Relationship between blood pressure, sleep K-complexes, and muscle sympathetic nerve activity in humans

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Tank, Jens, Andre Diedrich, Nanette Hale, Faiz E. Niaz, Raffaello Furlan, Rose Marie Robertson, and Rogelio Mosqueda-Garcia. Relationship between blood pressure, sleep K-complexes, and muscle sympathetic nerve activity in humans. Am J Physiol Regul Integr Comp Physiol 285: R208–R214, 2003; 10.1152/ajpregu.00013.2003.—Stage 2 sleep is characterized by the EEG appearance of “K-complexes” and blood pressure oscillations. K-complexes may be directly related to blood pressure changes or they may reflect central sympathetic activation. We analyzed the temporal relationship among K-complexes, heart rate (HR), blood pressure (BP), and muscle sympathetic nerve activity (MSNA) during sleep in eight healthy volunteers (3 men and 5 women, age 22–41 yr). Most K-complexes presented as single large complexes (56 ± 20%), followed by single small complexes (15 ± 14%) and as couplets or triplets (13 ± 6%). Single large K-complexes were preceded by a baroreflex-mediated increase of MSNA in approximately one-half (55%) of the cases. Detailed analysis of HR, BP, and MSNA was possible in 63 (45%) large single K-complexes not disturbed by preceding baroreflex-related changes. Systolic and diastolic BP and MSNA increased significantly after single events (22.5 ± 13, 5.2 ± 2.1, and 6.5 ± 3.0%). Mean sympathetic baroreflex latency was similar after the single large K-complexes compared with the mean value during stage 2 sleep (1,290 ± 126 vs. 1,279 ± 61 ms). The area under the burst was significantly increased after single large K-complexes (median 3.9 vs. 9.0 arbitrary units, P < 0.03). The results support the hypothesis that K-complexes express cortical activation leading to temporary facilitation of sympathetic outflow in a graded fashion. Their functional effects appear to be independent of baroreflex modulation of MSNA in ~50% of the cases.

cardiovascular physiology; autonomic nervous system; baroreflex

BLOOD PRESSURE (BP) regulation, particularly the decrease observed during sleep, is important to cardiovascular health (29). The changes in sympathetic activity and BP associated with different stages of the sleep process are complex and not well understood (5, 21, 26). Stage 2 of non-rapid eye movement (NREM) sleep is characterized by the EEG appearance of “sleep spindles” or “K-complexes” (17) and BP oscillations (12, 25). K-complexes are thought to be an expression of cortically generated slow oscillations of neuronal activity that may trigger or group other oscillations (7, 12, 20). However, the exact source of the K-complexes remains controversial (6, 10, 20, 27), with some evidence indicating a close relationship with autonomic function (7). K-complexes often occur synchronously with Mayer BP waves (12). Similarly, a concomitant increase in muscle sympathetic nerve activity (MSNA) with a latency of <1 s (5, 30) has been temporally associated with K-complexes. This MSNA latency is significantly shorter than the usual 1.0- to 1.5-s sympathetic reflex latency observed for this type of sympathetic fibers in the awake state and has been ascribed to a direct non-baroreflex-mediated activation of sympathetic centers that may reflect the effect of arousal stimulation on MSNA (5).

Two potential explanations have been advanced to explain the temporal association between MSNA and K-complexes. First, K-complexes may exclusively result from BP changes (12). Alternatively, K-complexes may reflect central sympathetic activation increasing MSNA independent of BP changes (5). However, studies (5, 12, 21, 26) supporting the second hypothesis have used indirect methods of BP determinations raising the possibility that changes in hemodynamics may have not been detected by these indirect methods of BP recording. In the present study, we tested the hypothesis that selected K-complexes not superimposed by Mayer BP waves induce changes in MSNA by measuring the temporal association among BP, MSNA, and heart rate (HR) in relationship to these K-complexes during stage 2 sleep in normotensive healthy volunteers using continuous intra-arterial recordings of BP.

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MATERIALS AND METHODS

Subjects

We studied eight normal subjects (5 women and 3 men, age 22–41 yr, body mass index 25 ± 4 kg/m², BP 125 ± 11/64 ± 5 mmHg, and HR 60 ± 12 beats/min) after obtaining their informed written consent. All abstained from caffeine-containing products and smoking for at least 3 days before the procedures. The subjects had negative medical histories and normal physical examinations. None had a sleep disorder. All subjects were given a 150 meq sodium and 70 meq potassium diet for at least 3 days before the study to reduce the influence on autonomic regulation. The subjects were admitted to the Vanderbilt General Clinical Research Center for two consecutive study nights.

Instrumentation and Data Acquisition

Polysomnography. EEG (with surface electrodes placed at the C4/A1, C3/A2, or O2/A1, O1/A2 points) recordings, eye movement recordings (left occulogram (LOG) and right occulogram (ROG), 1 cm above and below the outer canthi, respectively, against the reference electrode), electromyography at the C4/A1, C3/A2, or O2/A1, O1/A2 points) recordings, eye movements, and the use of the microneurography technique, as described previously (13, 15). In brief, the leg was immobilized with an orthopedic device and securely positioned to maximize successful recording. A unipolar tungsten electrode ( uninsulated bulb diameter of 1–5 μm) was inserted into the muscle nerve fascicles of the peroneal nerve at the fibular head for multiunit recordings. Nerve activity was amplified with a total gain of 100,000 and bandpass filtered (0.7–2 kHz). After rectification and integration (time constant 0.1 s) the integrated MSNA was obtained as mean voltage neurogram using a signal integrator (Univ. of Iowa, Biomedical Engineering Department) (3).

Respiratory movements, BP, and a second surface EKG were preamplified and filtered with the use of a Gould system (Gould Instrument Systems; Cleveland, OH) and then connected to an analog-to-digital converter for registration. The digital conversion was performed with a 14-bit analog-to-digital card (DAS 410, Datqa Instruments; Akron, OH) at 500 Hz by using the Windaq Pro+ software (Datqa Instruments) on a personal computer. The biosignals recorded with the two separate computers were synchronized using two separate EKG channels.

Experimental Protocol

Subjects were investigated in a comfortable supine position at a room temperature of 21–25°C during two consecutive nights. The first night was used for adaptation to the procedures. On the second night, the subjects were instrumented for polysomnography, intra-arterial BP measurements, and microneurography recordings. After 20 min of rest and a baseline recording of at least 15 min in the awake state, the subjects were allowed to sleep (~11 PM). Recordings were performed continuously irrespective of the specific sleep stage in a quite isolated room. The studies were terminated after several hours of recording (typically between 2 and 3 h of sleep).

Data Analysis

Sleep staging. The sleep stages were scored by two investigators for the complete records in 30-s epochs according to the criteria of Rechtschaffen and Kales (23) with the use of TWin software version 1.5 (Telefactor). Experiments during which subjects reached only stage 1 sleep were excluded from the analysis (one case).

EEG, BP, and MSNA analysis. The R-R intervals, diastolic BP (DBP), and systolic BP (SBP) values were defined with the use of PV-wave 6.1 software (Visual Numerics; Houston, TX). MSNA bursts were identified after filtering the signal and defining the baseline according to following criteria: 1) signal to noise ratio >2.5; 2) characteristic shape of the burst for MSNA compared with skin sympathetic nerve activity (skewness of the rising and falling parts of the bursts, width of the burst); 3) sympathetic baroreflex latency limit; 4) no preceding premature beats. The number of bursts per minute per 100 heart beats, the mean area under the burst, and the normalized mean area under the burst were calculated. To define the time delay between the decrease in BP and the following baroreflex-mediated increase in MSNA (sympathetic baroreflex latency) the time interval between the maximum of the burst and the corresponding R-peak in the EKG was determined. For the analysis of mean values, 5-min sequences toward the end of each stage 2 sleep were chosen for each volunteer.

K-complexes were marked manually in the EEG. Subsequently, K-complexes were subdivided as large K-complexes, multiple K-complexes, and small single K-complexes (5, 17). Large single K-complexes were defined as those that had a sharp onset, a duration of >0.5 s, no preceding or following premature beats, and no preceding or following other K-complexes within 8 s (5). Multiple K-complexes were defined as series of K-complexes. Small single K-complexes were defined as single K-complexes that did not fulfill the criteria for large K-complexes.

R-R interval, SBP, DBP, and MSNA were analyzed for four cardiac cycles before the large single K-complex and eight cardiac cycles after the large single K-complex as absolute values and in percent of the four preceding intervals. Differences were analyzed by using the Student t test. Clinical experiments (n = 3 subjects) were carried out with each of the subjects. The mean values were obtained from 5-min sequences toward the end of each stage 2 sleep.

Table 1. Heart rate, blood pressure, and muscle sympathetic nerve activity during stage 2 sleep compared with awake state

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Awake</th>
<th>Stage 2 Sleep</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR, beats/min</td>
<td>60 ± 12</td>
<td>56 ± 10*</td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>126 ± 11</td>
<td>123 ± 14</td>
</tr>
<tr>
<td>DBP, mmHg</td>
<td>64 ± 5</td>
<td>62 ± 5</td>
</tr>
<tr>
<td>Bursts/100 beats</td>
<td>30 ± 18</td>
<td>31 ± 19</td>
</tr>
<tr>
<td>Norm. area</td>
<td>9 ± 5</td>
<td>11 ± 8</td>
</tr>
</tbody>
</table>

Values are means ± SD. HR, heart rate; SBP, systolic arterial blood pressure; DBP, diastolic arterial blood pressure; norm. area, normalized area under the bursts. * Significant changes vs. baseline; $P < 0.05$, Wilcoxon-signed rank test.
ences between measurements were tested with one-way ANOVA for repeated measures. If the distribution was not Gaussian (Kolmogorov-Smirnov Test), the nonparametric Wilcoxon's matched pairs signed rank test was used. Results are presented as means ± SD. Nonparametric statistics are presented as median values. Significant differences were considered when $P < 0.05$.

RESULTS

In the presence of invasive monitoring for BP and microneurography, all volunteers except one reached stage 2 sleep, five reached stage 3 or 4 sleep, and no subject reached REM sleep. Therefore, we were able to analyze HR, intra-arterial BP, and MSNA responses after K-complexes during stage 2 sleep in 7 subjects. Mean HR was reduced during stage 2 sleep compared with baseline (56 ± 10 beats/min, $P < 0.05$). SBP and DB were similar during stage 2 sleep compared with the awake state (123 ± 14/62 ± 7 mmHg). The number of MSNA bursts per 100 heart beats and the normalized mean area under the burst did not change significantly (Table 1) during stage 2 sleep compared with baseline. However, BP and MSNA were significantly reduced during stage 3 and 4 sleep (Fig. 1).

Single and multiple K-complexes temporally associated with MSNA bursts occurred frequently during stage 2 sleep in all volunteers, but with high individual variability (Table 2). The majority of K-complexes presented as single, large complexes (56 ± 20%), followed by single, small complexes (15 ± 14%), and as couplets or triplets of K-complexes (13 ± 6%) (Fig. 2). Fifty-five (55%) of the single large K-complexes were superimposed by baroreflex-mediated sympathetic activation (Fig. 3). Sixty-three (45%) of the single large K-complexes were not associated with preceding changes in BP or baroreflex-related increases in MSNA (Fig. 4). MSNA (22.5 ± 13%), SBP (5.2 ± 2.1%), and DBP (6.5 ± 3.0%) increased significantly after single large K-complexes, compared with the values measured before the event. A prolonged R-R interval was observed at about three heartbeats after the onset of

Table 2. Absolute number of different K-complexes detected during stage 2 sleep

<table>
<thead>
<tr>
<th>ID</th>
<th>Events</th>
<th>Series</th>
<th>Couplet</th>
<th>Triplet</th>
<th>Small K-c</th>
<th>Large K-c</th>
<th>BP Decrease</th>
<th>Arousal</th>
<th>Analyzed</th>
</tr>
</thead>
<tbody>
<tr>
<td>BB</td>
<td>61</td>
<td>12(20)</td>
<td>9(15)</td>
<td>14(23)</td>
<td>26(43)</td>
<td>38(62)</td>
<td>8(13)</td>
<td>15(25)</td>
<td></td>
</tr>
<tr>
<td>PG</td>
<td>39</td>
<td>0(0)</td>
<td>3(8)</td>
<td>2(5)</td>
<td>34(57)</td>
<td>20(51)</td>
<td>3(8)</td>
<td>13(33)</td>
<td></td>
</tr>
<tr>
<td>LS</td>
<td>21</td>
<td>0(0)</td>
<td>2(10)</td>
<td>8(38)</td>
<td>11(52)</td>
<td>11(52)</td>
<td>0(0)</td>
<td>9(43)</td>
<td></td>
</tr>
<tr>
<td>ML</td>
<td>23</td>
<td>18(35)</td>
<td>5(22)</td>
<td>4(17)</td>
<td>6(26)</td>
<td>11(78)</td>
<td>5(22)</td>
<td>4(17)</td>
<td></td>
</tr>
<tr>
<td>HD</td>
<td>23</td>
<td>0(0)</td>
<td>2(9)</td>
<td>7(30)</td>
<td>14(61)</td>
<td>11(48)</td>
<td>0(0)</td>
<td>5(22)</td>
<td></td>
</tr>
<tr>
<td>DK</td>
<td>30</td>
<td>2(7)</td>
<td>4(13)</td>
<td>22(73)</td>
<td>14(47)</td>
<td>4(13)</td>
<td>7(23)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GA</td>
<td>56</td>
<td>18(32)</td>
<td>10(18)</td>
<td>0(0)</td>
<td>28(50)</td>
<td>30(54)</td>
<td>2(4)</td>
<td>10(18)</td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>253</td>
<td>40(16)</td>
<td>33(13)</td>
<td>39(15)</td>
<td>141(56)</td>
<td>135(53)</td>
<td>22(9)</td>
<td>63(25)</td>
<td></td>
</tr>
</tbody>
</table>

ID, volunteers initials; events, number of all detected EEG events during stage 2 sleep; series, more than three K-complexes; couplet, triplet, couplets or triplets of K-complexes; small K-c, single small K-complex; large K-c, single large K-complex; BP decrease, preceding decrease in diastolic blood pressure; arousal, K-complexes followed by awakening; analyzed, number of single, large K-complexes suitable for further detailed analysis. Percentage is given in parentheses.
the single large K-complex (3.0 ± 1.0%). The breathing pattern did not change significantly before, during, or after the occurrence of large single K-complexes. The latency measured between the onset of the single large K-complexes and the maximum of the following MSNA burst varied considerably (minimum 268 ms, maximum 3,750 ms, coefficient of variation 53.8%). The sympathetic baroreflex latency of the bursts immediately after the single large K-complexes (mean value of 1,290 ± 126 ms; minimum 905 ms, maximum 1,495 ms) was similar, compared with the mean sympathetic baroreflex latency also during stage 2 sleep (mean value of 1,279 ± 61 ms; minimum 1,186 ms, maximum 1,375 ms). However, the area under the burst was significantly higher for the MSNA bursts (Fig. 5) after single large K-complexes, compared with the mean value calculated for all bursts occurring during stage 2 sleep (median 3.9 vs. 9.0 arbitrary units·s⁻¹·100; 25–75% percentile 3.1–7.0 vs. 6.9–16.1 arbitrary units·s⁻¹·100, P < 0.03). Sympathetic baroreflex latency during sleep was similar compared with the awake state (mean value of 1,288 ± 61 ms).

**DISCUSSION**

K-complexes are EEG expressions of slow oscillations built upon rhythmic membrane depolarizations and hyperpolarizations of cortical neurons and reflect neuronal excitation (20). The main finding in our study is that not all the K-complexes appear to be secondary to changes in arterial BP because in approximately one-half of the large single K-complexes recorded, there was no preceding increase in MSNA, no preceding decrease in BP, and no subsequent K-complex within 8–10 s. In an earlier study, K-complexes reflected diffuse and variable cortical reaction during...
which large areas of the cortex may be active (20). A possible trigger would be acoustic stimuli or sensory stimuli of unknown origin (19) and unrecorded changes in other hemodynamic parameters, such as changes in central venous pressure.

Detailed analysis of the hemodynamic and micro-neurographic events in our study indicated a clear temporal association between single large K-complexes and MSNA bursts appearance during stage 2 sleep that is in agreement with the results reported by others (5, 21, 26, 28). However, the baroreflex-mediated increase in MSNA occurring frequently during sleep is likely to mask, or even abolish K-complex-evoked effects. Moreover, the simultaneous occurrence of K-complexes and clearly baroreflex-mediated changes in MSNA allowed the analysis of only 45% of the large single K-complexes, which were not preceded by changes in BP. However, this percentage was stable across the investigated subjects. Therefore, the baroreceptor input may modulate the effects of K-complexes on MSNA rather than causing K-complexes directly. Moreover, slow oscillations can trigger sleep rhythms, such as thalamic spindles as well as thalamic and cortical delta oscillations (1, 27).

Previously, some groups have described association of K-complexes with changes in respiration, sweating, and periodic leg movements (9, 11), as well as a relationship with BP oscillations (12). Additional support for the idea that the increase in MSNA after K-complexes during sleep may reflect direct central sympathetic activation comes from the analysis of the sympathetic baroreflex latency (5). The sympathetic baroreflex latency was constant at \( \frac{1}{10} \) s during sleep compared with the awake stage in our study, suggesting that pulse synchronicity was maintained as shown by other authors (5, 28). However, MSNA bursts occurring after single large K-complexes had a significantly increased area under the burst. This finding points to a modulating influence of K-complexes on MSNA, which in cases of very short latencies may even be a stimulus-response relationship between K-complexes and MSNA.

Interestingly, during the awake stage, unlike changes in BP and other hemodynamic factors, arousal stimulation has been reported not to affect MSNA while significantly increasing the activity of skin sympathetic nerve fibers (SSNA) (26, 28). This distinction has been used by some groups as one of the criteria helping to differentiate whether or not the microneurographic recording contains pure MSNA, pure SSNA, or a mixture of both (14, 24). Because bilateral blockade of the glossopharyngeal and vagus nerves or sur-

Fig. 4. Changes of MSNA (A), SBP (B), R-R interval (C), and DBP (D) during four cardiac cycles before the onset of single large K-complexes and eight cardiac cycles after the K-complexes. Values are normalized to the four cardiac cycles before the K-complexes. * \( P < 0.05 \), ANOVA. Means ± SE for 63 analyzed single large K-complexes not preceded by a baroreflex-mediated increase in MSNA.

Fig. 5. Area under the burst calculated in arbitrary units (au) as mean values for all bursts occurring during stage 2 sleep (stage 2) compared with the bursts occurring after single large K-complexes (K-c) in seven healthy volunteers. \( P < 0.03 \), matched-pairs Wilcoxon test. The median values are indicated by the vertical lines.
tween BP fluctuations and MSNA, with a final microelectrode pattern similar to the pattern observed with SSNA, the differences between SSNA and MSNA may be due to modulatory influences from afferent inputs as well as to changes in central integration and processing (4, 21). Similarly, changes in afferent inputs and in central integration and processing may therefore attenuate the differences between MSNA and SSNA during different sleep stages (4).

Moreover, K-complexes during NREM sleep are possibly associated with a slow negative potential related to cognitive process (18). In addition, some authors have proposed that the cardioinhibitory vagal baroreflex is interrupted after spontaneous single K-complexes (25). The results of our study suggest that the cardioinhibitory baroreflex is not invariably interrupted. We advance the idea that frequent and more pronounced increases in HR and BP after K-complexes not damped by the baroreflex may lead to awakening during stage 2 sleep. The habituation of evoked K-complexes to monotonous stimuli (2) during sleep supports the idea that K-complexes may represent a control element that modulates sympathetic activation during sleep in a graded fashion. Our results support the notion that K-complexes may be relevant in BP regulation during NREM sleep (25).


In summary, the present observations suggest that independent spontaneous oscillations of cortical activity influencing different pathways and originating from different sources affect MSNA. Furthermore, K-complexes may be involved in BP regulation during sleep. The nocturnal decrease in BP has a major impact on cardiovascular health. Indeed, absence or diminution of the decrease not only may suggest a secondary form of hypertension, but also predicts cardiovascular risk in its own right (2, 8, 21). The tendency of increased K-complex occurrence leading to a frequent increase in HR, BP, and MSNA may have impact on cardiovascular risk (8, 16, 22, 26).


