http://dx.doi.org/10.5665/sleep.1738

Abnormal Sleep-Cardiovascular System Interaction in Narcolepsy with Cataplexy: Effects of Hypocretin Deficiency in Humans

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Study Objective: Narcolepsy with cataplexy (NC) is associated with loss of hypocretin neurons in the lateral hypothalamus involved in the circadian timing of sleep and wakefulness, and many biologic functions including autonomic control. The authors investigated whether chronic lack of hypocretin signaling alters cardiovascular control during sleep in humans.

Design: Comparison of 24-hr circadian rhythms, day-night, time- and state-dependent changes of blood pressure (BP) and heart rate (HR) in drug-free patients with NC and control subjects.

Setting: University hospital.

Patients or Participants: Ten drug-free patients with NC (9 men, 1 woman) and 12 control subjects (9 men, 3 women).

Interventions: N/A.

Measurements and Results: Daytime BP was comparable in patients with NC and controls, but patients with NC displayed a nighttime nondipping BP pattern. The 24-hr circadian rhythmicity of BP and HR was normal in both groups. Systolic BP during nighttime rapid eye movement sleep was significantly increased in the NC group. The 24-hr HR was significantly higher in the NC group but the day-night and state-dependent HR modulations were intact. The nighttime BP pattern coupled in the NC group with increased sleep fragmentation and a higher prevalence of arousals, periodic limb movements in sleep (PLMS), and PLMS arousals. In an analysis of the sleep/cardiovascular interaction in the periods after sleep onset and preceding morning awakening, only PLMS were consistently associated with the blunted nighttime decrease in BP in the NC group.

Conclusions: Hypocretin deficiency in humans may couple with an altered nighttime BP regulation that can be associated with an increased cardiovascular risk. This finding may be the result not only of the hypocretinergic deficiency *per se* but also of the altered sleep/wake regulation characterizing NC.

Keywords: Narcolepsy, cardiovascular system, hypocretins, sleep

Citation: Grimaldi D; Calandra-Buonaura G; Provini F; Agati P; Pierangeli G; Franceschini C; Barletta G; Plazzi G; Montagna P; Cortelli P. Abnormal sleep-cardiovascular system interaction in narcolepsy with cataplexy: effects of hypocretin deficiency in humans. *SLEEP* 2012;35(4):519-528.

INTRODUCTION

Blood pressure (BP) and heart rate (HR) show circadian and short-term fluctuations resulting from changes in body posture, daily activity, and neurohumoral activity. In particular, the day-night oscillation of BP is strongly linked to the sleep-wake circadian rhythm and plays a dominant role in the observed 24-hr BP variation characterized by BP decrease to its lowest levels during nighttime sleep, a phenomenon generally referred to as dipping.[1] The nighttime BP decrease has major clinical implications, and the loss of normal reduction in BP during sleep (nondipping status, defined as < 10% decrease in BP during sleep) is considered one of the most sensitive predictors of cardiovascular mortality.[2-5]

Sleep-state transitions are also accompanied by changes in the cardiovascular system.[6] A marked reduction in BP, HR, and sympathetic activity occurs during non rapid eye movement (NREM) sleep, becoming more pronounced as sleep progresses from stage 1 to stage 4. In contrast, rapid eye movement (REM) sleep is characterized by a marked sympathetic activation associated with BP and HR instability, supporting the observation of increased prevalence of cardiovascular events in the early morning hours when transitions to REM sleep are more frequent.[7]

The hypocretins are hypothalamic neuropeptides that through connections with the suprachiasmatic nucleus and many state-regulatory brain regions have crucial involvement in the circadian timing of sleep and wakefulness.[8] More recent observations have shown that hypocretins also play a role in the regulation of many autonomic functions such as feeding, thermoregulation, energy expenditure, and neuroendocrine and autonomic control.[9,10] The loss of the hypothalamic neurons producing hypocretins results in the sleep disorder narcolepsy with cataplexy (NC),[11,12] whose major clinical features are excessive daytime sleepiness, cataplexy (a sudden bilateral loss of voluntary muscle tone provoked by emotions), and other REM sleep phenomena such as sleep paralysis and hallucinations.[13] Other key features of the narcoleptic syndrome are the fragmentation of the sleep-wake cycle disrupted by the frequent occurrence of REM sleep-onset episodes during daytime, the numerous awakenings during nocturnal sleep, and a higher incidence of periodic limb movements in sleep (PLMS).[14]

Given the role of hypocretins in mediating the complex interaction occurring between sleep and the cardiovascular system, we decided to test the hypothesis that the impairment of the

A commentary on this article appears in this issue on page 453.

*Dr. Montagna passed away December 2010.

Submitted for publication May, 2011
Submitted in final revised form November, 2011
Accepted for publication November, 2011

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hypocretin system might affect their interrelation. The aim of this study was to characterize the 24-hr circadian rhythms, the day-night, time- and state-dependent changes of the cardiovascular system, and the associated sleep parameters in a sample of drug-free patients with NC under controlled conditions.

METHODS

Subjects

Ten unrelated adult patients with NC (9 men; age 38 ± 12 yr; body mass index (BMI) 28 ± 4) consecutively referred to the Bologna Sleep Disorders Center (Italy) and meeting the International Classification of Sleep Disorders-II diagnostic criteria[13] were included in the study. All patients were positive for haplotype HLA DQB1*0602, and 9 of 10 had low hypocretin-1 levels in the cerebrospinal fluid (i.e., ≤ 110 pg/ml; not detectable in 5 patients; range 17-92 pg/ml in 4 patients). One patient refused lumbar puncture. The mean disease duration was 13.6 ± 5.5 yr. Exclusion criteria were an index of respiratory events (apneas + hypopneas) ≥ 10; cardiac, endocrine, metabolic, and renal diseases on the basis of history-taking, physical examination, and routine laboratory tests. All patients were drug-free at the time of the study: 3 of 10 were drug naïve, 7 were undergoing treatment with modafinil that was suspended at least 2 weeks before the study, and 1 also with clomipramine suspended 1 month before the study.

This group of patients with NC already has been the subject of 2 published papers on the control of body core temperature[15] and the analysis of cardiovascular reflexes and heart rate variability during wakefulness.[16]

Patients with NC were compared with 12 healthy control subjects (9 men; age 43 ± 12 yr; BMI 27 ± 4.5) in whom sleep disorders (motor and breathing disorders) were excluded by means of a structured interview and polysomnography (PSG). Patients with NC and controls did not differ significantly by age (unpaired 2-tailed *t*-test; P = 0.26) and BMI (P = 0.42). Patients with NC and controls were occasional alcohol consumers; they were physically active but none was on strength or sports training. Four of 10 patients with NC and 3 of 12 controls were mild to moderate cigarette smokers (up to 10 cigarettes/day).

Study Protocol

Systolic and diastolic blood pressure (SBP, DBP), HR, and wake-sleep cycle were continuously monitored for 44 hr from 12:00. SBP, DBP, and HR were monitored beat-to-beat with a Portapres portable recorder (Portapres® Model-2, Finapres Medical Systems, Paasheuvelweg, Amsterdam, The Netherlands). The sleep-wake cycle was monitored by an ambulatory polygraphic recorder (Albert Grass Heritage®, Colleague TM PSG Model PSG16P-1, Astro-Med, Inc, West Warwick, RI,) recording electroencephalogram (C3-A2, C4-A1), right and left electrooculogram, electrocardiogram, and electromyogram of the mylohyoideus and left and right anterior tibialis muscles. During the study, subjects were allowed to sleep *ad libitum*, living in a temperature- (24 ± 1°C) and humidity- (40-50%) controlled room, lying in bed except when eating, on a light-dark schedule (dark period: 11:00-07:00). The subjects were placed on a 1.800 kcal/day diet divided into 3 meals (8:00, 12:00, 06:00) and 3 snacks (10:00, 04:00, 11:00). From midnight preceding the monitoring, subjects were instructed to avoid alcohol and caffeinated beverages and to abstain from smoking.

The institutional review board of the Department of Neurological Sciences of the University of Bologna approved the project. All subjects recruited in the study gave their written informed consent.

Sleep Parameters

A 32-hr sleep-wake cycle starting from 11:00 (lights off) of the first day of PSG recording was visually scored in 30-sec epochs according to the standardized criteria of Rechtschaffen and Kales.[17] Total sleep time (TST), sleep efficiency (time spent asleep out of total recording time), and duration (in min) of NREM stages 1 and 2, slow-wave sleep (SWS), and of REM sleep were calculated for each subject over the 24-hr period (from 07:00 of the second day of PSG recording), the light period from 07:00 to 11:00 of the second day of PSG recording, and the dark period of the 2 consecutive nights (night 1 and night 2).

The arousal index (AI; number of arousals/hr of sleep) and the PLMS index (PLMSI; number of periodic limb movements/hr of sleep) with the PLMS arousal index (PLMS_AI: number of periodic limb movements associated with arousal/hr of sleep) were computed over the 2 nights (dark period) according to the scoring rules of the Sleep Disorders Atlas Task Force of the American Sleep Disorders Association[18] and the American Academy of Sleep Medicine Manual for the scoring of Sleep,[19] respectively.

Wake-sleep fragmentation was determined by calculating the frame shifts index, indicating the number of 30-sec sleep stage shifts occurring every 15 min throughout the 44-hr study.

Cardiovascular Parameters

Day-night pattern of SBP, DBP, and HR

To evaluate the nocturnal decline of SBP, DBP, and HR, daytime mean values (from 09:00 to 21:00 of day 2) and nighttime mean values (from 00:00 to 06:00 of day 1 and 2) were calculated.[20,21] The difference between nighttime and daytime values (ΔSBP, ΔDBP, ΔHR) was then calculated and also expressed as % decline of nighttime values over daytime values.

24-hr circadian rhythm of SBP, DBP, and HR

Rhythmicity was analyzed by evaluating the time series for SBP, DBP, and HR according to the single cosinor method, using a computerized procedure.[22] The procedure determined whether there was a rhythm within a 24-hr period (P < 0.05) and evaluated the following parameters of the cosinor function with their 95% confidence limits: the mesor (24-hr mean), the amplitude (AMP; one-half the peak to trough distance of the approximated waveform) and the acrophase (ACR; peak time referred to local midnight hour). For each subject we analyzed the 24-hr rhythmicity of 2 consecutive days, the first (day 1) starting at 12:00 and the second (day 2) starting at 08:00.

State-dependent changes in SBP, DBP, and HR during nighttime

The analysis was conducted on the data of the dark period of the 2 consecutive nights of PSG recording but not during the light period, given the unbalanced contribution to daytime sleep

phases of the 2 groups. The mean value of each variable in each sleep stage (NREM stage 1 and 2, SWS and REM sleep) was calculated. The difference (Δ) between the mean value of each variable in any sleep stage during the dark period and the mean value in wake (W) during the light period, considered as the reference value, was also calculated.

SBP, DBP, and HR pattern after sleep onset and before morning awakening

To better investigate the effects exerted by sleep on cardiovascular parameters, in each subject SBP, DBP, and HR were aligned with sleep onset (defined by 3 consecutive stage 1 epochs or 1 epoch of any other sleep stage, followed by a sustained sleep period of at least 10 min) and with the morning spontaneous awakening (defined by the last 30-sec epoch of any sleep stage followed by at least 10 min of relaxed wakefulness with subjects lying in bed, determined on the basis of PSG and video recordings). The 90 min after sleep onset and preceding the morning awakening were considered for the analysis, averaging the beat-to-beat values every 30 min. For SBP and DBP analysis, the difference (Δ SBP, Δ DBP) between each 30-mininterval mean value and the mean value of the last 10 min of relaxed wakefulness preceding sleep onset (baseline for data aligned with the sleep onset) and the mean value of the first 10 min of relaxed wakefulness of spontaneous awakening (baseline for data aligned with the morning awakening) was considered.

To investigate the sleep architecture associated with the cardiovascular changes, the prevalence (percentage) of sleep stages (NREM stage 1 and 2, SWS, REM) and W, the number of arousals, PLMS, PLMS arousals, and the 30-sec frame shifts index were calculated every 30 min in the 90 min after sleep onset and preceding morning awakening.

Statistics

To assess the between-group differences of sleep parameters, cosinor variables, ΔSBP, ΔDBP, and ΔHR values of day-night pattern, the unpaired 2-tailed *t*-test was adopted. State-dependent analysis of cardiovascular parameters was performed by fitting a mixed model where the factors were wake-sleep phases (W, NREM stage 1 and 2, SWS, REM) and group (control subjects versus patients with NC). The data aligned with sleep onset and morning awakening averaged every 30 min were analyzed using analysis of variance (ANOVA) (General Linear Model for repeated measure) to test the time × group interaction effect. Data not normally distributed (arousals, PLMS, PLMS arousals, frame shifts index) were analyzed after log10-transformation adding value 1 to all data sets given the presence of 0 observations. *Post-hoc* analysis was performed using an unpaired 2-tailed *t*-test. Wake-sleep stages prevalence every 30 min was analyzed using an unpaired 2-tailed t-test.

All the analyses were performed using Stata 9.0 (StataCorp LP, College Station, Texas, USA) and the significance level was set at $P < 0.05$. Data are reported as mean ± standard deviation (SD).

RESULTS

Sleep Parameters

Data concerning the sleep architecture of the 10 patients with NC and 10 of the 12 control subjects have been already published.[15] The analysis on this enlarged control population confirms previous findings: patients with NC displayed a significant increase in 24-hr TST compared with controls (NC group 626 ± 125 min; control group 405 ± 141 min; $P < 0.001$) mainly due to the longer amount of sleep during the light period (NC group 267 ± 97 min; control group 63 ± 73 min; $P < 0.0001$). During the dark period for both nights the 2 groups showed comparable durations of TST (night 1: NC group 362 ± 67 min; control group 374. ± 65 min; $P = 0.69$; night 2: NC group 360 ± 64 min; control group 342 ± 88 min; $P = 0.60$), stage 2 (night 1: NC group 151 ± 51 min; control group 165 ± 47 min; $P = 0.52$; night 2: NC group 136 ± 44 min; control group 148 ± 53 min; $P = 0.58$) and REM sleep (night 1: NC group 86 ± 30 min; control group 94 ± 27 min; $P = 0.50$; night 2: NC group 92 ± 35 min; control group 80 ± 28 min; $P = 0.34$). Patients with NC displayed a higher duration of stage 1 on both nights (night 1: NC group 63 ± 24 min; control group 25 ± 21 min; $P = 0.001$; night 2: NC group 56 ± 19 min; control group 21 ± 14 min; $P < 0.001$) and a mild reduction of SWS on night 1 (NC group 63 ± 29 min; control group 93 ± 26; $P = 0.02$) not detected on night 2 (NC group 76 ± 26 min; control group 94 ± 35 min; $P = 0.20$). The NC group was characterized by a significantly increased wake-sleep fragmentation throughout the 24 hr (frame shift index: NC group 312 ± 94; control group 123 ± 48; $P < 0.001$) that was more evident during the light period (frame shift index: NC group 139 ± 56; control group 21 ± 24; $P < 0.001$) compared with the dark period (frame shift index: NC group 173 ± 47; control group 100 ± 28; $P = 0.02$).

The analysis of the AI, PLMSI, and PLMS_AI during the dark period showed higher values in the NC group (AI 13 ± 4; PLMSI 23 ± 20; PLMS_AI 5 ± 4) compared with the control group (AI 9 ± 4; PLMSI 3 ± 6; PLMS_AI 1 ± 2) displaying P values of 0.01, < 0.001, and < 0.001, respectively.

The analysis of all sleep parameters after alignment with sleep onset and morning awakening is presented, together with the concomitant cardiovascular changes, in a separate paragraph.

Day-Night Pattern of SBP, DBP, and HR

The 24-hr pattern of SBP, DBP, and HR is shown in Figure 1. Daytime SBP mean values (NC group 128 ± 12 mmHg; control group 128 ± 12 mmHg) and DBP mean values (NC group 75 ± 7 mmHg; control group 75 ± 6 mmHg) on day 2 were comparable in the control and NC groups ($P = 0.91$ and $P = 0.98$, respectively) whereas mean HR values were higher in the NC group (NC group 78 ± 10 bpm; control group 70 ± 13 bpm; $P < 0.001$). ΔSBP and ΔDBP were significantly higher in the control group than in the NC group ($P < 0.001$), the nighttime SBP decline being 17 ± 7 mmHg (14% ± 5% decline) and 10 ± 6 mmHg (8 % ± 5% decline) in the control group and NC group, respectively and the nighttime DBP decline 10 ± 4 mmHg (13% ± 5% decline) and 6 ± 3 mmHg (8% ± 4% decline), respectively. ΔHR was comparable in the 2 groups ($P = 0.95$), at 11 ± 5 bpm (12% ± 6% decline) and 11 ± 7 bpm (16 % ± 9% decline) in the NC group and control group, respectively.

The 3 drug-naïve patients with NC included in the study displayed a similar 24-hr BP and HR profile: a nondipper SBP pattern (7% ± 3% decline) and DBP pattern (7% ± 3% decline) was

detected and associated with higher HR values (daytime values 78 ± 10 bpm) but with ΔHR values comparable with those of the control group (13% ± 4% decline).

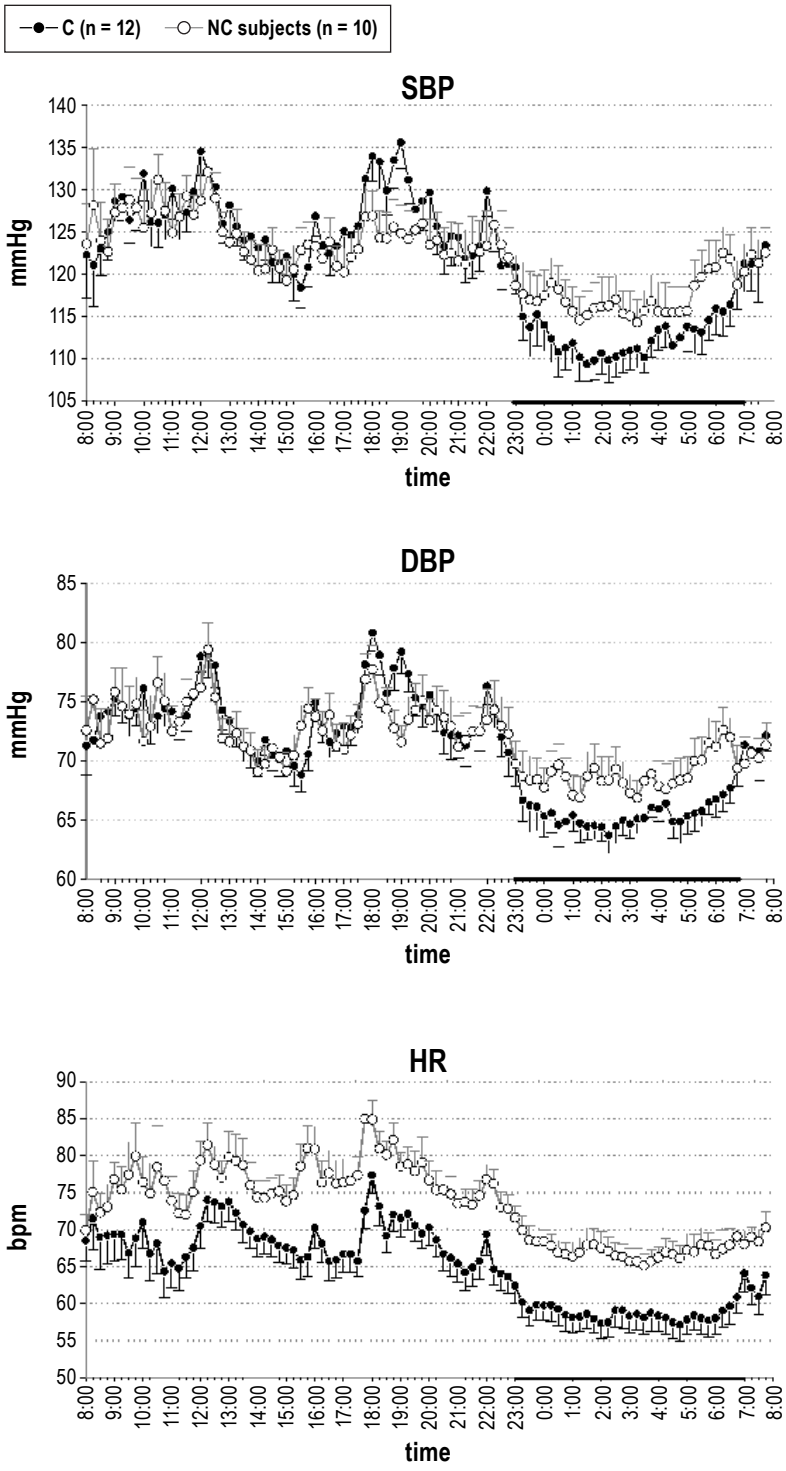

Figure 1—The 24-hr pattern of systolic blood pressure (SBP), diastolic blood pressure (DBP), and heart rate (HR) in control subjects (C) and patients with narcolepsy with cataplexy (NC). Each point from 8:00 to 12:00 in the morning represents the mean value every 15 min ± standard error (SE) considering for each subject the contribution of day 2 while each point in the following period from 12:00 to 8:00 represents the mean value every 15 min ± standard error (SE) considering for each subject the contribution of day 1 and day 2. Dark bar represents the dark period. SE has been reported in 1 direction for clarity.

24-Hr Circadian Rhythm of SBP, DBP, and HR

A significant circadian rhythmicity of SBP, DBP and HR was detected in patients with NC and in control subjects.

SBP mesor (NC group 122 ± 11 mmHg; control group 121 ± 10 mmHg; P = 0.79) and DBP mesor (NC group 72 ± 7 mmHg; control group 71 ± 7 mmHg; P = 0.58) were comparable in the 2 groups, whereas HR mesor was significantly increased in patients with NC (NC group 73 ± 9 bpm; control group 65 ± 10 bpm; P < 0.01).

The AMP of SBP and DBP rhythm was significantly reduced in patients with NC (SBP: NC group 6 ± 3; control group 9 ± 4; P < 0.01; DBP: NC group 4 ± 2; control group 6 ± 2; P = 0.02) and comparable in the 2 groups in HR rhythm (NC group 7 ± 4; control group 7 ± 4; P = 0.87). The ACR values of SBP (NC group 14, 3 ± 3, 1 hr, min; control group 14.5 ± 2. 3 h, min; P = 0.72), DBP (NC group 14.6 ± 4.1 hr, min; control group 15, 1 ± 2, 5 hr, min; P = 0.79) and HR (NC group 16.17 ± 2.2 hr, min; control group 15, 2 ± 1, 5 hr, min; P = 0.16) were comparable in the 2 groups.

State-Dependent Changes in SBP, DBP, and HR During Nighttime

Statistical analysis revealed a physiologic state-dependent modulation of BP and HR during the dark period in patients with NC and in control subjects (Figure 2) characterized by BP and HR reduction during NREM sleep with respect to values during W during the light period (SBP, DBP, and HR: P < 0.0001) and by their increase during REM sleep with respect to NREM sleep (SBP: P < 0.01; DBP: P = 0.03; HR: P = 0.04).[23] However, although comparable SBP and DBP values were detected in patients with NC and in controls during W during the light period (SBP: NC group 126 ± 11 mmHg; control group 127 ± 10 mmHg;, P = 0.76; DBP: NC group 75 ± 7 mmHg; control group 74 ± 5 mmHg; P = 0.78), HR values were significantly higher in the NC group (79 ± 11 bpm; control group 69 ± 11 bpm; P < 0.01).

The analysis, conducted on 20 nights in patients with NC and 24 nights in control subjects, showed stages of similar duration except for a higher representation of stage 1 in the NC group (59 ± 21 min; control group 23 ± 17 min) and a higher amount of SWS in the control group (NC group 69 ± 27 min; control group 93 ± 30 min). The ANOVA analysis did not detect a between-group interaction effect in DBP values (P = 0.42) in any sleep stage but showed a significant difference between the 2 groups in SBP during REM sleep (P = 0.02) that was higher in patients with NC (Figure 2). Because SBP and DBP during W in the light period were comparable in the 2 groups, the analysis of ΔSBP and ΔDBP values yielded comparable results.

To clarify the effect on SBP and DBP of REM sleep occurring early or late in the night in patients

with NC, we considered the data obtained during the 90-min period after sleep onset and preceding morning awakening. Patients with NC showed an increased amount of REM during the 90-min period preceding morning awakening (21 ± 8 min) with respect to that after sleep onset (15 ± 8 min), but comparable values of REM sleep-related SBP and DBP were detected during the period after sleep onset and preceding morning awakening (90-min period after sleep onset: SBP 121 ± 15 mmHg; DBP 70 ± 9 mmHg; 90-min period preceding morning awakening: SBP 121 ± 13; DBP 70 ± 8 mmHg).

As during daytime, HR remained significantly higher in the NC group in all sleep phases (P = 0.02) (Figure 2) but no between-group difference was detected in ΔHR values (P = 0.33).

An example of the altered sleep-cardiovascular interaction of 1 patient with NC compared with that of a control subject is shown in Figure 3; nighttime SBP surges, especially concomitant to REM sleep, are highlighted in the patient with NC.

Sleep and Cardiovascular Pattern After Sleep Onset and Before Morning Awakening

Data aligned with the sleep onset

The raw data of sleep parameters after alignment with sleep onset are reported in Table 1. The analysis of sleep stages prevalence displayed a significant increase in REM sleep in the NC group (P = 0.01) associated with a significant reduction of SWS (P = 0.001) in the first 30 min after sleep onset compared with the control group. A between-group interaction effect was detected in the number of PLMS and PLMS arousals that were significantly increased in patients with NC (P < 0.001). In particular, PLMS and PLMS arousals were higher in patients with NC in all 30-min intervals of the 90 min after sleep onset. No between-group interaction effect was detected in the number of arousals or in the frame shift index (P = 0.12 and P = 0.21, respectively).

The baseline values of SBP (NC group 118 ± 17 mmHg; control group 116 ± 12 mmHg; P = 0.44) and DBP (NC group 69 ± 10 mmHg; control group 67 ± 7 mmHg; P = 0.45) in the 10 min preceding sleep onset were comparable in the 2 groups, whereas basal HR was higher in patients with NC (NC group, 71 ± 8 bpm; control group 62 ± 10 bpm; P < 0.0001).

ANOVA showed a significant time × group interaction effect in Δ SBP (P = 0.006), higher in the control group, and HR (P < 0.0001) higher in the NC group. No between-group differences were detected in Δ DBP (P = 0.07). *Post hoc* analysis showed that ΔSBP was significantly reduced and HR significantly increased in patients with NC in all 30-min intervals of the 90 min after sleep onset (Figure 4).

Data aligned with morning awakening

The raw data of sleep parameters after alignment with morning awakening are reported in Table 1.

The prevalence of wake-sleep stages was comparable in the 2 groups, and no between-group interaction effect was detected in the number of arousals (P = 0.30) and PLMS arousals (P = 0.07). A between-group significant difference was present in the number of PLMS (P = 0.003) and in the frame shifts index (P = 0.001), both of which were increased in patients with NC in all 30-min intervals of the 90 min preceding morning awakening.

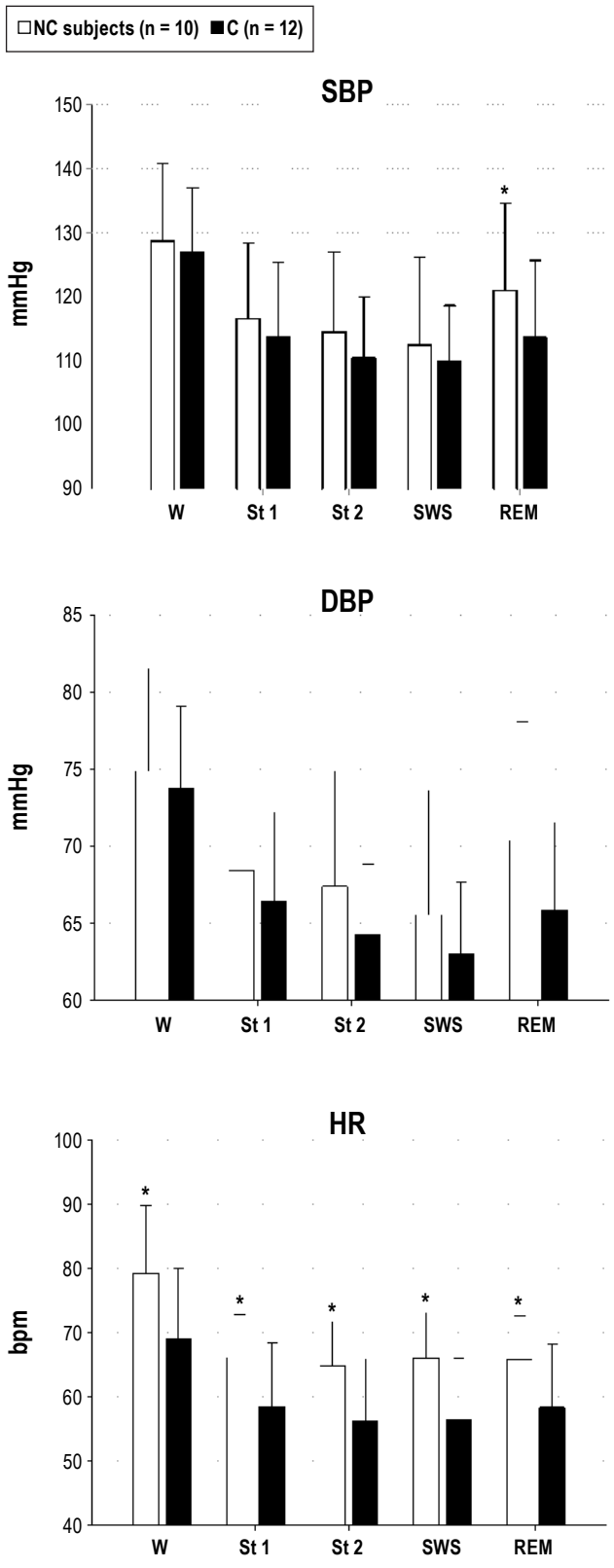

Figure 2—State-dependent changes in systolic blood pressure (SBP), diastolic blood pressure (DBP), and heart rate (HR) during the dark period (11:00-07:00) in control subjects (C) and patients with narcolepsy with cataplexy (NC) considering the contribution of night 1 and night 2. St 1, NREM stage 1; St 2, NREM stage 2; SWS, slow wave sleep; W, wake in the light period. Mean ± standard deviation. *P < 0.05.

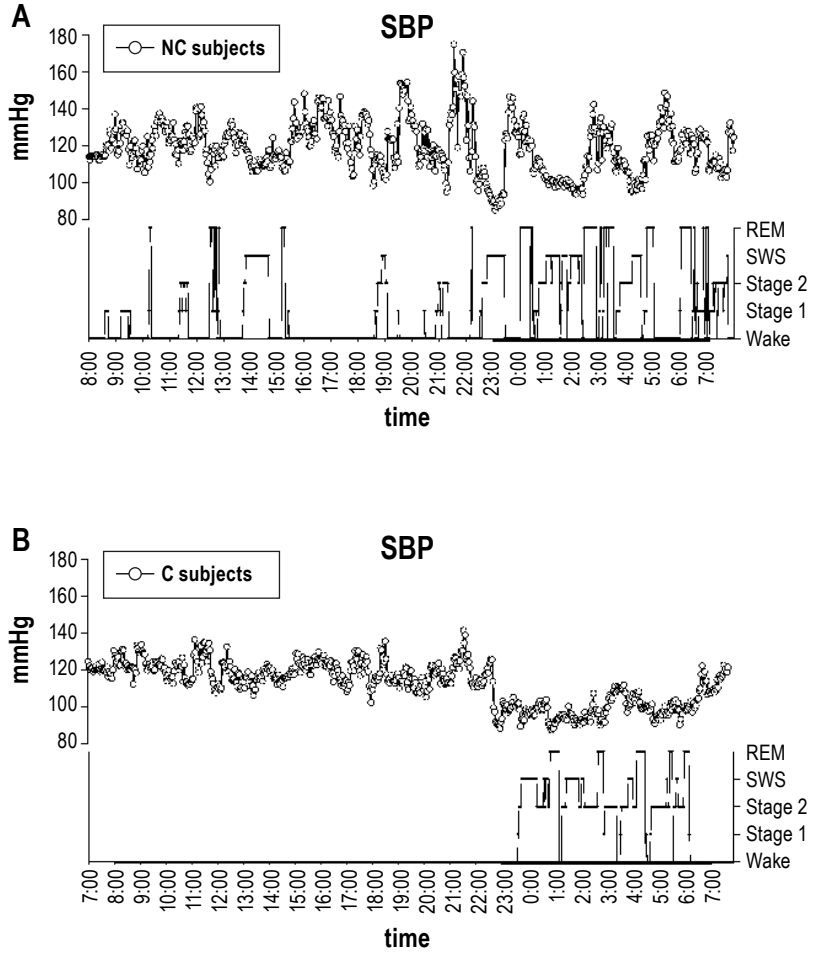

Figure 3—Systolic blood pressure (SBP) 24-hr profile and related hypnogram in **(A)**: a male patient, age 35 yr, with narcolepsy with cataplexy (NC) and **(B)**: a male control subject, age 30 yr. The figure highlights the fragmented wake-sleep architecture of the patient with NC and the nighttime SBP surges, especially concomitant with REM sleep, detectable in the patient with NC but not in the control subject. C: control, REM: rapid eye movement; SWS: slow-wave sleep.

The baseline values of morning awakening were comparable between patients with NC and controls for SBP (NC group 119 ± 13 mmHg; control group 121 ± 13; P = 0.65), DBP (NC group 70 ± 8 mmHg; control group 72 ± 7 mmHg; P = 0.49) and HR (NC group 72 ± 11 bpm; control group 67 ± 11 bpm; P = 0.09).

A significant time × group interaction effect was detected in Δ SBP (P = 0.02) and Δ DBP (P = 0.02) that was higher in controls and HR (P < 0.0001) that was higher in patients with NC. In particular (Figure 4), Δ SBP was blunted in patients with NC in the 60 min preceding the morning awakening and ΔDBP in all the 90 min preceding the morning awakening. HR was significantly increased in patients with NC in all 30-min intervals of the 90 min preceding morning awakening (Figure 4).

DISCUSSION

The main findings of this study were that in comparison with healthy control subjects, untreated patients with NC studied under controlled conditions displayed a blunted sleep-related decrease in BP and an increased SBP during nighttime REM sleep, whereas 24-hr circadian rhythmicity and diurnal behavior were unaffected. Interestingly, patients with NC displayed increased 24-hr HR with respect to controls but unlike BP regulation, the day-night and state-dependent HR modulation was intact. The observed BP night pattern in patients with NC was associated with an increased sleep fragmentation and a higher prevalence of arousals, PLMS, and PLMS arousals. These findings suggest that cardiovascular changes in patients with NC may be the result not only of the hypocretinergic deficiency *per se* or its associated compensatory mechanisms, but also of the altered sleep/wake regulation characterizing NC.

The Effect of Hypocretin Deficiency on Sleep Parameters

The analysis of sleep architecture in the 10 patients with NC who were evaluated confirmed previous reports[14] showing the typical changes of sleep/wake regulation representative of the disease. During the dark period, patients with NC behaved similarly to control subjects, showing on both consecutive nights a comparable TST and similar sleep architecture in terms of quantitative representation of sleep stages. During the light period, however, patients with NC had a longer amount of sleep compared with controls. As supported by previous findings,[24] these data were probably due to the bed-resting condition adopted in our protocol that has favored the sleep propensity of patients with narcolepsy. Finally, the NC group was characterized by an increased wake-sleep fragmentation throughout the 24 hr and increased AI, PLMSI, and PLMS_AI during the dark period.

The analysis of sleep parameters after alignment with morning awakening and sleep onset revealed a different qualitative representation of sleep stages between patients with NC and controls through the night. Patients with NC displayed a non-physiologic higher prevalence of REM sleep in the 30 min after sleep onset at the expense of a normal representation of SWS detectable in control subjects, whereas REM sleep representation in the 90-min period preceding the morning awakening was comparable in the 2 groups. PLMS were significantly increased in the NC group in the entire 90-min period after sleep onset and preceding morning awakening, this finding being in line with recent data from the literature demonstrating that PLMS represent an intrinsic feature of narcolepsy.[25,26] Interestingly, PLMS arousals also were significantly increased in patients with NC compared with controls in the 90 min after sleep onset, supporting their potential to disturb the consolidation of sleep in patients with NC at sleep onset.

The Effect of Hypocretin Deficiency on the Cardiovascular System

One of the original findings of this study was that compared to controls, patients with NC displayed a blunted sleep-related decrease in BP, and despite their different sleep architecture during the day-time, SBP and DBP values in this period were comparable to controls. These results are unexpected because the

Table 1—Sleep parameters after alignment with sleep onset and morning awakening in control subjects (n = 12) and patients with NC (n = 10)

	Alignment with Sleep Onset			Alignment with Morning Awakening		
	0-30 min mean ± SD	30-60 min mean ± SD	60-90 min mean ± SD	90-60 min mean ± SD	60-30 min mean ± SD	30-0 min mean ± SD
W (%)						
Controls	2.5 ± 4.7	10.0 ± 23.5	13.9 ± 24.7	25.5 ± 35.0	16.8 ± 28.6	14.51 ± 14.0
Patients with NC	3.6 ± 3.8	9.6 ± 16.1	20.0 ± 27.8	25.0 ± 23.4	24.3 ± 25.4	24.6 ± 15.9
St. 1-2 (%)						
Controls	52.4 ± 18.8	29.7 ± 22.7	33.0 ± 27.9	39.7 ± 32.6	45.0 ± 33.6	55.2 ± 27.3
Patients with NC	58.0 ± 29.0	39.33 ± 35.2	30.33 ± 20.8	53.00 ± 26.1	43.0 ± 28.5	41.0 ± 26.1
SWS (%)						
Controls	**44.4 ± 19.9§**	52.5 ± 30.3	19.7 ± 23.3	4.4 ± 11.2	4.6 ± 10.3	2.2 ± 6.1
Patients with NC	**20.3 ± 25.9**	43.3 ± 41.6	21.33 ± 34.7	5.3 ± 13.0	8.0 ± 21.3	6.6 ± 3.5
REM (%)						
Controls	**0***	7.78 ± 21.6	35.5 ± 28.7	31.3 ± 36.1	32.4 ± 36.6	27.4 ± 21.4
Patients with NC	**18.0 ± 33.0**	7.67 ± 18.39	25.67 ± 25.5	16.6 ± 18.9	24.6 ± 26.0	27.6 ± 22.3
Arousals (n°)						
Controls	3.1 ± 1.8	4.4 ± 3.0	4.1 ± 2.9	2.9 ± 3.0	4.4 ± 3.6	5.4 ± 4.0
Patients with NC	4.9 ± 5.1	5.7 ± 5.4	7.15 ± 3.94	3.4 ± 1.9	4.6 ± 3.7	5.2 ± 2.9
PLMS (n°)						
Controls	**1.3 ± 4.0§**	**1.8 ± 5.1§**	**3.3 ± 7.7***	**1.5 ± 3.9§**	**1.7 ± 3.8§**	**1.7 ± 3.7***
Patients with NC	**12.3 ± 16.9**	**17.2 ± 23.1**	**10.5 ± 13.7**	**9.7 ± 11.4**	**8.5 ± 12.3**	**8.7 ± 12.9**
PLMS_A (n°)						
Controls	**0§**	**0.3 ± 0.9§**	**0.8 ± 1.9***	0.9 ± 2.4	1.0 ± 2.7	1.1 ± 2.4
Patients with NC	**2.7 ± 5.0**	**3.5 ± 4.9**	2.7 ± 3.6	**1.2 ± 1.4**	1.7 ± 2.8	1.7 ± 2.9
30-sec Frame Shifts Index (n°)						
Controls	8.2 ± 3.6	7.7 ± 5.6	7.0 ± 3.8	**4.9 ± 3.4§**	**6.2 ± 3.6***	**7.7 ± 4.7§**
Patients with NC	8.15 ± 4.4	9.0 ± 7.1	12.0 ± 6.4	**10.6 ± 5.2**	**10.4 ± 6.4**	**12.4 ± 5.4**

NC, narcolepsy with cataplexy; PLMS, periodic limb movements in sleep; PLMS_A, arousals associated with PLMS; St. 1-2, NREM stages 1 and 2; SWS, slow wave sleep; 30-sec Frame Shifts Index, the number of 30-sec sleep-stage shifts occurring every 30 min; W, wake. *P < 0.05; §P ≤ 0.01.

current view of the mechanisms linking hypocretin signaling to cardiovascular control, mainly based on experimental models, suggests hypocretins enhance the sympathetic outflow.[27] Studies on hypocretin-ataxin 3 transgenic (TG) mice and hypocretin gene knockout (KO) mice, representing the experimental model of hypocretin deficiency, reported lower BP during wakefulness in both animal models,[28,29] and Schwimmer et al.[30] recently reported lower BP in both wakefulness and sleep conditions in TG rats. On the other hand, a recent study on TG and KO mice showed that both hypocretin-deficient mouse models had a reduction of the sleep-related decrease in BP, which was more evident during REM sleep, with BP during wakefulness comparable to that of control mice.[31] Interestingly, our findings on BP are similar to those reported by Bastianini et al.[31] showing that the NC group had SBP and DBP daytime values comparable to those of the control group but failed to show the physiologic night-time 10% decline in BP. This result may be of great clinical relevance because a nondipping nocturnal pattern in patients with and without hypertension is recognized as a strong independent risk factor for cardiovascular mortality.[2-5,32] Similar to what has been demonstrated in patients with hypertension,[33,34] a sympathetic activation might be determinant to the reduced nighttime decrease in BP detected in patients with NC, and this hypothesis would be in line with the sympathetic activation that we also observed in patients with NC during the daytime rest supine condition.[16]

Interestingly, the analysis of HR pattern is indicative of an increased sympathetic outflow in patients with NC who showed an increased HR during nighttime with respect to control subjects. However, unlike BP, an increased HR was also observed during daytime. All these findings support evidence that hypocretin deficiency in humans may not be unequivocally associated with a reduced sympathetic tone, as suggested by experimental models,[27] but through still-unknown mechanisms it may also be associated with a sympathetic activation.[16]

The hypocretin system might play a significant role also in the circadian control of cardiovascular parameters being a crucial relè between the dorsomedial nucleus of the hypothalamus and the suprachiasmatic nucleus that serves as the brain's master clock.[8] Our findings, however, suggest that circadian control of cardiovascular parameters is not affected by the hypocretin deficiency in patients with NC as we similarly demonstrated in the 24-hr circadian control of body core temperature.[15] In fact, patients with NC in our study displayed a significant 24-hr circadian rhythm of SBP, DBP, and HR coupled with a physiologic acrophase. As expected from the day-night pattern of SBP, DBP,

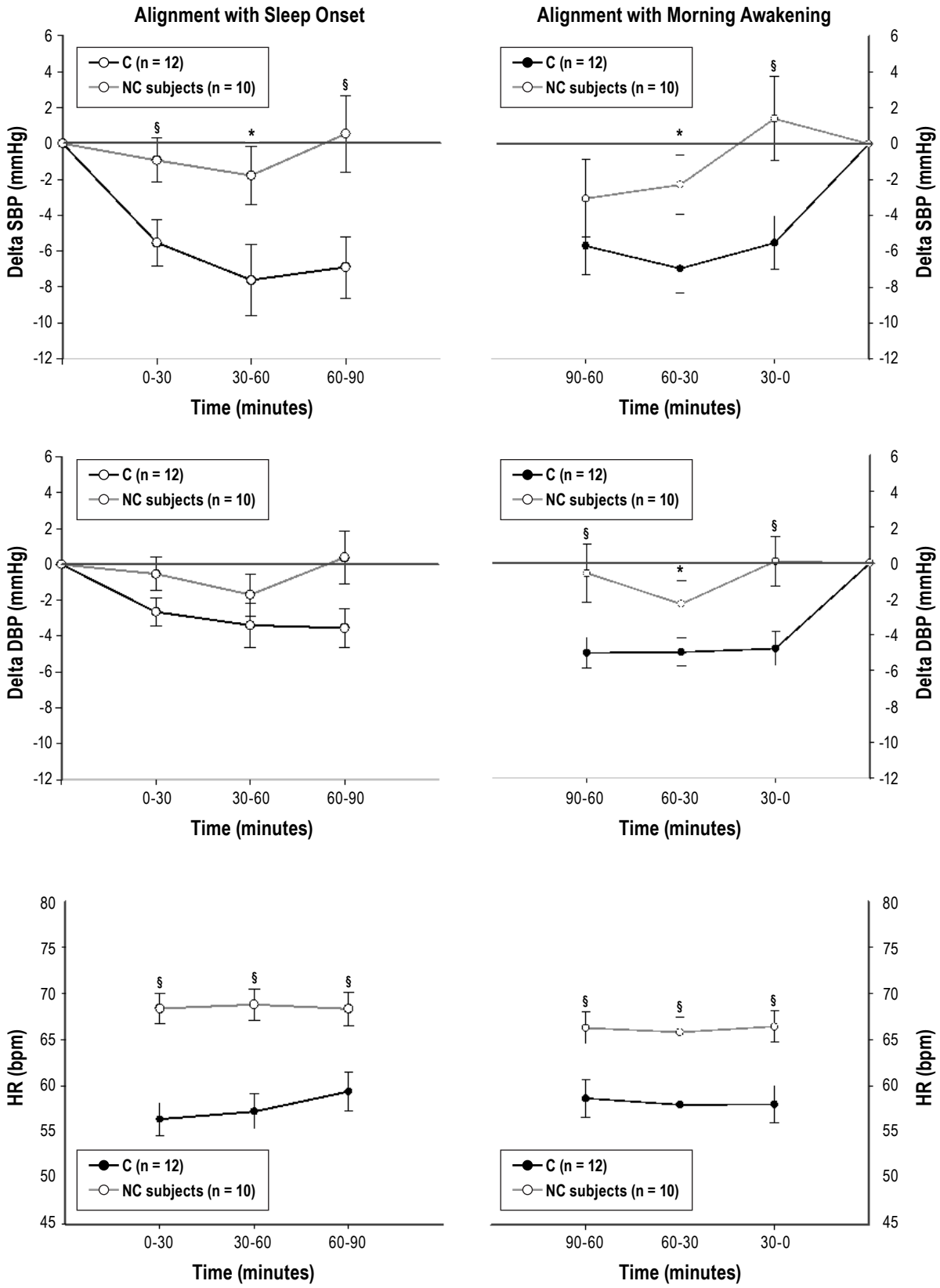

Figure 4—Δ SBP and Δ DBP were obtained by calculating the difference of each 30-min interval mean value with the mean value of the last 10 min of relaxed wakefulness preceding sleep onset for data aligned with sleep onset and the mean value of the first 10 min of relaxed wakefulness of spontaneous awakening for data aligned with morning awakening. Data are obtained for all subjects considering the 2 nights of polysomnographic recording. C, control; DBP, diastolic blood pressure; HR, heart rate; NC, narcolepsy with cataplexy; SBP, systolic blood pressure. Error bars represent SE. *P < 0.05; §P ≤ 0.01.

and HR, SBP and DBP amplitude were significantly reduced and the HR mesor significantly increased compared with control subjects. Detection of reduced BP amplitude in patients with NC likely reflects their blunted nighttime decrease in BP, and this has been shown to be highly representative of the nondipper status in patients with hypertension.[35] The higher HR mesor in the NC group is instead justified by the increase in 24-h HR.

Only one study investigated the nighttime state-dependent changes of BP in patients with narcolepsy, yielding negative results; however, it was conducted on a small sample of patients and without continuous BP measurements.[36] The BP state-dependent analysis we performed in our study during nighttime sleep showed a significant pressure effect of REM on SBP associated with a normal NREM effect on SBP and DBP in patients with NC. The pressor effect of REM sleep in patients with NC is in agreement with what was recently reported by Bastianini et al.[31] on TG and KO mice, and it cannot be explained by spending more time in this stage because the total duration of REM was similar between patients with NC and controls. Moreover, patients with NC showed a similar pressor effect during the early and the late night REM sleep, suggesting that it does not depend on when REM sleep appears and that it can be a hallmark of the narcoleptic REM sleep.

The state-dependent analysis of HR displayed higher values in patients with NC in all sleep stages but, in contrast with BP findings, HR modulation through sleep stages was comparable in patients with NC and controls, thereby supporting the hypothesis that HR reflects modulatory influences different from those controlling the BP pattern.[37,38]

The state-dependent analysis we performed did not take into account the time of the day and the length of sleep stages. However, the findings of Trinder et al.[37] demonstrating that BP is not affected by time during the sleep period and the statistical analysis that we performed considering the changes of BP and HR in different sleep stages also within the same subject, being this clearly affected by epochs length, support the consistency of our results.

The Effect of Hypocretin Deficiency on the Interaction Between Sleep and the Cardiovascular System

To better understand the effects exerted by sleep on cardiovascular control, we studied HR and BP pattern and sleep parameters after alignment with sleep onset and morning awakening, focusing our analysis on the 90 min after sleep onset and preceding morning awakening. We found that SBP was significantly higher in the NC group in all the 90-min period after sleep onset and in the 60 min preceding the morning awakening. DBP turned out to be higher in patients with NC only in the 90 min preceding morning awakening and, in line with the other findings of the study, HR was significantly increased in patients with NC in the 90-min periods after sleep onset and preceding morning awakening.

Of the sleep parameters evaluated, none of them was constantly associated with the blunted decrease in BP in patients with NC with respect to controls except for PLMS, the incidence of which increased in all the periods after sleep onset and preceding morning awakening. Because PLMS were demonstrated to be associated with heightened measures of sympathetic outflow and, presumably, cardiovascular consequences,[39] their increased incidence in NC may play a role in the altered sleep/cardiovascular interaction that we observed; their effects on the autonomic nervous system and their relationship with an increased cardiovascular risk are still a matter of debate.[40]

The major limitation of our study is the relatively small sample of patients, which increases the chance of type II errors. However, the controlled experimental conditions, the reproducibility of our results in 2 nights for patient, and the genetic characterizations of our drug-free patients with NC strengthen the reliability of our results. Another limitation of the study is that 7 of 10 patients with NC were undergoing treatment with stimulants that were suspended at least 2 wk before the study. Nevertheless, the washout time span we adopted and the fact the 24-hr BP and HR profile of the 3 drug-naïve patients with NC was comparable to that detected in the other patients makes a determinant causal role of the therapy on our results unlikely.

In conclusion, our data suggest that hypocretin deficiency in human NC is associated with an altered interaction between sleep and the cardiovascular system. Because this interaction is the opposite of what is expected from most experimental results, it may be of clinical relevance because it carries an increased cardiovascular risk, particularly when added to other conditions already suspected to increase the cardiovascular risk in patients with NC.[41-43] To our knowledge, information on the cardiovascular risk associated with NC in humans is lacking. Our work suggests the need for further studies to clarify this issue and the mechanisms through which the altered sleep/wake regulation characteristic of NC may affect the cardiovascular system.

ABBREVIATIONS

NC, narcolepsy with cataplexy
BP, blood pressure
SBP, systolic blood pressure
DBP, diastolic blood pressure
HR, heart rate
PSG, polysomnography
TST, total sleep time
NREM, nonrapid eye movement
REM, rapid eye movement
SWS, slow wave sleep
W, wake
SCN, suprachiasmatic nucleus
AI, arousal index (number of arousals/hrour of sleep)
PLMSI, periodic limb movements in sleep index (number of PLM/hr of sleep)
PLMS_AI, periodic limb movements in sleep arousal index (number of PLM associated with arousal/hr of sleep)
ACR, acrophase of the 24-hr circadian rhythm according to the single cosinor method
AMP, amplitude of the 24-hr circadian rhythm according to the single cosinor method

ACKNOWLEDGMENTS

This study was part of the research program of Daniela Grimaldi, PhD. The authors thank Professor Elio Lugaresi for his critical revision. Institution at which the work was performed: Clinica Neurologica, Dipartimento di Scienze Neurologiche, Alma Mater Studiorum - Università di Bologna, Italy.

DISCLOSURE STATEMENT

This was not an industry supported study. The authors have indicated no financial conflicts of interest.

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