameters were computed. Linear regression was used to assess dependence between the amplitudes of arousal-induced changes in the ECG interval, and Chi-square tests were used to examine differences in their incidence. Data were analyzed using StatView 5.0 (SAS Institute, Cary, NC). Group data are reported as means ± SD. P < 0.05 was considered significant.

RESULTS

Subject and EEG Arousal Characteristics

The subjects were young and of normal weight for height and did not differ in age or body mass index between genders (Table 1). The tone intensity associated with all tone-induced arousals was 63 ± 10 dB (range 58–69 dB) and was not different between genders (P = 0.846). The duration of arousal-related EEG changes was nearly identical for tone-induced vs. spontaneous arousals (7.0 ± 1.1 vs. 6.9 ± 1.0 s), and there were no gender or gender × arousal type interaction effects (P = 0.254 and P = 0.717, respectively).

Basal ECG Parameters

Values for the basal HR and for the PR and QT intervals are presented in Table 2. Females had a higher heart rate, longer QTcorr interval, and shorter Tpeak – Tend duration compared with males, but PR and QT intervals did not differ between males and females.

Changes in ECG Indices Associated With Arousal

As shown graphically in Fig. 2, there were strong time-dependent effects of arousal on heart rate (P < 0.001), PR (P = 0.006), and QT (P < 0.001) interval responses in the 20-s postarousal. There were also significant arousal type × time-

Table 1. Subject characteristics

<table>
<thead>
<tr>
<th></th>
<th>Males</th>
<th>Females</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>26.9 (7.3)</td>
<td>23.6 (6.3)</td>
<td>0.223</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>78.2 (11.2)</td>
<td>66.0 (11.4)</td>
<td>0.008</td>
</tr>
<tr>
<td>Height, cm</td>
<td>184.3 (6.2)</td>
<td>167.5 (5.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>23.0 (2.5)</td>
<td>23.4 (3.1)</td>
<td>0.666</td>
</tr>
</tbody>
</table>

Values are expressed as means (SD). n = 14 males and 14 females. BMI, body mass index.
dependent interaction effects for heart rate (P < 0.001) and QT interval (P = 0.014) and a trend for a similar interaction for PR interval (P = 0.093), with generally smaller responses for spontaneous compared with tone-induced arousals. The effect of gender was not significant, and there was no gender × arousal-type interaction. Arousals had no effect on Tpeak – Tend or late T-wave area.

Histograms showing the distribution of arousal-related ECG changes are shown in Fig. 3. For both types of arousal, the predominant HR response was transient increase in the HR (62% of spontaneous arousals and 89% of tone-induced arousals). When PR intervals changed (27% of spontaneous arousals and 44% of tone-induced arousals), the predominant direction of this change was prolongation. When QT intervals changed,
(31% of spontaneous arousals and 40% of tone-induced arousals), this was predominantly shortening. Of 54 arousals in which the QT interval shortened, this shortening started on the same cardiac cycle \((n = 6)\), one cycle after \((n = 32)\), or one cycle before \((n = 4)\) the beginning of changes in the R-R interval. The magnitude of changes in ECG intervals are presented in Table 2, and individual examples of arousal-induced ECG changes are illustrated in Fig. 4.

The pattern of arousal-related ECG changes varied between subjects, but for a given type of arousal, the pattern remained consistent within subjects. Linear regression analysis revealed that in females, but not in males, magnitude of tachycardic responses correlated with QT interval shortening, as illustrated in Fig. 5. This was true for both types of arousals. For both genders, there was no dependence between changes in HR and PR interval or between PR and QT intervals changes.

In some instances, PR interval prolongation was associated with transient changes in P-wave morphology (inversion, increase, or decrease in amplitude), as illustrated in Fig. 6. This happened during 11 sound-induced arousals and during 3 spontaneous arousal episodes. P-wave changes were of different duration (sometimes lasting just two or three beats) and clearly were not accompanied by changes in R- or T-wave morphology. In three cases, inversion or decrease of P-wave amplitude was associated with a small transient fall in R-wave amplitude (Fig. 6D).

The mean latency to QT shortening \((3.6 \pm 0.9 \text{ s})\) did not differ from the mean latency to HR increase \((4.1 \pm 0.9 \text{ s})\). The latency to PR interval change was longer \((5.2 \pm 1.3 \text{ s})\) and was significantly different from both HR and QT latency responses \((P < 0.01)\) (Table 2).

**Spontaneous Versus Tone-Induced Arousals**

The incidence of transient tachycardia and PR prolongation was significantly higher during tone-induced compared with spontaneous arousals \((P < 0.001; \text{PR prolongation: } P < 0.05)\). The incidence of QT interval shortening was not different between the two arousal types \((P = 0.277)\).

HR changes during tone-induced arousals were larger compared with spontaneous arousals \((+13 \pm 9 \text{ vs. } +9 \pm 8 \text{ beats per minute, } P < 0.05, n = 28)\) when measured for the whole experimental group. A small number of bradycardic responses did not affect this relationship. For all cases in which PR interval transiently increased, this increase was larger for tone-induced compared with spontaneous arousals \((+25 \pm 17 \text{ vs. } +14 \pm 11 \text{ ms, } P < 0.05)\). The magnitude of QT interval shortening was also substantially larger during tone-induced than spontaneous events \((-20 \pm 8 \text{ vs. } -12 \pm 5 \text{ ms, } P < 0.01)\).

**DISCUSSION**

This is the first study with a detailed beat-to-beat analysis of changes in atrio-ventricular conductance and ventricular repolarization (as detected by PR and QT interval duration) during arousal from sleep in humans. Our major aim was to test
whether arousal provokes any changes in these parameters. The most important and novel observation is that arousal-induced transient tachycardia often was associated either with a paradoxical prolongation of the PR interval, or with a rate-independent shortening of the QT interval, or with both. These results suggest that the cardiac pacemaker region, conducting system, and ventricular myocardium may be under independent neural control.

Mechanisms of Arousal-Induced Cardiac Changes

Several previous studies have examined cardiovascular changes during stimulus-evoked and spontaneous arousal from sleep in healthy humans (5, 7, 8, 17, 19, 22), and our observation of transient tachycardia shortly after an arousal stimulus is in full agreement with these reports. The short latency of cardiac changes observed in this and previous studies clearly indicates that these are neurally mediated responses.

In many instances increases in HR were associated with a transient QT interval shortening. QT interval is known to depend on HR, and shortens as HR increases. This rate-dependent shortening is not instantaneous and requires at least several cardiac cycles to develop. In our study, QT shortening occurred during the same or the subsequent cardiac cycle as an increase in HR, indicating that transient tachycardia was not the cause of QT alterations. Most likely, QT shortening reflects a genuine decrease in myocardial repolarization time elicited by sympathetically released noradrenaline.

Tachycardia is usually associated with a speeding in atrioventricular (AV) conductance that is reflected by PR interval shortening. Lack of such shortening in our subjects could mean that either relevant neural pathways were not activated or that PR changes were too small for detection. Our most interesting finding is that in many instances arousal-induced transient tachycardia was associated with PR interval prolongation, which was sometimes quite substantial. PR prolongation could not be attributed to software detection artifacts, as each ECG record was inspected visually following automatic processing. It is possible that PR prolongation was secondary to the rise in heart rate, a phenomenon well documented in humans during rapid atrial pacing (6, 20, 23). However, in this case, one would expect a correlation between the increase in HR and PR interval prolongation, and this was not observed in our study. Another possibility, albeit speculative, is that heart rate and AV conductance are under independent neural control.

Differences Between Spontaneous and Induced Arousals and Gender Differences

The intensity and incidence of cardiac changes were substantially higher during tone-induced arousals, which is possibly indicative of their adaptive role. Although spontaneous

Fig. 4. Changes in heart rate (HR), PR, and QT intervals during sound-induced arousals from sleep. A: illustration of changes in HR, PR, and QT observed in three different subjects (a, b, and c). Dashed lines indicate the time of acoustic stimulus. Bottom panels depict raw ECG traces from the case Aa. Dashed line in Ba and Bb is the same ECG complex recorded at 28 s, just before the arousal sound. Solid lines-ECG complexes with maximal QT (Ba) and PR (Bb) changes, recorded at times corresponding to the peaks on PR and QT traces, respectively, in Aa. Duration of all traces in B is 1 s.
awakening may or may not be associated with the onset of physical activity, forced arousal may indicate immediate threat. During the brief period after arousal from sleep, the normal baroreflex is suspended (31), perhaps in preparation for a flight-or-fight response. Horner (14) presents a view that the process of arousal represents a distinct physiological state, with reduced gating of sensory information. In this regard, it is interesting to note that auditory stimuli presented to awake volunteers habituate and produce much smaller cardiovascular effects compared with the situation when these same subjects are asleep (22).

Given that arousal-induced ventilatory changes followed a similar time course and were also larger after tone-induced compared with spontaneous arousals (15), it is possible that cardiac arousal responses are partly mediated by intrathoracic pressure and more delayed chemoreflex changes associated with the ventilatory arousal response. However, the very short latencies to cardiac changes as reported here indicate that they were unlikely to be secondary to ventilatory effects.

In full accord with a previous report by Lanfranchi et al. (18), we found that basal HR was higher, and basal QTcorr was longer in females compared with males. Interestingly, females had slightly shorter basal Tpeak – Tend interval. It is currently unknown whether this parameter is rate dependent (like QT-interval), making our finding difficult to interpret. The only gender-related difference in arousal-induced cardiac responses was that in females, but not in males, the amplitude of tachycardic response correlated with QT-interval shortening, for both spontaneous and induced arousals. The reasons for such differences are unclear and may at least, in part, reflect other cardiovascular influences unrelated to gender (e.g., differences in cardiovascular fitness). These data further support the growing evidence of gender differences in cardiovascular reactivity (9).

Relevance to Mechanisms of Arrhythmogenesis

Several types of cardiac arrhythmias are clearly associated with the sleep state (see Ref. 28 for a review). Sudden arousal from sleep by an alarm clock may precipitate potentially fatal polymorphic ventricular tachyarrhythmias (“torsades de pointes”) in patients with congenital long QT syndrome (29, 30). Sound-induced arousal from sleep is now a recognized arrhythmia trigger for the subjects with the LQT3 subtype of this syndrome (as opposed to LQT1 subtype in which physical exercise is a major trigger), and it is even recommended that they remove alarm clocks and phones from their bedrooms (26). Our present results indicate that sudden arousals possibly enhance myocardial noradrenaline release, a prerequisite for arrhythmogenesis (27).

Several previous studies have also reported an association between obstructive sleep apnea and cardiac arrhythmias (11, 13, 16). It may be that these arrhythmias are related, at least in part, to numerous arousals occurring during sleep in OSA patients. Such arousals are accompanied with changes in HR, albeit under quite different hemodynamic and ventilatory conditions.

It is unknown whether the changes in AV conductance reported here during arousal represent an additional proarrhythmic factor. We speculate that during increased sympathetic outflow to the ventricular myocardium, prolonged AV delay may potentially contribute to arrhythmogenesis by regarding the arrival of the normal ventricular excitation wave, thereby extending the “vulnerable” diastolic period.

We did not find any arousal-induced changes in Tpeak – Tend and late T-wave area, ECG indices of transmural dispersion of repolarization in the ventricular myocardium (32). This may indicate that either these changes are too small to be detected.
by our method or that in healthy individuals, sound-induced arousals from sleep do not affect transmural dispersion of repolarization.

We observed quite substantial interindividual variability in the magnitude of cardiac responses during arousals. It may be that more reactive individuals are at greater risk of developing cardiac arrhythmias compared with less reactive individuals. If so, assessment of cardiac reactivity during arousals from sleep may prove to be a useful approach for risk stratification.

**Perspectives**

At present, we can only speculate regarding the mechanisms underlying arousal-related changes in AV conductance. It is possible that arousal resulted in altered autonomic neural outflow to the AV node. Two possibilities exist. First, it may be that sympathetically activated transient tachycardia and shortening of ventricular repolarization were associated with increased vagal outflow to the conducting structures of the heart, resulting in an increase of the PR interval. Second, it is possible that a transient increase of autonomic outflow to the atria resulted in a spatial shift of the pacemaker active site (2, 4), so that the distance from this site to the AV node increased.

Transient changes in P-wave morphology in some of our subjects could represent a surface ECG manifestation of such a pacemaker shift. In most instances, these P-wave changes were not associated with any alterations in the shape or amplitude of the QRS complex or T-wave (Fig. 3, A–C), and it is thus unlikely that they were caused by respiration-related changes in cardiac axis. Clearly, further experiments with parasympathetic blockade are required to elucidate mechanism of the AV conductance slowing.

Whether arousal-induced increase in AV delay is a previously unknown adaptive physiological phenomena or a sign of pathology remains an open question. Auditory-induced arousals during non-rapid eye movement sleep are associated with a substantial fall in stroke volume, so that cardiac output also falls despite tachycardia (22). It thus seems entirely reasonable to suggest that in the physiologically alarmed state, such as the transition from sleep to wakefulness evoked by potentially dangerous external events, a longer interval between atrial and ventricular contractions may improve ventricular filling and thus counteract the decrease in the cardiac output.

The magnitude of the arousal-induced tachycardic response did not correlate with changes in AV conductance. Also, in

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**Fig. 6. Changes in P-wave morphology during arousals.** A–D: ECG records from four different subjects during sound-induced arousals from sleep. Dashed lines indicate the time of acoustic stimulus. Note that arousal was associated with P-wave inversion (A) or decrease (B) or increase (C and D) in the P-wave amplitude. E: EEG trace during arousal, with a typical K-complex and transient appearance of alpha rhythm.
males, change in heart rate did not correlate with changes in cardiac repolarization. Furthermore, in many instances, increase in heart rate was the only observable cardiac response to arousal. These results suggest that the cardiac pacemaker area, conductive system, and ventricular myocardium are controlled independently, possibly with simultaneous increase of sympathetic outflow to the pacemaker area and to the myocardium, and of vagal outflow to the conductive system. This, in turn, may indicate that separate subpopulations of cardiomotor neurons in the brain stem are responsible for the control of chronotropic, dromotropic, and inotropic function. Although such a possibility remains speculative with regard to sympathetic control, Gatti et al. (12) reported that functionally distinct preganglionic vagal motoneurons in the nucleus ambiguus independently control cardiac rate and AV conduction. Importantly, coactivation of vagal and sympathetic outflow to the heart was noted in several studies, in which the functional significance of such coactivation often remained unexplained [see review by Paton et al. (24)].

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