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RESEARCH ARTICLE

Neuroscience of Disease

Intranasal orexin A modulates sympathetic vascular tone: a pilot study in healthy male humans

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Abstract

Previous research suggests that the neuropeptide orexin A contributes to sympathetic blood pressure (BP) control inasmuch as hypothalamic injection of orexin A increases sympathetic vasomotor tone and arterial BP in rodents. In humans with narcolepsy, a disorder associated with loss of orexin-producing neurons, vasoconstrictive muscle sympathetic nerve activity (MSNA) is reduced. Since intranasally administered oligopeptides like orexin are known to modulate brain function, we investigated the effect of intranasal orexin A on vascular sympathetic baroreflex function in healthy humans. In a balanced, double-blind cross-over study, orexin A (500 nmol) and placebo, respectively, were intranasally administered to 10 lean healthy males (age 25.8 ± 4.6 yr). MSNA was assessed microneurographically before and 30–45 min after either substance administration. Additionally, baroreflex was challenged via graded infusions of vasoactive drugs before and after substance administration. Baroreflex function was defined as the correlation of BP with MSNA and heart rate. Intranasal orexin A compared with placebo induced a significant increase in resting MSNA from pre-to postadministration [Δburst rate, orexin A vs. placebo: + 5.8 ± 0.8 vs. + 2.1 ± 0.6 bursts/min, P = 0.007; total activity 169 ± 11.5% vs. 115 ± 5.0%; P = 0.002]. BP, heart rate, and sympathovagal balance to the heart, as represented by heart rate variability (HRV), as well as baroreflex sensitivity during the vasoactive challenge were not altered. Intranasally administered orexin A acutely induced vasoconstrictory sympathoactivation in healthy male humans. This result suggests that orexin A mediates upward resetting of the vascular baroreflex set point at centers superordinate to the mere baroreflex feedback loop.

NEW & NOTEWORTHY Our pilot study adds another important part to the complex network of neuroendocrine-sympathetic interaction. Our results demonstrate that intranasal orexin A elicits an excitatory effect on sympathetic vascular tone superordinate to mere baroreflex feedback regulation. This resetting of the baroreflex set point suggests an activation of hypothalamic core centers such as the paraventricular nucleus (PVN). The role of the orexinergic system in the development of neurogenic arterial hypertension warrants further investigations.

baroreflex; intranasal; muscle sympathetic nerve activity; orexin A; sympathetic nervous system

INTRODUCTION

Orexin A and orexin B, also referred to as hypocretin 1 and hypocretin 2, are hypothalamic neuropeptides derived from the same precursor peptide, prepro-orexin. Both orexins exert their effects via G protein-coupled receptors orexin-1 and orexin-2 receptors (1, 2). Synthesis of the orexins is almost exclusively localized in the hypothalamus. Orexin-containing axons and orexin receptors are widely distributed throughout the central nervous system (CNS) (2, 3). As to be

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assumed by this distribution pattern, the orexinergic system is involved in several physiological central nervous functions. Animal studies have shown that orexin A in particular is a potent regulator of food intake (4) as well as wakefulness and arousal (5, 6). Furthermore, experiments in rodents provided evidence of an interaction with sympathetic nervous blood pressure (BP) control: Intracerebrally injected orexin had differential effects on BP, heart rate (HR), sympathetic nerve activity (SNA), and sympathetic vasomotor tone depending on the injection site (7–11), whereas intravenous administration of orexin showed no or little effect on cardiovascular function (8, 12). Accordingly, key structures of central nervous BP control like the paraventricular nucleus (PVN) of the hypothalamus show an exceptionally high density of orexin receptors and orexin-containing fibers. The PVN is essentially involved in sympathetic-neuroendocrine interactions (3, 13, 14). Direct injection of orexin A into the PVN increases sympathetic activity, a sympathoexcitatory effect that is blunted by coadministration of an orexin-1 receptor antagonist (15). Furthermore, recent reviews stated the hypothesis that an overactivity of the orexinergic system might contribute to the development of neurogenic hypertension (16–18).

The blood-brain barrier (BBB) limits the distribution of systemically administered substances to the central nervous system (CNS). Intracerebral injection of neuroendocrine substances is a highly invasive technique and not suitable for human experimental studies. Intranasal administration is a noninvasive method that delivers drugs to the CNS, bypassing the BBB and minimizing systemic exposure and side effects (19). Intranasal administration of orexin has been successfully studied in animal models. Dhuria et al. (20) reported a rapid increase in brain and spinal cord tissue concentration of orexin after intranasal administration within 30 min in rats. Similarly, Deadwyler et al. (21) reported an increase of orexin levels in the cerebrospinal fluid (CSF) of primates ~10 min after intranasal administration. Although the distribution of intranasally administered orexins in brain parenchyma and/or CSF in humans has not yet been described, the feasibility and effectiveness of the intranasal approach for the delivery of neuropeptides into the human CNS have been repeatedly demonstrated (for reviews see Refs. 22–24).

In humans, the orexinergic system has been extensively studied in patients with narcolepsy. Narcolepsy with cataplexy is a rare sleep disorder that most likely originates from a loss of orexin-producing neurons in the lateral hypothalamus (25). In narcoleptic patients, intranasal orexin has stabilizing effects on sleep structure, especially regarding rapid eye movement (REM) sleep (26, 27). These observations and related functional changes suggest that intranasally administered orexin exerts central nervous effects in humans. In addition to excessive daytime sleepiness, patients with narcolepsy often suffer from autonomic dysfunctions (28). Notably, microneurographic assessment of muscle sympathetic nerve activity (MSNA) showed a significantly reduced sympathetic vasoconstrictive tone, combined with lower HR and BP during wakefulness in patients with the disorder compared to matched healthy control subjects. Moreover, decreased vasoconstrictive MSNA showed a correlation with the level of deficiency of orexin concentration in the CSF (29).

To investigate the contribution of orexin A to central nervous sympathetic BP control, we assessed set point and sensitivity of sympathetic baroreflex function after intranasal orexin A in healthy male volunteers according to a placebo-controlled crossover design. We specifically assessed MSNA in correlation with BP and HR. Additionally, plasma catecholamines and hormones of the hypothalamic-pituitary-adrenal (HPA) axis were measured. We hypothesized an increase in MSNA and/or BP after intranasal orexin indicating an upward shift of the BP set point of baroreflex regulation.

MATERIALS AND METHODS

Study Population

Twelve normotensive male volunteers without any history of chronic diseases participated in the experiments. The participants were aged between 20 and 33 yr (mean ± SD 25.8 ± 4.6 yr), had normal weight [body mass index (BMI) 23.2 ± 2.4 kg/m^2], were free of permanent medication, and were nonsmokers. Volunteers had to abstain from beverages containing alcohol and/or caffeine as well as intense physical efforts for at least 24 h before the experiment. Additionally, they were asked to fast after 2200 of the previous day. Shift work and substance abuse were exclusion criteria. The local Ethics Committee approved the study (University of Lübeck), and written informed consent was obtained from all participants.

Experimental Design and Protocol

In a balanced, double-blind placebo-controlled within-subject design, either orexin A or placebo was administered intranasally, i.e., half of the participants started with the orexin A condition and the other half with the placebo condition. All participants attended both experimental sessions, which were separated by at least 1 wk. Each experimental session consisted of measurements before and after the respective substance administration (Fig. 1), permitting comparisons within each experimental session and between sessions.

The experiments were performed at our neurophysiological laboratory starting at 7:30 AM. After the participants were briefly interviewed regarding their last meal and coffee and/or alcohol intake, they were investigated in a comfortable, supine position under standardized conditions (temperature, illumination, and background noises). The placement of two intravenous cannulas was followed by application of oscillometric BP (Vital Signs Monitor 300; Welch Allyn, Skaneateles Falls, NY) and continuous ECG monitoring. Subsequently, a tungsten electrode was inserted into the sympathetic fascicle of the peroneal nerve to obtain direct multiunit MSNA recordings and a reference electrode was placed into the subcutaneous tissue at a distance of 2–3 cm. This technique follows the broadly accepted practice of MSNA measurement. The technical details and evidence that the recorded activity is of muscle sympathetic origin have been published previously (30). Analog curves of HR and MSNA were continuously recorded throughout the experiment, digitized online, and stored for further computer-based analysis (PowerLab; ADInstruments, Colorado Springs, CO).

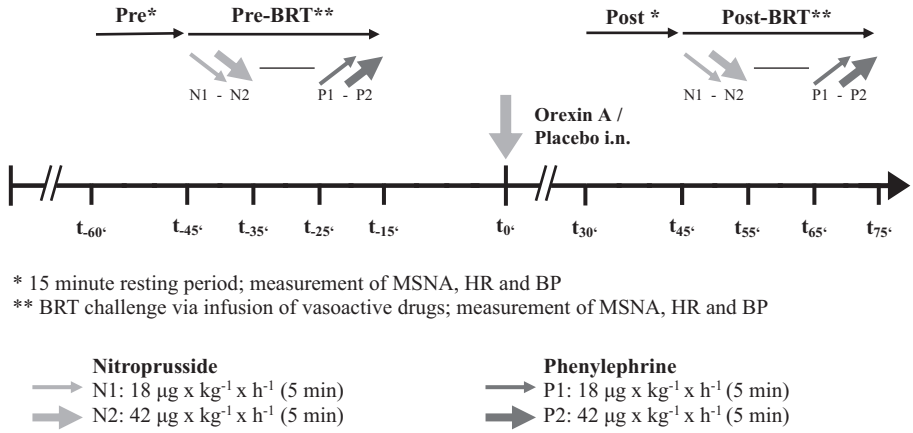

Figure 1. Schematic illustration of the experimental protocol consisting of 2 recording periods at unaffected rest (15 min): first the presubstance period, i.e., before orexin A or placebo intranasal (i.n.) administration, and then postsubstance periods starting from 30 min (postsubstance) after administration. Additionally, each experimental session included measurements during pharmacological baroreflex challenge before and after substance administration, with 2 incremental doses (thickness of arrows) of nitroprusside (N1, N2) and phenylephrine (P1, P2) separated by a washout interval. BP, blood pressure; BRT, baroreflex test; HR, heart rate; MSNA, muscle sympathetic nerve activity.

To determine MSNA, HR, and BP at unaffected rest, a 15-min recording period was carried out 60 min (t_{-60}) before the intranasal administration of either placebo or orexin A (presubstance). To define the vascular and cardiac baroreflex set point and sensitivity, baroreflex function was challenged with vasoactive drugs at incremental doses according to the well-established graded infusion protocol (31). In brief, sodium nitroprusside was infused intravenously in two steps (18 and 42 $\mu g \cdot kg^{-1} \cdot h^{-1}$), with each step lasting 5 min to obtain steady-state conditions at various BP levels. After a 10-min washout phase, phenylephrine was administered likewise (18 and 42 $\mu g \cdot kg^{-1} \cdot h^{-1}$). BP and HR measurement were repeated twice during the last 2 min of each step of both vasoactive substances. Furthermore, MSNA was assessed during the latter 2 min of the respective steady-state periods of baroreflex challenge. The preparation of the intranasal sprays containing orexin A ($C_{152}H_{243}N_{47}O_{44}S_4$, M_r 3,561.16; catalog number H-4172-GMP, Bachem Biochemica, Bubendorf, Switzerland) and placebo (vehicle) has been described elsewhere (26, 27). In brief, we administered orexin A at t_0, alternating to both nostrils over a time span of 5–10 min with a cumulative dosage of 500 nmol. Starting from ~30 min after sniffing (t_{30}), MSNA, HR, and BP measurements at unaffected rest as well as the baroreflex assessment protocol were repeated after administration of either substance (postsubstance period). Furthermore, short psychological assessments were performed once before and twice after sniffing of either substance. The schedule was predetermined according to the expected latency in increase of orexin A levels in the brain (20, 21, 27). Blood samples were drawn at t_{-60}, t_{30}, and t_{120}. The samples were immediately centrifuged and stored at −80°C for further analysis (32).

Data Analysis

Two investigators, blinded to the respective condition, independently identified sympathetic bursts by inspection of the mean voltage neurogram. Recordings of MSNA bursts were considered acceptable when the signal-to-noise ratio was at least three times above baseline noise level (Chart version 5.5.5; ADInstruments). The quality of recordings was further scrutinized by comparison of burst morphology and amplitude at inspiratory apnea, a strong sympathoexcitatory maneuver.

Sympathetic nerve activity was expressed as burst rate (bursts/min) as well as total activity (burst rate × burst amplitude). Burst rate and total activity of MSNA as well as HR and BP were assessed during 10 artifact-free minutes of each of the recordings at unaffected rest (presubstance and postsubstance).

Frequency domain measurements of heart rate variability (HRV) were calculated from 5-min recordings of the pre- and postsubstance periods at unaffected rest. The limits of low frequency and high frequency were defined according to international recommendations and expressed as normalized units.

For assessment of vascular and cardiac baroreflex function, the baroreflex set point was defined as the BP level in an awake unstressed individual at supine rest with the corresponding MSNA or HR (33). Under physiological conditions, reflex activation and/or deactivation oscillate around this BP threshold. Reflex changes of MSNA and HR in response to BP changes during baroreflex challenge via infusion of vasoactive drugs result in a stimulus-response curve of BP with MSNA or HR. Although this curve is principally sigmoid, the increment of the linear intercept around the set point determines baroreflex sensitivity, and the dose steps chosen fit well with the linear part. Moreover, baroreflex function was assessed from the last 2 min of each dosing step of vasoactive drug infusion.

Biochemical Analysis

All biochemical analyses were performed with commercially available and standardized assays. Blood samples were immediately centrifuged and aliquoted, and the serum or plasma was stored at −80°C until further analysis. Levels of cortisol, renin, and ACTH were determined by commercially available assays. Plasma levels of norepinephrine (NE) were analyzed by high-performance liquid chromatography (HPLC) (ClinRep; Recipe, Munich, Germany).

Psychometric Analysis

The feelings "hungry" and "tired" were rated on a visual analog scale (VAS; 0–100 mm, anchored at "not at all" and "extremely") before and ~30 (early) and 90 (delayed) min after intranasal administration of orexin A or placebo.

Statistical Analysis

The primary outcome measure was orexin A-related change in MSNA (burst/min and total activity). Individual data (BP, HR, MSNA, as well as blood parameters) were averaged and expressed as means ± SE. We expected changes and variations in MSNA similar to those observed after growth hormone releasing hormone (GHRH) delivery (32). Therefore, a sample size of $n = 12$ was estimated to be required to detect an effect with the size of 0.45 with a power of 0.8 at a two-sided α of 0.05 in a repeated-measures ANOVA, if repeated measures of increase had a correlation of 0.5, as the treatment × time interaction effect size was 0.8 in the GHRH study. Statistical analyses were performed on absolute values of BP, HR, and MSNA. Additionally, for each session postadministration MSNA recordings at rest were normalized with reference to the recording period before substance administration, to account for potential differences in baseline activity due to disparities in recording quality between sessions and to better discriminate substance-induced effects (32, 34), yielding resulting net changes (Δ values). Measures at unaffected rest (BP, HR, and MSNA burst rate and total activity) were analyzed by repeated two-way ANOVA with group factor treatment (orexin A vs. placebo) and repeated-measures factor time. Net changes were analyzed by paired Student's *t* test.

To evaluate the effects of BP on MSNA burst rate, mixed analysis of covariance (ANCOVA) with random intercepts and slopes was calculated to test the treatment × time interaction. In addition, modeling for a mediation analysis was performed to assess whether changes of MSNA were mediated by changes in BP or independent from BP changes. Starting from the triangular graph with orexin A changing both MSNA and BP and an effect of BP on MSNA, structural equation modeling (R 4.0.3 package lavaan 0.6-7) was used to estimate the direct effect of orexin A on MSNA by latent change score (35) for both MSNA and BP with one path coefficient linking BP and MSNA in both treatment settings (Supplemental Fig. S1; see https://doi.org/10.6084/m9.figshare.15000411.v1).

The individual data of baroreflex testing with vasoactive drugs were analyzed by ANCOVA with random effects. Moreover, the individual reflex changes of MSNA and HR were divided by the corresponding change of mean arterial pressure (MAP) at *dose step 2* of sodium nitroprusside or phenylephrine, respectively, to determine individual increments of baroreflex gain at either branch of baroreflex testing, i.e., vasodilation or vasoconstriction, separately. This quotient was compared by paired Student's *t* test. Biochemical parameters were analyzed by ANOVA.

A Greenhouse–Geisser-corrected P value of <0.05 was considered statistically significant. Post hoc testing was performed when overall analysis indicated significance. Statistical analyses were performed with SPSS software, version 26 (SPSS for Windows, Munich, Germany). Sample size calculations (power and effect size) were performed with G·Power 3.1.3 (Franz Faul, University of Kiel, Germany).

RESULTS

MSNA and Circulatory Parameters during Recordings at Unaffected Rest

Two of the recordings did not fulfill standard quality requirements (1 shift of MSNA baseline and 1 dislocation of the tungsten electrode). Accordingly, both respective participants had to be excluded from the final cohort. Therefore, in total, 10 participants were included in the final analysis (for raw data see Supplemental Fig. S2, https://doi.org/10.6084/m9.figshare.17818202.v1).

Before the intervention, MSNA, HR, and BP at rest were comparable between the experimental sessions (Table 1). MSNA burst rate (burst/min) was 26.2 ± 2.7 before intranasal orexin A administration (presubstance) and increased to 32.0 ± 2.7 (postsubstance) ~30 min after administration of orexin A. In the placebo condition, MSNA burst rate showed no significant changes. The increase in MSNA burst rate in the orexin A condition compared with the placebo condition was statistically significant as analyzed by ANOVA ($F = 12.1$, $P = 0.007$ for treatment × time) (Fig. 2*A*). The net changes of MSNA (ΔMSNA burst rate) increased by +5.8 ± 0.8 after orexin A, exceeding the change in the placebo condition (+2.1 ± 0.6; Student's *t* test: $P = 0.007$). Correspondingly,

Table 1. *MSNA and circulatory parameters of the respective experimental conditions at unaffected rest before (pre) and ~30–45 min after (post I) intranasal administration of either substance*

Parameter	Orexin A	Placebo	Δ Orexin A	Δ Placebo	*P* (ANOVA)
MSNA, burst/min					
Pre	26.2 ± 2.7	25.3 ± 2.5			
Post I	32.0 ± 2.7	27.4 ± 2.4	5.8 ± 0.8	2.1 ± 0.6	0.007
MAP, mmHg					
Pre	85.4 ± 1.7	86.6 ± 2.4			
Post I	87.9 ± 2.2	87.6 ± 2.5	2.5 ± 1.8	1.0 ± 1.5	0.49
Systolic BP, mmHg					
Pre	117.4 ± 2.4	120.9 ± 2.8			
Post I	120.8 ± 3.4	121.7 ± 3.6	3.3 ± 2.4	0.8 ± 1.7	0.20
Diastolic BP, mmHg					
Pre	69.4 ± 1.8	69.5 ± 2.6			
Post I	71.5 ± 2.1	70.5 ± 2.4	2.1 ± 2.0	1.1 ± 1.6	0.73
Heart rate, min^{-1}					
Pre	63.9 ± 1.7	63.1 ± 2.5			
Post I	62.8 ± 1.8	61.9 ± 2.0	−1.1 ± 1.3	−1.2 ± 1.8	0.92

Values are mean ± SE absolute values and postsubstance period net changes (Δ values) after either orexin A or placebo with reference to the corresponding presubstance period. BP, blood pressure; MAP, mean arterial pressure; MSNA, muscle sympathetic nerve activity.

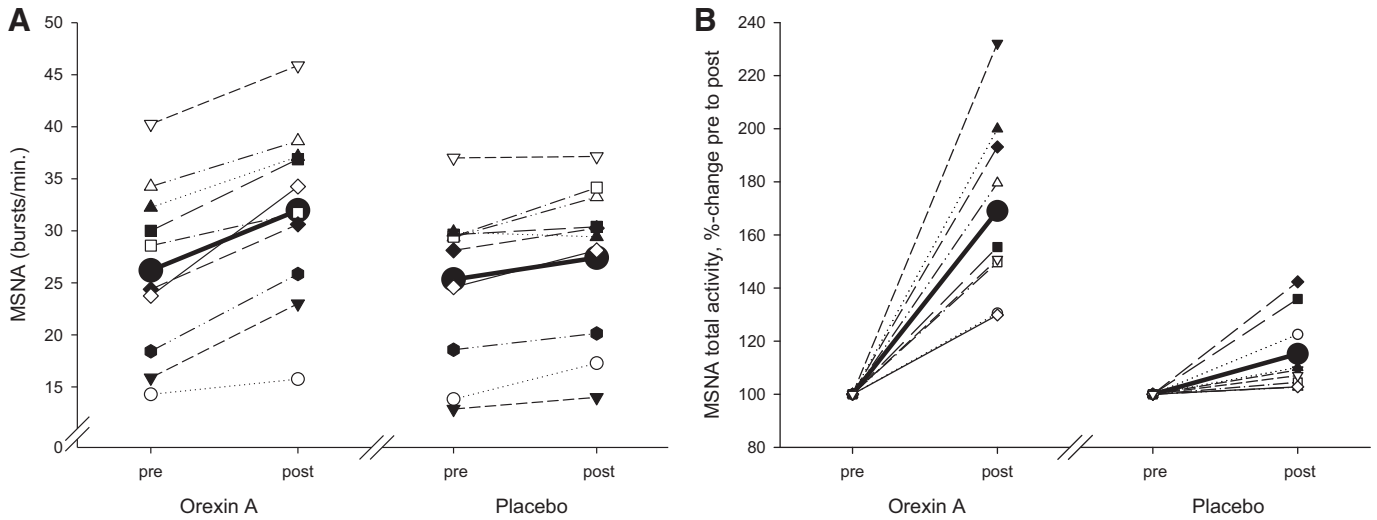

Figure 2. *A*: mean (thick line) and individual absolute muscle sympathetic nerve activity (MSNA) burst rate (burst/min) at unaffected rest before (pre) and ~30–45 min after (post) administration of either orexin A or placebo. *B*: mean (thick black circle) and individual % changes in MSNA total activity at unaffected rest ~30–45 min after (post) administration of either orexin A or placebo with reference to measurements before (pre) substance administration (100%).

MSNA total activity [burst rate × burst amplitude; represented as changes from presubstance period (100%)] increased significantly to 169 ± 11.5% after intranasal orexin A compared with 115 ± 5.0% in the placebo condition (ANOVA: $F = 19.3$, $P = 0.002$ for treatment × time) (Fig. 2*B*). Absolute values or net changes from pre- to postsubstance of circulatory parameters, i.e., HR and BP (diastolic, systolic, and MAP), showed no statistically significant differences between conditions.

To assess effects of hemodynamic parameters on the dependent variable MSNA, a mixed ANCOVA was performed. The ANCOVA confirmed that changes in MSNA burst rate were independent from changes in systolic BP ($F = 6.1$, $P = 0.02$), diastolic BP ($F = 7.7$, $P = 0.01$), MAP ($F = 7.0$, $P = 0.01$), and HR ($F = 8.0$, $P = 0.009$). Similarly, increases of MSNA total activity were confirmed to be independent from systolic BP ($F = 13.8$, $P = 0.001$), diastolic BP ($F = 14.0$, $P = 0.001$), MAP ($F = 13.9$, $P = 0.001$), and HR ($F = 13.6$, $P = 0.001$). A supplementary mediation analysis strengthened these results: The parameter estimate of the direct effect of orexin A on change of resting MSNA between the presubstance period and the postsubstance period was 6.4 [95% confidence interval (CI) 4.1 to 8.8], and the indirect effect was 2.5 × 0.01 = 0.025 if mediated by change in diastolic BP (Table 2, Supplemental Fig. S1). This again indicates that changes of MSNA following orexin were independent from BP changes.

Frequency domain measures of HRV did not differ at unaffected rest before and after intranasal substance administration or between the respective experimental versus control condition (see Supplemental Table S1, https://doi.org/10.6084/m9.figshare.17738639.v1).

Table 2. *Direct effect of orexin A on net changes of MSNA in a structural equation model of mediation by different net BP variables and HR*

	Direct Effect of Orexin A on ΔMSNA pre/post		
Mediator	**Estimate**	**95% CI**	***P* Value**
ΔDiastolic BP	6.4	4.1 to 8.8	<0.001
ΔSystolic BP	6.6	4.1 to 9.1	<0.001
ΔMAP	6.5	4.1 to 8.9	<0.001
ΔHeart rate	6.5	4.1 to 9.0	<0.001

BP, blood pressure; CI, confidence interval; MAP, mean arterial pressure; MSNA, muscle sympathetic nerve activity.

MSNA and Circulatory Parameters at Baroreflex Testing

Sodium nitroprusside infusion.

Both incremental doses of the direct vasodilator sodium nitroprusside caused a progressive decline in BP accompanied by a counterregulating increase in MSNA and HR, as expected. Absolute values of BP, HR, and MSNA were similar in both experimental conditions.

Phenylephrine infusion.

Both incremental doses of phenylephrine, an α_1-agonist, resulted in a progressive elevation of BP. This BP increase was accompanied by a reflexive decrease in MSNA and HR. Absolute values of BP, MSNA, and HR showed no statistical differences between pre- and postsubstance within each session as well as between corresponding periods of both conditions (Fig. 3, Table 3).

ANCOVA.

Mixed ANCOVA throughout baroreflex testing and subanalysis for each branch of the vasoactive baroreflex challenge (i.e., vasoconstriction and vasodilation) separately did not reveal any statistically significant treatment effects between presubstance and postsubstance baroreflex challenge depending on orexin A or placebo administration (Fig. 4 and Supplemental Fig. S3; see https://doi.org/10.6084/m9.figshare.17818925.v1). This indicates that there were no changes in baroreflex sensitivity ~45 min after intranasal orexin A. These findings were further confirmed by analysis of the individual increments at nitroprusside or phenylephrine infusion per Student's *t* test.

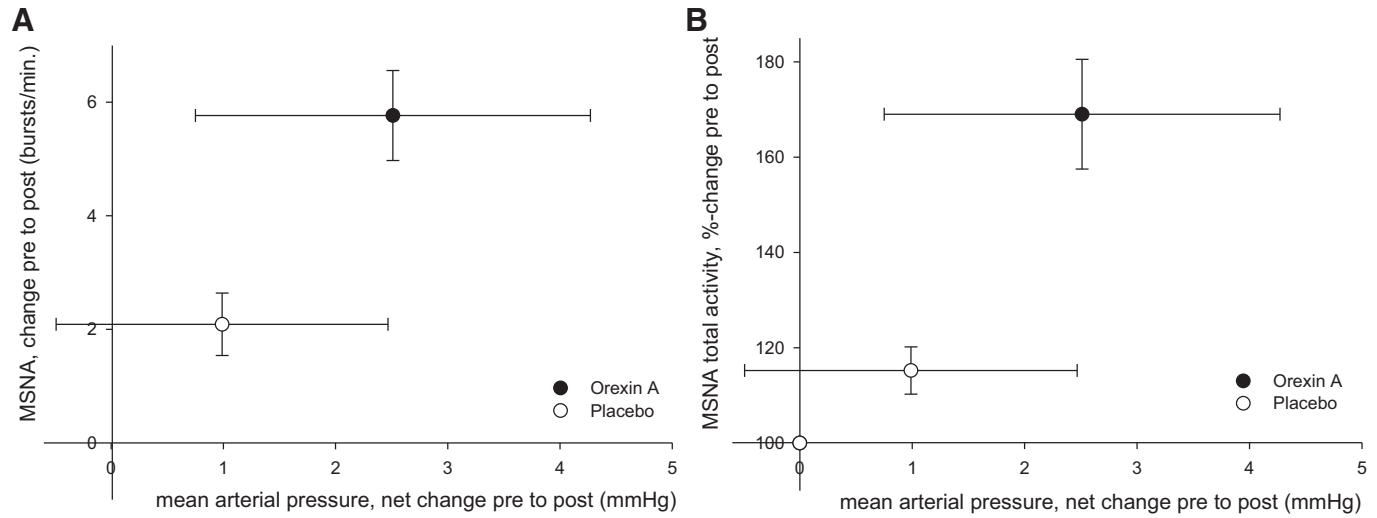

Figure 3. Resetting of baroreflex set point toward higher resting muscle sympathetic nerve activity (MSNA) burst rate (burst/min; *A*) and total activity (% changes; *B*) ~30–45 min after (post) intranasal orexin A without significant blood pressure (BP) alterations [mean arterial pressure (MAP)] in comparison to placebo; data in reference to the respective measurements at unaffected rest before (pre) administration of either substance (presubstance period). Values are means ± SE.

Blood Parameters

Baseline measurements of ACTH, cortisol, renin, and norepinephrine did not differ between experimental sessions at baseline, and there was no differential effect of orexin administration (Table 4).

Psychometric Parameters

The feeling of hunger significantly increased after orexin A from normalized presubstance ratings; however, this alteration did not differ significantly from corresponding placebo ratings. The feeling of tiredness significantly decreased at 30

Table 3. *Baroreflex testing with vasoactive drugs before and after intranasal administration of orexin A or placebo*

Baroreflex Condition	Parameter	Orexin A	Placebo	Δ Orexin A	Δ Placebo
		Baroreflex test presubstance			
Nitroprusside (max. dose)	MSNA, bursts/min	41.4 ± 3.2	40.8 ± 3.2	15.2 ± 2.9	15.4 ± 2.4
	MAP, mmHg	79.2 ± 1.4	77.6 ± 2.3	−6.2 ± 1.2	−9.0 ± 1.0
	Systolic BP, mmHg	112.1 ± 2.1	113.2 ± 2.4	−5.3 ± 1.6	−7.7 ± 1.3
	Diastolic BP, mmHg	62.7 ± 1.4	59.8 ± 2.6	−6.7 ± 1.2	−9.7 ± 1.2
	Heart rate, min^{-1}	79.1 ± 3.0	77.0 ± 3.3	15.2 ± 2.1	13.9 ± 2.4
	ΔMSNA/ΔMAP			1.0 ± 1.4	1.8 ± 0.3
	ΔHR/ΔMAP			1.2 ± 1.1	1.8 ± 0.4
Phenylephrine (max. dose)	MSNA, bursts/min	13.4 ± 2.5	13.9 ± 2.5	−12.8 ± 2.1	−11.5 ± 2.1
	MAP, mmHg	91.8 ± 2.1	89.4 ± 2.4	6.4 ± 1.8	2.8 ± 1.6
	Systolic BP, mmHg	122.3 ± 3.2	121.5 ± 3.1	4.8 ± 2.1	0.6 ± 1.8
	Diastolic BP, mmHg	76.1 ± 2.1	73.4 ± 2.4	6.7 ± 2.2	3.9 ± 1.7
	Heart rate, min^{-1}	58.0 ± 1.9	54.5 ± 2.2	−5.9 ± 2.2	−8.7 ± 1.7
	ΔMSNA/ΔMAP			4.2 ± 1.1	2.4 ± 1.2
	ΔHR/ΔMAP			1.8 ± 0.5	1.4 ± 0.8
		Baroreflex test postsubstance			
Nitroprusside (max. dose)	MSNA, bursts/min	47.1 ± 2.6	44.0 ± 2.4	15.2 ± 1.8	16.6 ± 1.8
	MAP, mmHg	77.8 ± 2.4	79.0 ± 3.1	−10.1 ± 1.4	−8.6 ± 2.2
	Systolic BP, mmHg	111.9 ± 3.0	116.7 ± 3.7	−8.9 ± 1.0	−5.1 ± 2.0
	Diastolic BP, mmHg	60.8 ± 2.5	59.8 ± 3.0	−10.7 ± 1.9	−10.8 ± 2.3
	Heart rate, min^{-1}	78.2 ± 2.8	77.3 ± 2.9	15.4 ± 1.4	15.4 ± 1.5
	ΔMSNA/ΔMAP			1.9 ± 0.4	2.2 ± 1.1
	ΔHR/ΔMAP			1.9 ± 0.3	2.0 ± 0.9
Phenylephrine (max. dose)	MSNA, bursts/min	14.0 ± 3.0	15.6 ± 2.8	−18.0 ± 3.0	−11.8 ± 1.6
	MAP, mmHg	92.5 ± 3.2	89.5 ± 3.3	4.6 ± 3.0	1.9 ± 1.2
	Systolic BP, mmHg	125.7 ± 4.7	123.1 ± 4.3	4.9 ± 4.4	1.4 ± 1.7
	Diastolic BP, mmHg	76.0 ± 2.9	73.0 ± 3.0	4.5 ± 2.7	2.5 ± 1.2
	Heart rate, min^{-1}	54.0 ± 1.5	53.8 ± 2.0	−8.8 ± 2.5	−8.1 ± 1.8
	ΔMSNA/ΔMAP			1.8 ± 3.5	2.3 ± 3.3
	ΔHR/ΔMAP			2.3 ± 2.2	0.1 ± 1.3

Values are mean ± SE absolute values and net changes (Δ orexin A and Δ placebo) of muscle sympathetic nerve activity (MSNA) and circulatory parameters related to respective preceding measurements at unaffected rest. BP, blood pressure; HR, heart rate; MAP, mean arterial pressure.

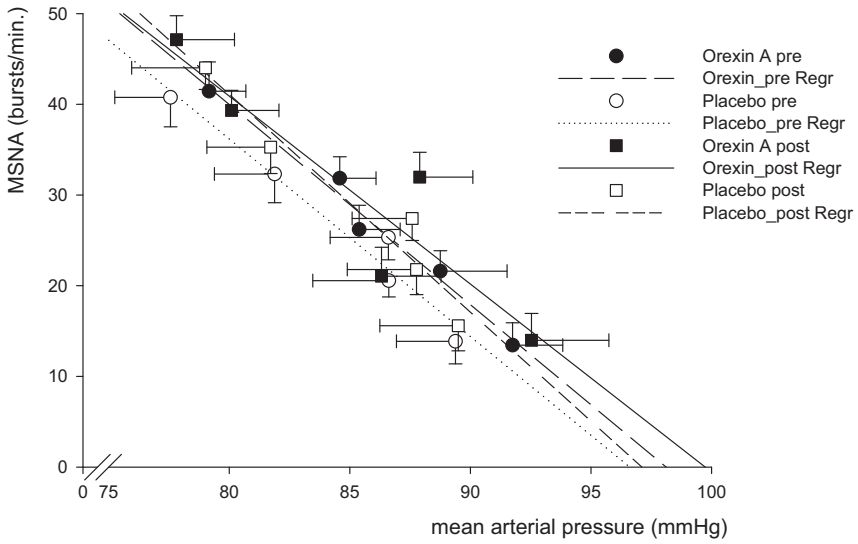

Figure 4. Stimulus-response curve of mean arterial pressure (MAP) and muscle sympathetic nerve activity (MSNA) burst rate during baroreflex testing. Analysis of covariance (ANCOVA) with random effects indicates that the individual baroreflex sensitivity, i.e., the increment of the linear slopes, was not altered by intranasal orexin A. Black circles and large dashed lines show values for the orexin A condition before administration (pre). Black squares and solid lines represent the values for the orexin A condition after administration (post). White circles and dotted lines represent values for the placebo before administration. White squares and small dashed lines represent values for the placebo condition after administration. Regr, regression.

min after orexin A compared with placebo [Supplemental Figs. S4 (see https://doi.org/10.6084/m9.figshare.17736524.v1) and S5 (see https://doi.org/10.6084/m9.figshare.17823080.v1) and Supplemental Table S2 (see https://doi.org/10.6084/m9.figshare.17737592.v1)].

DISCUSSION

In our study in young and healthy males, we were able to demonstrate specific sympathoexcitatory effects of intranasally administered orexin A on the vasculature of skeletal muscles as assessed via MSNA. Both MSNA burst rate and total activity were increased ~30–45 min after administration of orexin A compared with placebo, whereas alterations of BP and HR were absent. Mediation analysis and covariance analyses further confirmed that the orexin effect on MSNA emerged independently from BP. The increase in vascular sympathetic tone observed after intranasal orexin A was not accompanied by treatment-induced changes of HR, sympathovagal balance (represented by frequency domain analysis of HRV), and/or overall sympathetic activity as measured by plasma norepinephrine (NE). Therefore, our findings suggest that orexin A acutely mediates sympathoexcitation specifically on the vascular branch of the baroreflex. However, our findings do not point toward an equivalent effect on the cardiac and/or overall sympathetic activity.

BP and MSNA or HR are tightly regulated by feedback mechanisms of the baroreflex loop. Therefore, MSNA and HR always must be interpreted with regard to the prevailing BP. According to this simplified cybernetic baroreflex paradigm, MSNA or HR oscillates around the BP threshold of reflex activation and deactivation, called the set point. Thus, a similar increase in MSNA, as found after intranasal orexin A in the present study, usually reflects sympathetic counterregulation at baroreceptor and brain stem levels in response to a decreased BP. In contrast, the significant increase in MSNA after orexin A was not accompanied by a concomitant BP drop. Therefore, the increase in vascular sympathetic activity after orexin A administration implies a resetting of the baroreflex set point itself. In the present study, the rise in MSNA following intranasal orexin A did not translate into a significant BP elevation, yet the net effect is still sympathoexcitation beyond baroreflex level. Consistently, the slope of reflex changes of MSNA and HR in response to vasoactive drug challenge was not altered but was shifted in parallel toward higher sympathetic activity, indicating that baroreflex sensitivity was unaffected.

Physiologically, resetting of the baroreflex set point occurs according to instantaneous needs, e.g., during physical

Table 4. *Laboratory parameters in the orexin A and placebo conditions at respective sampling times*

Parameter	Time	Orexin A	Placebo	*P* (ANOVA) Treatment × Time
Cortisol, μg/dL	$t = -60$	13.5 ± 1.4	12.8 ± 1.2	
	$t = +30$	9.4 ± 0.7	9.2 ± 0.9	
	$t = +120$	9.8 ± 1.4	8.2 ± 0.7	0.55
ACTH, pg/mL	$t = -60$	30.6 ± 6.2	25.6 ± 3.1	
	$t = +30$	23.3 ± 3.8	21.9 ± 2.7	
	$t = +120$	26.7 ± 5.4	22.3 ± 2.7	0.25
Renin, pg/mL	$t = -60$	8.3 ± 1.8	8.6 ± 0.9	
	$t = +30$	7.7 ± 1.7	7.5 ± 0.8	
	$t = +120$	7.2 ± 1.2	6.9 ± 0.7	0.06
Norepinephrine, pg/mL	$t = -60$	225.7 ± 18.9	199.3 ± 25.1	
	$t = +30$	210.0 ± 22.1	207.3 ± 33.7	
	$t = +120$	220.9 ± 28.8	216.6 ± 18.3	0.50

Values are means ± SE. Times are before (−) and after (+) substance administration.

activity the baroreflex set point is upregulated (36) whereas sleep induces a reduction in sympathetic activity to heart and vasculature leading to BP dipping (37, 38). Such resetting of the baroreflex set point is mediated by feedforward commands from centers superordinate to the baroreflex feedback loop, like the hypothalamic PVN. Neuroendocrine oscillators modulate baroreflex resetting, e.g., leptin, oxytocin, and angiotensin (34, 39, 40). Our findings suggest a feedforward activation of hypothalamic sympathetic signals toward higher resting activity of the sympathetic vascular branch by intranasal orexin A without alterations of baroreflex sensitivity. The cardiac branch, which is coregulated by vagal circuits, appears to be unaffected because HR remained unaltered.

Our interpretation is supported by data from animal studies. Whereas intravenous administration of orexin A failed to affect cardiovascular and renal sympathetic nerve activity, equivalent doses of intracerebroventricular microinjections of orexin A elicited an increase in BP and plasma catecholamine concentrations in rabbits (12). Further experiments in narcoleptic dogs confirmed that only very high doses of intravenous orexin A were able to penetrate the BBB and produce short-lasting effects (41). Although sympathoexcitatory effects of intracerebral orexin A were consistently observed in rodents (8, 11), this needs further differentiation: When injected into the nucleus of the solitary tract (NTS), an integrative component of the baroreflex feedback loop at brain stem level (42), orexin A inhibits cardiac and vascular sympathetic activity (43). Opposite to these inhibitory effects, microinjections directly into the hypothalamic PVN had excitatory effects and exerted an increase in arterial BP and sympathetic vasomotor tone (15, 44, 45). Consistently, acute blockade as well as chronic downregulation of orexin-1 receptors in the PVN resulted in a decrease of BP and sympathetic activity in rats (15). The PVN is currently assumed to be a key center of autonomic circulatory control and shows an exceptionally high density of orexinergic neurons (13, 14). Accordingly, our findings support the hypothesis that intranasal orexin A in humans might preferentially affect hypothalamic sites like the PVN superordinate to the mere baroreflex feedback loop rather than baroreflex centers at brain stem level.

The intranasal route is a well-established noninvasive alternative for the delivery of small neuropeptides, such as orexin A, to the CNS in humans (19, 22–24). The most probable uptake and transmission route of intranasal orexin A seems to be via the trigeminal nerve or olfactory bulb (20, 46). Experiments in primates showed that intranasally administered orexin A led to significantly increased levels in the CSF (21). Furthermore, brain tissue concentrations in anesthetized rats were much higher after intranasal orexin A compared with equivalent intravenous doses, whereas orexin blood concentrations remained low (20). These increases were detected within 10–30 min after intranasal administration. Although brain tissue sampling is a very invasive procedure and not applicable for research in humans, related experiments have demonstrated increases in CSF concentrations of neuropeptides after intranasal delivery to healthy humans (19). In addition, the effectiveness of intranasally administered orexin A has been documented in functional studies in narcoleptic humans. This disorder is associated with a loss of hypothalamic orexin-producing neurons (25), and intranasal orexin A showed stabilizing and improving effects on dysfunctional sleep regulation (26, 27, 47). In narcoleptic patients, assessment of sympathetic vascular tone showed a correlation of reduced orexin A concentrations in the CSF with decreased MSNA (29). The involvement of the orexinergic system in the development of neurogenic arterial hypertension remains uncertain; however, our results point to a possible connection in which an increased sympathetic activity alters the set point of the baroreflex.

Our findings that intranasal administered orexin A might successfully penetrate the BBB and exert a central nervous-mediated sympathoactivation are further supported by the data of our psychometric tests. Ratings of "feeling tired" as assessed by VAS significantly decreased ~30 min after sniffing orexin A. This observation coincides with the MSNA increase at respective recordings at the postsubstance period. Orexin receptors are located with high density on sympathetic nuclei in the hypothalamus. However, arousal might inflict additional feedforward signals from superordinate centers on baroreflex function. Global autonomic response patterns that are elaborated in large portions of the midbrain, limbic forebrain, and cortex occur via rapid changes in cardiac output (CO) and regional arteriolar resistance. In most cases, these physiological adaptions facilitate gas and nutrient exchange in metabolically active tissues (e.g., muscles during exercise). We are not aware of any study in awake human subjects that correlates MSNA with the subjective feeling of tiredness/alertness (VAS). However, we cannot exclude that the observed increase in MSNA was in part mediated by arousal, confounding the direct effects of orexin A on hypothalamic sympathoexcitation.

There is extensive interaction of skeletal muscle metabolism and MSNA. This cross talk involves the metaboreflexes, where muscle metabolites modify afferent sympathetic signaling and in turn alter efferent sympathetic activity to meet altered perfusion needs, for example, during exercise (36, 48). Moreover, sympathoexcitation has lipolytic effects, and it is well known that intramuscular fat depots could have an important role in energy homeostasis. However, there is a marked heterogeneity between different skeletal muscle groups regarding lipolysis (49). To further explore this issue, MSNA recordings might be combined with microdialysis techniques and/or laser Doppler flowmetry in the respective skeletal muscle (50).

Limitations

Our study has important strengths and limitations to be addressed. First, microneurographic recordings of MSNA represent direct neural measurements of the sympathetic outflow to the muscle vascular bed, which is one of the most important vasoconstrictive stimuli to the resistance arteries. Therefore, measurement of MSNA enabled us to specifically assess the vascular branch of baroreflex function. Recordings of resting MSNA under constant experimental conditions commonly show high intraindividual reproducibility, whereas there is a high interindividual variability. Our placebo-controlled crossover design with pre- and postsubstance recordings permits within-subject

comparisons and controls for this variability (Pearson coefficient for cross-correlation of resting MSNA between both sessions at the presubstance period = +0.95). However, the baroreflex paradigm that we applied to interpret our results might only represent a simplified cybernetic input-output model for the vascular baroreflex. Other components like the cardiac branch were less specifically addressed, since HR is cross-regulated via vagal pathways. Furthermore, calculating the slopes and increments of MSNA, HR, and MAP from the resting period (set point) to the steady-state plateaus at *dose step 2* of vasodilation and vasoconstriction, respectively, is a simplified but solid and well-reproducible approach. This simplification was deliberately chosen to attain the best approximation of individual curve fitting. However, more complex and dynamic test methods such as the spontaneous baroreflex sensitivity could yield complementary results and prompt further insights on the sympathetic effects of orexin A. The assessment of the spontaneous baroreflex sensitivity requires invasive or noninvasive beat-to-beat BP measurements. Because of the well-known disadvantages of noninvasive techniques in experimental protocols with long duration (e.g., artifact-rich recordings and the associated need for higher sample size), we did not include this method in our present pilot study. Moreover, we did not measure total vascular resistance (TVR) or cardiac output (CO), invasive parameters known to be tightly connected to MSNA in healthy males (51, 52).

Second, the mechanisms of intranasal oligopeptide administration to bypass the BBB are still controversially discussed. There is a large body of evidence from animal and human studies supporting the hypothesis that intranasal administration is a feasible option for delivering neuropeptides directly into the brain (19, 24). However, information on the distribution pattern of intranasal orexin A in the human CSF or brain parenchyma is still pending. In animals, however, elevated CSF and brain tissue concentrations of orexin A were observed ~10–30 min after intranasal administration (20, 21). In accordance, we scheduled the postsubstance recording period of MSNA and circulatory parameters to start ~30 min after substance administration, which, however, does not permit further conclusions on dynamic, prolonged, or delayed sympathetic effects of intranasal orexin. Moreover, we did not measure CSF concentration of orexin A in the present study and therefore were not able to clarify delivery mechanisms and central nervous pharmacokinetics of intranasally administered orexin A. Also, we cannot exclude some direct peripheral effects of orexin A on BP regulation after permeation into the circulation via the nasal mucosa. Still, the present MSNA increase without any BP decreases following orexin A points toward central nervous resetting mechanisms and cannot be explained by mere peripheral effects.

The small sample size of only $n = 10$ is another limitation of our pilot study. For MSNA, an a priori statistical power analysis revealed a sufficient effect size with $n = 12$ volunteers, whereas changes of the cardiac baroreflex slope might have been underpowered. Unfortunately, two participants had to be excluded from analysis because of insufficient recording quality. Given the fact that MSNA has an excellent intraindividual reproducibility, our highly standardized crossover study design permitting within-subject comparison well compensates for small sample sizes. Furthermore, postsubstance data were normalized to resting values before administration of either substance. It is very unlikely that our highly significant MSNA findings (absolute data and net changes), which are in line with and well supported by numerous animal models, are mere random effects. Moreover, post hoc power analysis provided an effect size of 1.32 (Cohen's *d*), achieving an excellent power for change in MSNA (burst/min) with the included 10 subjects. For other parameters like HRV our pilot study might be underpowered.

Finally, our study included only male participants. Even though there is no direct evidence of sex-specific interactions of orexin A in humans, sex-specific differences were found in orexin-knockout mice concerning interaction with body weight and leptin (53, 54), which are both strong sympathoactivators (39, 55). Therefore, our results cannot easily be transferred to women, and this issue warrants further investigation.

SUPPLEMENTAL DATA

Fig. S1: https://doi.org/10.6084/m9.figshare.15000411.v1.
Fig. S2: https://doi.org/10.6084/m9.figshare.17818202.v1.
Fig. S3: https://doi.org/10.6084/m9.figshare.17818925.v1.
Fig. S4: https://doi.org/10.6084/m9.figshare.17736524.v1.
Fig. S5: https://doi.org/10.6084/m9.figshare.17823080.v1.
Table S1: https://doi.org/10.6084/m9.figshare.17738639.v1.
Table S2: https://doi.org/10.6084/m9.figshare.17737592.v1.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

M.M., J.V., A.K., F.M., and F.S. conceived and designed research; M.M., J.V., A.K., and F.M. performed experiments; M.M., J.V., A.K., F.M., R.V., and F.S. analyzed data; M.M., J.V., R.V., M.H., and F.S. interpreted results of experiments; M.M. and F.S. prepared figures; M.M., M.H., and F.S. drafted manuscript; M.M., J.V., A.K., F.M., R.V., M.H., and F.S. edited and revised manuscript; M.M., J.V., A.K., F.M., R.V., M.H., and F.S. approved final version of manuscript.

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