Antihypertensive Treatment and the Responsiveness to Glutamate in Ventrolateral Medulla

Takuya Tsuchihashi, Shuntaro Kagiyama, Yusuke Ohya, Isao Abe, Masatoshi Fujishima

Abstract—We have recently reported that the cardiovascular responses to excitatory amino acids are augmented in the rostral ventrolateral medulla of spontaneously hypertensive rats (SHR). In the present study, we investigated whether the responsiveness to excitatory amino acids would be normalized by antihypertensive treatment. Thus we treated 4-week-old SHR and age-matched Wistarr–Kyoto (WKY) rats with either enalapril (25 mg/kg per day in drinking water) or vehicle for 8 weeks. At 12 weeks of age, systolic blood pressure in the untreated SHR (248±9 mm Hg) was significantly (P<.01) higher than that in the enalapril-treated SHR (140±4 mm Hg), untreated WKY rats (148±4 mm Hg), and enalapril-treated WKY rats (117±1 mm Hg). The pressor responses to L-glutamate (2 nmol) microinjected into the rostral ventrolateral medulla were similar in enalapril-treated and untreated SHR. (40±5 and 47±3 mm Hg, respectively, NS), and these responses were significantly greater than that seen in the untreated WKY rats (24±2 mm Hg, P<.01). On the other hand, the pressor response to either N-methyl-D-aspartate, an ionotropic glutamate receptor agonist, or (1S,3R)-1-aminocyclopentane-1,3-dicarboxylic acid, a metabotropic glutamate receptor agonist, in the enalapril-treated SHR was slightly but significantly smaller than that in the untreated SHR but was still markedly greater than those in untreated and enalapril-treated WKY rats. These results suggest that the augmented responsiveness to excitatory amino acids in the rostral ventrolateral medulla of SHR may be at least partly genetically determined and cannot be normalized by the treatment with enalapril. (Hypertension. 1998;31[part 1]:73–76.)

Key Words: receptors ■ glutamate ■ ventrolateral medulla ■ blood pressure ■ SHR ■ antihypertensive treatment

Methods

Animal Preparation
All experiments were done in 4-week-old male SHR/Izm (n=12) and age-matched WKY/Izm rats (n=12) obtained from the Disease Model Cooperative Research Association (Kyoto, Japan). This experiment was reviewed and approved by the Committee of Ethics in Animal Experimentation of the Faculty of Medicine, Kyushu University. Both SHR and WKY rats were divided into two subgroups; one group received enalapril (25 mg/kg per day in drinking water, n=6) for 8 weeks, and the other group received tap water as a vehicle control (n=6). Systolic blood pressure was measured weekly by a tail-cuff method. The following experiment was carried out when the rats were 12 weeks of age. Rats were anesthetized with urethane (1.5 g/kg IP). A femoral artery and vein were cannulated for the measurement of arterial pressure and the injection of drugs, respectively. Body temperature was maintained at 37.5±0.5°C with a heating pad. Anesthetized rats were placed in the supine position with the head fixed in a stereotaxic frame (David Kopf Instruments). The trachea and esophagus were transected in the lower neck and reflected rostrally. The distal trachea was cannulated to facilitate ventilation. After retraction of the bilateral longus capitis muscles, the inferior occipital bone was removed to provide a 5×6 mm window to the surface of the ventral medulla oblongata. After the dura was incised and retracted, the exposed ventral surface of the medulla was kept moist with either artificial cerebrospinal fluid (pH 7.4) or endogenous cerebrospinal fluid. After paralysis was induced with d-tubocurarine (0.8 mg/kg IV), the tracheal cannula was connected to a ventilator (model 681D, Harvard Apparatus), and the rats were artificially
ventilated at a rate of 60 strokes/min with a tidal volume of 3.0 mL. Artificial ventilation maintained arterial blood gases and pH within physiological limits (pH, 7.35 to 7.45; P<sub>O</sub>₂, 100 to 140 mm Hg; P<sub>CO</sub>₂, 40 to 45 mm Hg).

**Microinjection Procedures**

Microinjections were made with the use of multibarrel micropipettes with tip diameters of 20 to 50 μm. The pipettes were made from calibrated microbore capillary glass tubing (Accu-Fill 90, Clay Adams). Unilateral injections (50 nL), performed over a 30-second period, were made using a hand-held syringe. The injection volume was measured by observation of the movement of the fluid meniscus along a reticule under a microscope.

The RVLM was identified after injection of L-glutamate (Glu, 2 nmol) based on the following criteria: (1) the latency to the onset of the change in blood pressure produced by Glu was no more than 5 seconds; (2) a response plateau occurred within 20 seconds after microinjection of Glu; and (3) the change in blood pressure was at least 20 mm Hg. The RVLM was restricted to injection sites located 0.6 to 1.0 mm rostral to the most rostral rootlet of the hypoglossal nerve, 1.7 to 1.9 mm lateral to the midline, and 0.5 to 0.8 mm below the ventral surface. Although histological examination was not performed in the present study, our previous observations suggested that the injection sites determined functionally according to the above-mentioned criteria were located in the area that encompassed the dorso-lateral aspect of the lateral paragangiotocellular nucleus and the region dorso-lateral to this nucleus.

All drugs were dissolved in artificial cerebrospinal fluid (in mmol/L: NaCl, 133.3; KCl, 3.4; CaCl₂, 1.3; MgCl₂, 1.2; NaH₂PO₄, 0.6; NaHCO₃, 32.0; and glucose, 3.4; pH 7.4).

Experimental Protocols

After the RVLM was determined by microinjection of Glu, an ionotropic glutamate receptor agonist, NMDA (20 pmol), and a metabotropic glutamate receptor agonist, (1S,3R)-ACPD (1 nmol), were microinjected in a randomized order into the unilateral RVLM. After completion of the microinjection studies, we injected norepinephrine intravenously at doses of 0.2 and 1 g/kg and compared the cardiovascular responsiveness with exogenous norepinephrine in the four groups.

Statistical Analysis

Data are expressed as mean±SEM. A one-way ANOVA followed by multiple comparisons with Duncan’s multiple range test was used to compare the results across the four subgroups. Data for multiple observations over time were analyzed by two-way ANOVA with repeated measures for overall treatment effect. Values of P<0.05 were considered statistically significant.

**Results**

Fig 1 shows the changes in body weight and systolic blood pressure during the treatment period. The time course of body weight was significantly (P<0.01) different between the untreated and the enalapril-treated SHR. In contrast, the untreated and the enalapril-treated WKY rats showed similar increases in body weight. More specifically, the enalapril-treated SHR from the age of 8 to 12 weeks were significantly smaller than the untreated SHR. Treatment with enalapril effectively prevented the elevation of blood pressure in SHR, and the blood pressure levels in the enalapril-treated SHR were comparable to those of untreated WKY rats. The enalapril-treated WKY rats also showed significantly lower blood pressure levels compared with the untreated WKY rats between the ages of 8 and 12 weeks. Baseline MAP and HR in anesthetized and artificially ventilated rats were 137±7 mm Hg and 394±17 beats per minute, respectively, in the untreated SHR. The enalapril-treated SHR showed significantly (P<0.01) lower MAP (82±7 mm Hg) and HR (327±8 beats per minute) than in the untreated SHR. Similarly, baseline MAP in the enalapril-treated WKY rats (60±2 mm Hg) was significantly (P<0.01) lower than in the untreated WKY rats (94±1 mm Hg). Baseline HR did not differ between the untreated and the enalapril-treated WKY rats (392±13 and 382±7 beats per minute, respectively). The cardiovascular response to Glu microinjected into the RVLM is shown in Fig 2. The increases in MAP were similar in the untreated and the enalapril-treated SHR, and both were significantly greater than those seen in WKY rats. The HR responses did not differ significantly among the four groups. The cardiovascular responses elicited by NMDA and (1S,3R)-ACPD are summarized in Fig 3. The increase in MAP in the enalapril-treated SHR was significantly less than that in the untreated SHR, but still significantly greater than those in the untreated and the enalapril-treated WKY rats.

The effect of intravenous injection of norepinephrine is shown in Fig 4. The pressor and bradycardic responses elicited by either

---

Selected Abbreviations and Acronyms

- EAA = excitatory amino acid
- HR = heart rate
- MAP = mean arterial pressure
- NMDA = N-methyl-D-aspartate
- RVLM = rostral ventrolateral medulla
- SHR = spontaneously hypertensive rats
- WKY = Wistar-Kyoto
- (1S,3R)-ACPD = (1S,3R)-1-amino-cyclopentane-1,3-dicarboxylic acid
- 2K1C = two-kidney, one-clip

---

**Figure 1.** Time courses of body weight (top) and systolic blood pressure (bottom) in untreated SHR (●), enalapril-treated SHR (▲), untreated WKY rats (■), and enalapril-treated WKY rats (○). Number of rats is 6 in each group. Values are mean±SEM. *P<0.05, †P<0.01 vs untreated WKY rats. ‡P<0.05, §P<0.01 vs untreated SHR.
0.2 or 1 μg/kg of norepinephrine were not different among the four groups.

**Discussion**

In the present study, we examined whether the augmented responsiveness of the RVLM to the stimulation of EAA receptors in SHR would be altered by the prevention of hypertension with antihypertensive treatment. Our findings that the pressor response to Glu in the enalapril-treated SHR was comparable to that of the untreated SHR and prominently greater than that of WKY rats indicate that the augmented responsiveness to EAA in the RVLM of SHR may be a genetically determined property of this strain. However, considering the finding that the pressor response evoked by the selective stimulation of either ionotropic or metabotropic glutamate receptors was slightly but significantly less in the enalapril-treated SHR than in the untreated SHR, the elevation of blood pressure may contribute in part to the augmented responsiveness to EAA seen in the untreated SHR.

The cardiovascular responsiveness to EAA in the RVLM has been examined in several experimental animal models of hypertension. In addition to our previous report on SHR, Bergamaschi et al reported that microinjection of Glu into the RVLM elicited an augmented pressor response in 2K1C hypertensive rats. Since the depressor response to microinjection of kynurenic acid, an ionotropic glutamate receptor antagonist, was also greater in 2K1C rats, they concluded that the activity of the EAA receptors in the RVLM has an important role in maintaining blood pressure in this model. Salt intake also may influence the sensitivity of RVLM neurons to EAA. Pawloski-Dahm and Gordon reported that normotensive Sprague-Dawley rats fed a high salt diet for 10 to 14 days showed enhanced responsiveness to microinjection of Glu into the RVLM without any associated change in baseline blood pressure. On the basis of this finding, we examined the responsiveness of the RVLM to EAA in Dahl salt-sensitive rats fed a high salt diet, but we failed to demonstrate any alteration. Thus the enhanced responsiveness of the RVLM may not

![Figure 2. Changes in mean arterial pressure (left) and heart rate (right) elicited by microinjection of L-glutamate (2 nmol) into the rostral ventrolateral medulla in untreated SHR (closed bars), enalapril-treated SHR (hatched bars), untreated WKY rats (open bars), and enalapril-treated WKY rats (shaded bars). Number of rats is 6 in each group. Values are mean±SEM. †P<.01 vs untreated WKY rats.](image)

![Figure 3. Changes in mean arterial pressure (left) and heart rate (right) elicited by microinjection of NMDA, 20 pmol (top), or (1S,3R)-ACPD, 1 nmol (bottom), into the rostral ventrolateral medulla in untreated SHR (closed bars), enalapril-treated SHR (hatched bars), untreated WKY rats (open bars), and enalapril-treated WKY rats (shaded bars). Number of rats is 6 in each group. Values are mean±SEM. †P<.05, †P<.01 vs untreated WKY rats. #P<.05, §P<.01 vs untreated SHR.](image)

![Figure 4. Changes in MAP (top) and HR (bottom) elicited by intra-venous injection of norepinephrine at doses of 0.2 and 1 μg/kg. Number of rats is 6 in each group. Values are mean±SEM.](image)
necessarily be associated with hypertension per se but may differ according to the pathogenesis of hypertension.

Although the pressor response to microinjection of either NMDA or (1S,3R)-ACPD in the enalapril-treated SHR was markedly greater than that in WKY rats, it was significantly less than that seen in the untreated SHR. This attenuated pressor response in enalapril-treated SHR may be attributable to the prevention of blood pressure elevation. An alternative explanation is that enalapril itself may have an influence on RVLM neurons to reduce their responsiveness to EAA. We have previously reported that angiotensin I as well as angiotensin II, microinjected into the RVLM, elicits pressor and sympathoexcitatory responses.8,9 There is also evidence that oral administration of enalapril reduces the ACE activity of the whole brain10 and the medulla oblongata.11 However, the possible contribution of the ACE inhibition within the RVLM to the altered responsiveness to EAA in the enalapril-treated SHR seems unlikely because of the following reasons: First, the pressor responses to NMDA and (1S,3R)-ACPD were not different between the untreated and the enalapril-treated WKY rats. Second, the exogenous injection of angiotensin I into the RVLM elicited comparable pressor responses between the untreated and the enalapril-treated WKY rats (data not shown). This observation may indicate that oral treatment with enalapril at the dose of 25 mg/kg per day for 8 weeks effectively reduces blood pressure, whereas it fails to inhibit the ACE activity in the RVLM. Taken together, the prevention of hypertension may have dominantly contributed to the attenuated pressor response in the enalapril-treated SHR.

Because microinjections were done unilaterally, one may argue that the baroreflex-mediated compensation through the contralateral RVLM could modify the pressor responses to EAA. If baroreflex function would be impaired in SHR, the pressor responses to EAA could be augmented compared with WKY rats with intact baroreflex function. In addition, the possible improvement of baroreflex function in SHR treated with enalapril12 could explain the attenuated pressor response to either NMDA or (1S,3R)-ACPD in this group. However, our finding that bradycardic responses to intravenous injection of norepinephrine did not differ among the four groups in the anesthetized condition (Fig 4) may not support a major role of the baroreflex-mediated compensation in the difference of pressor responses to EAA between SHR and WKY rats with or without the treatment with enalapril. We evaluated baroreflex function by observing the reflex bradycardia in response to norepinephrine-induced increase in blood pressure. Since norepinephrine acts at both α- and β-receptors, the heart rate response may not be explained simply by baroreflex. Further studies in the rats with sinoaortic denervation may be necessary to exclude the possible influence of baroreflex-mediated compensation.

In the present study, the developmental increase in body weight was significantly less in enalapril-treated SHR. A decrease in body weight as the result of treatment with enalapril11,14 or other ACE inhibitors15,16 has been also reported by other investigators. The mechanism of body weight reduction is not fully understood but may be explained in part by the natriuresis produced by ACE inhibitors, as has been suggested by Clozel et al.17

Because we did not measure sympathetic nerve activity in this study, we cannot exclude the possibility that the difference in pressor response evoked by microinjection of EAA into the RVLM may be due to the responsiveness of peripheral vessels rather than a difference in the sympathetic outflow. However, on the basis of our finding that the pressor response to intravenous injection of norepinephrine did not differ among the four groups, we conclude that the difference in the pressor response to microinjection of EAA is attributable to a difference in the sympathetic outflow rather than a difference in vascular responsiveness. Our previous report1 also demonstrated that the augmented pressor responsiveness to EAA is associated with enhanced sympathetic nerve activity in SHR.

In conclusion, the augmented responsiveness of the RVLM to EAA in SHR may be at least partly a genetically determined property of this strain and cannot be normalized by the prevention of hypertension with enalapril.

References

Antihypertensive Treatment and the Responsiveness to Glutamate in Ventrolateral Medulla
Takuya Tsuchihashi, Shuntaro Kagiyama, Yusuke Ohya, Isao Abe and Masatoshi Fujishima

Hypertension. 1998;31:73-76
doi: 10.1161/01.HYP.31.1.73

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1998 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/31/1/73

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/