—Original—

Relationship between Plasma Norepinephrine at Peak Exercise and ^{123}I-MIBG Imaging of the Heart and Lower Limbs in Heart Failure

Takaya Tsuchida[1], Nagaharu Fukuma[1], Keiko Oikawa[1], Kazuyo Kato[1],
Yuko Kato[1], Teruo Takano[1] and Shinichiro Kumita[2]

[1]Department of Functional Pathophysiology for Human Organs, Graduate School of Medicine, Nippon Medical School
[2]Department of Clinical Radiology, Graduate School of Medicine, Nippon Medical School

Abstract

Background: Past studies suggested that plasma norepinephrine during exercise originates in sympathetic nerve endings and that the main origin differs among pathophysiological conditions.

Aims: This study investigated the most important site of sympathetic terminals as an origin of plasma norepinephrine during exercise in patients with heart failure using ^{123}I-metaiodobenzylguanidine (MIBG) scintigraphy.

Methods and Results: Twenty patients with organic heart disease underwent exercise testing and ^{123}I-MIBG scintigraphy. Systemic ^{123}I-MIBG uptake was measured 4 hours after ^{123}I-MIBG injection, and the heart-to-brain (H/B) and lower limb-to-brain ratios (L/B) were calculated. Plasma norepinephrine concentration was measured at rest and at peak exercise. Subjects were divided into two groups: those with preserved left ventricular ejection fraction (LVEF≥45%, n=8) and those with reduced LVEF (<45%, n=12). Plasma norepinephrine at rest did not correlate with H/B or L/B. In the preserved LVEF group, plasma norepinephrine at peak exercise was correlated with H/B (r=0.722), but not with L/B. In the reduced LVEF group, the norepinephrine response to peak exercise correlated with L/B (r=0.642), but not with H/B.

Conclusion: The present findings suggest that norepinephrine concentration is regulated by sympathetic terminal function of working muscles in patients with impaired LVEF and by that of the heart in patients with preserved LVEF.
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Key words: exercise, norepinephrine, heart failure, sympathetic nerve,
[123]I-metaiodobenzylguanidine

Introduction

Circulating norepinephrine, that is, plasma norepinephrine, is known to be a determinant of prognosis in patients with heart failure[1]. This finding led to speculation that sympathetic overactivity exacerbates heart failure. However, it has been reported that circulating norepinephrine is not an accurate index of sympathetic nervous activity

because it is regulated by various factors[2-4]. This antithesis suggests that an increase in the concentration of circulating norepinephrine has a harmful effect on the state of heart diseases and the prognosis of heart diseases both through the mechanism of sympathetic activation and through an indirect pathway. An example of an acceptable interpretation of the mechanism is that an increase in circulating norepinephrine itself results in oxidative stress and, therefore, organ impairment[5]. Another example of a possible mechanism is that a factor other than sympathetic nervous activation that allows norepinephrine to increase might exacerbate heart failure. Inflammatory cytokines are reported to injure sympathetic nerve endings, which might result in an increase in plasma norepinephrine through impairment of reuptake[6]. Thus, an investigation into the mechanism for increases in circulating norepinephrine in patients with heart failure would be useful for elucidating the pathogenesis of heart failure. Further clinical studies are needed.

In the present study, we focused on the function of sympathetic nerve endings to clarify the factor that regulates the plasma norepinephrine level in patients with heart disease. We used systemic [123]iodine-metaiodobenzylguanidine (^{123}I-MIBG) imaging to evaluate sympathetic terminal function. A previous report has shown that cardiac uptake of ^{123}I-MIBG correlates with norepinephrine content in myocardial tissue in patients with heart failure and reflects the capacity to release norepinephrine from the myocardium[7,8]. Also, it has been reported that cardiac MIBG is related to increases in norepinephrine in drawn blood from the coronary sinus during exercise[9]. Therefore, studying the reaction of circulating catecholamines to exercise is important for investigating the meaning of circulating catecholamines in heart failure. In addition, MIBG is useful for tracing the origin of norepinephrine.

We examined the relation between plasma norepinephrine concentration and the function of sympathetic nerve terminals distributed to the heart and the right lower limb using ^{123}I-MIBG imaging in patients with heart disease. Previous investigations have not clarified the main origin of norepinephrine at rest and during intense exercise. In addressing this issue, we considered left ventricular systolic function and activity such as intense exercise.

Materials and Methods

Study Population

We studied 20 patients with heart disease without decompensated heart failure and without myocardial ischemia (15 men and 5 women; mean age, 51 ± 12 years). All subjects were clinically stable, and their medications had been unchanged for at least 2 weeks before the study. None had any significant lung disease or diabetes or took any β-adrenoreceptor blockers.

Transthoracic echocardiography (Sonos 5500, Hewlett-Packard, Palo Alto, CA, USA) was performed at rest, and the left ventricular ejection fraction (LVEF) was calculated. Subjects were divided into two groups on the basis of LVEF: a low LVEF group with chronic heart failure (LVEF<45%, n=12) and a preserved LVEF group with normal LV function (LVEF≧45%, n=8). Informed written consent for participation in this study was obtained from all subjects in accordance with the ethics committee of the institution.

Cardiopulmonary Exercise Testing

Symptom-limited cardiopulmonary exercise testing was performed with a bicycle ergometer (StrenghErgo.240, Mitsubishi Co., Tokyo, Japan) with the subject in a sitting position. After a 4-minute rest period, exercise began with a 4-minute warm-up at 10 W and 60 rpm, after which the intensity was increased by 1 W every 6 seconds according to the ramp protocol. Heart rate and 12-lead electrocardiogram were monitored continuously (ML-5000, Fukuda Denshi, Tokyo, Japan). During testing, blood pressure was measured every minute by an automatic indirect cuff manometer (STBD-780B, Nihon Collin Co., Ltd., Aichi, Japan).

Exercise was stopped due to exhaustion. No patient experienced angina, syncope, ischemic ST segment changes, or serious arrhythmia during exercise. Oxygen consumption (VO_2) was measured

Table 1 Comparison of background between the low LVEF and preserved LVEF groups

	Preserved LVEF (n=8)	Low LVEF (n=12)	p-value
Age (years)	57 ± 7	46 ± 13	ns
Sex (male : female)	5 : 3	10 : 2	ns
Etiology (IHD : CM)	7 : 1	8 : 4	ns
LVEF (%)	67 ± 6	33 ± 10	P<0.01

ns, not statistically significant; IHD, ischemic heart disease; CM, cardiomyopathy.

using a breath-by-breath gas analyzer (AE-300S, Minato Medical Science, Osaka, Japan).

Whole Body ^{123}I-MIBG Imaging

We performed whole body ^{123}I-MIBG imaging on the basis of past reports[10,11]. An anterior view of whole-body scintigraphy was obtained 4 hours (delayed image) after intravenous injection of ^{123}I-MIBG (MyoMIBG-I[123], Daiichi Radioisotope Laboratories, Tokyo, Japan) at a dose of 111 MBq using a γ-camera (Vertex, ADAC, Milpitas, CA, USA).

To assess the function of sympathetic nerve endings distributed to myocardium and skeletal muscle, we set the region of interest (ROI) on the heart, lower limb, and brain and measured the uptake of ^{123}I-MIBG in each region. We selected the brain as the reference for ^{123}I-MIBG uptake because ^{123}I-MIBG does not pass through the blood brain barrier. As there is little muscle on the skull, we chose a portion of the skull to be imaged as the standard for low accumulation of ^{123}I-MIBG. The ratio of ^{123}I-MIBG uptake of each target to brain area uptake was calculated. We considered these ratios to be variables reflecting the function of sympathetic nerve endings in each region. We then calculated the following two variables to assess the function of sympathetic nerve endings in the heart and skeletal muscle of the lower limb: the heart-to-brain ratio (H/B) and lower limb-to-brain ratio (L/B).

Plasma Norepinephrine Concentration

For measurement of norepinephrine, we collected blood samples into a tube containing EDTA from the antecubital vein when the patient was at rest

and during peak exercise. Blood samples in the tube were centrifuged at 3,000 rpm for 10 min, and plasma was extracted. The plasma concentration of norepinephrine was measured with high-performance liquid chromatography.

Statistical Analysis

All values are expressed as the means ± SD. Comparisons of variables between groups were performed using the unpaired Student's t-test and chi-square analysis. Simple linear regression analysis was used to assess the significance of the differences. Differences with p values less than 0.05 were considered statistically significant.

Results

There were no significant differences in age, sex, and etiology between the low LVEF and preserved LVEF groups (**Table 1**) . However, peak VO$_2$ (18.2 ± 2.0 vs. 25.7 ± 6.9 ml/min/kg; p=0.03) and workload (94 ± 18 vs. 128 ± 57 W; p<0.05) were significantly higher in the preserved LVEF group (**Table 2**) .

Plasma norepinephrine concentration at rest and at peak exercise did not differ significantly between the low LVEF and preserved LVEF groups. However, plasma norepinephrine concentration at peak exercise tended to be lower in the low LVEF group (**Table 2**) .

Ratio of ^{123}I-MIBG Uptake

The H/B and L/B ratios of ^{123}I-MIBG uptake did not differ significantly between groups, but tended to be lower in the low LVEF group, with the difference more obvious in the H/B ratio (**Table 3**) .

Table 2　Response to exercise in low LVEF and preserved LVEF groups

	Preserved LVEF (n=8)	Low LVEF (n=12)	p-value
Work load (W)	128 ± 57	94 ± 18	P<0.05
Peak VO₂ (ml/min)	25.7 ± 6.9	18.2 ± 2.0	P=0.03
Norepinephrine concentration (pg/ml)			
At rest	347 ± 160	403 ± 140	ns
At peak exercise	1,299 ± 467	987 ± 413	ns

Table 3　Comparison of ^{123}I-MIBG uptake between low LVEF and preserved LVEF groups

	Preserved LVEF (n=8)	Low LVEF (n=12)	p-value
H/B ratio	5.18 ± 1.82	3.83 ± 1.25	ns
L/B ratio	1.19 ± 0.33	1.05 ± 0.22	ns

Relationship between Norepinephrine and the H/B and L/B Ratios of ^{123}I-MIBG Uptake

At rest, the plasma concentration of norepinephrine did not correlate with the H/B or L/B ratios in either group (**Fig. 1, 2**). At peak exercise, the plasma concentration of norepinephrine correlated with only the H/B ratio in the preserved LVEF group (r=0.722, **Fig. 3**) and with only the L/B ratio in the low LVEF group (r=0.642, **Fig. 4**).

Discussion

Previous studies have reported the impairment of norepinephrine response to exercise in patients with heart failure[12]. However, the origin of plasma norepinephrine during exercise is still unclear. Therefore, we investigated the main derivative site of circulating norepinephrine using systemic ^{123}I-MIBG imaging. The relation between uptake of ^{123}I-MIBG to the heart and norepinephrine content in myocardial tissue in patients with heart failure has been clarified[7]. That uptake of ^{123}I-MIBG to the heart reflects the capacity to release norepinephrine from myocardium in heart failure patients has also been shown[8]. Although there have been several reports about ^{123}I-MIBG scanning of myocardium, to our knowledge there have been no investigations

concerning skeletal muscle. This is the first report on the relation between uptake of ^{123}I-MIBG to limbs and norepinephrine release under exercise stress.

Past studies[13,14] have found that the norepinephrine in granules in sympathetic nerve endings is released by sympathetic stimulation, a phenomenon termed "exocytosis". Nerve endings reuptake most of the released norepinephrine (uptake-1). As a result, the small quantity of norepinephrine without reuptake distributes to external nervous tissues (uptake-2), and the rest spills over to the blood (circulating norepinephrine). Based on the premise that the quantity of norepinephrine released from nerve endings through sympathetic excitation correlates with the quantity of norepinephrine spillover, it is thought that plasma norepinephrine can be used to evaluate sympathetic nervous activity[15,16]. However, plasma norepinephrine concentration does not accurately reflect sympathetic nervous activity. Plasma norepinephrine is regulated by various factors, such as local sympathetic activity, sympathetic terminal function, and clearance[2–4], and circulating norepinephrine has clinical significance other than evaluation of sympathetic activity in the clinical study of heart disease. Therefore, we attempted to determine which is the important organ as an origin of plasma norepinephrine during exercise in patients with heart failure using systemic ^{123}I-MIBG scintigraphy.

Methods of Assessment for Sympathetic Terminal Function in Heart Failure

Several clinical methods are available to examine the function of sympathetic nerves in various organs and tissues, such as skeletal muscle. The most representative method for determination of muscle
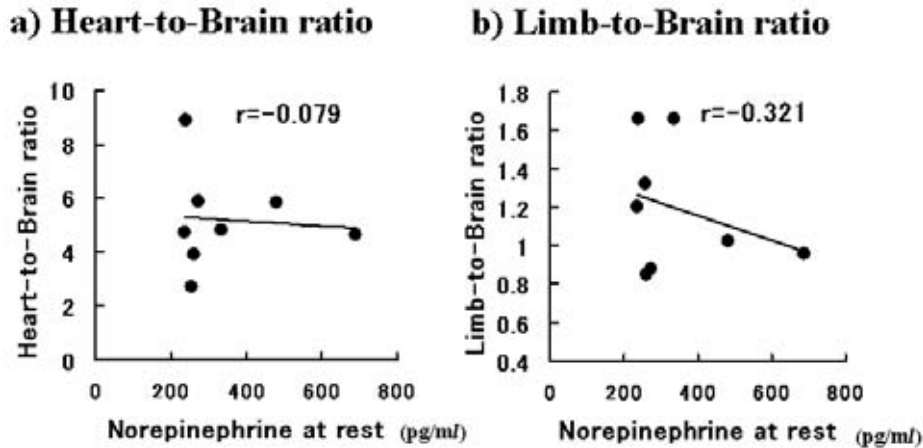
a) Heart-to-Brain ratio   b) Limb-to-Brain ratio

Fig. 1 Plasma norepinephrine concentration and ^{123}I-MIBG uptake at rest in the preserved LVEF group
Norepinephrine at rest, plasma norepinephrine concentration at rest. In the preserved LVEF group, plasma norepinephrine concentration at rest did not correlate with the H/B ratio (a) or the L/B ratio (b).
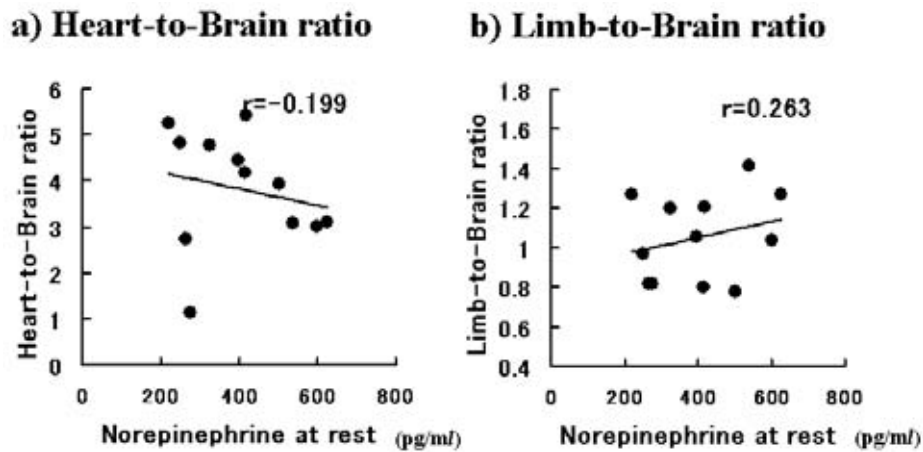
a) Heart-to-Brain ratio   b) Limb-to-Brain ratio

Fig. 2 Plasma norepinephrine concentration and ^{123}I-MIBG uptake at rest in the low LVEF group
Norepinephrine at rest, plasma norepinephrine concentration at rest. In the low LVEF group, plasma norepinephrine concentration at rest did not significantly correlate with the H/B ratio (a) or the L/B ratio (b).

sympathetic nerve activity (MSNA) should be mentioned. MSNA is recorded microneurographically by the insertion of a tungsten microelectrode into the nerve innervating the muscle. Many clinical studies utilize MSNA for the clinical assessment of sympathetic nervous function in skeletal muscle. The burst frequency of MSNA is considered a useful parameter to characterize sympathetic vasomotor tone innervating skeletal muscle during exercise[17]. However, the method for determining MSNA is invasive and cannot be used solely for evaluating the function of sympathetic nerve endings or evaluating sympathetic function in organs and tissues other than skeletal muscle. Several reports have described the determination of sympathetic nerve terminal function in skeletal muscle using ^{123}I-MIBG scintigraphy[10,11]. Taki et al. have shown with whole-body ^{123}I-MBG scintigraphy in hypertrophic cardiomyopathy that a difference exists between the heart and other organs, including skeletal muscle[10]. Hirayama et al. have investigated ^{123}I-MIBG uptake and washout in femoral skeletal

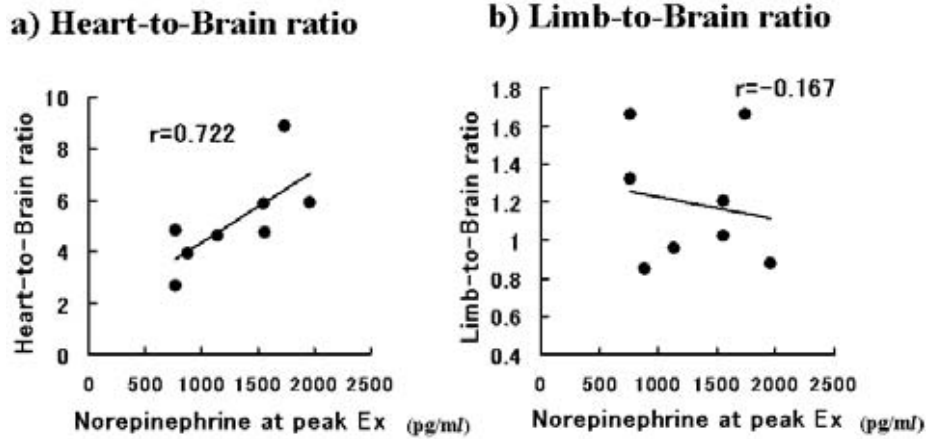## a) Heart-to-Brain ratio

b) Limb-to-Brain ratio

Fig. 3 Plasma norepinephrine and ^{123}I-MIBG uptake at peak exercise in preserved LVEF group
Norepinephrine at peak Ex, plasma norepinephrine concentration at peak exercise. In the preserved LVEF group, plasma norepinephrine concentration at peak exercise significantly correlated with the H/B ratio (**a**, r=0.722) but not with the L/B ratio (**b**, r=−0.167).

a) Heart-to-Brain ratio
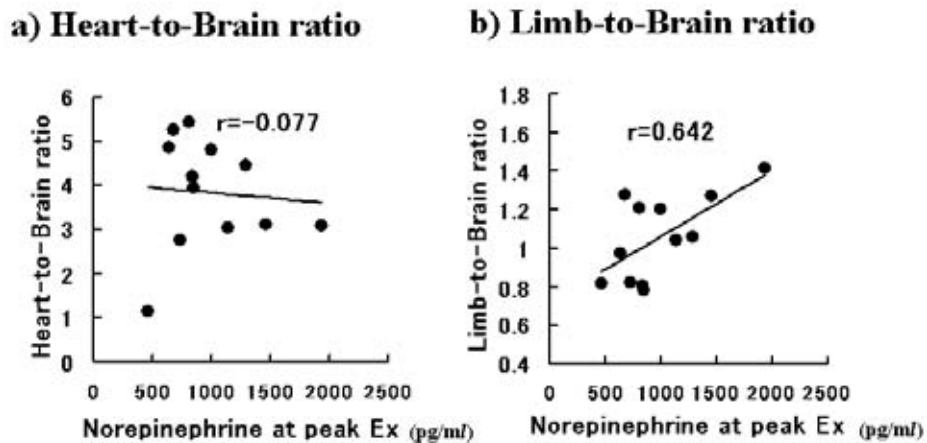
b) Limb-to-Brain ratio

Fig. 4 Plasma norepinephrine and ^{123}I-MIBG uptake at peak exercise in low LVEF group
Norepinephrine at peak Ex, plasma norepinephrine concentration at peak exercise. In the preserved LVEF group, plasma norepinephrine concentration at peak exercise significantly correlated with the L/B ratio (**b**, r=0.642) but not with the H/B ratio (**a**, r=−0.077).

muscle in patients with autonomic failure and concluded that the main site of ^{123}I-MIBG uptake in skeletal muscle is peripheral vessels[11]. These reports suggest that ^{123}I-MIBG scintigraphy of the extremities can be used for evaluating sympathetic function of peripheral vessels in skeletal muscle. Compared with MSNA, whole-body ^{123}I-MIBG scintigraphy is easier to perform and less invasive for quantitative evaluation of sympathetic terminal function. In the present study, we evaluated the sympathetic terminal function in heart and skeletal

muscles by analyzing ^{123}I-MIBG uptake in the whole body. The acquired indexes are thought to reflect the storage of norepinephrine in sympathetic nerve endings on myocardium and working muscles on the basis of previous reports[10,11].

We did not analyze the washout rate of systemic ^{123}I-MIBG. Although delayed ^{123}I-MIBG images of skeletal muscle have been examined by several investigators, the ^{123}I-MIBG washout rate in skeletal muscle has not been studied. Therefore, the clinical significance of the ^{123}I-MIBG washout rate in skeletal

muscle remains unknown. If the ^{123}I-MIBG washout rate of working muscle has a meaning similar to that in cardiac muscle[18,19], the interpretation of this index is controversial. Another report has indicated that the ^{123}I-MIBG washout rate in patients with heart failure does not reflect only sympathetic activity[20]. For these reasons, we did not evaluate the washout rate.

Norepinephrine at Rest

Plasma norepinephrine does not represent the total amount of released norepinephrine from sympathetic nerve endings, as it is influenced by other mechanisms, such as reuptake. This phenomenon concerning the regulation of circulating norepinephrine is termed "spillover". Although plasma norepinephrine represents about 1% of norepinephrine secreted from sympathetic nerve endings in healthy people, the release of norepinephrine from nerve endings increases with excitation of the sympathetic nervous system, resulting in an elevation in norepinephrine concentration. Therefore, plasma norepinephrine should reflect sympathetic nervous activity.

In patients with heart disease, however, plasma norepinephrine does not accurately reflect the status of sympathetic excitation. One reason is that the sympathetic terminal as an origin of norepinephrine is impaired by the pathogenesis associated with heart disease, such as oxidative stress. This phenomenon has been reported in patients with heart failure[21]. Examination of norepinephrine dynamics from the source to the spillover of norepinephrine, has shown that sympathetic nerves in patients with heart failure are supplied with a greater quantity of tyrosine and DOPA as raw materials for norepinephrine than are those in healthy subjects. However, the store of norepinephrine in sympathetic nerve endings is decreased in patients with heart failure because of the increased norepinephrine release from sympathetic nerve endings and the decreased efficacy of reuptake. This inability to maintain norepinephrine in sympathetic terminals leads to an increase in norepinephrine spillover to the blood. Consequently, it is understood that an increase in

plasma norepinephrine concentration in patients with heart failure both reflects the activity of sympathetic nerves and indicates sympathetic terminal dysfunction.

In the present study, norepinephrine concentration at rest did not show a significant relation to ^{123}I-MIBG uptake of either myocardium or skeletal muscle regardless of cardiac function. This finding shows that norepinephrine storage in sympathetic nerve endings, which is indicated by ^{123}I-MIBG, does not accurately reflect the dynamics of norepinephrine at rest. The reason is unclear. As a possible mechanism, however, we considered regulation by a factor such as sympathetic excitation rather than the norepinephrine store.

Norepinephrine during Exercise

Although it has been reported that plasma norepinephrine during exercise originates mainly in working muscle, that finding was based on results with healthy subjects[24]. According to an investigation of patients with heart failure, cardiac sympathetic activity is accelerated and plasma norepinephrine during exercise originates mainly in the heart[9]. Our results indicate that the plasma norepinephrine at peak exercise in the preserved LVEF group is related to ^{123}I-MIBG of myocardium but not to that of skeletal muscle. In the low LVEF group, however, plasma norepinephrine at peak exercise was shown to be related to ^{123}I-MIBG of the lower limb as a working muscle but not to that of myocardium. It can be considered that the circulating norepinephrine during intense exercise in the low LVEF group was dominated by that of working muscle, similar to findings in healthy subjects, although the main origin of plasma norepinephrine was the heart in the preserved LVEF group.

In the design of the present study, we felt that classifying subjects according to the LVEF would have the same meaning as classification by function of cardiac sympathetic terminals. In subjects with severely injured myocardium, sympathetic nerve endings should be greatly impaired and the plasma norepinephrine derived from the heart would be decreased. Although we did not recognize a

significant difference in myocardial ^{123}I-MIBG uptake between the low LVEF and preserved LVEF groups, the uptake of ^{123}I-MIBG to myocardium tended to be lower in the LVEF group. This finding supports the possibility that myocardial damage results in a decrease in norepinephrine release from the heart. Consequently, it is speculated that the origin of norepinephrine changes from the working muscle to myocardium, depending on the severity of heart failure. However, if the heart failure is further aggravated, the origin of norepinephrine is thought to return to skeletal muscle.

Conclusion

The present results indicated that plasma norepinephrine originates mainly in myocardium in patients with heart disease and preserved LVEF and originate in skeletal muscle in patients with low LVEF. This is the first clinical investigation of this topic. It is possible that further investigation into the origin of circulating norepinephrine will lead to a novel approach to the treatment of heart failure. The control of plasma concentration of norepinephrine in patients with heart failure[22,23] is thought to have the potential to improve prognosis. In the future, we intend to investigate treatments to modify plasma norepinephrine through medicines or exercise therapy.

Limitations

We are aware of two limitations in the present work. The first concerns the study population. We did not recruit healthy subjects as a control group. Therefore, we could not fully interpret the clinical significance of the correlation of ^{123}I-MIBG uptake with norepinephrine response to exercise. It is still unclear whether the origin of norepinephrine in healthy subjects is similar to that in patients with heart disease and preserved EF or in patients with low EF. However, it is difficult to get permission for injection of radioisotopes in healthy subjects. On the basis of a previous report[24], we can speculate that plasma norepinephrine during exercise in healthy people originates mainly from working muscle.

Therefore, it is thought that the plasma norepinephrine in patients with low LVEF is regulated by the norepinephrine released from sympathetic terminals in skeletal muscle as in normal subjects, although the norepinephrine at peak exercise originates from the sympathetically activated heart in patients with preserved EF.

The second limitation is that the ^{123}I-MIBG uptake was estimated at rest, but not during exercise. Therefore, we could not examine the relationship between plasma norepinephrine and ^{123}I-MIBG dynamics during exercise. However, information on the dynamics of ^{123}I-MIBG uptake during exercise may not be necessary to assess the norepinephrine response. It has been reported that[9] ^{123}I-MIBG uptake of the heart at rest correlates with values for norepinephrine from blood samples obtained from the coronary sinus during exercise. It has been suggested that impairment of the norepinephrine store of sympathetic nerve endings in the heart results in the limitation of increases in plasma norepinephrine in the coronary sinus. Thus, it can be thought that information on the dynamics of ^{123}I-MIBG during exercise is not needed to investigate the mechanism of norepinephrine response to exercise.

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