

## RESEARCH ARTICLE

## Open Access



# Inhaled $\beta$ -agonist does not modify sympathetic activity in patients with COPD

Helge Haarmann<sup>1</sup>, Cordula Mohrlang<sup>2</sup>, Uta Tschiesner<sup>2</sup>, David B Rubin<sup>5</sup>, Thore Bornemann<sup>1</sup>, Karin Rüter<sup>1</sup>, Slavtcho Bonev<sup>3</sup>, Tobias Raupach<sup>1</sup>, Gerd Hasenfuß<sup>1</sup> and Stefan Andreas<sup>1,4\*</sup>

## Abstract

**Background:** Neurohumoral activation is present in COPD and might provide a link between pulmonary and systemic effects, especially cardiovascular disease. Because long acting inhaled  $\beta$ -agonists reduce hyperinflation, they could reduce sympathoexcitation by improving the inflation reflex. We aimed to evaluate if inhaled therapy with salmeterol reduces muscle sympathetic nerve activity (MSNA) evaluated by microneurography.

**Methods:** MSNA, heart rate, blood pressure, and respiration were continually measured. After baseline recording of 20 minutes, placebo was administered; after further 45 minutes salmeterol (50  $\mu$ g) was administered which was followed by a further 45 minutes of data recording. Additionally, lung function, plasma catecholamine levels, arterial pulse wave velocity, heart rate variability, and baroreflex sensitivity were evaluated. Following 4 weeks of treatment with salmeterol 50  $\mu$ g twice daily, measurements were repeated without placebo administration.

**Results:** A total of 32 COPD patients were included. Valid MSNA signals were obtained from 18 patients. Change in MSNA (bursts/100 heart beats) following acute administration of salmeterol did not differ significantly from the change following placebo ( $-1.96 \pm 9.81$  vs.  $-0.65 \pm 9.07$ ;  $p = 0.51$ ) although hyperinflation was significantly reduced. Likewise, no changes in MSNA or catecholamines were observed after 4 weeks. Heart rate increased significantly by  $3.8 \pm 4.2$  ( $p < 0.01$ ) acutely and  $3.9 \pm 4.3$  bpm ( $p < 0.01$ ) after 4 weeks. Salmeterol treatment was safe and well tolerated.

**Conclusions:** By using microneurography as a gold standard to evaluate sympathetic activity we found no change in MSNA following salmeterol inhalation. Thus, despite an attenuation of hyperinflation, the long acting  $\beta$ -agonist salmeterol does not appear to reduce nor incite sympathoexcitation.

**Trial registration:** This study was registered with the European Clinical Trials Database (EudraCT No. 2011-001581-18) and ClinicalTrials.gov (NCT01536587).

**Keywords:** Chronic obstructive pulmonary disease, Sympathetic activity, Beta agonist, Salmeterol

## Background

Patients with chronic obstructive pulmonary disease (COPD) are at increased risk of death and disability from various extra pulmonary complications [1,2]. In COPD patients, cardiovascular diseases are the most common comorbidities [3], are one of the leading causes of hospitalization and one of the main causes of death [4].

It is well known that inhaled  $\beta$ 2-agonists induce adverse effects, such as increased heart rate and tremor [5]. This is

thought to be due to spillover of the  $\beta$ -agonist into the systemic circulation thereby causing direct activation of  $\beta$ 2 and  $\beta$ 1 receptors within the cardiovascular system. Furthermore systemic  $\beta$ -agonists (e.g. adrenaline) have excitatory effects on the autonomic nervous system such as direct activation of sympathetic nerve activity [6,7]. Long-acting  $\beta$ 2-agonists (LABA) have an acceptable safety profile, although there is still controversy as to whether LABA may increase the risk of asthma mortality [8] or new cardiovascular events in older COPD patients [9].

A striking elevation of sympathetic tone is present in COPD and might provide a link between COPD and systemic effects, specifically cardiovascular disease [10-14]. Several mechanisms related to lung function impairment

\* Correspondence: [sandreas@lungenfachklinik-immenhausen.de](mailto:sandreas@lungenfachklinik-immenhausen.de)

<sup>1</sup>Clinic for Cardiology and Pneumology, University Medical Center Göttingen, Göttingen, Germany

<sup>4</sup>Lung Clinic Immenhausen, Immenhausen, Krs. Kassel, Germany  
Full list of author information is available at the end of the article

such as chemoreflexes [15], impaired baroreflexes [16] and hyperinflation [17] contribute to sympathetic activation in COPD [18,19].

In COPD  $\beta_2$ -agonists improve lung function, quality of life and exacerbations [5]. Since these drugs reduce bronchoconstriction and hyperinflation, it is conceivable that inhaled  $\beta_2$ -agonists reduce sympathetic activation in COPD. Despite their widespread use, the effects of inhaled  $\beta_2$ -agonists on sympathetic activity have never been investigated.

Previous investigations have demonstrated that muscle sympathetic nerve activity (MSNA) evaluated by microneurography reflects short-term changes in sympathetic activity, is highly reproducible, and correlates closely with cardiac norepinephrine spillover [20], therefore being considered the gold standard to evaluate sympathetic activity [21].

This study investigates the effects of LABA therapy on lung function and sympathetic activation in COPD. The latter involved microneurographic recordings of MSNA as well as non-invasive assessments of heart rate variability (HRV), catecholamine levels, and spontaneous baroreflexes. Arterial pulse wave velocity (aPWV) is linked to MSNA, is elevated in COPD and indicates risk for cardiovascular disease and mortality. Accordingly aPWV was also assessed.

## Methods

### Study design

This was a partially blinded, single-arm, single centre study in subjects with COPD GOLD stage II or III. The study included a screening visit and two study visits (visit 1 at week 0 and visit 2 at week 4), and one telephone contact at week 2. At the screening visit, eligibility of the subjects was assessed and written informed consent obtained. At visit 1 a complex measurement period with continuous recordings of MSNA, blood pressure, tidal volume, respiratory rate, oxygen saturation and transcutaneous  $\text{CO}_2$  were conducted while placebo and 50  $\mu\text{g}$  salmeterol were inhaled by the subjects in a sequential manner. Following visit 1, all subjects were treated with salmeterol 50  $\mu\text{g}$  twice daily for 4 weeks. At visit 2, these observations were repeated without placebo inhalation. The subjects were recruited in a single study centre with the help of two local chest physicians and newspaper advertisements.

Male or female patients aged between 40 and 80 years were eligible to enter the study. A prerequisite for inclusion was COPD GOLD class II or III with a post-bronchodilator spirometry forced expiratory volume in one second ( $\text{FEV}_1$ ) <70% predicted, and current or prior history of  $\geq 10$  pack-years of cigarette smoking. The main exclusion criteria were concomitant active lung diseases other than COPD, exacerbation or

hospitalisation due to COPD six weeks prior to screening, frequent exacerbations necessitating therapy with inhaled glucocorticosteroids, non-invasive ventilation, treatment with drugs affecting sympathetic tone (e.g. theophylline), use of systemic glucocorticoids or antibiotics, uncontrolled sleep apnoea syndrome, left heart failure with a left ventricular ejection fraction <40%, polyneuropathy, or insulin dependent diabetes mellitus.

The study was performed in a partially blinded manner: The patient was blinded to the sequence of study drug administration (i.e., to the fact that lactose was administered as placebo before salmeterol) at visit 1. Furthermore, the researchers evaluating the recorded data were blinded to the subject, visit and intervention. This was done by pseudonymisation of the recorded data sets of each of the five measurement periods (baseline, placebo, salmeterol at visit 1; baseline and salmeterol at visit 2) and removing the time stamps from each recorded file.

### Study protocol

Subjects were studied two hours after a low-calorie meal free from caffeine-containing beverages. They had been instructed not to smoke any cigarettes for 8 hours before the clinic visit. Study participants were requested to take their usual medication except for diuretics which were to be withheld on the morning of the study visit. The 2-hour measurement period comprised the continuous recording of MSNA, ECG, blood pressure, respiration, oxygen saturation, and transcutaneous  $\text{CO}_2$  with the patient lying in a supine position. About 20 minutes after a stable MSNA signal had been obtained ('baseline'), one dose of placebo was administered; after a further recording period of 45 minutes one dose of salmeterol (50  $\mu\text{g}$ ) was administered which was followed by a further 45 minutes of data recording. We analyzed the last 10 minutes of each of these recording sections (baseline, placebo, salmeterol).

### Muscle sympathetic nerve activity

Sympathetic tone was measured using microneurographic recordings of efferent muscle sympathetic nerve activity in the peroneal nerve [22]. A tungsten microelectrode (shaft diameter 200  $\mu\text{m}$ , tip diameter 1-5  $\mu\text{m}$ , FHC, Bowdoin, USA) was inserted into the nerve. The signal was amplified and integrated (662C-4 Nerve Traffic Analysis System, Absolute Design and Manufacturing Services, Iowa, USA). Intraobserver variation in identifying bursts of efferent sympathetic nerve activity was  $\sim 5\%$  in previous studies done in our laboratory [15,23].

### Hemodynamics, baroreflex sensitivity, and heart rate variability

ECG (Datex AS/3, Datex Ohmeda, WI, USA) and continuous blood pressure measurements (Portapres, Finapres Medical Systems, Amsterdam, Netherlands) were used to obtain data from which heart rate variability (HRV) and baroreflex sensitivity (BRS) were computed [24,25].

### Catecholamine and BNP measurements

During MSNA measurement, blood samples of plasma norepinephrine, epinephrine and brain natriuretic peptide (BNP) were taken at least 20 minutes after insertion of an intravenous line into an antecubital vein at baseline, after placebo and after salmeterol inhalation.

### Lung function, pulse wave velocity, and echocardiography

Lung function was assessed before and after the MSNA measurement at visit 1 and visit 2 (at least 60 min. after salmeterol inhalation) via body plethysmography and helium dilution in the sitting position. Carotid-femoral aPWV was evaluated via SphygmoCor (AT Cor Medical, Itasca, USA) before MSNA measurement at visit 1 and visit 2.

Transthoracic echocardiography was performed before visit 1 and visit 2. Because patients need to be completely relaxed during MSNA recordings, lung function tests, aPWV and echocardiography could not be performed during these measurements.

### Sample size calculation

The primary endpoint was the difference in MSNA change elicited by either salmeterol or placebo inhalation (compared to baseline MSNA levels) on visit 1. According to a sample size calculation using data from previous studies (standard deviation of intra-individual difference in MSNA between 9 and 10 bursts/100 heart beats following interventions such as oxygen administration or slow breathing [16,23]), 24 subjects needed to be investigated in order to be able to detect a 10% difference in MSNA change at a significance level of 5% and with 80% power. Under the assumption that adequate microneurographic recordings would only be obtained in 75% of all subjects studied in our lab, 32 subjects needed to be enrolled.

### Statistical analysis

The statistical analysis was performed using SAS-software, version 9.2 (SAS institute, Cary, North Carolina, USA). Data are presented as percentages or means  $\pm$  standard deviations, as appropriate. Changes in continuous variables were assessed using paired t-tests, and Pearson's coefficient was calculated for correlations. The significance level was set to 5% (two-sided tests). The safety analysis was based on the analysis of adverse events (AEs).

### Clinical trial registration

The study was registered with BfArM, the European Clinical Trials Database (EudraCT No. 2011-001581-18) and www.clinicaltrials.gov (NCT01536587) and approved by the Ethics Committee of Göttingen University (Application number 11/12/11).

More information on the methods of this study is available as supplementary material (see Additional file 1).

### Results

A total of 32 subjects were enrolled. Two subjects discontinued the study prematurely due to an adverse event (nasopharyngitis and dyspnea; bronchitis).

Demographic and baseline characteristics are summarized in Table 1. Concomitant diseases were reported in 30 subjects (93.8%). Most common were cardiovascular disorders (17 subjects, 53.1%) with hypertension being the condition most frequently reported. Concomitant non-COPD medications were reported in 28 subjects (87.5%). Most common were anti-thrombotic agents (15 subjects, 46.9%), and medications for cardiovascular disease (14 subjects, 43.8%).

**Table 1 Demographic and baseline characteristics**

<b>Gender</b>	Male, n (%)	21 (65.6)
	Female, n (%)	11 (34.4)
<b>Age [years]</b>		61.2 $\pm$ 8.4
<b>Body weight [kg]</b>		81.5 $\pm$ 19.8
<b>Height [cm]</b>		174.6 $\pm$ 7.6
<b>Body mass index [kg/m<sup>2</sup>]</b>		26.5 $\pm$ 5.2
<b>First diagnosis of COPD [months before screening]</b>		59.0 $\pm$ 62.3
<b>GOLD stage</b>	II (moderate COPD), n (%)	14 (45.2)
	III (severe COPD), n (%)	17 (54.8)
<b>COPD symptoms</b>	Breathlessness, n (%)	31 (96.9)
	Cough, n (%)	24 (75.0)
	Sputum production, n (%)	21 (65.6)
<b>FEV<sub>1</sub> [L]</b>		1.6 $\pm$ 0.4
<b>FEV<sub>1</sub> [% predicted]</b>		50.2 $\pm$ 9.9
<b>FVC [L]</b>		2.9 $\pm$ 0.8
<b>FEV<sub>1</sub>/FVC [%]</b>		54.5 $\pm$ 9.2
<b>Smoking status</b>	Previous smoker, n (%)	13 (40.6)
	Smoker, n (%)	19 (59.4)
<b>Cessation of smoking [months before screening]</b>		54.0 $\pm$ 47.4
<b>Pack years</b>		43.1 $\pm$ 17.7

COPD = Chronic obstructive pulmonary disease; FEV<sub>1</sub> = forced expiratory volume in one second; FVC = forced vital capacity; GOLD = Global Initiative for Chronic Obstructive Lung Disease. Values are expressed as mean  $\pm$  SD unless otherwise stated.

### Medication adherence

As calculated on the basis of the residual doses in the returned dose dispensers  $97.5 \pm 9.0\%$  of the medication was used by the patients. Use of rescue medication between weeks 2 and 4 was reported by 23 subjects (74.2%,  $N = 31$ ) at visit 2.

### Muscle sympathetic nerve activity

A valid MSNA signal for visit 1 was obtained in 18 patients. In 14 patients a valid MSNA signal could either not be obtained or was lost in the course of the examination. Intraobserver variation in identifying bursts was  $4.01 \pm 0.02\%$ .

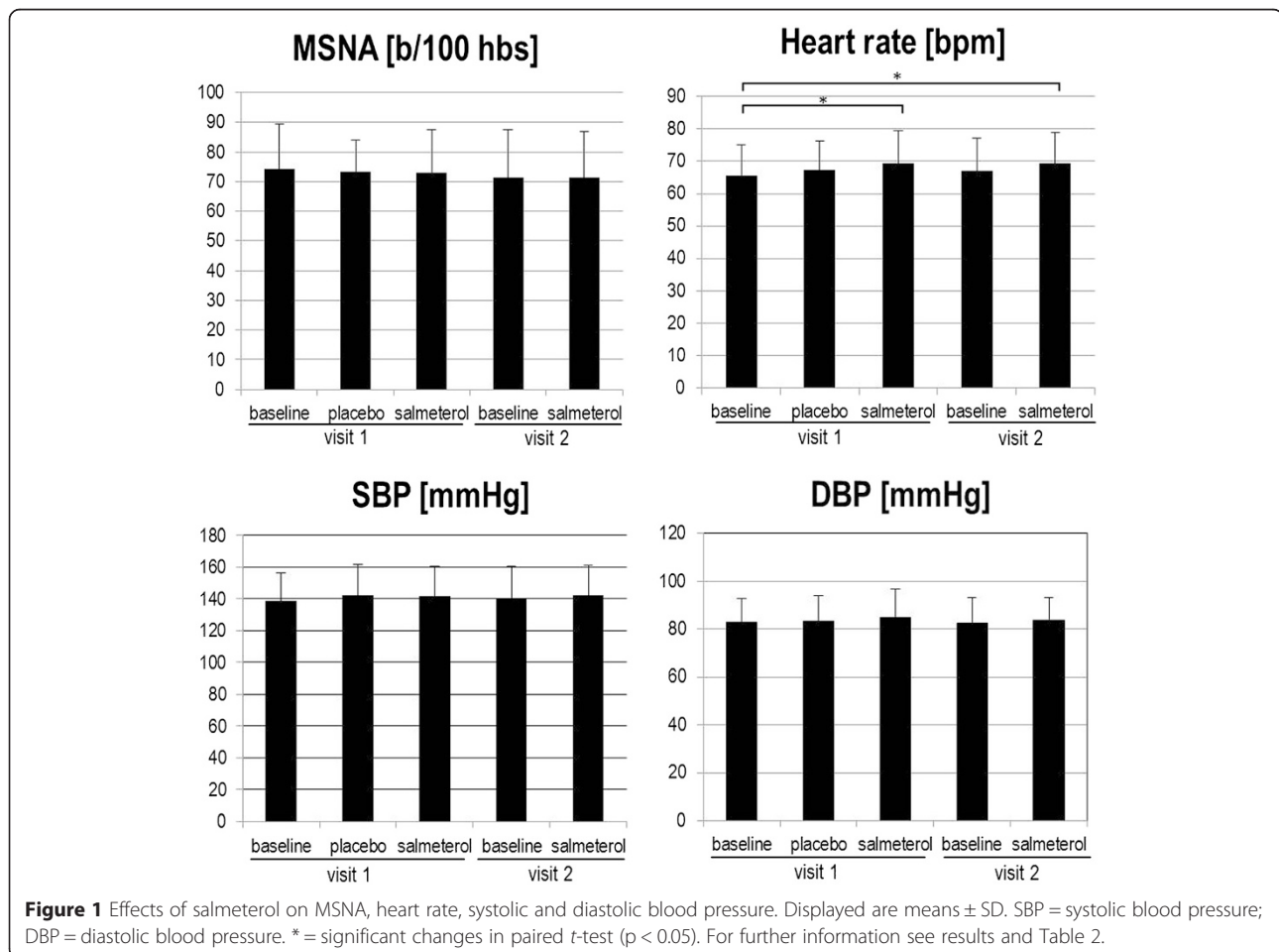
Baseline MSNA was  $74.4 \pm 16.3$  bursts/100 heart beats. This was higher as compared to historical controls [16] of similar age and BMI. No significant effect of smoking status on baseline MSNA was observed (smokers:  $75.91 \pm 15.44$  vs. non-smokers:  $71.58 \pm 15.20$  bursts/100 heart beats;  $p = 0.54$ ).

As shown in Figure 1, both placebo and salmeterol inhalation elicited minor changes in MSNA compared to baseline levels. The change in MSNA (bursts/100 heart

beats) following acute administration of salmeterol did not differ significantly from the change following placebo ( $-1.96 \pm 9.81$  vs.  $-0.65 \pm 9.07$ ;  $p = 0.51$ ). MSNA after salmeterol inhalation at visit 2 was not significantly different from MSNA at baseline at visit 1 (change by  $-3.07 \pm 11.59$  bursts/100 heart beats;  $p = 0.38$ ,  $n = 12$ ).

### Hemodynamics, baroreflex sensitivity, heart rate variability, ventilation and pulse wave velocity

Systolic and diastolic blood pressure as well as BRS showed no relevant changes (Figure 1; Table 2). Heart rate was significantly increased after acute salmeterol inhalation at visit 1 and visit 2 as compared to baseline (Figure 1; Table 2). Resting heart rate after 4 weeks of salmeterol treatment increased from  $65.6 \pm 9.4$  at baseline to  $67.1 \pm 9.9$  bpm at visit 2 (before the administration of salmeterol;  $p = 0.04$  in paired  $t$ -test). As an acute effect of salmeterol, low frequency power (LF) of HRV, reflecting sympathetic tone, was significantly elevated while high frequency power (HF), reflecting parasympathetic tone, decreased. There was no correlation between changes in heart rate, HRV, BRS and changes in MSNA.



**Table 2 Heart rate variability and baroreflex sensitivity**

	Heart rate [beats/min]	SDNN [ms]	RMSSD [ms]	LF [normalized units]	HF [normalized units]	Baroreflex sensitivity [ms/mmHg]
<b>Visit 1 (Week 0)</b>						
	Mean $\pm$ SD; p (two-sided)					
Baseline	65.6 $\pm$ 9.4	59.4 $\pm$ 38.8	64.5 $\pm$ 61.9	53.6 $\pm$ 21.8	46.4 $\pm$ 21.8	6.3 $\pm$ 3.7
After placebo	67.2 $\pm$ 9.0	66.9 $\pm$ 48.1	75.1 $\pm$ 81.6	52.9 $\pm$ 22.2	47.1 $\pm$ 22.2	6.8 $\pm$ 5.9
After salmeterol	69.4 $\pm$ 10.1	57.6 $\pm$ 33.2	53.0 $\pm$ 49.4	62.1 $\pm$ 20.3	37.9 $\pm$ 20.3	6.2 $\pm$ 3.9
Change 'after salmeterol' – Baseline	3.7 $\pm$ 4.2; p = <0.01	-1.7 $\pm$ 26.4; p = 0.71	-11.5 $\pm$ 36.3; p = 0.08	8.4 $\pm$ 14.5; p < 0.01	-8.4 $\pm$ 14.5; p < 0.01	0.1 $\pm$ 1.9; p = 0.84
<b>Visit 2 (Week 4)</b>						
Before salmeterol	67.1 $\pm$ 9.9	57.0 $\pm$ 43.3	55.4 $\pm$ 70.4	59.9 $\pm$ 19.0	40.1 $\pm$ 19.0	7.7 $\pm$ 5.1
After salmeterol	69.4 $\pm$ 9.4	60.2 $\pm$ 48.8	60.8 $\pm$ 84.4	55.3 $\pm$ 22.8	44.7 $\pm$ 22.8	6.5 $\pm$ 5.2
Change 'after salmeterol' – Baseline	3.9 $\pm$ 4.3; p < 0.01	0.1 $\pm$ 53.9; p = 0.99	-5.2 $\pm$ 96.1; p = 0.76	2.5 $\pm$ 23.3; p = 0.55	-2.5 $\pm$ 23.3; p = 0.55	0.2 $\pm$ 2.6; p = 0.62

Baseline = value at visit 1 before any inhalation; HF = High frequency component; LF = Low frequency component; SDNN = Standard deviation of the NN interval; RMSSD = Root mean square of the successive differences.

The course of lung function and respiratory parameters is shown in Table 3. Mean tidal volume and mean respiratory minute volume increased after salmeterol inhalation at visit 1. This effect could not be observed after 4 weeks of salmeterol treatment. TLC, RV and FRC significantly decreased after acute and long-term salmeterol inhalation, while FEV<sub>1</sub> was unchanged. Capillary blood gas analysis (Table 4), continuous measurements of oxygen saturation and transcutaneous pCO<sub>2</sub> revealed no relevant changes.

Echocardiographic data (not shown) and carotid-femoral aPWV was unchanged after 4 weeks of salmeterol treatment (9.17  $\pm$  2.06 vs. 8.83  $\pm$  2.23 m/s; p = 0.39).

#### Catecholamine and BNP measurements

The levels of plasma norepinephrine, epinephrine and brain natriuretic peptide did not change following salmeterol inhalation as compared to placebo. However, there was a significant correlation between changes in plasma norepinephrine levels and changes in MSNA after salmeterol administration at visit 2 as compared to visit 1 (r = 0.72; p = 0.01; Figure 2).

#### Discussion

For the first time the effect of an inhaled  $\beta$ 2-agonist on MSNA was evaluated. The primary endpoint, a significant difference in MSNA change following salmeterol inhalation versus placebo inhalation was not met. However, MSNA and other measures of neurohumoral activation such as plasma norepinephrine, BRS and aPWV did not change following acute or long-term salmeterol inhalation. These findings are of interest, since previous work in patients with heart failure indicates that neurohumoral activation and sympathoexcitation are linked to mortality [26-28].

#### Effects on heart rate

Our results showing an increase in heart rate of about 4 beats/minute following inhalation of a sympatho-mimetic drug corroborate older studies. Cazzola and colleagues found an increase in heart rate of about 4 beats/minute with salmeterol in COPD patients with cardiovascular disease [12]. Blood pressure was not evaluated in that study. In a recent study using inhaled fluticasone and the long-acting  $\beta$ 2-agonist vilanterol in asthma patients, heart rate was acutely increased by 3.4 beats/minute. Long-term use yielded a decrease in heart rate that was greater with placebo [29]. In COPD patients, the same combination had no effect on heart rate or blood pressure [30]. Similarly, the long acting  $\beta$ 2-agonist indacaterol at standard doses had no effect on heart rate or blood pressure in COPD patients [31]. The fact that salmeterol treatment was associated with an increase in heart rate without an increase in MSNA in our study suggests that the increase in heart rate is caused by activation of cardiac  $\beta$ -receptors rather than efferent excitatory traffic of the autonomic nervous system.

Increased resting heart rate is related to mortality in patients with COPD [32] and heart failure [33]. Salmeterol as well as other LABA have been successfully used for inhalation therapy in large studies and for a long time [34]. In controlled randomised trials,  $\beta$ 2-agonists improve lung function, quality of life and exacerbations in COPD, but a reduction in mortality was never demonstrated [5]. Interestingly, among older COPD patients, starting LABA treatment was associated with an increased risk of cardiovascular events in a recent nested case control analysis [9].

#### Sympathetic activation

MSNA was increased in our patients and as high or even higher as compared to previous studies in COPD

**Table 3 Lung function and ventilation**

	FEV <sub>1</sub> [L]	FVC [L]	FRC [L]	FRC (helium dilution) [L]	TLC [L]	RV [L]	Respiratory rate [/min]	Tidal volume [mL]	Respiratory minute volume [mL/min]
<b>Visit 1 (Week 0)</b>	<b>Mean ± SD; p (two-sided)</b>								
Baseline	1.35 ± 0.46	2.60 ± 0.69	4.65 ± 1.33	3.64 ± 0.97	6.61 ± 1.39	3.90 ± 1.28	15.66 ± 3.34	549 ± 200	8362 ± 3333
After salmeterol	1.31 ± 0.40	2.58 ± 0.62	3.86 ± 1.68	3.21 ± 0.78	5.88 ± 1.89	3.16 ± 1.64	15.17 ± 3.45	610 ± 250;	8934 ± 3942
Change 'after salmeterol' – Baseline	-0.04 ± 0.23; p = 0.35	-0.02 ± 0.35; p = 0.72	-0.90 ± 1.66; p < 0.01	-0.52 ± 0.73; p < 0.01	-0.82 ± 1.71; p = 0.01	-0.85 ± 1.79; p = 0.01	-0.49 ± 1.62; p = 0.09	60 ± 118; p = 0.01	572 ± 1530; p = 0.05
<b>Visit 2 (Week 4)</b>									
Before salmeterol	1.38 ± 0.44	2.68 ± 0.64	4.20 ± 1.61	3.57 ± 0.86	6.23 ± 1.70	3.46 ± 1.61	15.76 ± 3.74	564 ± 197	8773 ± 3369
After salmeterol	1.32 ± 0.46	2.64 ± 0.71	3.67 ± 1.80	3.38 ± 0.77	5.67 ± 1.83	2.90 ± 1.79	15.48 ± 4.02	593 ± 217	8829 ± 3629
Change 'after salmeterol' – Baseline	-0.04 ± 0.21; p = 0.33	0.04 ± 0.35; p = 0.51	-1.26 ± 1.75; p < 0.01	-0.27 ± 0.83; p = 0.18	-1.17 ± 1.84; p < 0.01	-1.30 ± 1.80; p < 0.01	-0.06 ± 2.63; p = 0.90	56 ± 184; p = 0.15	671 ± 2963; p = 0.28

Baseline = value at visit 1 before any inhalation; FVC = Forced vital capacity; FRC = Functional residual capacity; TLC = Total lung capacity; RV = Residual volume.

**Table 4 Capillary blood gas analysis**

	pH	SO <sub>2</sub> c [%]	pCO <sub>2</sub> [mmHg]	pO <sub>2</sub> [mmHg]
	Mean ± SD; p (two-sided)			
visit 1 (Week 0)	7.43 ± 0.03	92.06 ± 4.79	39.56 ± 3.77	65.69 ± 10.56
visit 2 (Week 4)	7.43 ± 0.03	92.10 ± 4.80	39.26 ± 2.98	65.10 ± 9.83
Change visit 2 – visit 1	-0.002 ± 0.02; p = 0.61	0.03 ± 2.60; p = 0.95	-0.29 ± 2.95; p = 0.59	-0.68 ± 9.39; p = 0.69

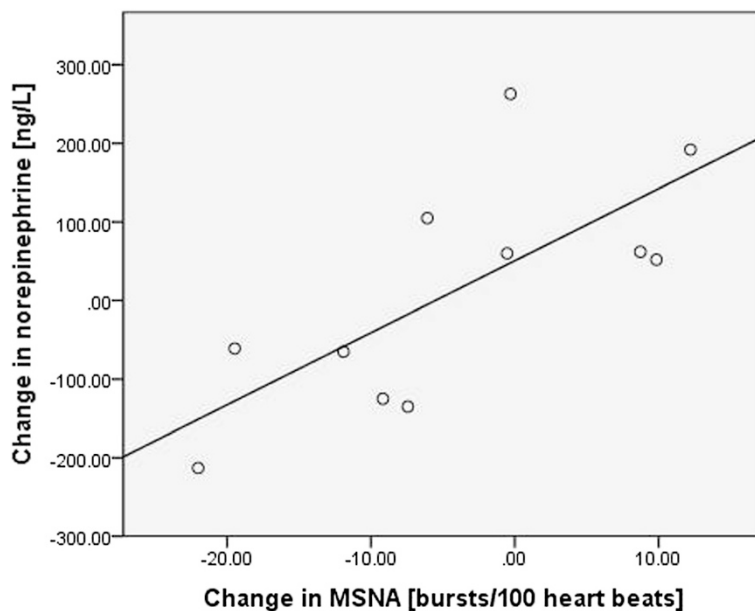
SaO<sub>2</sub> = oxygen saturation; pCO<sub>2</sub> = carbon dioxide tension; pO<sub>2</sub> = oxygen tension.

patients [16,35]. In a setting of increased MSNA, previous interventional studies using oxygen or slow breathing demonstrated a significant decrease in MSNA [16,35]. However, in the present study no effect of the intervention on MSNA was noted. For more insight into the effects of inhaled β-agonists on sympathetic activity it is helpful to discuss older work on intravenous administration of catecholamines. Adrenaline infusion (with α1-receptor activity causing vasoconstriction and β-receptor activity) did cause an increase in MSNA [6]. Intravenous dobutamine (a predominant β1-agonist and weak β2-agonist without relevant α1 activity) did not increase MSNA in heart failure patients or matched controls [36]. While there was no effect on heart rate or blood pressure in heart failure patients, healthy matched controls experienced an increase in heart rate but not blood pressure. In contrast, in an unmatched group of younger healthy subjects blood pressure increased, and consecutively MSNA decreased following dobutamine

infusion [36]. In another study intravenous dobutamine reduced cardiac norepinephrine spillover in patients with heart failure but not in subjects with normal ejection fraction [37]. Similarly, in healthy young subjects the increase in heart rate following salmeterol was explained mainly by a decrease in total peripheral resistance [38,39]. Our study contributes to the understanding of these data by the comprehensive evaluation of respiration, cardiovascular function and MSNA: In our older patients with increased sympathetic activity we did not observe a change in blood pressure or MSNA following salmeterol.

The observed changes in HRV are suggestive of a decrease in parasympathetic tone. Similar results were observed in a small study using the SABA salbutamol [38]. However, given the absence of significant changes in MSNA and baroreflex sensitivity, the HRV findings are difficult to interpret [40]. Furthermore, changes in ventilation and heart rate as present in our study impact on HRV thus rendering interpretation even more problematic.

Sympathoexcitation in COPD is thought to be caused by several mechanisms such as hypoxia, chemoreflexes [15], impaired baroreflexes [16] and lung hyperinflation [17,18]. Lung hyperinflation affects the autonomic nervous system by activation of intrapulmonary non-myelinated afferents and by increased end-expiratory pressure with consecutive impaired left ventricular filling and reduced stroke volume [17,41,42]. Furthermore hyperinflation increases the work of breathing [43] and thus impacts on muscle metaboreceptors



**Figure 2** Scatter plot of change in norepinephrine against change in MSNA. Displayed are changes from visit 1 (week 0) to after salmeterol inhalation at visit 2 (week 4). (r = 0.72; p = 0.01; n = 11).

[4,16,44]. Thus we speculated that the improvement of lung function and hyperinflation following inhaled therapy reduces sympathetic activation. However, despite reducing hyperinflation, salmeterol did not impact on sympathetic tone in our study. It is possible that the positive effects mediated via bronchodilatation were too small to elicit a reduction in sympathetic activity. It is also possible, that any positive effect on sympathetic activity were offset by consequences of systemic  $\beta$ -receptor activation. Our study is not able to further dissect these possibilities. In this context it would be interesting to investigate an inhaled therapy with stronger bronchodilator activity.

We studied not only the acute but also the long-term effects of salmeterol over one month. As in the acute setting, MSNA and other measures of autonomic nervous system activity were unchanged. Desensitization or saturation of  $\beta$ 2-adrenoceptors that follows regular use of  $\beta$ 2-agonist treatment is believed to be responsible for the resolution of hemodynamic findings after the first days and weeks and may also prevent long-term effects of salmeterol on MSNA [5]. However, in our study the acute effects were comparable to the effects after 4 weeks. Specifically the increase in heart rate was similar in the acute and long-term setting.

### Strengths and limitations

In this study, COPD patients underwent a comprehensive protocol including evaluation of the cardiovascular, respiratory and autonomic nervous system. We used microneurography as the gold standard to measure sympathetic activity. The good internal validity of our data is demonstrated by the positive correlation between the change in MSNA and the change in plasma norepinephrine levels. The calculated sample size of 24 patients for the primary endpoint was missed by 6 subjects ( $n = 18$ ), as we overestimated the rate of successful MSNA registrations. The per visit success rate of MSNA recordings is reported as 70% in literature [45]. We had a 56% success rate on visit 1. The difficulty to keep a stable MSNA signal for a data registration period of almost two hours was underestimated, as previous studies [15,16] had distinct shorter registration periods. As a consequence, the power of the study was slightly decreased.

Although we implemented a placebo application, no parallel design was used. Thus we cannot control for time effects. Nevertheless it is unlikely that the increase in heart rate with constant MSNA and baroreflexes as main findings can be explained by the effect of time. A double blind protocol was not feasible. However, blinded investigators performed evaluation of MSNA, heart rate, HRV, BRS, tidal volume and respiratory rate.

In our study salmeterol was a weak bronchodilator because measures of hyperinflation but not FEV<sub>1</sub> were improved. This finding may be explained by the supine position the patients had to maintain during evaluation of MSNA and that deep inhalation of the study drug is difficult in the supine position. Some other studies were also unable to detect an increase in FEV<sub>1</sub> with salmeterol despite positive findings concerning hyperinflation or exercise physiology [46].

### Conclusions

By using microneurography as a gold standard to evaluate sympathetic activity we found no change in MSNA following acute or chronic salmeterol inhalation. Thus a relevant negative effect of LABA inhalation on sympathetic activation is highly unlikely. Further studies with stronger bronchodilators are warranted.

### Additional file

**Additional file 1: Supplementary information on the methods of the study.**

### Abbreviations

BNP: Brain natriuretic peptide; BRS: Baroreflex sensitivity; COPD: Chronic obstructive pulmonary disease; ECG: Electrocardiogram; FEV<sub>1</sub>: Forced expiratory volume in 1 second; FRC: Functional residual capacity; FVC: Forced vital capacity; GSK: GlaxoSmithKline; GOLD: Global Initiative for chronic obstructive lung disease; HRV: Heart rate variability; LABA: Long-acting beta-agonist; MSNA: Muscle sympathetic nerve activity; pCO<sub>2</sub>: Arterial carbon dioxide tension; RV: Residual volume; SABA: Short-acting beta-agonist; SaO<sub>2</sub>: Arterial oxygen saturation; tCO<sub>2</sub>: Transcutaneous carbon dioxide; TLC: Total lung capacity; VC: Vital capacity.

### Competing interests

HH has received a travel grant from Pfizer. CM, UT, DR are employees at GSK and own stock in GSK. TB, KR and SB declare no conflict of interests. TR undertakes research and consultancy and receives fees for speaking from companies that develop and manufacture smoking cessation medications (Pfizer, Novartis, GlaxoSmithKline, Astra Zeneca, and Roche). GH has received honorarium for presentations from the following companies: Servier, Impulse Dynamics, Astra Zeneca, and Bayer. He has furthermore received consultant fees from the following companies: Servier, Impulse Dynamics, Novartis, CircuLite, and DC Devices. SA has received support from GSK to conduct this study, fees for Advisory Boards from GSK, Pfizer and Almirall, and fees for lectures from GSK, Almirall, Boehringer Ingelheim, Novartis, Nycomed and Pfizer.

### Authors' contributions

HH is subinvestigator, performed data collection, contributed to study conception, data analysis, and manuscript writing. CM contributed to protocol writing and reviewed the manuscript. UT contributed to data analyses and manuscript writing. DR contributed to study conception and reviewed the manuscript. TB contributed to data collection and data analysis. KR performed echocardiography. SB contributed to HRV and BRS data. TR contributed to data interpretation and manuscript writing. GH contributed to data interpretation. SA is principal investigator, guarantor of the manuscript, developed the study conception, contributed to data analysis, and drafted the manuscript. All authors read and approved the final draft before submission.

### Acknowledgments

We thank the statisticians Norbert Barnik, Bernd Westermayer and Michael Hennig for their grateful contributions to study design and data analyses. We also thank Prof. Dr. X.P. Nguyen and his co-workers (Mannheim Biomedical Engineering



Laboratories, Medical Faculty at Heidelberg University) for developing the BEM A/D converter and B2B analysis tools. The work was supported by GlaxoSmithKline, Munich, Germany (Clinical Study Identifier SCO114520).

#### Author details

<sup>1</sup>Clinic for Cardiology and Pneumology, University Medical Center Göttingen, Göttingen, Germany. <sup>2</sup>GlaxoSmithKline (GSK), Munich, Germany. <sup>3</sup>Mannheim Biomedical Engineering Laboratories, Medical Faculty at Heidelberg University, Mannheim, Germany. <sup>4</sup>Lung Clinic Immenhausen, Immenhausen, Krs. Kassel, Germany. <sup>5</sup>GlaxoSmithKline, Research Triangle Park, NC, USA.

Received: 20 July 2014 Accepted: 22 April 2015

Published online: 30 April 2015

#### References

- Decramer M, Janssens W, Miravittles M. Chronic obstructive pulmonary disease. *Lancet*. 2012;379(9823):1341–51.
- Sin DD, MacNee W. Chronic obstructive pulmonary disease and cardiovascular diseases: a “vulnerable” relationship. *Am J Respir Crit Care Med*. 2013;187(1):2–4.
- Divo M, Cote C, de Torres JP, Casanova C, Marin JM, Pinto-Plata V, et al. Comorbidities and risk of mortality in patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med*. 2012;186(2):155–61.
- Stone IS, Barnes NC, Petersen SE. Chronic obstructive pulmonary disease: a modifiable risk factor for cardiovascular disease? *Heart*. 2012;98(14):1055–62.
- Cazzola M, Page CP, Rogliani P, Matera MG. beta2-agonist therapy in lung disease. *Am J Respir Crit Care Med*. 2013;187(7):690–6.
- Persson B, Andersson OK, Hjemsdahl P, Wysocki M, Agerwall S, Wallin G. Adrenaline infusion in man increases muscle sympathetic nerve activity and noradrenaline overflow to plasma. *J Hypertens*. 1989;7(9):747–56.
- Andreas S. Computerised Polysomnography. *Thorax*. 1994;49:528.
- Weatherall M, Wijesinghe M, Perrin K, Beasley R. Long-acting beta-agonists and asthma death: how useful are different study designs to evaluate the potential association? *J Asthma*. 2010;47(4):434–8.
- Gershon A, Croxford R, Calzavara A, To T, Stanbrook MB, Upshur R, et al. Cardiovascular safety of inhaled long-acting bronchodilators in individuals with chronic obstructive pulmonary disease. *JAMA Intern Med*. 2013;173(13):1175–85.
- Barnes PJ, Celli BR. Systemic manifestations and comorbidities of COPD. *Eur Respir J*. 2009;33(5):1165–85.
- Fatouleh R, Macefield VG. Respiratory modulation of muscle sympathetic nerve activity is not increased in essential hypertension or chronic obstructive pulmonary disease. *J Physiol*. 2011;589(Pt 20):4997–5006.
- Cazzola M, Imperatore F, Salzillo A, Di Perna F, Calderaro F, Imperatore A, et al. Cardiac effects of formoterol and salmeterol in patients suffering from COPD with preexisting cardiac arrhythmias and hypoxemia. *Chest*. 1998;114(2):411–5.
- Wouters EF. The systemic face of airway diseases: the role of C-reactive protein. *Eur Respir J*. 2006;27(5):877–9.
- Canning BJ. Reflex regulation of airway smooth muscle tone. *J Appl Physiol*. 2006;101(3):971–85.
- Heindl S, Lehnert M, Criée CP, Hasenfuß G, Andreas S. Marked sympathetic activation in patients with chronic respiratory failure. *Am J Respir Crit Care Med*. 2001;164:597–601.
- Raupach T, Bahr F, Herrmann P, Luethje L, Heusser K, Hasenfuss G, et al. Slow breathing reduces sympathoexcitation in COPD. *Eur Respir J*. 2008;32(2):387–92.
- Macklem PT. Therapeutic implications of the pathophysiology of COPD. *Eur Respir J*. 2010;35(3):676–80.
- Andreas S, Anker SD, Scanlon PD, Somers VK. Neurohumoral activation as a link to systemic manifestation of chronic lung disease. *Chest*. 2005;128:3618–24.
- van Gestel AJ, Kohler M, Clarenbach CF. Sympathetic overactivity and cardiovascular disease in patients with chronic obstructive pulmonary disease (COPD). *Discov Med*. 2012;14(79):359–68.
- Wallin BG, Esler M, Dorward P, Eisenhofer G, Ferrier C, Westerman R, et al. Simultaneous measurements of cardiac noradrenaline spillover and sympathetic outflow to skeletal muscle in humans. *J Physiol Lond*. 1992;453:45–58.
- Grassi G. Assessment of sympathetic cardiovascular drive in human hypertension: achievements and perspectives. *Hypertension*. 2009;54(4):690–7.
- Delius W, Hagbarth KE, Hongell A, Wallin BG. General characteristics of sympathetic activity in human muscle nerves. *Acta Physiol Scand*. 1972;84:65–81.
- Andreas S, Reiter H, Luthje L, Delekat A, Grunewald RW, Hasenfuss G, et al. Differential effects of theophylline on sympathetic excitation, hemodynamics, and breathing in congestive heart failure. *Circulation*. 2004;110(15):2157–62.
- Del Colle S, Milan A, Caserta M, Dematteis A, Naso D, Mulatero P, et al. Baroreflex sensitivity is impaired in essential hypertensives with central obesity. *J Hum Hypertens*. 2007;21(6):473–8.
- Heart rate variability. Standards of measurement, physiological interpretation, and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. *Eur Heart J*. 1996;17(3):354–81.
- Swedberg K, Eneroth P, Kjekshus J, Wilhelmens L. Hormones regulating cardiovascular function in patients with severe congestive heart failure and their relation to mortality. CONSENSUS Trial Study Group *Circulation*. 1990;82(5):1730–6.
- Schrier RW, Abraham WT. Hormones and hemodynamics in heart failure. *N Engl J Med*. 1999;341(8):577–85.
- Grassi G, Seravalle G, Quarti-Trevano F, Dell’oro R. Sympathetic activation in congestive heart failure: evidence, consequences and therapeutic implications. *Curr Vasc Pharmacol*. 2009;7(2):137–45.
- Busse WW, O’Byrne PM, Bleecker ER, Lotvall J, Woodcock A, Andersen L, et al. Safety and tolerability of the novel inhaled corticosteroid fluticasone furoate in combination with the beta2 agonist vilanterol administered once daily for 52 weeks in patients >=12 years old with asthma: a randomised trial. *Thorax*. 2013;68(6):513–20.
- Hanania NA, Feldman G, Zachgo W, Shim JJ, Crim C, Sanford L, et al. The efficacy and safety of the novel long-acting beta2 agonist vilanterol in patients with COPD: a randomized placebo-controlled trial. *Chest*. 2012;142(1):119–27.
- Beier J, Chanez P, Martinot JB, Schreurs AJ, Tkacova R, Bao W, et al. Safety, tolerability and efficacy of indacaterol, a novel once-daily beta(2)-agonist, in patients with COPD: a 28-day randomised, placebo controlled clinical trial. *Pulm Pharmacol Ther*. 2007;20(6):740–9.
- Jensen MT, Marrott JL, Lange P, Vestbo J, Schnohr P, Nielsen OW, et al. Resting heart rate is a predictor of mortality in COPD. *Eur Respir J*. 2013;42(2):341–9.
- Castagno D, Skali H, Takeuchi M, Swedberg K, Yusuf S, Granger CB, et al. Association of heart rate and outcomes in a broad spectrum of patients with chronic heart failure: results from the CHARM (Candesartan in Heart Failure: Assessment of Reduction in Mortality and morbidity) program. *J Am Coll Cardiol*. 2012;59(20):1785–95.
- Vestbo J, Anderson JA, Calverley PM, Celli B, Ferguson GT, Jenkins C, et al. Adherence to inhaled therapy, mortality and hospital admission in COPD. *Thorax*. 2009;64(11):939–43.
- Heindl S, Dödt C, Krahwinkel M, Hasenfuss G, Andreas S. Short term effect of continuous positive airway pressure on muscle sympathetic nerve activity in patients with chronic heart failure. *Heart*. 2001;85(2):185–90.
- Velez-Roa S, Renard M, Degaute JP, van de Borne P. Peripheral sympathetic control during dobutamine infusion: effects of aging and heart failure. *J Am Coll Cardiol*. 2003;42(9):1605–10.
- Al-Hesayen A, Azevedo ER, Newton GE, Parker JD. The effects of dobutamine on cardiac sympathetic activity in patients with congestive heart failure. *J Am Coll Cardiol*. 2002;39(8):1269–74.
- Cekici L, Valipour A, Kohansal R, Burghuber OC. Short-term effects of inhaled salbutamol on autonomic cardiovascular control in healthy subjects: a placebo-controlled study. *Br J Clin Pharmacol*. 2009;67(4):394–402.
- Guhan AR, Cooper S, Osborne J, Lewis S, Bennett J, Tattersfield AE. Systemic effects of formoterol and salmeterol: a dose-response comparison in healthy subjects. *Thorax*. 2000;55(8):650–6.
- Eckberg DL. Sympathovagal balance: a critical appraisal. *Circulation*. 1997;96(9):3224–32.
- Vassaux C, Torre-Bouscoulet L, Zeineldine S, Cortopassi F, Paz-Diaz H, Celli BR, et al. Effects of hyperinflation on the oxygen pulse as a marker of cardiac performance in COPD. *Eur Respir J*. 2008;32(5):1275–82.
- Barr RG, Bluemke DA, Ahmed FS, Carr JJ, Enright PL, Hoffman EA, et al. Percent emphysema, airflow obstruction, and impaired left ventricular filling. *N Engl J Med*. 2010;362(3):217–27.

43. Krieger BP. Hyperinflation and intrinsic positive end-expiratory pressure: less room to breathe. *Respiration*. 2009;77(3):344–50.
44. Easton PA, Hawes HG, Doig CJ, Johnson MW, Yokoba M, Wilde ER. Parasternal muscle activity decreases in severe COPD with salmeterol-fluticasone propionate. *Chest*. 2010;137(3):558–65.
45. Gomes ME, Aengevaeren WR, Lenders JW, Verheugt FW, Smits P, Tack CJ. Improving myocardial perfusion by percutaneous coronary intervention reduces central sympathetic activity in stable angina. *Clin Cardiol*. 2010;33(6):E16–21.
46. Man WD, Mustafa N, Nikolettou D, Kaul S, Hart N, Rafferty GF, et al. Effect of salmeterol on respiratory muscle activity during exercise in poorly reversible COPD. *Thorax*. 2004;59(6):471–6.

**Submit your next manuscript to BioMed Central  
and take full advantage of:**

- Convenient online submission
- Thorough peer review
- No space constraints or color figure charges
- Immediate publication on acceptance
- Inclusion in PubMed, CAS, Scopus and Google Scholar
- Research which is freely available for redistribution

Submit your manuscript at  
[www.biomedcentral.com/submit](http://www.biomedcentral.com/submit)

