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Beneficial effects of γ-aminobutyric acid on right ventricular pressure and pulmonary vascular remodeling in experimental pulmonary hypertension

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ABSTRACT

Aims: It has been reported that activation of the sympathetic nervous system and increase in plasma norepinephrine (NE) levels are observed in patients with pulmonary hypertension (PH). γ-Aminobutyric acid (GABA) is one of the major inhibitory neurotransmitters in the central nervous system and suppresses peripheral sympathetic neurotransmission. This study investigated whether chronic treatment with GABA prevents the development of monocrotaline (MCT)-induced PH. To elucidate the relationship between the development of PH and sympathetic nerve activity, hemodynamic parameters, cardiac functions, and plasma NE concentrations as well as cardiac endothelin-1 (ET-1) contents of MCT-induced PH rats were evaluated with or without GABA treatment.
Main methods: Rats were injected with MCT (60 mg/kg) or saline subcutaneously and these rats were randomly divided into GABA (500 mg/kg/day for 4 weeks)- or vehicle-treated groups, respectively.
Key finding: MCT-treated rats had higher right ventricular systolic pressures, right ventricle-to-left ventricle plus septum weight ratios, pulmonary arterial medial thickening, and plasma NE levels than those of saline-injected rats. MCT-induced alternations were significantly attenuated by treatment with GABA. In MCT-induced PH rats with or without GABA treatment, plasma NE levels were positively correlated with right ventricular systolic pressure. Right ventricular endothelin-1 (ET-1) contents were increased by MCT injection, but these increments were not affected by treatment with GABA.
Significance: These results suggest that plasma NE levels play an important role in the development of MCT-induced PH in rats and that GABA exerts a preventive effect against MCT-induced PH by suppressing the sympathetic nervous system but not the cardiac ET-1 system.

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Introduction

Pulmonary hypertension (PH) is characterized by elevated pulmonary arterial pressure, pulmonary arterial remodeling, and right ventricular hypertrophy. Among the types of PH, idiopathic PH has an especially poor prognosis and is difficult to manage clinically (Simonneau et al., 2009). Multiple factors are involved in the pathogenesis of idiopathic PH. It has been reported that activation of the sympathetic nervous system and increases in plasma norepinephrine (NE) levels are observed in patients with PH and the experimental animal model of PH in rats (Ciarka et al., 2010; Bogaard et al., 2010). In animals, α/β-adrenergic receptor blockers, such as carvedilol and arotinolol, are known to prevent the development of monocrotaline (MCT)-induced PH (Usui et al., 2006; Ishikawa et al., 2009). Chemical sympathectomy has also been shown to reduce right ventricular hypertrophy induced by MCT in rats (Tucker et al., 1983). These findings suggest that the sympathetic nervous system plays an important role in the development of PH in both humans and animals. However, precise mechanisms underlying the contribution of the sympathetic nervous system and/or plasma NE in the pathogenesis of PH remain to be clarified.

γ-Aminobutyric acid (GABA) serves as a major inhibitory neurotransmitter within the central nervous system (Curtis and Johnston, 1974), and it is also found in peripheral tissues (Jessen et al., 1979). Treatment with GABA plays an important role in the modulation of cardiovascular functions (Gillis et al., 1980) by acting not only within the central nervous system but also within the peripheral tissues (Defeudis et al., 1981; Defeudis, 1982). In the paraventricular nucleus of the hypothalamus, $GABA_A$ and $GABA_B$ receptors are involved in tonic regulation of sympathetic outflow and modulate cardiac function (Wang et al., 2009). Moreover, GABA reportedly modulates vascular tone via suppression of NE release in the isolated rabbit ear

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artery, rat kidney, rat mesenteric arterial bed, and the pulmonary artery of cats through stimulation of prejunctional GABA receptors (Manzini et al., 1985; Fujimura et al., 1999; Hayakawa et al., 2002; Starke and Weitzell, 1980). These findings indicate that GABA inhibits peripheral sympathetic neurotransmission. Thus, GABA may prevent the development of PH by suppressing activation of the sympathetic nervous system.

The aim of the present study is to investigate whether GABA prevents the development of MCT-induced PH in rats. Moreover, the possible involvement of alterations in plasma NE levels and cardiac endothelin-1 (ET-1) contents in GABA's action was evaluated.

Materials and methods

Animals

Male Sprague–Dawley rats (220–250 g, 8 weeks old, Japan SLC, Shizuoka, Japan) were used in this study. Animals were housed in a light-controlled room with a 12-hour light/dark cycle and were allowed access to food and water ad libitum. Animals were maintained at the Departmental Animal Care Facility of Osaka University of Pharmaceutical Sciences in accordance with recommendations of the Declaration of Helsinki. Experimental protocols and animal care methods were approved by the Experimental Animal Research Committee of Osaka University of Pharmaceutical Sciences. Rats were randomized to MCT (60 mg/kg) or 0.9% saline subcutaneous injection and further separated to distilled water- or GABA (500 mg/kg/day for 4 weeks, given in drinking water)-treatment groups. This protocol resulted in the creation of four groups: saline-injected rats given distilled water (vehicle group), MCT-injected rats given distilled water (MCT group), saline-injected rats treated with oral GABA (vehicle + GABA group), and MCT-injected rats treated with oral GABA (MCT + GABA group). The dose and administration method of GABA were based on the previous studies (Sasaki et al., 2006, 2007).

Separately, time-course experiments using the MCT group and MCT + GABA group to examine time-dependent alterations of pathology were also performed.

Experimental protocol

Four weeks after injection of MCT or saline, each rat was artificially ventilated under anesthesia with sodium pentobarbital (40 mg/kg, i.p.). A polyethylene catheter, connected to a pressure transducer, was inserted into the right carotid artery to measure systolic arterial blood pressure and heart rate recorded by a polygraph system (RM 6000, Nihon Koden, Tokyo, Japan). Another polyethylene catheter was inserted into the right jugular vein to measure right ventricular systolic pressure. A 18-gauge needle was inserted into the abdominal aorta for blood sampling to determine plasma NE concentrations. The heart and lungs were excised, weighed, and used for morphometric analysis. A portion of the right ventricle was frozen separately to determine the ET-1 content.

In separate experiments, animals were sacrificed every week to examine the time-course changes of pathology.

Histological studies

Excised left lungs were processed for light microscopic observation according to standard procedures (Nishida et al., 2004a). Lungs were fixed in phosphate-buffered 10% formalin, chopped into small pieces, embedded in paraffin, cut into 3-μm slices, and stained using the Elastica-van-Gieson technique. Pulmonary arteries were identified as vessels with two clearly defined elastic laminae, with a layer of smooth muscle cells between the two laminae. The external diameter and medial wall thickness were measured for 15 to 20 muscular arteries (in size ranges of 50–100 and 100–150 μm in external diameter) per lung section. For each artery, the percent wall thickness was calculated using the following formula: percent wall thickness = (medial thickness × 2)/(external diameter) × 100. Wall thickness was determined using an image analyzer (AE-6905C, ATTO, Tokyo, Japan).

ET-1 measurement

ET-1 was extracted from the right ventricle, as described elsewhere (Fujita et al., 1995; Nishida et al., 2004b). Briefly, right ventricular tissue was homogenized in 4 ml of ice-cold organic solution (chloroform/methanol, 2:1, including 1 mM N-ethylmaleimide). Homogenates were left overnight and 0.09% trifluoroacetic acid was then added. Homogenates were centrifuged and the supernatant was stored. Aliquots of the supernatant were diluted 1/10 with 0.09% trifluoroacetic acid solution and applied to Sep-Pak C18 cartridges. Eluates were dried in a centrifugal concentrator, and the dried residue was reconstituted in assay buffer for radioimmunoassay. The clear solution was subjected to radioimmunoassay.

NE measurement

NE was extracted from plasma by the alumina absorption method, and NE concentration was measured by high-performance liquid chromatography with an amperometric detector (HTEC-500: Eicom, Kyoto, Japan), as previously reported (Hayashi et al., 1991).

Drugs

Monocrotaline and GABA were obtained from Sigma Chemicals (St. Louis, MO, USA). All other chemicals were purchased from Nacalai Tesque (Kyoto, Japan) and Wako Pure Chemical (Osaka, Japan).

Statistical analysis

Each value represents the mean ± S.E.M. For statistical analysis, we used a one-way analysis of variance followed by the Tukey–Kramer multiple comparisons. Differences were considered significant at $P<0.05$.

Results

Body, heart, and lung weight, and systemic hemodynamics

Results of the body, heart, lung weights and systemic hemodynamics 4 weeks after MCT treatment are shown in Table 1. MCT treatment did not affect systolic arterial pressure or heart rate. MCT-induced PH was evaluated by measuring right ventricular systolic pressure. MCT treatment produced significant increases in right ventricular systolic pressure ($P<0.01$). Daily administration of GABA for 4 weeks attenuated increases in right ventricular systolic pressure ($P<0.05$). There was no significant difference in right ventricular systolic pressure between the vehicle group and vehicle + GABA group. Body weight gain in the vehicle group was significantly greater than that in the MCT group.

MCT-induced right ventricular hypertrophy was evaluated by measuring right ventricle-to-left ventricle plus septum weight ratio. MCT induced an increase in the right ventricle-to-left ventricle plus septum weight ratio, whereas right ventricular hypertrophy was suppressed by administration of GABA for 4 weeks ($P<0.05$). There was no difference in the right ventricle-to-left ventricle plus septum weight ratio between the vehicle group and vehicle + GABA group. Lung weight-to-body weights were higher in animals who received an MCT injection than in vehicle-treated animals ($P<0.01$). The change in lung weight-to-body weight was not suppressed by GABA. MCT treatment did not affect left ventricle or septum weight-to-body weight.

Table 1
Comparative data on body, heart, and lung weights, hemodynamics, medial thickness, RV ET-1 content, and plasma norepinephrine levels.

	Vehicle		MCT	
Drug	(−)	GABA	(−)	GABA
BW (g)	385 ± 8**	393 ± 4	331 ± 4	329 ± 7
RV/BW (g/kg)	0.55 ± 0.03**	0.48 ± 0.05	0.90 ± 0.04	0.76 ± 0.02*
LV + S/BW (g/kg)	1.75 ± 0.03	1.97 ± 0.04	1.85 ± 0.02	1.95 ± 0.07
Heart/BW (g/kg)	2.44 ± 0.05**	2.54 ± 0.03	2.94 ± 0.07	2.91 ± 0.08
Lung/BW (g/kg)	3.90 ± 0.09**	4.06 ± 0.17	6.15 ± 0.25	6.16 ± 0.18
RV/LV + S	0.32 ± 0.01**	0.24 ± 0.03	0.49 ± 0.02	0.39 ± 0.01*
SBP (mm Hg)	128 ± 3	114 ± 7	116 ± 5	122 ± 5
HR (beat/min)	418 ± 15	359 ± 40	408 ± 14	350 ± 27
RVSP (mm Hg)	30 ± 2**	21 ± 4	57 ± 3	43 ± 3*
Medial thickness (%) <50–100 μm>	15 ± 2**	14 ± 1	30 ± 2	22 ± 1**
Medial thickness (%) <100–150 μm>	15 ± 1**	14 ± 2	25 ± 2	19 ± 1**
RV ET-1 content (ng/g tissue)	0.09 ± 0.01	0.06 ± 0.02	0.15 ± 0.03	0.14 ± 0.01
Plasma norepinephrine concentration (pg/ml)	176 ± 34**	175 ± 12	262 ± 24	131 ± 13**

MCT: monocrotaline, SBP: systolic blood pressure, HR: heart rate, RVSP, right ventricular systolic pressure, BW: body weight, RV: right ventricular, LV: left ventricular, S: septum. ET-1: endothelin-1, medial wall thickness (%) of small pulmonary arteries: external diameters 50–100 mm and 100–150 mm.
Values represent the mean ± S.E.M. (n = 6–8). *P<0.05, **P<0.01 compared with MCT + vehicle.

Lung vascular morphology, right ventricular ET-1 content, and plasma NE concentrations

The lung vascular morphology of MCT-treated rats revealed significantly larger medial thicknesses of pulmonary arteries, with diameters that ranged from 50 to 100 μm and 100 to 150 μm, than the lungs of vehicle-treated animals (Table 1). MCT-induced increases in the medial thickness of the pulmonary arteries were significantly suppressed by GABA treatment (P<0.01, respectively). There was no difference between vehicle groups and the vehicle + GABA group. Representative micrographs of each group were shown in Fig. 1. In addition, right ventricular ET-1 contents tended to be increased 4 weeks after MCT treatment, irrespective of GABA administration. On the other hand, plasma NE levels were significantly elevated by treatment with MCT, and this elevation was abolished by GABA administration (Table 1).

Time-course changes in right ventricular systolic pressure, right ventricular hypertrophy, pulmonary arterial medial thickening and plasma NE concentrations

Time-course changes in right ventricular systolic pressure and plasma NE concentrations are shown in Fig. 2. Right ventricular systolic pressure was gradually increased after the MCT injection, and these increments were markedly suppressed by daily treatment with GABA. Plasma NE levels also increased gradually after the MCT injection. These increases in plasma NE levels were completely suppressed by administration of GABA. Furthermore, similar time-course changes in right ventricular hypertrophy and pulmonary arterial medial thickening were observed (Figs. 3 and 4).

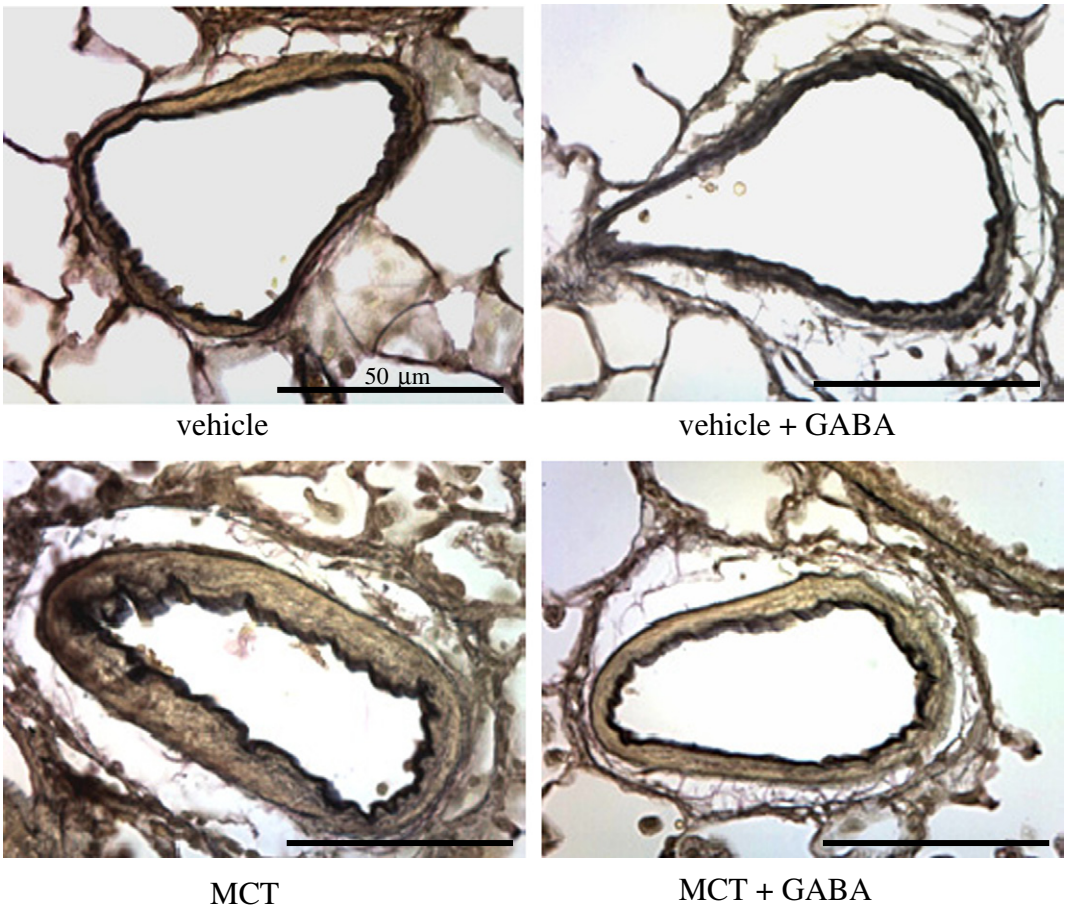

Fig. 1. Representative micrographs of small pulmonary arteries. MCT-induced increases in the medial thickness of the pulmonary artery were markedly suppressed by GABA treatment. There was no difference between vehicle group and vehicle + GABA group.

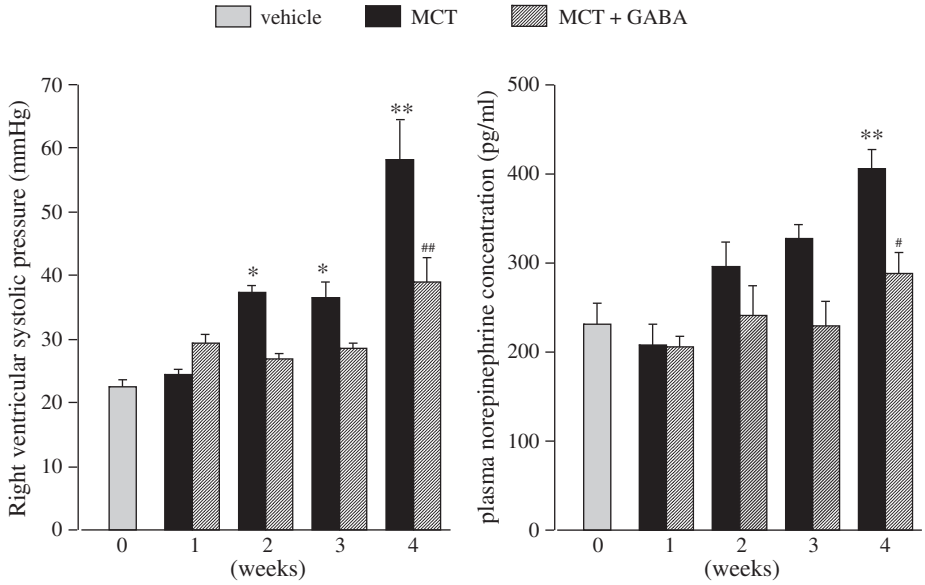

Fig. 2. Time-course changes in right ventricular systolic pressure (left panel) and plasma NE concentration (right panel). Each column and bar represent the mean ± S.E.M. (n = 5). *P<0.05, **P<0.01, compared with vehicle, and #P<0.05, ##P<0.01, compared with MCT at the same week.

In the MCT and MCT + GABA groups, there was a positive correlation between plasma NE concentrations and right ventricular systolic pressure ($r = 0.6541$, $y = 4.3986 + 123.71$, $P < 0.01$; Fig. 5), thereby suggesting that changes in plasma NE concentrations are closely related to the pathogenesis of MCT-induced PH.

Time-course changes in right ventricular ET-1 content

Time-course changes in right ventricular ET-1 content in the MCT group exhibited a gradual increase, compared with the vehicle group (MCT: 0.09 ± 0.02, 0.10 ± 0.02, 0.12 ± 0.01, 0.15 ± 0.03 ng/g tissue for 1–4 weeks, respectively vs. vehicle: 0.10 ± 0.01), although it is statistically not significant. These increases in right ventricular ET-1 content were not suppressed by daily administration of GABA (0.09 ± 0.01, 0.11 ± 0.01, 0.13 ± 0.01, 0.14 ± 0.01 ng/g tissue for 1–4 weeks, respectively) (Fig. 6).

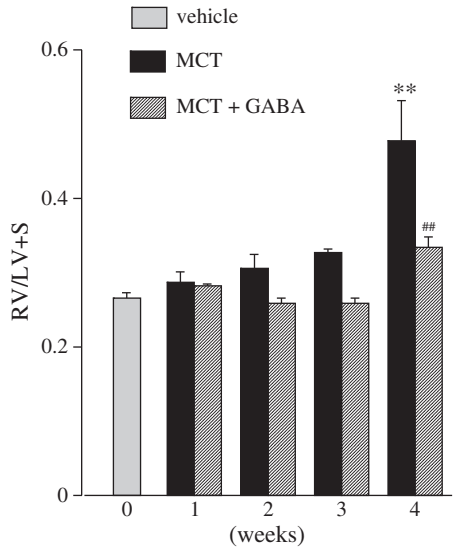

Fig. 3. Time-course changes in the right ventricle-to-left ventricle plus septum weight ratio. Each column and bar represent the mean ± S.E.M. (n = 5). **P<0.01, compared with vehicle, and ##P<0.01, compared with MCT at the same week.

Discussion

Multiple factors, such as vasoconstriction, remodeling of pulmonary vessels, and thrombosis are involved in the pathogenesis of PH (Simonneau et al., 2009). Sympathetic nerve activity is also one of the important causal factors of development of PH (Usui et al., 2006; Ishikawa et al., 2009; Tucker et al., 1983). In an animal model of PH, the activity of the sympathetic nervous system is markedly increased and neurohumoral derangements participate in excessive muscularization and fibrosis of the pulmonary artery (Faber et al., 2007).

GABA is one of the major inhibitory neurotransmitters in the central nervous system. Intracerebroventricular injection of GABA modulated cardiovascular functions in chronic heart failure via suppression of sympathetic nerve activity (Wang et al., 2009). Moreover, systemically administered GABA could reduce blood pressure in both spontaneously hypertensive rats and human subjects by inhibiting NE release from sympathetic nerve fibers and by decreasing peripheral sympathetic nerve activity (Kimura et al., 2002; Hayakawa et al., 2004; Yamakoshi et al., 2007; Li and Pan, 2010). However, it is unclear whether chronic treatment with GABA prevents the development of MCT-induced PH.

In the present study, we demonstrated that GABA treatment suppressed MCT-induced right ventricular hypertrophy, PH, pulmonary arterial hypertrophy, and elevations in plasma NE levels, thereby indicating that GABA effectively prevented the development of MCT-induced PH. Moreover, it has been reported that increases in plasma NE levels are observed in PH patients and animal models (Ciarka et al., 2010; Bogaard et al., 2010). The neuronal reuptake of NE was impaired in MCT-induced PH (Kimura et al., 2007), and continuous administration of NE promoted cardiac hypertrophy and augmented the decreased cardiac function in congestive heart failure rats (Kimura et al., 2010), indicating that the spillover of NE by increasing sympathetic nerve activity aggravates cardiac hypertrophy and cardiac dysfunction. Thus, we investigated time-course changes of MCT-treated rats to elucidate the relationship between plasma NE levels and development of PH. MCT-treated rats showed time-dependent increases in right ventricular systolic pressure, right ventricular hypertrophy, pulmonary arterial medial thickening, right ventricular ET-1 content, and plasma NE levels. On the other hand, GABA treatment markedly suppressed elevated plasma NE levels and pulmonary hypertensive lesions in

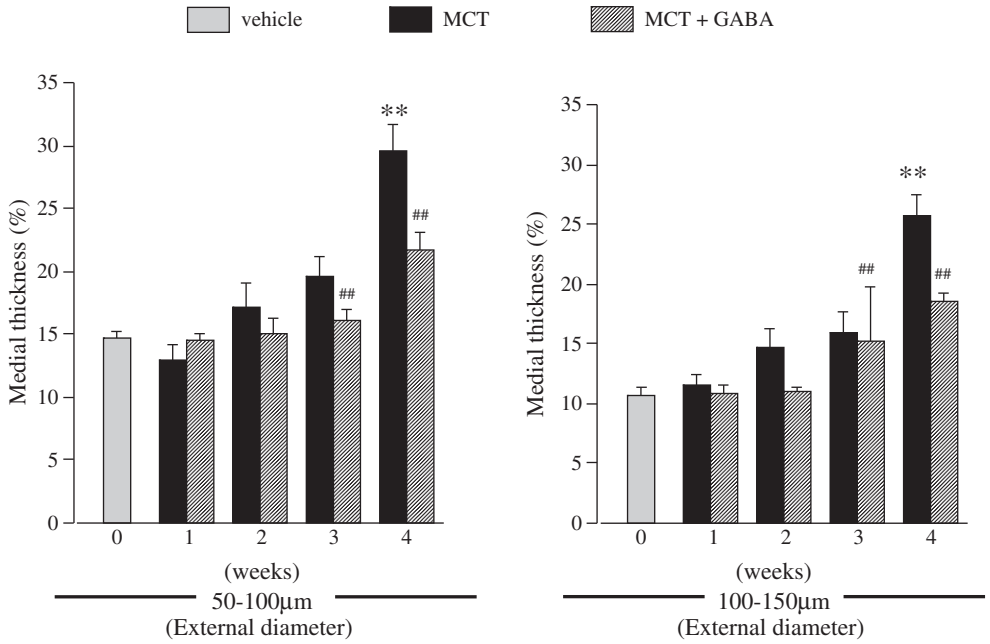

Fig. 4. Time-course changes in the medial wall thicknesses of pulmonary arteries, with diameters that ranged from 50 to 100 μm and 100 to 150 μm. Each column and bar represent the mean ± S.E.M. (n = 5). **P<0.01, compared with vehicle, ##P<0.01, compared with MCT at the same week.

MCT-induced PH rats. Although the elevated right ventricular ET-1 content was not decreased by GABA treatment, plasma NE levels positively correlated with right ventricular systolic pressure, suggesting that alterations in plasma NE concentrations play an important role in the development of MCT-induced PH in rats and cardioprotective effects of GABA.

The blood–brain barrier is impermeable to GABA (Kuriyama and Sze, 1971), and intravenous or intraperitoneal administration of GABA is due to its actions within the peripheral tissues. It has been reported that GABA is able to modulate vascular tone by suppressing NE release in the isolated rabbit ear artery and rat kidney (Manzini et al., 1985; Monasterolo et al., 1996; Fujimura et al., 1999). GABA has antihypertensive effects due to its inhibition of NE release from sympathetic nerves via presynaptic $GABA_B$ receptors (Hayakawa et al., 2002). Recently, we found that intravenous injection of GABA prevented the development of ischemia/reperfusion-induced acute kidney injury by suppressing enhanced renal sympathetic nerve activity during ischemia and increased NE overflow from renal sympathetic nerve endings (Kobuchi et al., 2009). Interestingly, these actions were abolished by intracerebroventricular injection of a $GABA_B$ receptor antagonist (Kobuchi et al., 2011). It has been reported that the concentration of GABA in the cerebrospinal fluid is increased by intravenous infusion of GABA (Awadi et al., 2006). Taken together, it seems likely that peripherally-administered GABA exerts its action through not only the decreasing effect on NE release from sympathetic nerve endings but also the suppression of central sympathetic outflow. However, at present, the precise mechanisms, sites, and receptor subtypes underlying the GABA-induced decreasing effect on plasma NE concentrations of MCT rats cannot be determined.

ET-1 plays an important role in the progression of PH (Michel et al., 2003), and both selective ET_A and nonselective ET_A/ET_B receptor antagonists are currently available for the treatment of PH patients. In MCT-treated rat PH models, ET-1 mRNA expression and ET-1

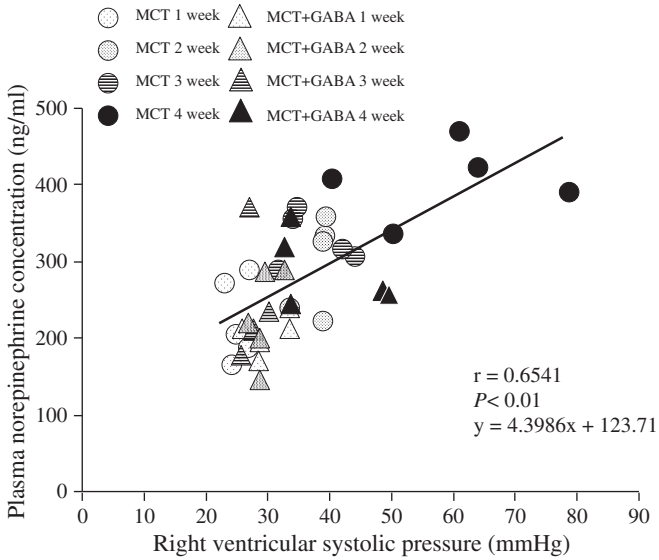

Fig. 5. Correlation between plasma NE concentration and right ventricular systolic pressure.

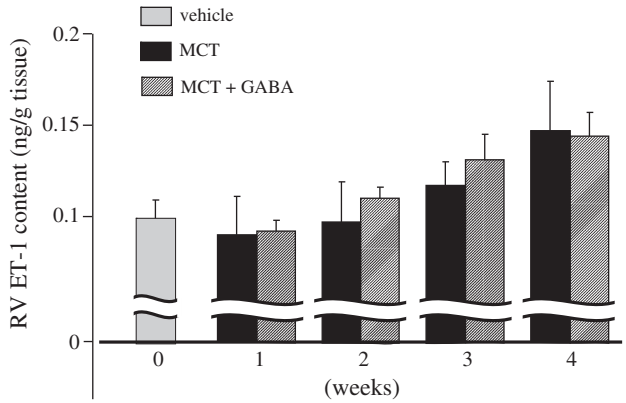

Fig. 6. Time-course changes in the right ventricular ET-1 content. Each column and bar represent the mean ± S.E.M. (n = 5).

peptide levels are elevated in the right ventricle (Giaid et al., 1993; Miyauchi et al., 1993; Nishida et al., 2004b). It has been reported that the preventing effects of nonselective ET_A/ET_B receptor antagonists against the development of MCT-induced PH are accompanied by decreases in sympathetic nerve activity (Uchino et al., 2008) and plasma NE levels (Clozel et al., 2006). Although there is no direct evidence of the relationship between the ET-1 system and sympathetic nervous system in the pathogenesis of PH, ET receptor antagonists may improve the progression of PH by suppressing the ET-1-enhanced sympathetic nervous system. However, in the present study, it was noted that GABA treatment suppressed MCT-induced elevations in plasma NE levels in PH rats without affecting right ventricular ET-1 contents, suggesting the absence of a direct relationship between the ET-1 system and sympathetic nervous system in the pathogenesis of MCT-induced PH. Further studies are needed to clarify the precise mechanisms underlying the beneficial effect of GABA against MCT-induced PH in rats.

Conclusion

Chronic treatment with GABA exerts a preventative effect on the development of MCT-induced PH, pulmonary arterial remodeling, and right ventricular hypertrophy, at least in part, via the suppression of the sympathetic nervous system but not the cardiac ET-1 system. However, we must keep in mind that this study is just a basic and preliminary research and that further investigations are required to apply these findings in a clinical setting.

Conflict of interest statement

The authors declare no conflict of interest.

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