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Title: **The effect of treatment for sleep apnea on determinants of blood pressure control**

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METHODS

Trial Design

A double-blind, placebo-controlled, randomized, crossover study was conducted in patients newly diagnosed with hypertension and OSA (NCT02398032). The study protocol was approved by the institutional ethics committee (CEIC Hospital Universitario La Paz, PI 99/0252), and all participants provided written informed consent to participate in the study.

Participants

Participants were recruited between May 2013 and September 2015 from the sleep unit of a teaching hospital (La Paz University Hospital, Madrid, Spain). Patients were initially eligible for participation in the study if they were between 18 and 75 years of age, had a recent diagnosis of OSA (apnea-hypopnea index > 10 h^{-1}), and had a diagnosis by 24-h ambulatory monitoring of blood pressure (AMBP) of INH (nighttime blood pressure of ≥120 mm Hg systolic or 70 mm Hg diastolic and a daytime blood pressure <135/85 mm Hg) or D-NSH (nighttime blood pressure of ≥120 mm Hg or 70 mm Hg diastolic and a daytime blood pressure of ≥135 mm Hg systolic) [1].

Subjects who met any of the following criteria were excluded: (1) current use of CPAP treatment or antihypertensive drugs; (2) severe hypertension (>180/120 mmHg) or previous diagnosis of secondary hypertension; (3) severe daytime sleepiness (Epworth sleepiness score >15); (4) renal insufficiency (creatinine >1·4 mg/dl); (5) previous diagnosis of chronic obstructive pulmonary disease, asthma, bronchiectasis, lung cancer, restrictive lung disease, chest wall disease or thoracic surgery; (6) previous diagnosis or clinical evidence of heart disease, neuromuscular disease or thyroid dysfunction; (7) morbid obesity (body mass index >40 Kg/m^2); (8) respiratory infection in the last two months; (9) treatment with systemic corticosteroids in the last two years; (10) excessive alcohol intake (>40 g/day).

Study Procedures

All subjects underwent overnight attended polysomnography, which included the following variables: electroencephalogram (F3, F4, C1, C3, O1, O2, M1, M2), electro-oculogram (LEA2, REA1), chin and leg electromyograms, electrocardiogram, and airflow (with an oronasal thermistor and nasal prong). Chest and abdominal efforts were recorded by belt sensors, body position by an accelerometer, and arterial oxyhemoglobin saturation by finger pulse oximetry. All variables were recorded by an 18-channel polysomnograph (Sleep Screen, Viasys Healthcare, Hoechberg, Germany). The PSG records were manually scored using conventional criteria. Sleep staging was scored according to the criteria of American Academy of Sleep Medicine [2]. Arousals were scored as defined in the ASDA Atlas Task Force Report on EEG Arousals [3]. Apnea was defined as a reduction of the measured parameter of airflow to 10% of baseline or less, with a duration ≥10 seconds. Hypopnea was defined as a 30% to 90% airflow reduction lasting 10 seconds or more, which was accompanied by ≥3% decrease in measured oxygen saturation, or a contiguous micro arousal from sleep [4]. Apneas or hypopneas were defined as obstructive events if oronasal airflow was decreased as described above, but the thoracic and abdominal breaths continued at the baseline amplitude, or increased in amplitude at any time during the period of decreased airflow. The apnea-hypopnea index (AHI) was calculated as the total number of apnea and hypopnea episodes per hour of electroencephalographic sleep. A patient was classified as having OSA when the obstructive component was dominant and AHI was ≥10 per hour.

Information regarding sleepiness (ESS score), lung function and 24-h blood pressure were recorded for each patient at inclusion. 24-hour ABPM was carried out using the oscillometric method with a portable Spacelabs 90202 device (Redmond, Wash., USA). An appropriate cuff was used and placed on the non-dominant arm. The monitor was attached in the morning and removed after 24 hours. The subjects were told to go about their usual daily activities and to go to bed no later than 23:00 h. The device was programmed to perform measurements every 30 min during the day (08:00 to 23:00) and every 60 min at night (23:00 to 08:00) on a workday. The data were considered valid when monitoring was conducted for a minimum period of 21 hours, with a minimum number of

twenty measurements during day and eight at night. Systolic BP measurements above 260 mmHg and below 70 mmHg, and diastolic BP measurements above 150 mmHg and below 40 mmHg were automatically excluded from the analysis. The limits for heart rate detection were between 20 and 200 bpm. The test was acceptable if at least 75% of the measurements taken throughout the 24-hour period had been performed successfully. INH and D-NSH were defined according to the above criteria [1]. A nocturnal decrease in BP of 10% or more was defined as dipper status.

Baseline spirometry and plethysmography were performed with a MasterLab Body 6.0 device (Vyasis, Würzburg, Germany), according to European Respiratory Society standardization [5,6]. European Coal and Steel Community predicted values were used [7]. Maximal static inspiratory pressure (PImax) was measured using a differential pressure transducer (M-163; Sibelmed, Barcelona, Spain). Patients, comfortably seated and wearing a noseclip, performed maximal inspiratory efforts at residual volume against an obstructed mouthpiece with a small leak (internal diameter, 0·7 mm) to minimize oral pressure artifacts. The maneuvers were repeated until three measurements, sustained for at least 3 s and with less than 5% variability, were recorded. The highest value obtained was used for analysis [8].

Randomization and Intervention

A randomized block design was employed with block sizes of 4 and stratification (according to baseline diagnosis of INH or D-NSH), using an online statistical computing web program (www.randomization.com) to generate the randomization schedule. Patients were allocated to receive either effective CPAP or sham CPAP for two 12-week periods. Immediately afterwards, the other treatment was applied with a washout period of 5-7 days. The sham CPAP device consisted of a conventional CPAP device in which the area of the exhalation port was amplified, thereby nearly cancelling nasal pressure; an orifice resistor was connected between the tubing and the CPAP unit that loads the blower with the same airflow resistance as in effective CPAP (Carburos Metálicos, Air Products Group, Madrid, Spain).

Optimal CPAP pressure was titrated by an auto CPAP device (AutoSet, ResMed, Sydney, Australia), according to a previous validation of the Spanish Sleep Network [9]. The prescribed fixed CPAP pressure was based on the 95% percentiles of pressures over at least three consecutive nights. The CPAP device used (S9, ResMed) recorded all the data from the 6 months of use. Adherence was considered adequate if mean CPAP use was at least 4 hours per night and more than 5 days per week [10]. Patients remained blinded as to whether they were receiving CPAP or sham, and systemic hypertension was not treated with drugs during the study period. Moreover, the researchers who performed the study determinations were unaware of both the subject's group and the patient's treatment assignment at each visit.

Outcome Assessment and Follow-up

The primary study outcome was the change in the nocturnal increase of peripheral chemosensitivity (withdrawal response after sleep – withdrawal response before sleep). Secondary outcomes included change in peripheral chemosensitivity and blood gases before and after sleep as well as in RAAS activity, urinary catecholamines, biomarkers of endothelial dysfunction and blood pressures.

Patients were evaluated at baseline and 3 and 6 months afterwards. At each visit, anthropometric characteristics, ESS score, and adherence to CPAP or sham were recorded. Simultaneously, a 24-h ABPM with urine collection for determination of catecholamines were performed. Subjects were instructed to maintain their activities of daily life. Before going to bed (20:00-21:00 h) and immediately after waking, peripheral chemosensitivity and blood gases were measured. The next morning, immediately after waking, the same measurements were repeated with a venous blood extraction to analyze the renin-angiotensin-aldosterone system and endothelial biomarkers.

Measurements

Peripheral chemosensitivity evaluation

Arterial blood gas values breathing room air (Rapidpoint 405, Bayer, Munich, Germany) were measured, immediately after which peripheral chemosensitivity was detected as a fall in ventilation following sudden elimination of mild hypoxia [11,12]. At the beginning of the test, V'I and $PETCO_2$ were measured while the subject was breathing room air in a rubber bag. N_2 and CO_2 were then added to obtain a $PETO_2$ of 60 mmHg and $PETCO_2$ 5 mmHg higher than the control. Two breaths of O_2 were then given by turning a three-way stopcock near the inlet of the respiratory valve to raise $PETO_2$ higher than 200 mmHg. $PETCO_2$ was also decreased by 2-3 mmHg because inspired PCO_2 ($PICO_2$) decreased to zero at this time [11]. After two breaths of 100% O_2, the inspiratory gas was switched back to the hypercapnic hypoxic gas. The V'I during room air breathing was defined as VI,N. The V'I before breathing 100% O_2 during the mildly hypercapnic hypoxic state was defined as VI,0. The V'I between 5 and 20 s after changing the inspiratory gas to 100% oxygen was defined as VI,5-20. The difference between VI,0 and VI,5-20 was defined as the withdrawal response (ΔVI), and %ΔVI (ΔVI/VI,0 x 100) was used as an index of the peripheral chemoreceptor activity. The withdrawal test was performed three or more times at intervals of 20 min. The subject breathed room air between tests to avoid the effects of hypoxic ventilatory depression [12]. To eliminate the effects of body size and sex, the indices of each ventilatory response were corrected by body surface area (BSA) in square meters.

Urinary catecholamines

Subjects were requested to collect separate urine samples from 08:00 to 23:00 (diurnal) and from 23:00 to 07:00 (night). Urine specimens for each sample were collected in polyethylene containers, acidified with HCl 6M as preservative and stored at -40°C before analysis. Urine samples were processed by HPLC (high performance liquid chromatography) in an Agilent analyzer (1100 series LC, Agilent Technologies) with electrochemical detection in a coulometric detector (ESA model Coulochem II, USA), using Bio Rad reagents. Acidified urine was mixed with internal standard. The

mixture was then applied onto an ion exchange column. Interfering substances were washed off the column with an elution reagent and distilled water. In the next step, the analytes were eluted with another reagent. An aliquot of the eluate was injected into an isocratic HPLC system. The samples were separated on a reversed phase cartridge, electrochemically detected and evaluated quantitatively with help of the internal standard. Intra-assay coefficients of variation were 1·6%-2·1% for norepinephrine, and 1·7%-2·2% for epinephrine. Inter-assay coefficients of variation were 2·5%-3% for norepinephrine and 2·2%-2·9% for epinephrine.

Renin-angiotensin aldosterone system and biomarkers

Plasmatic renin concentration was measured in EDTA human plasma by chemiluminescent immunoassay (CLIA) using commercial kits (Dia Sorin in LIAISON analyzer). Normal range sitting at rest, on a normal sodium diet, was 0·23–3·32 ng/ml/h; intra-assay and interassay coefficients of variation (CVs) were within 2·12%-4·87% and 6·81%-13·03%, respectively. Plasma renin activity was obtained using a conversion factor (1ng/ml/h= 12 mU/L) .The assay for plasma aldosterone concentration (PAC) was performed by chemiluminescent immunoassay (CLIA) (Dia Sorin in LIAISON analyzer). Normal range was 1·8–23·2 ng/dl supine on a normal sodium diet; intra-assay and interassay CVS were 1·8%-4·2% and 5·6%-10·5%, respectively; the cross-reactivity of the antibody for aldosterone for other adrenal steroids was < 0·02%.

Enzyme-linked immunosorbent assays (ELISA) from R & D Systems (Minneapolis, USA) were used to measure soluble Intracellular Adhesion Molecule-1 (sICAM-1) and soluble Vascular Cell Adhesion Molecule-1 (sVCAM-1). The detection limits were 0·254 and 1·26 ng/ml, respectively. The intra-assay coefficients of variation for both sICAM-1 and sVCAM-1 in this study were 5·2%. Concentrations of endothelin-1 in plasma were measured using an ELISA kit according to the manufacture's protocol (R&D Systems, Abingdon, UK). The detection limit of this assay was 0·087 pg/ml. The coefficients of variation were 5·8% (interassay) and 4·5% (intra-assay).

Statistical Analysis

Sample size calculation was performed assuming a peripheral chemosensitivity of 24·0 ± 3·6% in OSA patients [12]. Based on a 2-tailed test with an α=0·05, ß=0·20 and an expected dropout rate of 15%, 16 subjects were necessary in each group to recognize as statistically significant a difference greater than or equal to 4 units with the intervention in the active versus the control group.

Data are summarized as mean ± SD or median (interquartile range) for continuous variables, while frequencies (percentages) are used for categorical variables. The normality of the variable distribution was tested using the Kolmogorov-Smirnov test.

Outcomes analysis used intention-to-treat principle and included all participants as randomized. No imputation of the missing data was performed for the main or secondary outcome measurements. A sensitivity analysis using different approaches including no imputation, baseline observation carried forward, last observation carried forward, and multiple imputation showed similar results for the primary outcome and the treatment effect. Between-group baseline comparisons were based on 2-sample *t* tests or the Mann-Whitney test for continuous variables and chi-square test with a Yates continuity correction (or Fisher exact test if the expected frequencies were less than 5) for categorical variables. The intragroup differences from the beginning to the end of the study were evaluated with a paired *t* test. Comparisons of effects of the treatment over time were made with repeated-measures ANOVA, with treatment as a within-subject factor and order as a between-subject factor. When ANOVA results showed significant differences between treatment conditions, post hoc multiple comparisons were performed with the Bonferroni test. A per protocol analysis based on data from patients with adequate adherence to CPAP was also performed without imputation.

Relationships between variables were determined by Pearson's correlation. Those variables that reached statistical significance in univariate correlation analysis were then introduced in a stepwise multiple linear regression analysis to identify independent relationships. Stepwise criteria were a probability of F-Snedecor test to enter <0·05 and a probability of F-Snedecor test to remove >0·10.

The assumptions of linearity and distributional normality were controlled for all variables. Homoscedasticity was explored by scatter plots of the standardized residuals on the standardized predicted values and by Levene's test for equality of variances.

A 2-sided P value less than 0·05 was considered significant. Data management and statistical analyses were performed using Stata, version 11, and SPSS software (IBM), version 13.

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Table S1. Changes from baseline in the peripheral chemosensitivity index and arterial gases before and after sleep*

		Before sleep				After sleep			
		Sham CPAP		CPAP		Sham CPAP		CPAP	
		Mean difference† (95%CI)	P	Mean difference† (95%CI)	p	Mean difference† (95%CI)	p	Mean difference† (95%CI)	p
ΔV'I, %									
	INH	-0·4 (-3·6 to 2·8)	NS	-0·5 (-4·3 to 3·3)	NS	-1·8 (-7·3 to 3·7)	NS	-7·4 (-12·2 to -2·6)	0·005
	D-NSH	-0·6 (-3·3 to 2·1)	NS	-3·0 (-5·6 to -0·5)	0·023	1·1 (-3·3 to 5·5)	NS	-5·7 (-9·3 to -2·2)	0·003
	Overall	0·5 (-2·4 to 1·5)	NS	-1·8 (-4·0 to 0·4)	NS	-0·3 (-3·6 to 3·0)	NS	-6·5 (-9·3 to -3·7)	<0·001
pH									
	INH	-0·018 (-0·047 to 0·012)	NS	-0·019 (-0·042 to 0·004)	NS	-0·007 (-0·030 to 0·016)	NS	-0·013 (-0·033 to 0·006)	NS
	D-NSH	0·003 (-0·022 to 0·028)	NS	-0·001 (-0·023 to 0·020)	NS	0·008 (-0·013 to 0·029)	NS	0·009 (-0·012 to 0·030)	NS
	Overall	-0·007 (-0·025 to 0·011)	NS	-0·010 (-0·025 to 0·005)	NS	0·001 (-0·014 to 0·016)	NS	-0·002 (-0·016 to 0·012)	NS
PaO_2, mmHg									
	INH	0·6 (-2·3 to 3·5)	NS	1·1 (-2·0 to 4·2)	NS	0·4 (-2·0 to 2·8)	NS	1·3 (-2·6 to 5·2)	NS
	D-NSH	1·0 (-1·1 to 3·1)	NS	-1·2 (-3·7 to 1·3)	NS	2·5 (-1·4 to 6·5)	NS	-0·3 (-2·7 to 2·1)	NS
	Overall	0·8 (-0·9 to 2·4)	NS	-0·1 (-2·0 to 1·8)	NS	1·5 (-0·7 to 3·8)	NS	0·5 (-1·7 to 2·6)	NS
$PaCO_2$, mmHg									
	INH	0·2 (-1·1 to 1·5)	NS	0·7 (-1·5 to 2·9)	NS	0·1 (-1·3 to 1·4)	NS	-0·5 (-2·1 to 1·0)	NS
	D-NSH	-0·5 (-1·1 to 0·1)	NS	-1·3 (-2·5 to 0·02)	NS	-1·3 (-3·2 to 0·5)	NS	-1·2 (-3·1 to 0·8)	NS
	Overall	-0·2 (-0·8 to 0·5)	NS	-0·4 (-1·6 to 0·8)	NS	-0·7 (-1·8 to 0·4)	NS	-0·9 (-2·1 to 0·3)	NS

Definition of abbreviations: NS=not significant; CPAP=continuous positive airway pressure; ΔVI=withdrawal response or index of the peripheral chemoreceptor activity; PaO_2=oxygen arterial pressure; $PaCO_2$=carbon dioxide arterial pressure
†Adjusted for treatment order

Table S2. Changes from baseline in the nocturnal increase of peripheral chemosensitivity (ΔV'I, %). Per protocol analysis

	Sham CPAP		CPAP	
	Mean difference† (95%CI)	**P**	**Mean difference† (95%CI)**	**p**
INH (n=14)	-2.2 (-9.0 to 4.5)	NS	-7.9 (-13.9 to -1.9)	0.013
D-NSH (n=14)	1.1 (-2.8 to 4.9)	NS	-2.9 (-7.0 to 1.2)	NS
Overall (n=28)	-0.5 (-4.1 to 3.1)	NS	-5.3 (-8.8 to -1.9)	0.004

Definition of abbreviations: NS=no significance; CPAP=continuous positive airway pressure; ΔVI=withdrawal response or index of the peripheral chemoreceptor activity
†Adjusted for treatment order

Table S3. Changes from baseline in the peripheral chemosensitivity and arterial gases before and after sleep*. Per protocol analysis

	Before sleep				After sleep			
	Sham CPAP	**CPAP**	**Sham CPAP**	**CPAP**	**Sham CPAP**	**CPAP**	**Sham CPAP**	**CPAP**
	Mean difference† **(95%CI)**	*P*	**Mean difference†** **(95%CI)**	*P*	**Mean difference†** **(95%CI)**	*P*	**Mean difference†** **(95%CI)**	*P*
$\Delta V'I$, %								
INH (n=14)	-0.2 (-3.6 to 3.3)	NS	0.5 (-3.0 to 3.9)	NS	-2.4 (-8.2 to 3.4)	NS	-7.5 (-12.6 to -2.3)	0.008
D-NSH (n=14)	0.1 (-2.5 to 2.6)	NS	-2.9 (-5.7 to -0.2)	0.038	1.1 (-3.6 to 5.8)	NS	-5.9 (-9.7 to -2.0)	0.006
Overall (n=28)	-0.04 (-2.0 to 1.9)	NS	-1.3 (-3.4 to 0.8)	NS	-0.6 (-4.1 to 3.0)	NS	-6.6 (-9.6 to -3.6)	<0.001
pH								
INH (n=14)	-0.020 (-0.051 to 0.011)	0.180	-0.023 (-0.042 to -0.005)	0.019	-0.006 (-0.029 to 0.017)	NS	-0.012 (-0.034 to 0.009)	NS
D-NSH (n=14)	0.002 (-0.026 to 0.030)	NS	-0.002 (-0.017 to 0.013)	NS	0.015 (-0.004 to 0.035)	NS	0.014 (-0.008 to 0.035)	NS
Overall (n=28)	-0.09 (-0.028 to 0.011)	NS	-0.012 (-0.024 to -0.003)	0.046	0.005 (-0.010 to 0.019)	NS	0.000 (-0.014 to 0.015)	NS
PaO_2, mmHg								
INH (n=14)	-0.2 (-3.0 to 2.5)	NS	0.8 (-2.4 to 4.0)	NS	-0.1 (-2.5 to 2.4)	NS	1.0 (-3.3 to 5.4)	NS
D-NSH (n=14)	0.2 (-1.5 to 1.8)	NS	-2.2 (-4.4 to 0.1)	NS	0.6 (-1.7 to 2.9)	NS	-0.9 (-3.2 to 1.4)	NS
Overall (n=28)	0.0 (-1.5 to 1.5)	NS	-0.7 (-2.6 to 1.3)	NS	0.3 (-1.2 to 1.8)	NS	0.1 (-2.2 to 2.4)	NS
$PaCO_2$, mmHg								
INH (n=14)	0.1 (-1.3 to 1.5)	NS	0.5 (-1.8 to 2.9)	NS	-0.1 (-1.5 to 1.4)	NS	-0.6 (-2.3 to 1.2)	NS
D-NSH (n=14)	-0.6 (-1.2 to 0.05)	NS	-1.1 (-2.5 to 0.2)	NS	-1.6 (-3.5 to 0.2)	NS	-1.4 (-3.4 to 0.6)	NS
Overall (n=28)	-0.3 (-0.9 to 0.4)	NS	-0.4 (-1.6 to 0.9)	NS	-0.9 (-2.0 to 0.3)	NS	-1.0 (-2.3 to 0.2)	NS

Definition of abbreviations: NS=not significant; CPAP=continuous positive airway pressure; ΔVI=withdrawal response or index of the peripheral chemoreceptor activity; PaO_2=oxygen arterial pressure; $PaCO_2$=carbon dioxide arterial pressure
†Adjusted for treatment order

Table S4. Changes from baseline in urinary cathecholamines, activity of renin-angiotensin aldosterone system and systemic biomarkers in the study groups*. Per protocol analysis

	Sham CPAP		CPAP	
	Mean difference† (95%CI)	p	Mean difference† (95%CI)	p
Diurnal norepinephrine, µg/g				
INH (n=14)	-1.8 (-5.7 to 2.2)	NS	-10.5 (-17.6 to -3.4)	0.007
D-NSH (n=14)	1.0 (-6.3 to 8.4)	NS	-6.4 (-12.0 to -0.9)	0.027
Overall (n=28)	-0.3 (-4.3 to 3.7)	NS	-8.4 (-12.7 to -4.2)	<0.001
Diurnal epinephrine, µg/g				
INH (n=14)	0.4 (0.01 to 0.7)	0.038	1.7 (-0.9 to 4.3)	NS
D-NSH (n=14)	-0.1 (-1.4 to 1.1)	NS	0.2 (-1.1 to 1.5)	NS
Overall (n=28)	0.1 (-0.5 to 0.7)	NS	0.9 (-0.4 to 2.3)	NS
Nocturnal norepinephrine, µg/g				
INH (n=14)	0.1 (-4.7 to 4.8)	NS	-9.3 (-15.6 to -2.9)	0.008
D-NSH (n=14)	0.8 (-2.1 to 3.8)	NS	-5.0 (-9.8 to -0.2)	0.044
Overall (n=28)	0.5 (-2.1 to 3.0)	NS	-7.1 (-10.8 to -3.3)	0.001
Nocturnal epinephrine, µg/g				
INH (n=14)	0.6 (-1.8 to 3.0)	NS	-0.9 (-2.1 to 0.2)	NS
D-NSH (n=14)	0.5 (0.04 to 1.0)	0.036	0.4 (0.0 to 1.8)	NS
Overall (n=28)	0.5 (-0.6 to 1.7)	NS	-0.2 (-1.1 to 0.7)	NS
Plasma renin activity, ng/ml/h				
INH (n=14)	0.05 (-0.05 to 0.1)	NS	0.2 (-0.1 to 0.4)	NS
D-NSH (n=14)	0.1 (-0.1 to 0.3)	NS	0.1 (-0.2 to 0.3)	NS
Overall (n=28)	0.1 (-0.1 to 0.2)	NS	0.1 (-0.1 to 0.3)	NS
Aldosterone, ng/dl				
INH (n=14)	-0.5 (-1.5 to 0.6)	NS	0.4 (-1.7 to 2.4)	NS
D-NSH (n=14)	-0.3 (-1.2 to 0.7)	NS	-1.7 (-3.6 to 0.1)	NS
Overall (n=28)	-0.4 (-1.0 to 0.3)	NS	-0.7 (-2.1 to 0.6)	NS
Aldosterone/renin ratio				
INH (n=14)	-1.0 (-2.0 to 0.1)	NS	-0.4 (-3.0 to 2.1)	NS
D-NSH (n=14)	-1.0 (-3.1 to 1.1)	NS	-1.4 (-4.0 to 1.2)	NS
Overall (n=28)	-1.0 (-2.1 to 0.2)	NS	-0.9 (-2.6 to 0.8)	NS
Endothelin 1, pg/ml				
INH (n=14)	0.03 (-0.1 to 0.2)	NS	-0.07 (-0.3 to 0.1)	NS
D-NSH (n=14)	-0.07 (-0.3 to 0.1)	NS	-0.03 (-0.2 to 0.1)	NS
Overall (n=28)	-0.02 (-0.1 to 0.1)	NS	0.07 (-0.05 to 0.2)	NS
sVCAM-1, ng/ml				
INH (n=14)	264 (-446 to 973)	NS	492 (-355 to 1338)	NS
D-NSH (n=14)	482 (49.4 to 914)	0.032	-147 (-826 to 532)	NS
Overall (n=28)	377 (-5 to 958)	NS	161 (-356 to 677)	NS
sICAM-1, ng/ml				
INH (n=14)	40 (-71 to 151)	NS	0.06 (-0.01 to 0.02)	NS
D-NSH (n=14)	20 (-104 to 145)	NS	16 (-19 to 52)	NS
Overall (n=28)	30 (-48 to 108)	NS	8 (-9 to 25)	NS

Definition of abbreviations: NS=not significant; CPAP=continuous positive airway pressure; INH=isolated nocturnal hypertension; D-NSH=day-night sustained hypertension; sVCAM=soluble vascular cell adhesion molecule; sICAM=soluble intercellular adhesion molecule

†Adjusted for treatment order

Table S5. Parameters related with nighttime blood pressure in OSA patients with isolated nocturnal hypertension

	Nighttime systolic BP, mmHg			Nighttime diastolic BP, mmHg			Nighttime mean BP, mmHg		
	r	95%CI	*p*	*r*	95%CI	*p*	*r*	95%CI	*P*
REM phase, %	0·062	-0·447 to 0·541	NS	-0·640	-0·862 to -0·211	0·008	-0·374	-0·734 to 0·149	0·153
Apnea-hypopnea index, h^{-1}	0·764	0·432 to 0·914	0·001	0·070	-0·441 to 0·547	NS	0·523	0·037 to 0·809	0·037
Nocturnal norepinephrine, µg/g	0·365	-0·160 to 0·729	NS	0·439	-0·072 to 0·768	NS	0·517	0·029 to 0·806	0·040
Nocturnal epinephrine, µg/g	0·526	0·041 to 0·810	0·036	0·441	-0·070 to 0·769	NS	0·619	0·178 to 0·853	0·011
ΔVI after sleep, %	0·715	0·340 to 0·894	0·002	0·515	0·026 to 0·805	0·041	0·769	0·442 to 0·916	0·001
Nocturnal increase in ΔVI, %	0·533	0·051 to 0·814	0·033	0·454	-0·054 to 0·775	NS	0·623	0·184 to 0·855	0·010

Definition of abbreviations: *r*=Pearson correlation coefficient; CI=confidence interval; NS=not significant; REM=rapid eye movement; ΔVI=peripheral chemosensitivity assessed by the withdrawal response

Table S6. Parameters related with daytime blood pressure in OSA patients with isolated nocturnal hypertension

	Daytime systolic BP, mmHg			Daytime diastolic BP, mmHg			Daytime mean BP, mmHg		
	r	95%CI	*p*	*r*	95%CI	*p*	*r*	95%CI	*P*
Sleep efficiency, %	-0·173	-0·616 to 0·353	NS	-0·597	-0·843 to -0·144	0·015	-0·549	-0·821 to -0·073	0·028
REM phase, %	-0·449	-0·773 to 0·060	NS	-0·478	-0·787 to 0·023	NS	-0·526	-0·810 to -0·041	0·036
Diurnal norepinephrine, µg/g	0·634	0·202 to 0·860	0·008	0·057	-0·451 to 0·538	NS	0·260	-0·271 to 0·669	NS
Nocturnal norepinephrine, µg/g	0·628	0·192 to 0·857	0·009	0·167	-0·358 to 0·612	NS	0·341	-0·186 to 0·716	NS

Definition of abbreviations: *r*=Pearson correlation coefficient; CI=confidence interval; NS=not significant; REM=rapid eye movement

Table S7. Parameters related with nighttime blood pressure in OSA patients with day-night sustained hypertension

	Nighttime systolic BP, mmHg			Nighttime diastolic BP, mmHg			Nighttime mean BP, mmHg		
	r	**95%CI**	*p*	*r*	**95%CI**	*p*	*r*	**95%CI**	*P*
Apnea-hypopnea index, h^{-1}	0·374	-0·149 to 0·734	NS	0·569	0·102 to 0·830	0·022	0·538	0·058 to 0·816	0·032
Diurnal norepinephrine, µg/g	0·452	-0·056 to 0·774	NS	0·603	0·153 to 0·846	0·013	0·596	0·142 to 0·843	0·015
Diurnal epinephrine, µg/g	-0·471	-0·784 to 0·032	NS	-0·687	-0·882 to -0·290	0·003	-0·663	-0·872 to -0·249	0·005
Nocturnal norepinephrine, µg/g	0·478	-0·023 to 0·787	NS	0·505	0·012 to 0·800	0·046	0·548	0·072 to 0·821	0·028
Nocturnal epinephrine, µg/g	0·459	-0·048 to 0·778	NS	0·627	0·190 to 0·857	0·009	0·614	0·170 to 0·851	0·011
Aldosterone, ng/dl	0·615	0·172 to 0·851	0·011	0·290	-0·240 to 0·687	NS	0·472	-0·031 to 0·784	NS
ΔVI after sleep, %	0·318	-0·211 to 0·703	NS	0·701	0·315 to 0·888	0·002	0·594	0·139 to 0·842	0·015

Definition of abbreviations: r=Pearson correlation coefficient; CI=confidence interval; NS=not significant; ΔVI=peripheral chemosensitivity assessed by the withdrawal response.

Table S8. Parameters related with daytime blood pressure in OSA patients with day-night sustained hypertension

|	Daytime systolic BP, mmHg			Daytime diastolic BP, mmHg			Daytime mean BP, mmHg		
	r	95%CI	*p*	*r*	95%CI	*P*	*r*	95%CI	*p*
Sleep efficiency, %	-0·609	-0·849 to -0·162	0·012	-0·146	-0·598 to 0·377	NS	-0·478	-0·787 to 0·023	NS
Slow-wave sleep, %	-0·584	-0·837 to -0·124	0·017	-0·465	-0·781 to 0·040	NS	-0·611	-0·849 to -0·165	0·012
Apnea-hypopnea index, h^{-1}	0·345	-0·182 to 0·718	NS	0·638	0·208 to 0·861	0·008	0·527	0·042 to 0·811	0·036
Diurnal norepinephrine, µg/g	0·622	0·183 to 0·854	0·010	0·803	0·510 to 0·929	<0·001	0·772	0·448 to 0·917	<0·001
Nocturnal norepinephrine, µg/g	0·507	0·015 to 0·801	0·045	0·528	0·044 to 0·811	0·035	0·591	0·135 to 0·840	0·016
Nocturnal epinephrine, µg/g	0·421	-0·094 to 0·758	NS	0·799	0·502 to 0·927	<0·001	0·633	0·200 to 0·859	0·008
Aldosterone, ng/dl	0·606	0·158 to 0·847	0·013	0·534	0·052 to 0·814	0·033	0·639	0·210 to 0·862	0·008
Aldosterone-renin ratio	0·547	0·070 to 0·820	0·028	0·704	0·320 to 0·889	0·002	0·682	0·281 to 0·880	0·004

Definition of abbreviations: r=Pearson correlation coefficient; CI=confidence interval; NS=not significant
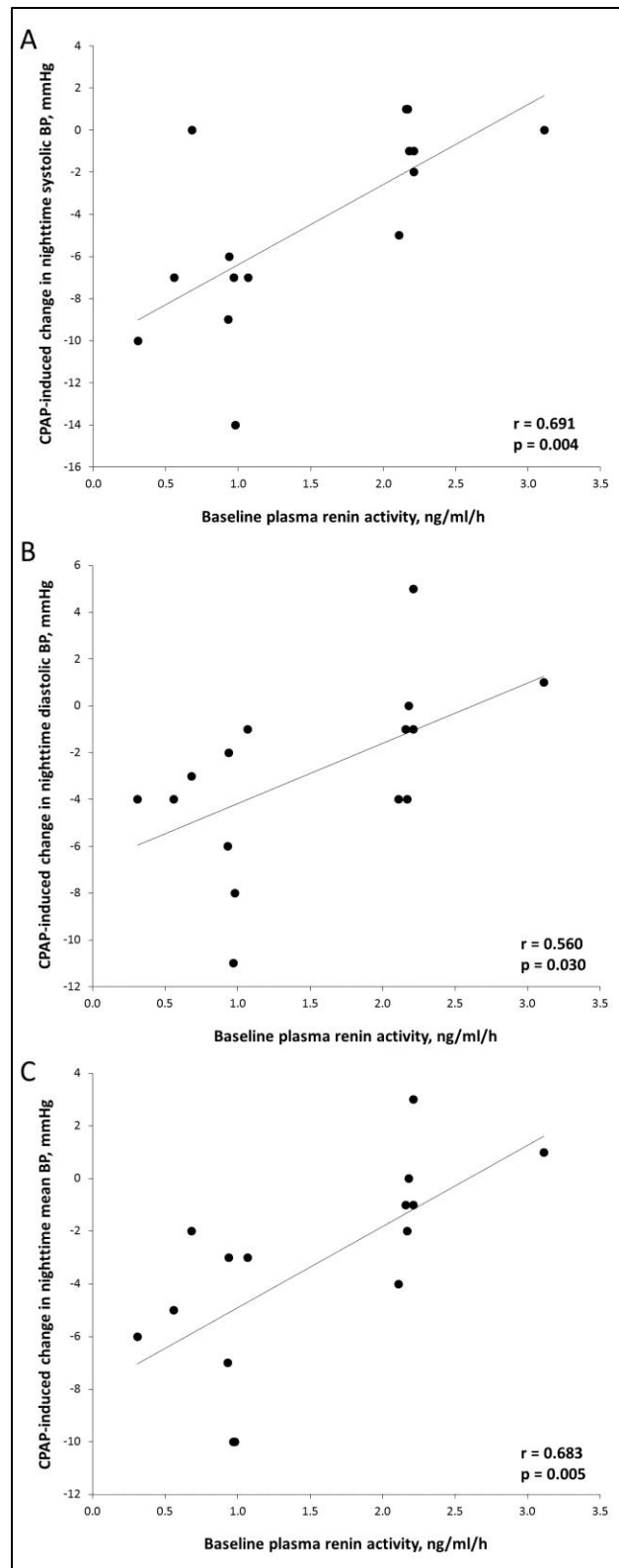
FIGURES

Figure S1. Nocturnal evolution of peripheral chemosensitivity at baseline and after sham CPAP and CPAP in OSA patients with isolated nocturnal hypertension (INH) and day-night sustained hypertension (D-NSH). The group mean ± SEM are shown beside the individual values of each subject. Before and after refer to the values before and after sleep. ΔVI=withdrawal response or index of the peripheral chemoreceptor activity. Significance of comparisons between values after and before sleep: * $p<0.05$; †$p<0.005$
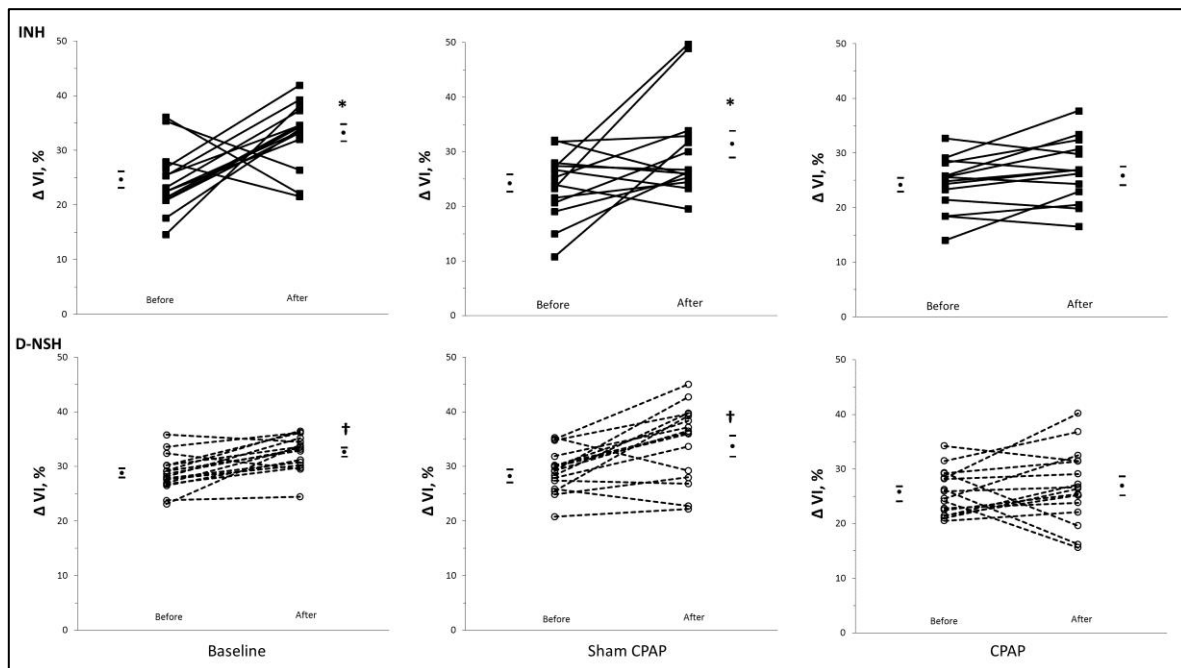
Figure S2. Relationship between the CPAP-induced changes from baseline in peripheral chemosensitivity and urinary excretion of catecholamines in OSA patients with isolated nocturnal hypertension
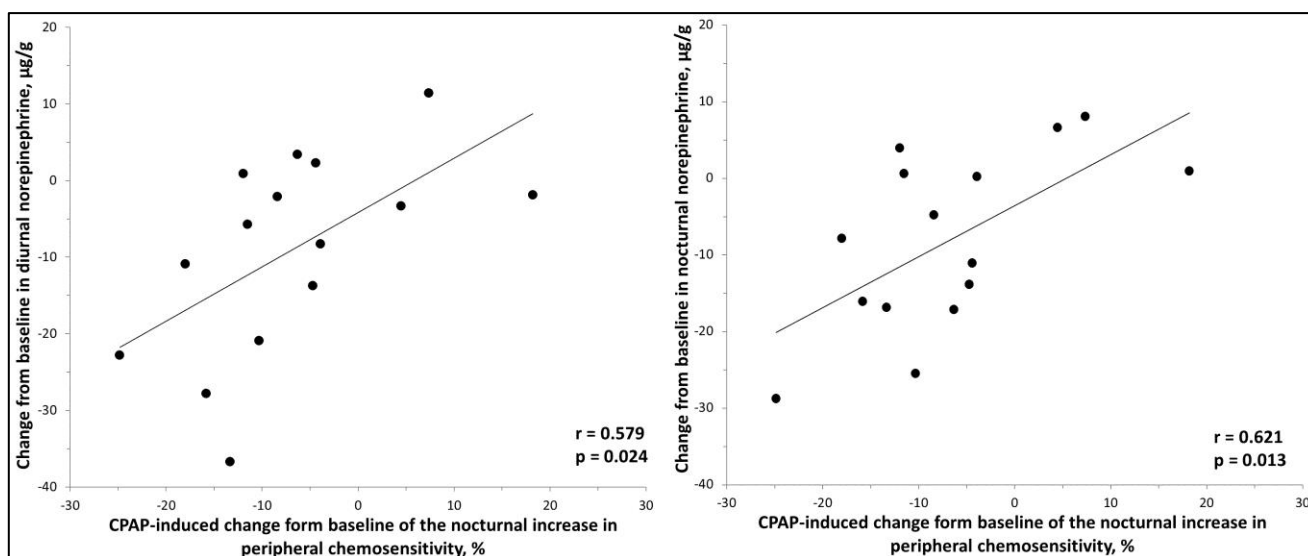
Figure S3. Baseline parameters independently related with CPAP-induced change from baseline on nighttime blood pressures in OSA patients with isolated nocturnal hypertension

Figure S4. Baseline parameters independently related with CPAP-induced change from baseline on diurnal and nighttime mean blood pressures in OSA patients with day-night sustained hypertension