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Clonidine, moxonidine, folic acid, and mecobalamin improve baroreflex function in stroke-prone, spontaneously hypertensive rats

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Key words

clonidine; moxonidine; folic acid; mecobalamin; arterial baroreflex; stroke-prone spontaneously hypertensive rats

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Abstract

Aim: To investigate the effect of clonidine, moxonidine, folic acid, and mecobalamin on arterial baroreflex (ABR) function in stroke-prone spontaneously hypertensive rats (SHR-SP) and the possible mechanisms involved. **Methods:** Eighty-one SHR-SP were divided into 7 groups. Four groups were designated for the intragastric (ig) administration of clonidine (1.0 and 10.0 μg/kg), moxonidine (0.1 and 1.0 mg/kg), folic acid (1.0 mg/kg), and mecobalamin (1.0 mg/kg). Three groups were for the intracerebroventricular (icv) injection of clonidine (4 μg/4 μL), moxonidine (5 μg/4 μL), and mecobalamin (20 μg/4 μL). Blood pressure (BP) was recorded in the conscious state for 30 min and baroreflex sensitivity (BRS) was determined respectively before and after drug administration. **Results:** Clonidine and moxonidine significantly decreased BP, prolonged the heart period (HP), and increased BRS when administered as either ig or icv injections. Both BP and HP were unchanged by ig folic acid or mecobalamin injection. However, BRS was significantly increased by both. **Conclusion:** Clonidine, moxonidine, folic acid, and mecobalamin improved impaired ABR function in SHR-SP. The central mechanism was involved in this effect of either clonidine or moxonidine. Mecobalamin improved ABR function through the peripheral mechanism.

Introduction

Arterial baroreflex (ABR) is one of the most important regulatory mechanisms in the cardiovascular system. Baroreflex function, expressed as baroreflex sensitivity (BRS), is an important determinant for many cardiovascular diseases. Clinically, it has been proven that ABR dysfunction is associated with sudden death in patients with acute myocardial infarction and mortality in patients with congestive heart failure[1-4]. Our previous works demonstrated that ABR function was closely related to atheroscleosis and end-organ damage in hypertension[5,6]. Furthermore, ABR function also plays an important role in the onset and prognosis of stroke. There is established evidence of abnormal ABR function in both animal models of stroke[7,8] and patients with acute stroke[9-11]. Recently, a study within our department indicated that ABR function affected the survival time of stroke-prone spontaneously-hypertensive rats (SHR-SP). The study also found that restoring impaired ABR function could prevent stroke in hypertension, suggesting that BRS is a new and important predictor for stroke incidence and that the restoration of ABR function is a new target for the prevention of stroke[12]. Since ABR function is associated closely with stroke, it is of great clinical importance to discover drugs with the ability to improve impaired ABR function.

Clonidine and moxonidine, representing the first and second generation of antihypertensive drugs, act on the central nervous system. It was demonstrated that clonidine improved ABR function in conscious mice through parasympathetic activation[13]. However, little is known about the effect of moxonidine. Both folic acid (vitamin B_9) and mecobalamin (methyl-vitamin B_{12}) belong to the vitamin B group. During the past decade, interest in the health benefits of these has increased considerably. Recent interest of

B vitamin focused on their beneficial effects on preventing cardiovascular diseases. Clinical studies indicated that folic acid improved ABR function in hypertensive patients[14]. In many cases, folic acid involved biochemical processes that required the participation of vitamin B_{12}[15]. Therefore, we postulated that mecobalamin might improve ABR function in conscious SHR-SP.

This study was designed to examine the effects of clonidine, moxonidine, folic acid, and mecobalamin on impaired ABR function in SHR-SP and the possible mechanisms involved.

Materials and methods

Animals and drugs Female SHR-SP rats, aged 20–24 weeks, were provided by the Animal Center of the Second Military Medical University (Shanghai, China). The rats were housed under controlled temperatures (23–25 °C) and lighting (8:00–20:00 light, 20:00–8:00 dark) with free access to food and tap water. All the animals used in the experiment received humane care in compliance with institutional guidelines for health and care of experimental animals.

Clonidine (Sigma Chemical Co, St Louis, MO, USA), moxonidine (Yahu Pharmaceutical Co, Shanghai, China), folic acid (Fushu Pharmaceutical Co, Shanghai, China), mecobalamin (Shenyang Wanlong Pharmaceutical Co, Shenyang, China). All were dissolved with sterile saline and stored at 0–4 °C.

Blood pressure and heart period measurement Systolic blood pressure (SBP) and diastolic blood pressure (DBP), and heart period (HP) were continuously recorded using a previously described technique[16,17]. Briefly, the rats were anesthetized with a combination of ketamine (50 mg/kg, ip) and diazepam (5 mg/kg, ip). A floating polyethylene catheter was inserted into the lower abdominal aorta via the left femoral artery for blood pressure (BP) measurement. Another catheter was placed into the left femoral vein for BRS measurement. A stomach catheter was inserted when intragastric (ig) administration was needed or a stainless steel cannula (0.7 mm OD) was stereotaxically (1.0 mm posterior to bregma, 1.6 mm lateral from the midline, 4.0 mm below the surface of the skull) implanted into the lateral cerebral ventricle for intracerebroventricular (icv) injection. All the catheters were exteriorized through the interscapular skin. After a 2 d recovery period, the animals were placed in individual cylindrical cages containing food and water for BP recording. The aortic catheter was connected to a BP transducer via a rotating swivel that allowed the animals to move freely in the cage. After about 4 h habituation, the BP signal was digitized by a microcomputer and beat-to-beat SBP, DBP, and HP values were determined online. The mean values during a period of 30 min were calculated and served as the SBP, DBP, and HP.

Baroreflex sensitivity measurement BRS was determined using a previously described method[18]. The principle of this method was to measure the prolongation of HP in response to an elevation of BP. Briefly, a bolus injection of phenylephrine (2–5 mg/kg) was used to induce an elevation of SBP between 20–40 mmHg. HP was plotted against SBP for a linear regression analysis and the slope of SBP–HP was expressed as BRS (ms/mmHg). The mean value of 2 measurements with the proper dose served as the final result.

Experimental protocol Eighty one SHR-SP were randomly divided into 7 groups. Four groups were designated for the ig administration of clonidine (1.0 and 10.0 μg/kg), moxonidine (0.1 and 1.0 mg/kg), folic acid (1.0 mg/kg), and mecobalamin (1.0 mg/kg). Three groups were used for the icv injection of clonidine (4 μg/4 μL), moxonidine (5 μg/4 μL), and mecobalamin (20 μg/4 μL). After 4 h habituation, basal (predrug) BP was recorded continuously for 30 min and BRS was determined. Thereafter, a single dose of drug was given via an ig catheter or stainless steel cannula as designated. One hour (ig moxonidine) or 30 min (the other drugs) later, the BP was recorded for another 30 min and BRS was determined again. The mean values of SBP, DBP, HP and BRS served as post-drug values. For the ig administration of clonidine or moxonidine, 2 doses were conducted in 1 rat, that is, the lower dose was administrated and post-drug values were calculated; 30 min later, the higher dose was administered and the same protocol was performed.

Statistical analysis All data are expressed as mean±SEM. Statistical analysis was performed with Student's paired *t*-test. $P<0.05$ was considered statistically significant.

Results

Effects of clonidine on BP, HP, and BRS in SHR-SP Figure 1 illustrates the effects of clonidine (ig) on BP, HP, and BRS in SHR-SP. SBP and DBP were significantly decreased (–16±3 mmHg and –12±3 mmHg) and HP was prolonged dramatically by 10.0 μg/kg clonidine. DBP was significantly decreased after 1.0 μg/kg clonidine while SBP and HP were not obviously affected. BRS was enhanced markedly from 0.30±0.03 ms/mmHg to 0.43±0.04 ms/mmHg by 1.0 μg/kg clonidine and from 0.30±0.03 ms/mmHg to 0.53±0.09 ms/mmHg by 10.0 μg/kg clonidine.

Clonidine (4 μg, icv; Figure 2) significantly decreased SBP and DBP (–21±3 mmHg and –15±4 mmHg), but HP was

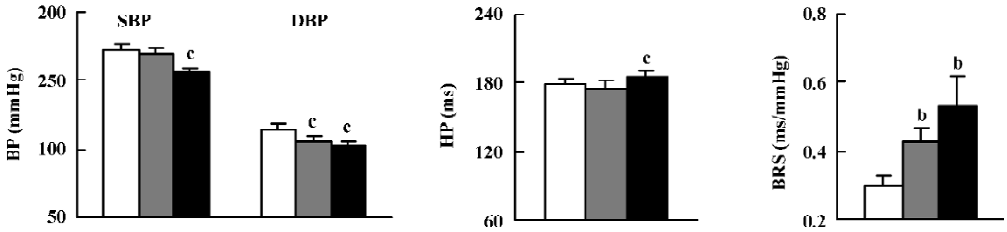

Figure 1. Effects of a single dose of clonidine (ig) on blood pressure (BP), heart period (HP) and baroreflex sensitivity (BRS) in SHR-SP. White colume, pre-clonidine; Gray colume, clonidine 1.0 μg/kg; Black colume, clonidine 10.0 μg/kg. SBP, systolic blood pressure; DBP, diastolic blood pressure. n=9. Mean±SEM. $^{b}P<0.05$, $^{c}P<0.01$ compared with pre-clonidine.

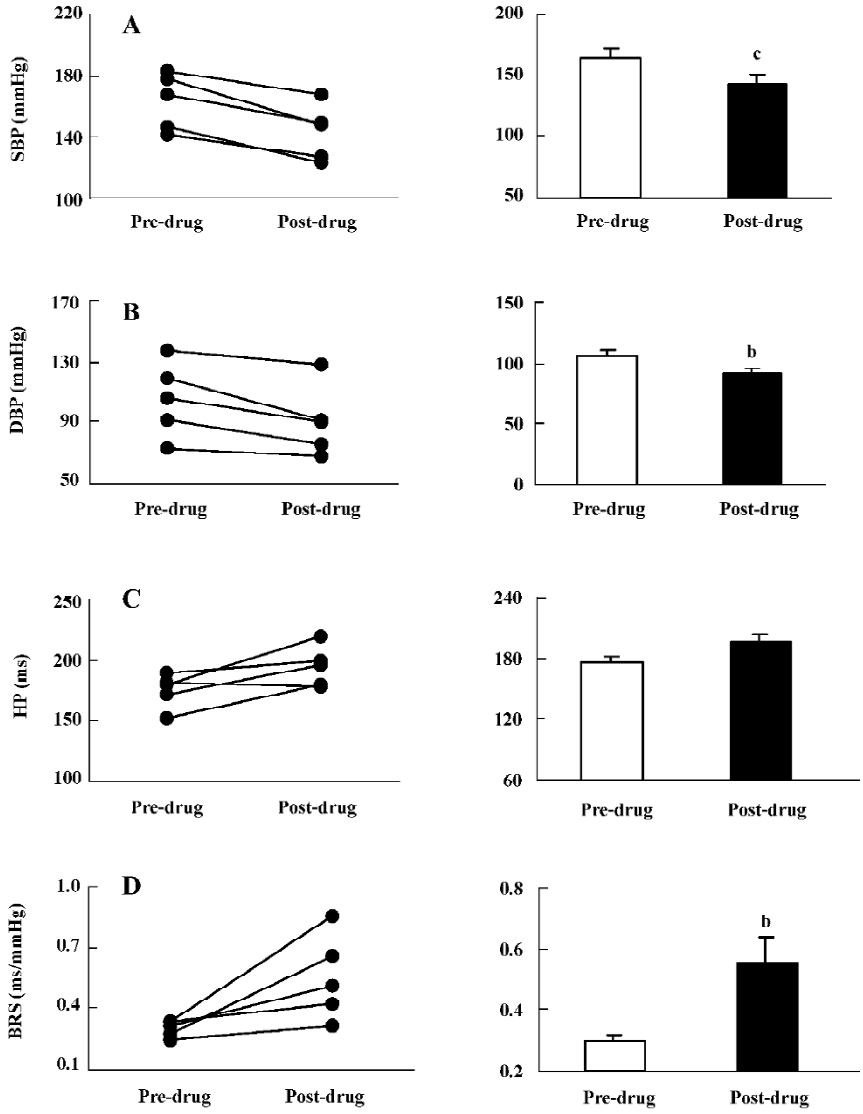

Figure 2. Effects of a single dose of clonidine (icv) on blood pressure (BP, A and B), heart period (HP, C), and baroreflex sensitivity (BRS, D) in SHR-SP. White colume, preclonidine; Black colume, 4 μg/rat clonidine. n=9. Mean±SEM. $^{b}P<0.05$, $^{c}P<0.01$ compared with preclonidine.

not significantly prolonged. BRS markedly increased from 0.30±0.02 ms/mmHg to 0.55±0.09 ms/mmHg.

Effects of moxonidine on BP, HP and BRS in SHR-SP As shown in Figure 3, both SBP and DBP were significantly

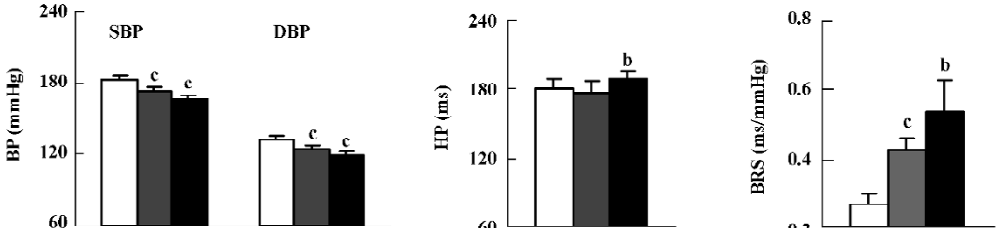

Figure 3. Effects of a single dose of moxonidine (ig) on blood pressure (BP), heart period (HP), and baroreflex sensitivity (BRS) in SHR-SP. White colume, premoxonidine; Gray colume, 0.1 mg/kg moxonidine; Black colume, 1.0 mg/kg moxonidine. n=13. Mean±SEM. ^{b}P<0.05, ^{c}P< 0.01 compared with premoxonidine.

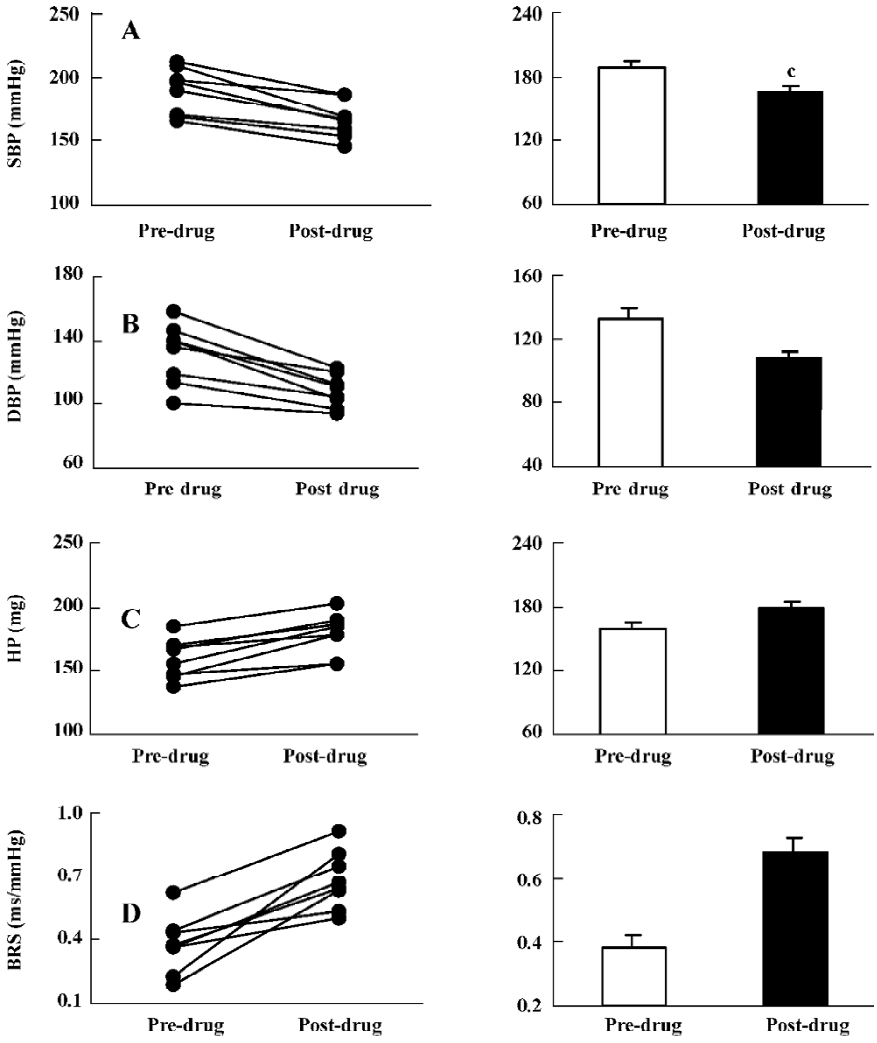

Figure 4. Effects of a single dose of moxonidine (icv) on blood pressure (BP, A and B),heart period (HP, C), and baroreflex sensitivity (BRS, D) in SHR-SP. White colume, premoxonidine; Black colume, 5 μg/rat moxonidine. n=8. Mean±SEM. ^{c}P<0.01 compared with premoxonidine.

decreased by 2 doses of moxonidine (ig). The reduction in SBP was 10±2 mmHg by 0.1 mg/kg and 17±3 mmHg by 1.0 mg/kg moxonidine. HP was significantly prolonged by 1.0 mg/kg moxonidine. BRS was significantly improved (0.27±0.03 *vs* 0.43±0.03 ms/mmHg and 0.27±0.03 *vs* 0.54±0.09 ms/mmHg) by 0.1 and 1.0 mg/kg moxonidine (ig).

The effects of moxonidine (icv) are shown in Figure 4. Moxonidine (5 μg) significantly decreased SBP (189±7 *vs* 166±5 mmHg) and DBP (132±7 *vs* 108±4 mmHg). HP was markedly prolonged. BRS was significantly enhanced from 0.38±0.04 ms/mmHg to 0.68±0.05 mmHg. These results indicated that clonidine and moxonidine improved ABR function through central mechanisms.

Effects of mecobalamin on BP, HP, and BRS in SHR-SP

Figure 5 shows that 1.0 mg/kg mecobalamin (ig) did not cause changes in SBP, DBP, and HP. BRS was increased significantly (0.33±0.02 *vs* 0.44±0.04 ms/mmHg). Similar to the ig administration, the icv administration of 20 μg mecobalamin (Figure 6) did not affect SBP, DBP, and HP. However, BRS was not changed by icv injection. These results revealed that mecobalamin increased BRS through peripheral mechanisms.

Effects of folic acid on BP, HP, and BRS in SHR-SP

Similar to the ig administration of mecobalamin, 1.0 mg/kg of ig-administered folic acid (Figure 7) increased BRS without influence on the SBP, DBP, and HP levels. Icv injec-

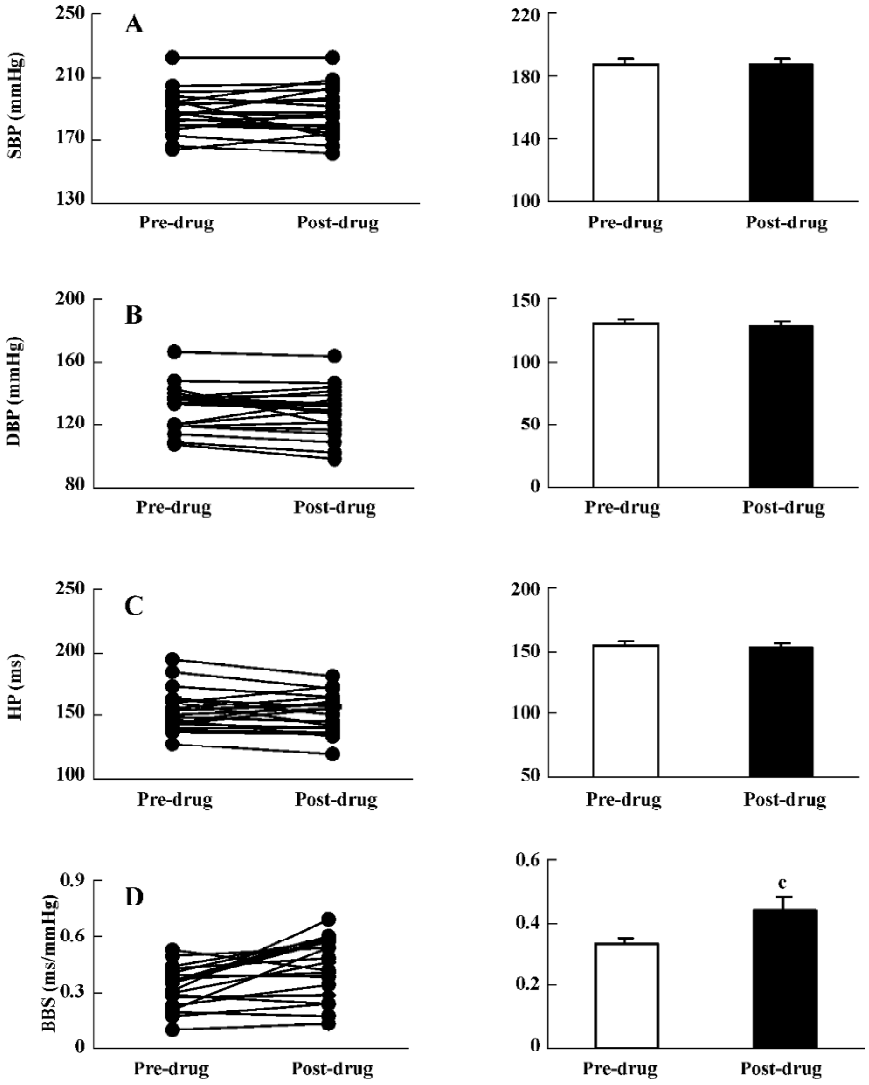

Figure 5. Effects of a single dose of mecobalamin (ig) on blood pressure (BP, A and B),heart period (HP, C), and baroreflex sensitivity (BRS, D) in SHR-SP. White colume, premecobalamin; Black colume, 1.0 mg/kg mecobalamin. n=20. Mean±SEM. $^cP<0.01$ compared with premecobalamin.

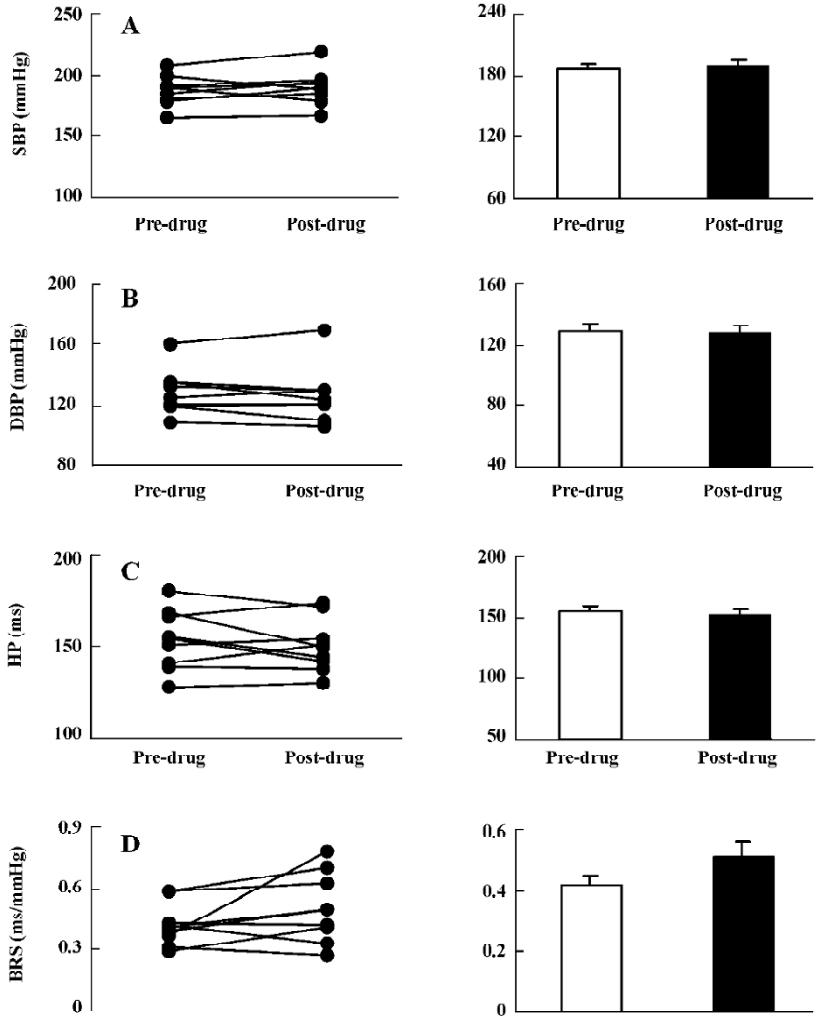

Figure 6. Effects of a single dose of mecobalamin (icv) on on blood pressure (BP, A and B), heart period (HP, C), and baroreflex sensitivity (BRS, D) in SHR-SP. White colume, premecobalamin; Black colume, 20 μg/rat mecobalamin. n=9. Mean±SEM.

tion was not conducted because folic acid could not reach the concentration needed in this study.

Discussion

The present study clearly demonstrated that clonidine, moxonidine, folic acid, and mecobalamin all improved the damaged ABR function in SHR-SP, whether or not the BP levels were changed or unchanged. It is well known that ABR function, damaged in many cardiovascular diseases, including myocardial infarction, heart failure, atherosclerosis, diabetes, and end-organ damage in hypertension, plays a key role in the regulation of cardiovascular acti-vities. Since the end of 1980s, the pathological importance of ABR function has attracted the attention of many investigators. Nowadays, more and more studies have demonstrated that ABR function is closely associated with stroke. Robinson *et al* reported a significant reduction in cardiac BRS after acute stroke in patients[10]. Thereafter, they reported that post-stroke patients with impaired BRS values (≤5.0 ms/mmHg) had a significantly poorer prognosis than patients without impaired BRS (>5.0 ms/mmHg). Based on these results, they suggested therapeutic strategies for stroke by increasing BRS activity with drugs[11].

Given the clinical importance of BRS in stroke therapy, we believe it is necessary to find drugs that are effective in

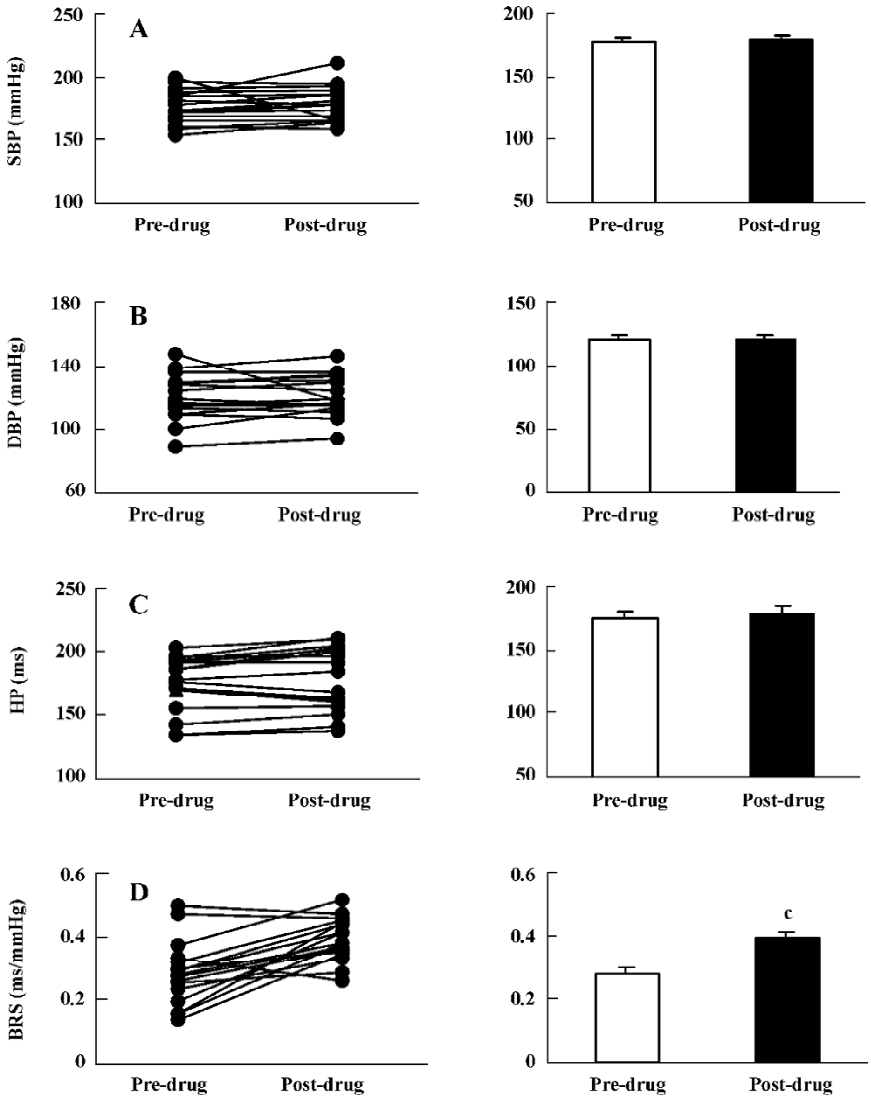

Figure 7. Effects of a single dose of folic acid (ig) on on blood pressure (BP, A and B), heart period (HP, C), and baroreflex sensitivity (BRS, D) in SHR-SP. White colume, prefolic acid; Black colume, 1.0 mg/kg folic acid. n=17. Mean±SEM. ^cP<0.01 compared with prefolic acid.

restoring damaged ABR function in stroke patients. The application of these drugs for the prevention of stroke incidence may be a new strategy in stroke therapy. In this study, SHR-SP were used. It was reported that SHR-SP had damaged ABR function compared with normotensive Wistar–Kyoto rats[19]. At the same time, SHR-SP are a useful experimental model for examining the pathogenesis of stroke as well as their treatment because the cerebrovascular lesions in these animals are similar to that in humans[20].

Clonidine, a centrally–acting, antihypertensive drug, is an agonist for both I_1-imidazoline receptors and α_2-adrenoceptors. Tank *et al* reported that clonidine improved spontaneous BRS in conscious mice[13]. In this study, we found that clonidine enhanced BRS significantly after ig administration in SHR-SP. To determine the possible mechanism involved in this effect, icv-administered clonidine was conducted. The results indicated that icv-administered clonidine also improved BRS significantly in SHR-SP. These results suggested that the BRS-improving effect induced by clonidine was mediated through the central mechanism. Furthermore, 1.0 μg/kg clonidine did not decrease SBP, but increased BRS significantly, indicating that this BRS-improving effect was not secondary to a decrease in BP.

Moxonidine, an agent of the second generation, antihy-

pertensive drugs acting on the central nervous system, mediates hypotensive effects by activating central I_1-imidazoline receptors and subsequently decreasing sympathetic nerve activity[21]. In the present study, the effect of moxonidine on ABR function in conscious SHR-SP was tested. Our study was the first to find that ig–administered moxonidine significantly increased BRS in SHR-SP. Furthermore, icv-administered moxonidine was performed and the results indicated that BRS was also enhanced significantly, demonstrating that the central mechanism was involved in the BRS-improving effect of moxonidine. However, both 0.1 and 1.0 mg/kg moxonidine decreased SBP and increased BRS significantly, so it is unclear whether this effect was dependent on the decrease of blood pressure or not. Further studies are needed to testify this mechanism.

Folic acid was considered to have potential protection against cardiovascular diseases due to its homocysteine-lowering effect[22]. It was also suggested that folate might have a direct antioxidant role *in vivo*, which was independent of any indirect effects through the lowering of homocysteine levels[23]. Abundant attention has been focused on folic acid with its role in the prevention of cardiovascular disease[24,25]. Bechir *et al*[14] proved that folic acid improved *i*mpaired BRS in hypertensive patients. They speculated that the positive effects of folic acid seemed to be mediated by a reduction of oxidative stress because oxidative stress directly interfered with nerve endings of baroneurons in the arterial wall to damage ABR function[26]. They also found that folic acid had antioxidative properties[27]. In the present study, we examined effect of folic acid on ABR function in SHR-SP. It was found that BRS was significantly enhanced after ig administration of folic acid under the condition that both BP and HP were unchanged. The BRS-improving effect of folic acid in SHR-SP might be conducted through its antioxidative properties. As well as this, folic acid was reported to enhance endothelial function and increase the production of nitric oxide (NO)[28,29]; NO played an important role in the regulation of ABR function[30]. This might be another contribution to BRS improvement induced by folic acid. In this study, an icv injection was not conducted because folic acid could not reach the concentration needed in this study. Therefore, it was unclear whether the central mechanism was involved in this BRS-improving effect and further studies are needed.

Mecobalamin is one of the active analogs of vitamin B_{12}. It is the essential cofactor for methionine synthase. Deficiency in folic acid and vitamin B_{12} leads to the elevation of the plasma homocysteine level, which is considered an independent risk factor in the pathogenesis of atherosclerosis[31], acute myocardial infarction[32], stroke[33], and hypertension[34]. All of these diseases were characterized by poor ABR function. We speculated that vitamin B_{12} might affect BRS. In the present study, the effect of vitamin B_{12} on ABR function was examined in SHR-SP. It was found that ig-administered mecobalamin increased BRS markedly, but icv-administered mecobalamin did not. These results suggested that the central mechanism was not involved in this BRS-improving effect. The exact mechanism of this effect by vitamin B_{12} is still unclear. Visontai *et al* reported a negative correlation between the homocysteine concentration and BRS in exfoliation syndrome or exfoliation glaucoma patients[35]. At the same time, folic acid and vitamin B_{12} decreased blood plasma homocysteine[36]. So we postulated that the reduction of the homocysteine concentration might contribute to the BRS-improving effect of folic acid and mecobalamin.

In conclusion, this study is the first to directly demonstrate that clonidine, moxonidine, folic acid, and mecobalamin all improve impaired BRS in SHR-SP. The central mechanism was involved in this effect of either clonidine or moxonidine and mecobalamin improved ABR function through the peripheral mechanism.

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