Central and Baroreflex Control of Heart Rate During the Wake-Sleep Cycle in Rat

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Abstract: Spontaneous fluctuations in Heart Period (HP) and Mean Arterial Pressure (MAP) make it possible to evaluate baroreceptor-heart rate reflex sensitivity (BRS). 30-s sequences of HP and MAP beat-to-beat values were not considered in the different wake-sleep states (Wake, W; Quiet Sleep, QS; Active Sleep, AS) in rats to assess whether 1) BRS changes between states and 2) the different indexes supply consistent BRS measures. BRS indexes were calculated according to validated literature procedures as regression coefficients of HP vs. MAP 1) within all ramps of increasing or decreasing MAP of four beats or more, with HP and MAP changing in the same direction (baroreflex-mediated fluctuations, BRSP), 2) within all such ramps irrespective of the relative direction of HP and MAP changes (baroreflex + non-baroreflex, i.e. non-homeostatic centrally driven, fluctuations, BRSA), HP vs. MAP regression coefficient along the entire 30-s sequence (bHPMAP) was also calculated. Results: BRSP did not change among states, BRSA decreased from QS to W to AS, bHPMAP decreased from QS to W and became negative in AS. Conclusions: 1) as indicated by BRSP, baroreflex sensitivity is state independent, 2) BRSA to BRSP are increasingly affected by non-baroreflex fluctuations, BRSA being most apt to measure BRS, 3) non-homeostatic MAP and HP fluctuations increase from QS to W and prevail in AS. These potentially harmful fluctuations are normally buffered by baroreflexes: in the case of baroreflex impairment, circulatory risk may arise in conditions like AS, when they prevail.

Key words: Sleep; heart rate; baroreflex; circulation

INTRODUCTION

HEART RATE IS NEURALLY REGULATED THROUGH THE INTERPLAY OF THE AUTONOMIC OUTPUT TO THE HEART AND VESSELS, via either a direct effect on heart rate or an indirect effect mediated by the baroreceptor-heart rate reflex. As a consequence of this regulation, beat to beat values of Arterial Pressure (AP) and Heart Period (HP, the R-R time interval) undergo simultaneous changes, equal or opposite in sign: 1) if the baroreflex influence of changes in AP prevails in HP control, an increase in AP will be accompanied by an increase in HP; 2) if the effects of direct autonomic stimulation on heart and vessels prevail, an increase in AP will be accompanied by a decrease in HP. Thus, sequences of AP and HP values may provide information on the direct effects of centrally driven changes in autonomic output to the heart and vessels and/or the baroreflex mediated AP effects on HP.

Classically, the influence of AP on HP (i.e. the baroreceptor-heart rate reflex and its sensitivity [BRS]), have been studied by applying pharmacological or mechanical treatments affecting AP (for a review, see 1). On the other hand, the analysis of spontaneous sequences of AP and HP may yield useful estimates of BRS, relevant for the heartbeat control in different functional conditions. Evaluating BRS from spontaneous AP and HP fluctuations avoids administration of vasoactive drugs or mechanical maneuvers that, by altering blood pressure, might also alter BRS itself. Moreover, measures can be taken over long periods of time within physiological AP and HP ranges without disturbing the functional condition.

Spontaneous sequences of AP and HP beat-to-beat values have been used to evaluate the sensitivity of the baroreceptor-heart rate reflex.2-4 In these studies, time domain techniques were applied and the BRS indexes considered were validated through surgical or pharmacological procedures. In other studies, frequency domain techniques were also applied5,6 (see Appendix B and C). Whichever the computational procedures adopted, the relation between AP and HP does not depend on the baroreceptor-heart rate reflex alone, but centrally driven influences also affect the relationship between AP and HP, so that it may be difficult to separate baroreflex from non-baroreflex contributions to HP control.

Cardiovascular function is strongly affected by wake-sleep states (W, Wakefulness; QS, Quiet Sleep; AS, Active Sleep) and the relative weight of centrally driven influences may change in different states. The different BRS indexes considered may be differently affected by non-baroreflex, centrally driven, influences acting on HP. As a consequence, if different BRS values are observed in different states, this does not necessarily indicate that a change in baroreceptor sensitivity has occurred. This may account for the divergent results found across species in measuring BRS during sleep: 1) a decrease in BRS in AS vs. QS11 or vs. W12 was reported in cat; 2) no change in AS vs. W13 or vs. QS and W14 was found in lambs; 3) BRS resulted higher in AS with respect to W in humans15-18.

In the present study we analyzed spontaneous sequences of beat-to-beat HP and AP values during W, QS and AS and utilized different time domain indexes of HP vs. AP statistical dependence, in order to gain information on the changes occurring in heart regulation during the wake-sleep cycle. We found that the relative importance of non-baroreflex influences on HP increased in AS with respect to W and QS, while the sensitivity of the baroreceptor-heart rate reflex, BRS, did not change between states. We also showed that the different BRS indexes considered provide complementary information on heart regulation, since they are affected to different extents by baroreflex and non-baroreflex influences on the heart.

Rats were chosen as experimental animals because their high heart rate makes it possible to easily adjust for the time delay...
between changes in AP and HP in the evaluation of HP vs. AP statistical dependence.

METHODS

The following protocol was approved by the Bologna University ethical committee on animal experimentation. The experiments were carried out on six male Sprague-Dawley rats (250-300 g).

Surgery

Under general anesthesia (1% halothane, 30% O2, balance N2O), electrodes were chronically implanted for standard electroencephalographic (EEG) and electromyographic (EMG) recordings. In particular, four miniature stainless steel screws were soldered to copper insulated wire and implanted into the skull (1.0 mm anterior and 2.0 mm lateral to bregma, and 0.0 anterior and 2.0 mm lateral to lambda). In addition, two teflon coated platinum stranded wire electrodes were inserted bilaterally into the dorsal nuchal muscle to record EMG activity. A Silastic catheter (0.30 mm i.d., 0.64 mm o.d) was positioned in the abdominal aorta via the right femoral artery for arterial pressure and heart period measurements and withdrawal of blood samples. Calcium heparin (~15 IU/100g/day) was continuously administered through an osmotic pump (ALZET, Palo Alto, CA) implanted s.c. in the interscapular region.

Recording Procedure

After one week’s recovery, each animal was habituated for one day to the recording apparatus in a thermoregulated box (22°C). Food and water were available ad libitum. On the day of the experiment the arterial catheter was connected to a Statham P23 transducer for pressure signal recording and blood gases were measured (Instrumentation Laboratory, Model 1304). EEG and pressure signals were low pass filtered at 60 Hz, EMG signal at 1 KHz. A personal computer equipped with an analog to digital converter was used for data collection, online analysis, screen display, and storage on hard disk.

Data Analysis

On line analysis included calculating HP and AP (systolic, diastolic and mean arterial pressure) values over each cardiac cycle, as well as their mean values over the last two-second period. EEG spectral components were calculated over the last eight-second period. The wake-sleep state identification was automatically performed algorithmically by the software utilized. The mean value of EEG root mean square over the last two-second period, as well as EEG spectral components over the last eight-second period, were considered. High EMG and high theta/delta ratio indicated W, low EMG and low theta/delta ratio indicated QS, low EMG and high theta/delta ratio indicated AS. Reference values for sleep stage identification were set for each rat during a preliminary session and readjusted at the end of each recording session. State identification was also done by the operator on the basis of the physiological variables displayed on the screen as well as the animal posture and recorded through the keyboard. Both classifications were stored and only periods with concurrent classifications were taken into account in the following offline analysis. Paper recording was also performed (Grass Polygraph). The experimental sessions lasted from 10:00 to 17:00 for five to seven days.

From EEG, EMG, and AP continuous recordings, 30-second artifact-free sequences were selected during W, QS, and AS episodes (in AS, the sequence was positioned at the beginning of the episode) and sequential beat-to-beat values of HP and mean arterial pressure (MAP) were taken into account. MAP instead of systolic or diastolic pressure was utilized in the analysis because, especially in long term recordings, MAP is more reliable than both systolic and diastolic pressures. The 30-second duration was chosen as a compromise between the length of the sequence and the absence in the same sequence of movement artifacts in W and spontaneous awakenings in QS and AS. The selected length of 30-second restricted the analysis to frequencies>0.033Hz, thus neglecting the peak frequency (0.025 Hz) of the open loop gain determined for the baroreceptor-blood pressure reflex in rat by Dworkin et al., but nonetheless encompassing most of the frequency range they considered.

To evaluate the sensitivity of the baroreceptor-heart rate reflex, we chose time instead of frequency domain techniques, because time domain techniques yield separate BRS measures from MAP and HP ramps with positive (baroreflex ramps, BRSP index) and positive+negative (baroreflex+non-baroreflex ramps, BRSA index) HP vs. MAP slope. On the contrary, BRS indexes evaluated in the frequency domain are based on all HP and MAP data in the considered sequence and no separation between baroreflex and non-baroreflex components can be made.

After linear detrending, spectral analysis was performed and the LFHP/HFHP sympathovagal index calculated within each 30-second sequence of MAP and HP beat to beat values (see Appendix A). The sequence of MAP and HP beat to beat values was then low pass filtered and utilized to calculate three time domain indexes of HP vs. MAP statistical dependence (see Appendix B): two baroreflex indexes, BRSP and BRSA, and the regression coefficient of HP vs. MAP fluctuations (bHPMAP). BRSP was evaluated according to2,20,21 by averaging only positive regression coefficients of HP vs. MAP within increasing or decreasing ramps of MAP values. BRSA was evaluated according to3 by averaging all ramp regression coefficients, irrespective of their sign. This was done on the assumption that HP and MAP changes are either baroreflex-mediated changes or they “occur randomly, thus only adding noise to the detection system.” BRSP and BRSA have been validated by surgical and pharmacological procedures (see Appendix C). bHPMAP, at variance with the previous indexes, is based on all the data within each sequence and takes into account all fluctuations occurring within a 30-second period (i.e. within a period much longer than both BRSP and BRSA). A positive significant value in bHPMAP is compatible with a baroreflex link between HP and MAP; a negative significant value is not compatible with either baroreflex or random influences on HP and MAP.

Statistical Analysis

All variables calculated on HP and MAP sequences were averaged over the day and wake-sleep stage, so that for each animal and variable only one value per day per stage was retained for further analysis. Statistical significance of inter-state differences.
was evaluated by two-way ANOVA, with factors Animal (six levels) and Stage (three levels, W, QS and AS), together with modified t-test and Bonferroni's method.22 Association between variables was studied by the linear correlation technique on standardized values (within animal Z-scores) of the different variables.

RESULTS

Mean values of HP, MAP, and the sympathovagal index LFHP/HFHP in the different states of the wake-sleep cycle were presented in Table 1. Values of sympathovagal index are in agreement with the literature.8 Mean values of time domain indexes of statistical dependence of HP on MAP (i.e. baroreflex indexes BRSp and BRSA, as well as the regression coefficient of HP vs. MAP fluctuations bHHP/HFHP), are reported in Table 2a. Two of these indexes, namely BRSA and bHHP/HFHP, are affected by the wake-sleep state, whereas BRSp is not. In AS, BRSA is significantly lower than in QS, while bHHP/HFHP becomes negative. Furthermore, bHHP/HFHP is positively correlated with BRSp and BRSA in W and QS, but not in AS (Table 2b), thus indicating that in QS and W, but not in AS, it could be taken as a baroreflex index. On the contrary, in AS but not in W and QS, bHHP/HFHP is negatively and significantly correlated with the LFHP/HFHP sympathovagal index, thus suggesting that increased slow fluctuations of autonomic output in AS are responsible for both the increased value of the sympathovagal index and the negative value in bHHP/HFHP. In conclusion, bHHP/HFHP reflects the prevailing baroreflex (positive bHHP/HFHP) or non-baroreflex (negative bHHP/HFHP) influence on the heart.

The number of times bHHP/HFHP tested positive or negative in any of the 30-second sequences was also evaluated (the total number of sequences was 824, distributed among W, QS and AS) and is presented in Table 3 for the different states. Chi-square statistics was highly significant (p<0.001), indicating an uneven distribution of positive and negative values between the states. The positive/negative ratio progressively decreases from QS to W to AS, where negative values prevail.

It can be finally seen that LFHP/HFHP, BRSp, BRSA, and bHHP/HFHP show monotonic changes from QS to W to AS. From the point of view of cardiovascular regulation, W represents a state sharing intermediate features between QS and AS.

DISCUSSION

Sequences of HP and MAP beat-to-beat values 30-second long were selected during W, QS, and AS and different indexes of statistical dependence of HP and MAP were considered in the time domain: the mean value of HP vs. MAP regression coefficients in ramps showing parallel changes in MAP and HP (BRSp), the mean value of HP vs. MAP regression coefficients in all ramps (BRSA), as well as the regression coefficient of HP vs. MAP fluctuations along the entire 30-second sequence (bHHP/HFHP) (Table 2a). These indexes were calculated after low pass filtering to remove the effects of fluctuations likely linked to respiration (Appendix

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| Table 1—Heart Period (HP), Mean Arterial Pressure (MAP), and the sympathovagal index LFHP/HFHP in the different states of the wake-sleep cycle. |
|-------------------------------|-----------------|-----------------|-----------------|
|                             | W               | QS              | AS              |
| HP (ms)                     | 164.9±1.7       | 170.2±1.2       | 169.3±1.2       |
| MAP (mmHg)                  | 103.3±1.1       | 102.2±1.1       | 104.6±1.0       |
| LFHP/HFHP                   | 4.61±0.63       | 3.17±0.43       | 6.50±0.61       |

W, QS, and AS: Wakefulness, Quiet Sleep, and Active Sleep, respectively. Values are expressed as Means±SEM. Statistical significance of differences with respect to QS is reported (modified t-test and Bonferroni’s method; *, **, and NS for p<0.05, p<0.001 and not significant, respectively).

| Table 2 a, b—Indexes of statistical dependence of heart period vs. mean arterial pressure (BRSp, BRSA, and bHHP/HFHP) (a) and correlation coefficients of bHHP/HFHP vs. BRSp, BRSA, and the sympathovagal index LFHP/HFHP (b) during the wake-sleep cycle. |
|-----------------|-----------------|-----------------|-----------------|
|                 | W               | QS              | AS              |
| BRSp (ms/mmHg)  | 0.484±0.037     | 0.463±0.027     | 0.488±0.028     |
| BRSA (ms/mmHg)  | 0.305±0.041     | 0.339±0.021     | 0.203±0.037     |
| bHHP/HFHP (ms/mmHg) | 0.163±0.060 | 0.253±0.042 | -0.217±0.056   |

Means±SEM are presented for the different states of the wake-sleep cycle (Wake, W; Quiet Sleep, QS, and Active Sleep, AS). BRSp and BRSA: indexes of Baroreflex Sensitivity; bHHP/HFHP: regression coefficient of HP vs. MAP fluctuations over 30-s time intervals; r (bHHP/HFHP vs. BRSp), r (bHHP/HFHP vs. BRSA), and r (bHHP/HFHP vs. LFHP/HFHP): correlation coefficients of bHHP/HFHP vs. BRSp, BRSA, and LFHP/HFHP, respectively, calculated on standardized variables (within-animal Z-scores). Table 2a lists the statistical significance of differences with respect to QS (modified t-test and Bonferroni’s method); all mean values are significantly different from zero with p<0.001, with the exception of bHHP/HFHP in W, where p<0.05. Table 2b lists the statistical significance of correlation coefficients. Significance level: **, *** and NS for p<0.01, p<0.001 and not significant, respectively.
seen in Table 3, showing the number of times b HPMAP tested pos-
sively noise, as hypothesized,\textsuperscript{3} even if a noise component (i.e., HP
positive or negative in the different wake-sleep states.

Thus, in QS, and to a lesser extent in W, baroreflex-mediated
control prevails on centrally driven commands to the heart. As a con-
sequence, all indexes of HP vs. MAP statistical dependence
are positive. In AS, centrally driven commands prevail over
baroreflex-mediated influences to the heart and opposite changes
occur in both MAP and HP. As a consequence, in AS, BRS\textsubscript{A} is
reduced and b\textsubscript{HPMAP} becomes negative (Table 2a). This is clearly
seen in Table 3, showing the number of times b\textsubscript{HPMAP} tested posi-
tive or negative in the different wake-sleep states.

The non-baroreflex MAP and HP fluctuations are not neces-
sarily noise, as hypothesized,\textsuperscript{3} even if a noise component (i.e., HP
and MAP fluctuations uncorrelated between them) cannot be
ruled out. In fact, in states where non-baroreflex events prevail,
as in AS, b\textsubscript{HPMAP} does not approach zero, as would to be expect-
ed for uncorrelated HP and MAP fluctuations, but becomes nega-
tive and significantly different from zero (p<0.001) (Table 2a).
Non-baroreflex responses may be determined by feed-forward\textsuperscript{23,24} or positive feedback\textsuperscript{25} loops in cardiovascular neural
regulation (see also Hughson et al.\textsuperscript{4}). Positive feedback mecha-
isms rely on both sympathetic and parasympathetic outflow and
dynamically interact with negative feedback mechanisms.\textsuperscript{26}

The effects of prevailing baroreflex or non-baroreflex influ-
ences on the HP vs. MAP relation can be visually appreciated in
Figure 1, where two 30-second HP and MAP sequences are dis-
played for QS and AS, respectively. In QS, HP and MAP fluctu-
ations are parallel, whereas in AS they are almost opposite to
each other. They are also slower than the parallel ones: this
agrees with the fact that in AS b\textsubscript{HPMAP} is significantly and nega-
tively correlated with the sympathovagal LF\textsubscript{HP}/HF\textsubscript{HP} index (Table
2b). This suggests that slow fluctuations in centrally driven
commands to the heart and vessels are responsible for both negative
values in b\textsubscript{HPMAP} and high values in the sympathovagal index.

The results of the present study confirn a prevailing homeo-
static mode of operation of physiological controls in QS:\textsuperscript{27,28} the
value of the regulated variable, HP, mostly results from baro-
reflex loop activity. In AS, on the contrary, a non-homeostatic logic
is apparent:	extsuperscript{27,28} centrally generated phasic neural events (demon-
strated in sino-aortic deafferented animals)\textsuperscript{29} may overcome the
underlying baroreflex regulation. Single unit recordings, lesion
and stimulation experiments demonstrated the crucial role of
brainstem structures in initiating and controlling AS phe-
nomenology.\textsuperscript{30} This notwithstanding, our results indicate that the
mode of operation of the baroreflex neural circuit is resistant to
noise from other sources.

It is worth remembering that: 1) the salient features of the
baroreceptor-heart rate reflex are also shared by the barorece-
ptor-arterial pressure reflex; as a consequence, the protective role of
baroreflexes is exerted on both HP and MAP, by dampening the
changes induced by non-homeostatic regulations; 2) this role is
actually most important when baroreceptor function might
appear less effective (i.e., when disturbances on the controlled
variables HP and MAP are most relevant and the regression coe-

Table 3—Frequency of occurrence of positive and negative val-
ues in the regression coefficient of HP vs. MAP fluctuations
(b\textsubscript{HPMAP}) over 30-second time intervals in the different functional
states.

<table>
<thead>
<tr>
<th></th>
<th>W</th>
<th>QS</th>
<th>AS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>103</td>
<td>256</td>
<td>110</td>
</tr>
<tr>
<td>Negative</td>
<td>82</td>
<td>74</td>
<td>199</td>
</tr>
<tr>
<td>Positive / Negative</td>
<td>1,256</td>
<td>3,459</td>
<td>0.553</td>
</tr>
<tr>
<td>Chi-Square = 114, df = 2, p&lt;0.001</td>
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W, QS, and AS: Wakefulness, Quiet Sleep, and Active Sleep, respective-
ly. Statistical significance is obtained by Pearson Chi-square.

B). It is worth recalling that HP and MAP may show either par-
ellel or opposite spontaneous changes. If prevailing fluctuations
occur in MAP and induce baroreflex-mediated changes in HP,
MAP and HP will change in the same direction. On the contrary,
if centrally driven commands act equally on both blood vessels
and the heart, MAP and HP will change in opposite directions.
As far as the different indexes are concerned, the following con-
siderations can be made in our experimental conditions: 1) BRSP
is always positive and does not change between states (Table 2a).
If BRSP is taken as a measure of baroreflex sensitivity, the pre-
sent data indicate that baroreflex sensitivity does not change
among the different wake-sleep states. 2) BRS\textsubscript{A} is always posi-
tive, but is significantly reduced in AS (Table 2a). If BRS\textsubscript{A} were
taken as a measure of baroreflex sensitivity, the present data
would indicate, at variance with BRSP, that baroreflex sensitivity
is reduced in AS. 3) b\textsubscript{HPMAP} is positive in QS, decreases not sig-
nificantly in W and becomes negative in AS. b\textsubscript{HPMAP} is posi-
tively correlated with BRSP and BRS\textsubscript{A} in W and QS, but not in AS
(Table 2b). Thus, b\textsubscript{HPMAP} could be taken as a baroreflex index in
QS and W, but not in AS, where it shows a negative value.

The variable values and changes between states shown by
these indexes can be explained on the basis of the following as-
sumptions: 1) baroreflex sensitivity per se is independent of the
wake-sleep state, 2) the different indexes used to evaluate this
sensitivity are affected to an increasing extent, from BRSP to
BRS\textsubscript{A} to b\textsubscript{HPMAP}, by non-baroreflex influences on the heart
and vessels; as a consequence, BRSP appears to be most apt to evalu-
ate the baroreceptor-heart rate sensitivity in all the states, while
b\textsubscript{HPMAP} becomes negative when non-baroreflex influences pre-
vail, and 3) fluctuations in centrally driven commands to the
heart and vessels, responsible for the appearance of non-baro-
reflex HP and MAP fluctuations, increase from QS to W to AS.

Thus, in QS, and to a lesser extent in W, baroreflex-mediated
control prevails on centrally driven commands to the heart. As a con-
sequence, all indexes of HP vs. MAP statistical dependence
are positive. In AS, centrally driven commands prevail over
baroreflex-mediated influences to the heart and opposite changes
occur in both MAP and HP. As a consequence, in AS, BRS\textsubscript{A} is
reduced and b\textsubscript{HPMAP} becomes negative (Table 2a). This is clearly
seen in Table 3, showing the number of times b\textsubscript{HPMAP} tested posi-
tive or negative in the different wake-sleep states.

The non-baroreflex MAP and HP fluctuations are not neces-
sarily noise, as hypothesized,\textsuperscript{3} even if a noise component (i.e., HP
and MAP fluctuations uncorrelated between them) cannot be
ruled out. In fact, in states where non-baroreflex events prevail,
ficient between HP and MAP is most negative).

The protective role of the baroreflex function is emphasized by the recent results reported by Dworkin et al., namely that spontaneous neurally mediated fluctuations in blood pressure occur continuously, even in the absence of skeletal muscle activity and postural or behavioral changes; they are dampened by the baroreflexes and increase after sino-aortic denervation. This protective role becomes particularly important when homeostatic regulations are altered as in AS: the increase in blood pressure normally occurring in AS is enhanced in sino-aortic denervated rats.

The results of the present study have major implications in the field of sleep pathophysiology and medicine: baroreflex sensitivity is depressed in pathological conditions across fields as diverse as neurology (Pure Autonomic Failure, Alcoholic Neuropathy), metabolic disorders (Diabetes, Renal Failure, Chronic Liver Disease), cardiology (Myocardial Infarction); altered baroreflex response may also result from occasional events like surgery or trauma (cf. ). In all these conditions, AS may represent a specific risk state (in Diabetes, nocturnal sudden deaths have been described) in that centrally originated phasic neural disturbances are inadequately buffered by the impaired baroreflex response.

ACKNOWLEDGMENTS

This study was supported by the grants from the European Union CII*-CT93-0002.

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ABBREVIATIONS

ANOVA: Analysis of variance
AP: Arterial Pressure
AS: Active Sleep
bHPMAP: Regression Coefficient of HP vs. MAP fluctuations
BRS: Baroreflex Sensitivity
BRS_A: Baroreflex Sensitivity calculated on all ramps, whichever the sign of HP vs. MAP regression coefficient
BRS_P: Baroreflex Sensitivity calculated on ramps with positive HP vs. MAP regression coefficient
EEG: Electroencephalogram
EMG: Electromyogram
HF: High Frequency
HP: Heart Period
LF: Low Frequency
LFHP/HFHP: Sympathovagal Index
MAP: Mean Arterial Pressure
QS: Quiet Sleep
W: Wakefulness

APPENDIX A

Spectral Analysis

Linear detrending of HP and MAP sequences was first performed to avoid the contribution of the linear trend to low frequency power: HP and MAP values were regressed vs. time and the expected value was subtracted. Spectral analysis was then performed on HP beat-to-beat values by FFT (0.033 Hz resolution) on the 30-second interpolated, Welch windowed sequences. Low and high frequency components of HP variability, namely LF_HP and HF_HP, were calculated within frequency bands 0.03 to 0.8 Hz for LF and 0.81 to 2.50 Hz for HF (cf. 35). The sympathovagal index LF_HP/HF_HP was evaluated in each sequence and retained for further analysis.

APPENDIX B

Indexes of HP vs. MAP statistical dependence and baroreflex sensitivity evaluated in the time domain

After performing spectral analysis on HP (see above), 30-second sequences of MAP and HP beat-to-beat values were low pass filtered by a moving average of 10 beats applied to both HP and MAP. This was done to eliminate the relatively fast fluctuations likely related to respiratory cycle.3 Ramps of increasing or decreasing MAP of four beats or more were sought and, when found, the slope of HP vs. MAP was assessed with three, four, and five beats delay. For each ramp, BRS was calculated as the average value of these three slopes. As far as the baroreflex sensitivity index for the entire 30-second sequence is concerned, a first index, BRS_P, was evaluated according to 2,20,21 by averaging positive ramp BRS’s only. A second index, BRS_A, was evaluated according to 3 by averaging all ramp BRS’s, irrespective of their sign. A third index was also considered, bHPMAP, consisting of the regression coefficient of HP vs. MAP over the entire 30-second sequence. It is worth noting that BRS_P is based only on part of the data (i.e. the ramps of increasing or decreasing MAP with positive HP vs. MAP slope); BRS_A is based on all the ramps, while bHPMAP is based on all the data in the 30-second sequence.