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Neuroendocrine Dysregulation in Irritable Bowel Syndrome Patients: A Pilot Study

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Abstract

Background/Aims

Irritable bowel syndrome (IBS) is a multifactorial disorder, involving dysregulation of brain-gut axis. Our aim was to evaluate the neuroendocrine activity in IBS.

Methods

Thirty IBS and 30 healthy subjects were enrolled. Psychological symptoms were evaluated by questionnaires. Urinary 5-hydroxyindoleacetic acid (5-HIAA), plasma serotonin, endothelin, neuropeptide Y (NPY), plasma, and urinary cortisol levels were evaluated. Fourteen IBS subjects underwent microneurography to obtain multiunit recordings of efferent postganglionic muscle sympathetic nerve activity (MSNA).

Results

Prevalent psychological symptoms in IBS were maladjustment (60%), trait (40%) and state (17%) anxiety, obsessive compulsive-disorders (23%), and depressive symptoms (23%). IBS showed increased NPY (31.9 [43.7] vs 14.8 [18.1] pmol/L, $P = 0.006$), serotonin (214.9 [182.6] vs 141.0 [45.5] pg/mL, $P = 0.010$), and endothelin [1.1 [1.4] vs 2.1 [8.1], $P = 0.054$], compared to healthy subjects. Moreover, plasma NPY, endothelin, cortisol and serotonin, and urinary 5-HIAA were associated with some psychological disorders ($P < 0.05$). Despite a similar resting MSNA, after cold pressor test, IBS showed a blunted increase in MSNA burst frequency (+4.1 vs +7.8 bursts/minute, $P = 0.048$; +30.1% vs +78.1%, $P = 0.023$). Baseline MSNA tended to be associated with urinary cortisol ($\rho = 0.557$, $P = 0.059$), and moreover, changes in heart rate and MSNA after mental stress were associated with urinary ($\rho = 0.682$, $P = 0.021$) and plasma cortisol ($\rho = 0.671$, $P = 0.024$), respectively.

Conclusion

Higher concentrations of endothelin, NPY, and serotonin were found to be associated with some psychological disorders in IBS patients together with an altered cardiovascular autonomic reactivity to acute stressors compared to healthy subjects.

Key Words

Autonomic nervous system; Endothelin-1; Irritable bowel syndrome; Neuropeptide Y; Serotonin

Introduction

Irritable bowel syndrome (IBS) is the most prevalent functional digestive disorder. It negatively affects the quality of life and is associated with a significant economic burden related to direct and indirect annual health-care costs.[1]

A recent review shows in the general population a worldwide IBS (diagnosed by the Rome III criteria) prevalence of 1.1-29.2%.[2] IBS is characterized by abdominal pain and/or discomfort associated with a change of shape and/or consistency of stool. The pathophysiology of IBS is still unclear, but certainly, it involves a dysregulation of the brain-gut axis.[3-6] It has been clearly demonstrated that IBS patients show an increased reactivity to psychosocial stressors, with subsequent hypersensitivity leading to distension and abnormal motor pattern of the intestine.[3,7]

Approximately 60% of patients with IBS have concomitant mood and anxiety disorders; conversely, 75% of patients with major depression and panic disorder report some IBS symptoms.[8] An increased intestinal response to mental stress and clinical response to drugs affecting the central nervous system have been detected in IBS patients,[4] suggesting the presence of an alteration in the neuroendocrine pathway.

Neuropeptide Y (NPY) is a polypeptide present in the central and peripheral nervous system and in the digestive tract. In healthy subjects, there is a positive correlation between serum cortisol and NPY, which are used as activation markers of the hypothalamic-pituitary-adrenal (HPA) axis and sympathetic fibers of autonomic nervous system (ANS) axis, respectively.[9,10] NPY can also act as a modulator between the autonomic nervous system and mast-cells,[11] linking the psycho-emotional state to the symptoms of patients with IBS. In fact, NPY can modulate the activity of mast cells through specific receptors. This activation may lead to a dysregulation of permeability and visceral sensitivity of the intestine.[4]

Another mediator in both the central nervous system and peripheral tissues is serotonin (5-HT), which acts through its interaction with specific receptors.[6] In the gastrointestinal (GI) tract, 5-HT regulates sensory, secretory, and motor functions through the intrinsic and extrinsic nervous system. In particular, in a previous study,[5] we observed that IBS patients showed a significant association between specific psychological characteristics and neuroendocrine markers, especially plasma cortisol and NPY.

For example, endothelin-1 (ET-1) exerts vasoconstrictor and mitogenic properties on smooth muscle cells, but has also potent effects on GI smooth muscle, leading to contraction of the esophagus, stomach, and intestine.[12] Indeed, Nowicki et al[13] showed that the ET-1 tissue concentration is greater in human preterm intestine with histologic evidence of necrotizing enterocolitis. Interestingly, ET-1 is able to modulate sympathetic activity in humans through type A receptors.[14,15] To date, this interaction has been demonstrated only in hypertensive patients,[15] but we cannot exclude the possibility that a greater susceptibility to the sympatho-excitatory effect of ET-1 might be present in other conditions characterized by autonomic dysregulation, such as IBS. Taken together, these data suggest that a dysregulation in sympathetic activity, possibly due to neurohormonal alterations, might be implied in the pathophysiology of both vascular dysfunction and GI motility disorders. Based on these premises, the objective of this study was to assess the neuroendocrine activity in patients with IBS.

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Materials and Methods

1. Patients

Thirty patients (9 men, 21 women; mean age 38.43 ± 11.82 years; age range 19-67 years) with IBS according to the Rome III criteria[7] who were referred to the Gastroenterology outpatients services of a single tertiary center in Pisa, Italy, were enrolled in this study. Out of 30 IBS patients, 7 patients (2 men and 5 women) had mixed diarrhea and constipation; 12 (6 men and 6 women) had diarrhea; 11 (1 man and 10 women) had constipation. We also enrolled 30 healthy volunteers (HVs) (14 men, 16 women; mean age 47.79 ± 15.56 years; age range 27-75 years). The matter of the research was described to both the patients and the HVs. The Local Ethics Committee approved the study. Each study participant delivered written informed consent before beginning the study, in accordance with the principles of the Declaration of Helsinki (Sixth Revision, Seoul 2008).

2. Psychological Assessment

The assessment of anxiety, personality dimensions, psychophysiological features, fears of the subjects, symptoms of depression, obsessions, and compulsions were performed using the questionnaires that were part of the Cognitive Behavioral Assessment (CBA, version 2.0.).[16]

These questionnaires were validated on Italian populations of healthy subjects and patients with various diseases. Therefore, healthy controls who scored with significant psychological symptoms were excluded.

3. Neuroendocrine Markers

Venous blood samples of patients and HVs were taken after an overnight fasting. As described previously,[5] levels of 5-HT, cortisol, and NPY were measured in the venous blood samples, and levels of cortisol and 5-hydroxyindoleacetic acid (5-HIAA) were also determined in one sample of 24-hour urine excretion from all patients and HVs. Endothelin (ELISA; Biomedica Medizinprodukte GmbH & Co KG, Vienna, Austria) was measured in the venous blood samples.

4. Microneurography Assessment

Fourteen out of 30 patients (8 men/6 women) agreed to undergo microneurography. Microneurography was used to obtain multiunit recordings of efferent postganglionic muscle sympathetic nerve activity (MSNA), as previously described.[15,17]

All experimental sessions were performed in the morning, in a quiet and comfortable room. The microelectrodes for the MSNA recording and the other measuring devices were positioned. After 10 minutes, non-invasive beat-to-beat blood pressure (BP) (Portapres; Finapres Medical System, Enschede, The Netherlands), heart rate (Biotach; Gould Electronics, Chandler, AZ, USA), and MSNA were continuously monitored and recorded, using a digital acquisition system (ACQ-16; Gould Electronics) and a dedicated computer software (Ponemah; Data Sciences Interntional, New Brighton, MN, USA), for a 10-minute interval to obtain baseline levels. Then, acute reactivity to stress was evaluated using a mental arithmetic stress test and cold pressor test.

During the verbally administered mental arithmetic stress test, IBS patients were asked to subtract two-digit numbers from four–digit numbers as quickly and accurately as possible. Throughout the mental arithmetic stress test, the subjects were urged to work more quickly and more accurately.[18,19] The cold pressor test was performed by immersing the left hand of the subject in ice water up to the wrist for 2 minutes.[20] MSNA was analyzed by visual inspection by a single investigator (R.M.B.). MSNA was quantified as the MSNA burst frequency (expressed in bursts/min) and incidence (expressed in bursts/100 heart beats). Absolute (D) and relative changes from baseline (D%) in MSNA, BP, and heart rate were calculated.

5. Statistical Methods

Statistical analysis was performed using STATA 12 software (Stata Statistical Software, College Station, TX, USA). The data were not normally distributed, therefore all results were expressed as the median and interquartile range. Correlation analysis between 2 variables was performed using the Spearman rank correlation coefficients (ρ). Comparisons of continuous data were performed using the Wilcoxon signed-ranks test applied to two-sample (for comparisons between HV and IBS) or to paired samples (for comparisons between baseline and acute stressors). Statistical significance was assigned to a P-value < 0.05.

Results

1. Psychological Assessment

The most prevalent psychological symptoms in IBS patients were: social maladjustment (60%), trait anxiety (40%), obsessive compulsive-disorders (23%), depressive symptoms (23%), state anxiety (17%).

2. Neuroendocrine Markers

Data are summarized in Table 1. Significant differences between IBS patients and HVs were found in the plasma levels of neuroendocrine markers. In particular, baseline plasma levels of NPY (31.9 [43.7] vs 14.8 [18.1] pmol/L, P = 0.006) and serotonin (214.9 [182.6] vs 141.0 [45.5] pg/mL, P = 0.01) were significantly higher in IBS patients than in controls. Plasma endothelin showed also a trend to increased values in IBS patients.

3. Neuroendocrine Markers and Psychological Features

There were significant associations of NPY with "maladjustment" ($P < 0.01$). Endothelin had a significant association with “fears for repellent animals” ($P = 0.040$) and “fears for natural disasters” ($P = 0.040$). Plasma cortisol had significant associations with “obsessive disorders” ($P = 0.010$), “checking” ($P = 0.040$), and “fears for repellent animals” ($P = 0.04$). Plasma serotonin showed significant associations with “state anxiety,” “trait anxiety,” and psychophysical disorders ($P < 0.01$). 5-HIAA had a significant association with “doubting/ruminating” ($P = 0.020$). Urinary cortisol had significant associations with “trait anxiety.” The associations between neuroendocrine markers and psychological features are shown in Table 2.

4. Neuroendocrine Markers and Microneurography

Hemodynamic and sympathetic variables in IBS patients at baseline and after the mental arithmetic stress test and cold pressor test are shown in Table 3. In 3 of 14 IBS patients, MSNA recordings during the stress tests were not readable because of artifacts due to movements and muscle contractions. IBS patients showed a significant increase in mean BP and HR after both mental stress and cold pressor test. Conversely, a significant rise in MSNA frequency and incidence was found only after mental stress, thus suggesting a blunted response to cold pressor test. Baseline burst frequency tended to be positively associated with urinary cortisol ($\rho = 0.557$, $P = 0.059$). Absolute and relative changes in heart rate after mental stress were also positively associated with urinary cortisol (D HR $\rho = 0.636$, $P = 0.035$; D% HR $\rho = 0.682$, $P = 0.021$). Absolute and relative changes in burst incidence after mental stress were also positively associated with plasma cortisol (D MSNA $\rho = 0.551$, $P = 0.079$; D% MSNA $\rho = 0.671$, $P = 0.024$). Plasma serotonin tended to be inversely associated with BP response to cold pressor test (D mean BP $\rho = -0.582$, $P = 0.060$; D% mean BP $\rho = -0.510$, $P = 0.090$).

Furthermore, we compared individuals with diarrheic IBS subtype (n = 6) to the non-diarrheic IBS subtype (defined grouping patients with either mixed diarrhea and constipation or

constipation only): microneurographic parameters at baseline were similar in the two groups (see Supplementary Table). IBS individuals with non-diarrheic subtype had instead a blunted MSNA frequency increase after the cold pressor test compared to the diarrheic subtype (median value: 0.9 vs +6.7 bursts/min, P = 0.055; 19% vs +42%, P = 0.130). Furthermore, individuals with diarrheic subtype tended to have a blunted % MSNA incidence increase after mental stress compared to non-diarrheic subtype (median value: +19% vs +46%, P = 0.090), but a similar absolute change (+3.2 vs +3.5 bursts/100 hour, P = 0.450).

In order to demonstrate whether the present findings were specific of IBS patients, we compared microneurographic features of patients enrolled in this study to those of a group of healthy individuals. We selected among HVs participating to previously published studies[15,17] or unpublished protocols a group of 12 age-, sex-, and BP-matched individuals (6 men, age 40.7 ± 10.4 years), who performed a similar microneurographic protocol.

MSNA, mean BP and HR in resting conditions, as well as their response to mental stress, was similar in IBS individuals and in HVs (Table 3). Interestingly, after cold pressor test IBS patients had a blunted increase MSNA burst frequency (median value: +4.1 vs +7.8 bursts/minute, P = 0.048; +30.1% vs +78.1%, P = 0.023) and incidence (median value: +3.3 vs +8.1 bursts/100HR, P = 0.034; 15.1% vs 58.7%, P = 0.023) than HVs, while differences in changes in mean BP (median value + 10 vs +17 mmHg, P = 0.18; +13 vs +22%, P = 0.160) and HR (median value: +4 vs +7 bpm, P = 0.91; +7 vs +12%, P = 0.860) did not reach statistical significance. When different IBS subgroups were analyzed, only individuals with a non-diarrheic IBS subtype had a blunted MSNA frequency increase after cold pressor test in comparison to HVs (median value: +0.9 vs +7.8 bursts/minute, P = 0.003).

Discussion

To our knowledge, this is the first study that evaluated endothelin levels in IBS patients and found increased levels compared with HVs. Furthermore, this is the first time that muscle sympathetic nerve activity in IBS patients was studied by using direct intraneural recordings. Although we could not demonstrate any significant increase in resting sympathetic activity in IBS patients, autonomic reactivity to cold pressor test in the vascular district was blunted, particularly in the non-diarrheic IBS subtype. Furthermore, cardiovascular autonomic reactivity to mental stress positively correlated with cortisol levels, suggesting an association between HPA and sympathetic activation in this population.

The secretion of the glial cell-derived neurotrophic factor, nerve growth factor, and transforming growth factor-beta contributes to the maintenance of epithelial integrity; the secretion of endothelins might be involved in vasoregulation.[21] Some studies have demonstrated an altered autonomic function in IBS patients and suggest that IBS patients have increased sympathetic nervous system and cardiovascular activation.[22,23] The endothelin system seems to exert an increased vasoconstrictive and sympathoexcitatory role in conditions characterized cardiovascular risk such as hypertension.[15] Moreover, some studies demonstrated that ET-1 mediates smooth muscle contraction in GI tract.[12]

According to previous studies,[5] NPY levels at baseline are higher in IBS patients than in controls. It is possible that NPY levels change during psychological distress. In fact, in the present study, NPY showed a significant association with "fears for natural disasters" and with “obsessive-compulsive disorders”. Fear occurs in response to a known threat that is external, defined, or otherwise non-conflictual in origin; anxiety occurs as a response to a threat that is unknown, internal, vague, or conflictual in origin.[24]

Measurement of plasma NPY levels could play an important role in the diagnosis of some functional disorders and their psychological comorbidities that are very frequently present because,

in most cases, the other neuroendocrine and immune markers have only a paracrine effect. In fact, the local release of some neuropeptides into the enteric nervous system could play a role in the pathophysiology of IBS, but these neuropeptides cannot be assayed in the serum because they spread in a paracrine way. As suggested by Forbes et al[25] in a mouse model, NPY seems to directly inhibit the colonic motor response induced by the corticotrophin-releasing factor (CRF_1) receptor activation during stress. Given that the local 5-HT release in the GI tract is involved in excitatory action on vagal afferent fibers and on contraction of smooth colonic muscle,[6] it is possible that indirect high levels of NPY try to maintain normal colonic motor response also during psychological distress, counteracting the effect of 5-HT release. Indeed, via the gut-brain axis, local 5-HT secretion contributes to central pain perception, visceral hypersensitivity and transit modification of the GI motility pattern; however, several studies demonstrated alterations in plasma 5-HT in a specific subgroup of IBS patients but not in all IBS patients.[5,6,26]

In this study, the mean levels of plasma cortisol were not significantly different between IBS patients and controls, suggesting an uncoupling between the HPA and ANS axes. Our data are consistent with a previous study by Fukudo et al[27] that observed increased plasma levels of adrenocorticotropic hormone but normal levels of cortisol in response to corticotropin releasing hormone injection in IBS patients. However, our results suggest a more complex relationship between the HPA and ANS axes in IBS, with a facilitator role of the HPA axis on ANS that manifests only during conditions of acute stress. Indeed, baseline cortisol levels, a possible index of distress in this population, are associated with an increased cardiovascular autonomic reactivity to stress, as represented by the increase of MSNA during mental challenge. This result is in line with previous findings showing that in humans, injection of corticotropin increases MSNA,[28] while a correlation between resting MSNA and morning salivary cortisol has been observed in healthy young individuals.[29] Finally, a preliminary analysis according to IBS subtypes suggest that a blunted response to the cold pressor test is present in the non-diarrheic subtype. A hypo-reactivity of sympathetic central integration and/or sympathetic efferent pathways might reflect a generalized

hyporesponsiveness of the autonomic nervous system to stimuli, possibly underlying the clinical feature of IBS in this subgroup.

It is important to outline the methodological limitations of the study. First, IBS patients had, as expected, a significantly higher prevalence of psychological disorders in comparison to healthy individuals. Therefore, it is not possible to demonstrate whether the altered neuroendocrine pattern shown in IBS is disease-specific or it is rather related to the psychological comorbidity. Furthermore, the control group for MSNA substudy was not recruited on purpose for this study, adding another possible source of bias, and had a small sample size; for this reason, MSNA results should be considered preliminary and interpreted with caution. It is mandatory that these aspects should be investigated in further studies with adequate sample size and appropriately selected control group in order to confirm the findings of this pilot study.

In conclusion, this study demonstrated for the first time that endothelin levels are increased in IBS patients compared to controls, suggesting a possible involvement of endothelin in autonomic disorders present in IBS patients. Furthermore, circulating levels of cortisol are related to autonomic cardiovascular reactivity to stress in this population, thus establishing a new link between the ANS and HPA axes in IBS pathophysiology. Though our study failed to demonstrate an altered sympathetic traffic directed to the vasculature, a blunted sympathetic response to cold pressor test was shown, particularly in the non-diarrheic subtypes, suggesting that multiple autonomic dysregulations might be present in this condition. Finally, higher concentrations of NPY were demonstrated in IBS patients than in controls. If this relationship is confirmed in a larger sample of IBS patients, the use of this neuropeptide could be considered as a possible diagnostic marker for IBS or could at least help to detect possible IBS psychological comorbidities.

Supplementary Material

Note: To access the supplementary table mentioned in this article, visit the online version of *Journal of Neurogastroenterology and Motility* at http://www.jnmjournal.org/, and at https://doi.org/10.5056/jnm16155.

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Tables

Table 1. Neuroendocrine Markers in the Study Population

Parameter	Healthy volunteers		IBS patients		*P*-values
	Median	IQR	Median	IQR	
Neuropeptide Y (pmol/L)	14.8	18.1	31.9	43.7	0.006
Serum cortisol (μg /dL)	15.8	2.9	16.4	9.0	0.718
Urinary cortisol (μg /24 hr)	27.0	27.0	35.2	23.5	0.487
Plasma serotonin (ng/mL)	141.0	45.5	214.9	182.6	0.012
5-hydroxyindoleacetic acid (mg/24 hr)	9.3	5.9	8.1	4.9	0.468
Endothelin (pg/mL)	1.1	1.4	2.1	8.1	0.054

IQR, interquartile range.

Table 2. Association Between Neuroendocrine Markers and Psychological Features in Irritable Bowel Syndrome Patients

Parameters	State anxiety	Trait anxiety	PD	Fears for natural disasters	Fears for repellent animals	OCD	Checking	Doubting/rumi nating	Maladjustment
Neuropeptide Y (31.95 [43.7])	ns	ns	ns	ns	ns	ns	ns	ns	$\rho = 0.5$ $P < 0.01$
Serum cortisol (16.4 [9.0])	ns	ns	ns	ns	$\rho = -0.4$ $P = 0.040$	$\rho = 0.4$ $P = 0.010$	$\rho = 0.4$ $P = 0.040$	ns	ns
Plasma 5-HT (214.85 [182.6])	$\rho = 0.5$ $P < 0.01$	$\rho = 0.6$ $P < 0.01$	$\rho = 0.5$ $P < 0.01$	ns	ns	ns	ns	ns	ns
Endothelin (2.1 [8.1])	ns	ns	ns	$\rho = -0.4$ $P = 0.040$	$\rho = 0.4$ $P = 0.040$	ns	ns	ns	ns
5-hydroxyindoleacetic Acid (8.1 [4.9])	ns	ns	ns	ns	ns	ns	ns	$\rho = 0.5$ $P = 0.020$	$\rho = 0.4$ $P = 0.020$
Urinary cortisol (35.2 [23.5])	ns	$\rho = 0.4$ $P = 0.040$	ns	ns	ns	ns	ns	ns	ns

PD, psychophysical disorders; OCD, obsessive compulsive disorders; ns, no statistical association; Rho= ρ, Spearman rank correlation coefficient; 5-HT, serotonin.

Values of neuroendocrine markers are expressed as median and interquartile range.

Table 3. Hemodynamic and Sympathetic Variables in Irritable Bowel Syndrome Patients at Baseline and After Mental Stress and Cold Pressor Test

	Baseline		Mental stress		Cold pressor test	
IBS patients	Median	IQR	Median	IQR	Median	IQR
Mean BP (mmHg)	79.2	11.6	90.2[a]	19.8	89.8[b]	12.3
Heart rate (bpm)	69.9	16.2	87.0[a]	29.0	81.5[b]	33.8
MSNA burst frequency (bursts/min)	14.9	10.4	18.3[a]	15.3	19.5	12.0
MSNA burst incidence (bursts/100 heart beats)	22.6	12.1	23.2[a]	17.4	27.2	9.5
Healthy individuals	Median	IQR	Median	IQR	Median	IQR
Mean BP (mmHg)	84.7	6.5	95.7[a]	16.7	102.9[a]	18.1
Heart rate (bpm)	66.6	10.0	75.0[a]	23.5	72.8[a]	18.2
MSNA burst frequency (bursts/min)	10.3	8.5	15.5[a]	10.0	19.2[a]	18.0
MSNA burst incidence (bursts/100 heart beats)	14.7	14.3	20.2[a]	5.5	25.5[a]	28.3

[a]$P < 0.01$ vs baseline.

[b]$P < 0.05$ vs baseline.

IBS, irritable bowel syndrome; IQR, interquartile range; BP, blood pressure; MSNA, muscle sympathetic nerve activity.

Supplementary Material

Supplementary Table. Microneurography results in non-diarrheic and diarrheic irritable bowel syndrome patients.

	Baseline	Mental stress		Cold pressor test	
IBS patients: non-diarrheic subtype		D	D%	D	D%
Mean BP (mmHg)	77 (19)	+12 (18)	+15 (31)	+10 (21)	+13 (37)
Heart rate (bpm)	68 (21)	+6 (41)	+8 (66)	+13 (26)	+16 (42)
MSNA burst frequency (bursts/min)	8.2 (21.7)	+6.0 (14.6)	+57 (42)	+0.9 (7.6)	+19 (42)
MSNA burst incidence (bursts/100 heart beats)	13.3 (27.2)	+3.5 (11.7)	+46 (35)	-0.9 (12.8)	-12 (50)
IBS patients: diarrheic subtype					
Mean BP (mmHg)	79 (19)	+6 (15)	+8 (14)	+8 (31)	+13 (28)
Heart rate (bpm)	70 (13)	+14 (16)	+19 (22)	+3 (29)	+5 (42)
MSNA burst frequency (bursts/min)	14.9 (2.7)	+6.1 (5.7)	+41 (47)	+6.7 (11.6)	+42 (102)
MSNA burst incidence (bursts/100 heart beats)	22.7 (6.4)	+3.2 (6.3)	+19 (26)	+5.9 (15.6)	+27 (88)

IBS, irritable bowel syndrome; D, absolute change; D%, relative change from baseline; BP, blood pressure; MSNA, muscle sympathetic nerve activity. Data are expressed as median value (interquartile range).