Sympathetic hyper-reactivity to air-jet stress in the chromosome 1 blood pressure quantitative trait locus congenic rats

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Short title: Sympathetic response to air-jet stress in congenic rats

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Abstract

A chromosome 1 blood pressure quantitative trait locus (QTL) was introgressed from the stroke-prone spontaneously hypertensive rats (SHRSP) to Wistar-Kyoto (WKY) rats. This congenic strain (WKYpch1.0) showed an exaggerated pressor response to both restraint and cold stress. In this study, we evaluated cardiovascular and sympathetic response to an air-jet stress and also examined the role of the brain renin-angiotensin system (RAS) in the stress response of WKYpch1.0. We measured mean arterial pressure (MAP), heart rate (HR) and renal sympathetic nerve activity (RSNA) responses to air-jet stress in WKYpch1.0, WKY and SHRSP. We also examined effects of intracerebroventricular (ICV) administration of candesartan, an angiotensin II type 1 receptor blocker, on MAP and HR responses to air-jet stress. Baseline MAP in the WKYpch1.0 and WKY were comparable, while it was lower than that in SHRSP. Baseline HR did not differ among the strains. In WKYpch1.0, air-jet stress caused greater increase in MAP and RSNA than in WKY. The increase in RSNA was as large as that in SHRSP while the increase in MAP was smaller than in SHRSP. ICV injection of a non-depressor dose of candesartan inhibited the stress-induced pressor response to a greater extent in WKYpch1.0 than in WKY. Intravenous injection of phenylephrine caused a presser effect comparable between WKYpch1.0 and WKY. These results suggest that the chromosome 1 blood pressure QTL congenic rat has a sympathetic hyper-reactivity to an air-jet stress, which causes exaggerated pressor responses. The exaggerated response is at least partly mediated by a brain RAS.
Key words

Blood pressure, renal sympathetic nerve activity, Wistar-Kyoto rat, stroke-prone spontaneously hypertensive rats, brain renin-angiotensin system
INTRODUCTION

Hypertension is a multifactorial disease, resulting from the interaction of a number of genetic and environmental factors (8). On the way of identifying genes contributing to the pathogenesis of hypertension, genome-wide linkage analyses of inbred rat models of hypertension have identified multiple quantitative trait loci (QTLs) for blood pressure (10,24). The construction of congenic strains for the QTLs is an essential step to identify genes responsible both for hypertension per se and for ‘intermediate phenotypes’ of hypertension (7,23). One important intermediate phenotype associated with hypertension is sympathetic hyperactivity. The development of hypertension in humans (6) and various animal models (17) was associated with increase in sympathetic nerve activity.

Hypertensive human and animals with sympathetic hyperactivity exhibit enhanced pressor and sympathetic responses to different types of stress (15,20,30,32). Air-jet stress is an environmental stress thought to be a pure psychoemotional stress and causes the characteristic pattern of the classic defense reaction with an increase in mean arterial pressure (MAP), heart rate (HR), renal sympathetic nerve activity (RSNA) and a decrease in urinary sodium excretion (9,12). In acute response to this stress, spontaneously hypertensive rats (SHR) exhibit increased cardiovascular and sympathetic responses and abnormal renal vascular responses (4,21).

Accumulating evidence suggests that the brain renin-angiotensin system (RAS) has a key role in modulating the stress-induced cardiovascular responses (13). Intracerebroventricular (ICV) injection of angiotensin (Ang) II-receptor blockers
attenuated cardiovascular responses induced by an immobilization stress in the rat (14,25), and the microinjection of an Ang II type 1 receptor blocker into the rostral ventrolateral medulla decreased the pressor response to an air-jet stress in the rabbit (19).

Nabika et al constructed a congenic rat for a chromosome 1 blood pressure QTL (23). A segment of chromosome 1 from stroke-prone SHR (SHRSP) was introgressed into Wistar-Kyoto (WKY) rat by repeated backcrossing. This congenic rat showed only a small increase in blood pressure measured continuously by radiotelemetry but an exaggerated pressor response to both restraint (2) and cold stress (3). Since these exaggerated response was abolished by chemical sympathectomy (2,3) and urinary excretion of norepinephrine during cold stress was greater in the congenic rat than in the WKY (3), a hyper-reactivity of the sympathetic system might be present in the congenic rat. However, the previous studies did not measure sympathetic nerve activity directly. Further, mechanisms of the hyper-reactivity of the sympathetic system remain unknown.

In the present study, we therefore examined the RSNA in addition to the responses of blood pressure and HR to air-jet stress in this congenic strain. We also examined effects of ICV administration of candesartan, an angiotensin II type 1 receptor blocker, on the cardiovascular responses to air-jet stress. Our observation suggests the role of brain RAS in the exaggerated pressor response in the congenic rat.

METHODS
Animals

Fourteen-week-old male rats were used for the experiments. The SHRSP/Izm and WKY/Izm rats were distributed from the Disease Model Cooperative Research Association (Kyoto, Japan). The congenic rats (WKYpch1.0) were maintained at the Animal Center in University of the Ryukyus. Rats were fed a standard laboratory rat chow and tap water ad libitum and kept in a room maintained at constant temperature (24±2 Celsius) and humidity (55±10%) under a 12 hr light period between 8:00 and 20:00. After 7 days of adaptation to these conditions, the experimental procedures were performed. Experiments were performed in random order on WKYpch1.0, WKY or SHRSP. All procedures were in accordance with the National Institutes of Health guidelines for the care and use of laboratory animals. The protocol was approved by the Animal Care and Use Committee, University of the Ryukyus.

Construction of congenic rats

A congenic strain, WKY.SHRSP-(D1Wox29-D1Arb21)/Izm (abbreviated here as WKYpch1.0) was constructed as described previously (3). In brief, a segment of chromosome 1 from SHRSP/Izm was introgressed into WKY/Izm by 10 generations of repeated backcrossing. Five genetic markers inside were monitored to keep the region between D1Wox29 and D1Arb21 heterozygous. A congenic strain was established through the final brother-sister mating. This region covered the 100: 1 confidence interval for the blood pressure QTL. More than 99.9% of the background genome was expected to be homozygous for original WKY rats in the congenic strain (2,3).
Implantation of arterial and venous catheters and the renal nerve electrode

Rats were anesthetized with an intraperitoneal injection of 50 mg/kg of sodium pentobarbital, and vascular catheters were inserted through the femoral artery and vein for blood pressure recording and drug administration, respectively. The renal nerves were exposed through a retroperitoneal approach. A branch of the nerves was separated from surrounding connective tissue and a bipolar silver wire electrode (no. 7855; A-M Systems, Carlsborg, WA) was placed under the nerve branch. When an optimal neurogram was obtained, the nerve and an electrode were embedded in silicone gel (Semicosil 932; Wacker, Munich, Germany) and allowed to harden. Catheters and lead wires from the electrode were exteriorized at the interscapular region through a subcutaneous tunnel. After surgery, each rat received an intramuscular injection of 40,000 U/kg body weight of penicillin-G for prophylaxis.

Implantation of guide cannula for ICV injection

Separate groups of WKY and WKYpch1.0 were anesthetized and the rats were placed on a stereotaxic frame (Narishige Scientific Instruments, Tokyo, Japan) in a prone position. The incisor bar was set at 4 mm below the interaural line. The skin overlying the midline of the skull was incised, and a small hole was drilled to the dorsal surface of the cranium according to the following coordinates; 0.8 mm posterior to the bregma, 1.5 mm lateral to the midline. A 23-gauge stainless-steel guide cannula was lowered 2 mm vertically from the skull surface toward the lateral ventricle. The guide cannula was fixed to the skull with screws and hardened together with cyanoacrylate adhesive. Before
and after the surgery, each rat received an intramuscular injection of 20,000 U/kg body weight of penicillin G for prophylaxis.

**Experimental protocol 1: Effect of air-jet stress on blood pressure, HR, and RSNA**

At least 18 hr after the implantation of the renal nerve electrode, the rat was placed in an 18 cm diameter plastic bowl and was allowed to move freely. The amplified nerve pulses and pulsatile blood pressure, MAP, and HR were recorded. RSNA was rectified and integrated (reset every 1 s) for analysis. After recording baseline MAP, HR, and RSNA, each rat had a continuous air-jet stress delivered from nozzles 8-10 cm apart directed to the face of the rat for 20 seconds. This stress was applied two times with an interval of at least 10 minutes. The strength of the stimulus was adjusted so that the rats would not increase gross locomotor activity. In response to air-jet stress, rats normally take a defensive posture and stand ready to get out from the air-jet. In most case, rats stayed in same place during 20 sec of the stress, however, some time rats moved around. Responses of MAP, HR, and RSNA were recorded. The data of air-jet stress from each rat were averaged for analysis. We deleted rats, which had more than 10 sec of motion artifact on RSNA during the air-jet stress from further data analysis. We also deleted rats from further analysis when RSNA neurogram was strongly influenced by respiration, because this type of RSNA could have more influence of electromyogram. At the end of experiments, 40 mg/kg of hexamethonium were administered intravenously to examine the effect of ganglionic blockade on blood pressure and to determine background noise level of nerve pulses.
**Experimental protocol 2: Effect of ICV injection of an Ang II type 1 receptor blocker to air-jet stress**

At least 4 days after the implantation of guide cannula for ICV injection, the rat had a surgery to implant the arterial and venous catheters. The following day, the experiment was performed on WKYpch1.0 or WKY in conscious, unrestrained condition. Before ICV injection, each rat had 20 seconds of air-jet stress and MAP and HR were recorded. An injector needle, which extended 2 mm beyond the tip of the guide cannula, was inserted through the guide cannula. Each injection of drug was given over a period of one minute in a 2 µl volume. Rats had ICV injection of either 1 µg of candesartan or normal saline as a vehicle. This dose of candesartan reported to lack a centrally-mediated antihypertensive effect in SHR and to abolish changes in MAP and HR in response to ICV injection of Ang II (100 ng) (1). Thirty minutes after the ICV injection, the rats had another 20 seconds of air-jet stress. At least 10 minutes after the stress, 20 ng of Ang II was injected into the lateral ventricle of the rat. Finally, each rat received a bolus intravenous injection of 3 µg of phenylephrine, an alpha 1 adrenoceptor agonist. At the end of the ICV experiments, rats were anesthetized and received an ICV injection of 5% solution of methylene blue to verify the position of ICV cannula. The animal preparations and procedures have been described in detail elsewhere (26-28,31).
Statistical analysis

Values are expressed as the mean±SEM. Differences among the groups were tested by two-way analysis of variance. Scheffe's F test was used for subsequent analysis. A value of p < 0.05 was considered to be significant.

RESULTS

Effect of air-jet stress on blood pressure, HR, and RSNA

Air-jet stress was performed on WKYpch1.0 (n=6), WKY (n=6) and SHRSP (n=6). Baseline values of MAP and HR are shown in Table 1. Baseline MAP in WKYpch1.0 was lower than that in SHRSP, while comparable to that in WKY. Baseline HR was not different among the strains. Typical traces of pulsatile arterial pressure, MAP, HR and RSNA of each strain in response to air-jet stress were shown in Figure 1. Air-jet stress caused larger responses in MAP, HR and RSNA in SHRSP than in WKY. In WKYpch1.0, larger responses in MAP (35±3 mmHg vs. 22±2 mmHg, p<0.005) and RSNA (216±51% vs. 78±20%, p<0.05) were observed when compared with those in WKY. The increase in RSNA in WKYpch1.0 was comparable with SHRSP (261±36%). Although the MAP change in WKYpch1.0 was significantly smaller than that in SHRSP (47±1 mmHg, p<0.005, see Figure 2), percent change in MAP was similar between WKYpch1.0 (33±3%) and SHRSP (28±2%). To estimate the basal sympathetic tone in the three strains, intravenous injection of 40 mg/kg of hexamethonium was performed. Ganglionic blockade by hexamethonium caused greater decrease in MAP of SHRSP
when compared with WKY or WKYpch1.0. Decrease in MAP by the blockade was not different between WKYpch1.0 and WKY (Table 1). Consequently, attained levels of MAP after the blockade were comparable among the strains.

Effect of ICV injection of an Ang II type 1 receptor blocker on the stress response

ICV injection of candesartan was performed on WKYpch1.0 and WKY. Twelve rats of each strain were divided into two groups, which received either 1 µg of candesartan (n=6) or vehicle (n=6). Baseline values of MAP and HR are shown in Table 2. Baseline MAP and HR were similar between the strains. Air-jet stress caused greater increase in MAP (36±2 mmHg vs. 23±2 mmHg, p<0.001) in WKYpch1.0 than in WKY while increase in HR (105±12 bpm vs. 85±8 bpm) was not significantly different. ICV injection of either vehicle or 1 µg of candesartan caused negligible effects on blood pressure and HR in WKYpch1.0 and WKY (Table 2). ICV injection of candesartan but not of vehicle significantly attenuated the increases in MAP and HR caused by air-jet stress in the both strains (Figure 3). Interestingly, the attenuation of the pressor response by candesartan was significantly greater in WKYpch1.0 than in WKY (10±1 mmHg vs 3±1 mmHg, p<0.05). In all vehicle-injected rats, ICV injection of Ang II caused a pressor and a drinking response. The drinking behavior was initiated within 5 min after the injection. The pressor response to Ang II injected into the lateral ventricle was not different significantly in WKYpch1.0 and in WKY (Table 2). ICV injection of candesartan abolished both the pressor effect and the stimulatory effect on drinking, which were induced by Ang II injection.
Bolus intravenous injection of phenylephrine caused a comparable pressor response in the both strains (Table 2).

DISCUSSION

The principal observation in the present study was that an air-jet stress caused the greater increase in RSNA as well as MAP in the congenic rat than in the WKY. In addition, the greater attenuation in the pressor response to air-jet stress was observed after ICV injection of candesartan, an Ang II type 1 receptor blocker, in this congenic rat. These results suggested that 1) the congenic rats exhibited the sympathetic hyper-reactivity to an air-jet stress, which caused exaggerated cardiovascular response; 2) the exaggerated response in the congenic rat was at least partly mediated by the brain RAS.

The most interesting observation in the present study was an exaggerated RNSA as well as a blood pressure response in the congenic rat under an air-jet stress. This congenic rat has been reported to have an exaggerated blood pressure elevation to environmental stresses such as restraint (2) and cold stress (3). In the present study, we observed the exaggerated blood pressure response to another type of stress in this congenic rat. Air-jet stress is thought to be a pure psychoemotional stress, which is largely different from ‘physical’ stresses like cold stress (18). The pressor responses caused by air-jet stress in the present study were comparable to our previous observations in SHR and WKY (21). In addition, we clearly showed that the exaggerated response of blood pressure was accompanied with the hyper-activation of the RSNA in this strain.
(see Figure 1 and 2). In addition, since intravenous injection of phenylephrine caused a pressor response comparable between WKYpch1.0 and WKY, difference in the peripheral vascular reactivity to catecholamines can not be an interpretation for the exaggerated blood pressure response to the stress. Therefore, our results strongly suggest that this congenic rat showed an exaggerated response in blood pressure to the stresses through the hyper-reactivity of the sympathetic nervous system.

In the present study, the increase in RSNA in response to air-jet stress was similar in congenic rat to that SHRSP, whereas the increase in MAP was smaller in congenic rat than in SHRSP. There was a discrepancy between responses of RSNA and MAP. Baseline MAP was markedly higher in SHRSP than WKY and WKYpch1.0. We assessed MAP response to air-jet stress by % change in MAP. Percent change in MAP was similar between WKYpch1.0 and SHRSP. There are possibility that the absolute values of MAP response to air-jet stress were influenced by the baseline MAP. Another possibility caused this discrepancy is a difference in baroreflex function. SHRSP is known to have an impaired baroreflex function (16). This impairment might be contributed to the discrepancy between responses of RSNA and MAP to air-jet stress in congenic rat and in SHRSP.

It is notable that the increase in RSNA to air-jet stress in WKYpch1.0 was as large as that in SHRSP (see Figure 2). This result implied that a gene or genes located in the QTL region on chromosome 1 exerted a major effect on the sympathetic reactivity. As WKYpch1.0 showed an exaggerated RSNA reactivity comparable with that in SHRSP despite of its 'normal' blood pressure, this strain may be a useful model for the study of
reactivity of the sympathetic nervous system without regarding secondary effects of blood pressure.

Accumulating evidence has established that the brain RAS plays an important role in the control of cardiovascular functions (22,29) including stress-induced cardiovascular responses (13). Subfornical organ, paraventricular nucleus, and rostral ventrolateral medulla are proposed to take parts in the emotional pressor circuit using Ang II as a major neurotransmitter (13). We observed a greater effect of an Ang II type I receptor blocker in the congenic rat, attenuating the pressor response to air-jet stress (see Figure 3). This observation implied that the exaggerated stress response in the congenic rat was at least partly mediated by the brain RAS. Therefore, we speculate that the brain RAS contributes to the sympathetic hyper-reactivity to the stress in this congenic rat.

Careful discussion is, however, necessary on the relationship between ‘hypertension’ genes and the exaggerated sympathetic response observed in the present study. A previous study demonstrated a small but significant elevation in blood pressure in WKYpch1.0 when compared with WKY (2), while another report could not replicate the significant difference in blood pressure between the congenic rat and WKY at the baseline (3). Thus, it remains still controversial whether this congenic rat had an elevated blood pressure under a resting condition. In the present study, blood pressure as well as HR of WKYpch1.0 was not different significantly from that of WKY. In addition, the decrease in MAP by the ganglionic blockade was comparable between the two strains. These observations suggested that the sympathetic and cardiovascular hyperactivities in the resting condition were not obvious in the congenic rat. The discrepant results for the blood pressure among studies may be due to differences in experimental conditions such
as the different methods used in blood pressure measurement and influence of the surgery. In any case, it appears that the increase of blood pressure in WKYpch1.0 was quite small if any. As a reciprocal congenic strain for the same QTL showed a large decrease in blood pressure (11), the effect of the QTL on blood pressure seems dependent on the genetic background of rats. This is quite a contrast to the air-jet stress-induced increase in the RSNA, which was as large in WKYpch1.0 as in SHRSP (Figure 2). These results might suggest that a gene (or genes) in the chromosome 1 QTL had a major influence on the stress-induced activation of RSNA, although synergistic effects of other genetic factors were necessary to cause an evident increase in blood pressure. Another interpretation that should be considered is that a gene regulating RSNA was different from that responsible for high blood pressure. The QTL region isolated in the congenic strain was still quite large. It harbors hundreds of genes and it is sometime quite difficult to show that one of them is responsible for high blood pressure (5). The sub-congenic rats with a narrower segment of the QTL would help to identify candidate genes.

There is some limitation to interpret the RSNA data in the present study. Since we observed the sympathetic nerve activity under conscious condition, it is unable to remove artifacts due to motion and electromyogram from RSNA entirely. It is also unable to control a startle motion in response to air-jet stress, which might cause the artifacts on RSNA. We did not observe a noticeable difference in the motion artifact between WKY and WKYpch1.0 during air-jet stress. However, SHRSP might have more motion artifact than other two strains. This strain-specific difference in the artifact due to startle motion might influence the RSNA results. However, we believe that we could
minimize these influences by excluding the data, which contained large motion artifacts, from the analyses.

In summary, an air-jet stress caused greater increase in RSNA as well as MAP in the congenic rat for the QTL on chromosome 1. In addition, injection of an Ang II type 1 receptor blocker elicited a greater attenuation of the pressor response to air-jet stress in the congenic rat. These results suggest that the blood pressure QTL on chromosome 1 harbored a gene or genes influencing the sympathetic hyper-reactivity to the environmental stress, which caused exaggerated cardiovascular response. Contribution of the brain RAS to the exaggerated response was suggested. This congenic rat may be a good model for ‘stress-induced hypertension’, which was described in humans as well (18).
Acknowledgements

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References


Figure legends

Fig. 1. Typical traces of pulsatile arterial pressure (AP), mean arterial pressure (MAP), heart rate (HR), and renal sympathetic nerve activity (RSNA) in response to 20 seconds of air-jet stress of WKY, WKYpch1.0, and SHRSP. Slashed bars show a period of air-jet stress. WKY, Wistar-Kyoto rat; WKYpch1.0, the chromosome 1 blood pressure QTL congenic rat; SHRSP, stroke-prone spontaneously hypertensive rat. bpm, beats/min.

Fig. 2. Cardiovascular and sympathetic responses to air-jet stress in WKY, WKYpch1.0, and SHRSP.

Fig. 3. Increase in MAP and HR in response to air-jet stress, before and after intracerebroventricular injection of candesartan or vehicle.
Table 1. Baseline values of MAP and HR, and change in value of MAP after ganglionic blockade

<table>
<thead>
<tr>
<th>Strain</th>
<th>n</th>
<th>MAP, mmHg</th>
<th>HR, beats/min</th>
<th>ΔMAP, mmHg</th>
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<tbody>
<tr>
<td>WKY</td>
<td>6</td>
<td>108±3</td>
<td>317±14</td>
<td>-49±5</td>
</tr>
<tr>
<td>WKYpch1.0</td>
<td>6</td>
<td>107±2</td>
<td>321±11</td>
<td>-48±6</td>
</tr>
<tr>
<td>SHRSP</td>
<td>6</td>
<td>175±6*</td>
<td>287±5</td>
<td>-102±5*</td>
</tr>
</tbody>
</table>

MAP, mean arterial pressure; HR, heart rate; RSNA, WKY, Wistar-Kyoto rat; WKYpch1.0, congenic rat for chromosome 1 blood pressure QTL; SHRSP, stroke-prone spontaneously hypertensive rat; C6, hexamethonium (40 mg/kg iv).

*P<0.05 vs WKY and WKYpch1.0, values are mean±SE.
Table 2. Baseline values of MAP and HR, change in value of MAP and HR after ICV injection of saline or candesartan and change in value of MAP after ICV injection of Ang II and IV injection of PHE

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Saline or Candesartan ICV</th>
<th>Ang II ICV</th>
<th>PHE IV</th>
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<tbody>
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<td></td>
<td>n</td>
<td>MAP, mmHg</td>
<td>HR, beats/min</td>
<td>ΔMAP, mmHg</td>
</tr>
<tr>
<td>WKY, saline ICV</td>
<td>6</td>
<td>107±4</td>
<td>306±12</td>
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<tr>
<td>WKY, candesartan ICV</td>
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<td>6</td>
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<td>329±13</td>
<td>-2±3</td>
</tr>
</tbody>
</table>

MAP, mean arterial pressure; HR, heart rate; WKY, Wistar-Kyoto rat; WKYpch1.0, congenic rat for chromosome 1 blood pressure QTL; ICV, intracerebroventricular injection; IV, intravenous injection; Ang II, angiotensin II (20 ng); PHE, phenylephrine (3 µg).

*P<0.05 vs same strain with saline ICV, values are mean±SE.