Tonic Excitatory Input to the Rostral Ventrolateral Medulla in Dahl Salt-Sensitive Rats

Satoru Ito, Kazutoshi Komatsu, Kazuyoshi Tsukamoto, Alan F. Sved

Abstract—The goal of the present study was to test the hypothesis that the balance of tonic excitation and inhibition of vasomotor neurons in the rostral ventrolateral medulla (RVLM) driven by excitatory amino acid (EAA)–mediated inputs to the RVLM is shifted toward excitation in Dahl salt-sensitive (DS) rats compared with Dahl salt-resistant (DR) rats. Glutamate and the EAA antagonist kynurenic acid were microinjected into the RVLM of chloralose-anesthetized DS and DR rats maintained on diets containing either 0.3% NaCl or 8.0% NaCl. DS rats had a higher arterial pressure than DR rats, and this difference was greatly exaggerated by high dietary salt intake. Bilateral injection of kynurenic acid (2.7 nmol) into the RVLM decreased mean arterial pressure by 16±2 mm Hg in DS rats fed a diet containing 0.3% NaCl, and this effect was significantly larger in DS rats fed the high-salt diet (40±2 mm Hg). In contrast, injections of kynurenic acid into the RVLM did not significantly decrease arterial pressure in DR rats fed either diet. In DR rats, the pressor response elicited by the injection of glutamate into the RVLM was potentiated in rats fed the high-salt diet. The glutamate-evoked pressor response was greater in DS rats compared with DR rats, and the response in DS rats was not influenced by the salt content of the diet. These data suggest that tonically active EAA inputs to the RVLM may contribute to salt-sensitive hypertension in the Dahl model. (Hypertension. 2001;37[part 2]:687-691.)

Key Words: brain stem ■ glutamic acid ■ kynurenic acid ■ hypertension

The rostral ventrolateral medulla (RVLM) is a key site in the regulation of cardiovascular function.1 RVLM neurons projecting to the spinal cord provide tonic excitation of sympathetic vasomotor outflow and are essential for the maintenance of normal arterial blood pressure (AP). Inhibition of neuronal activity in the RVLM results in a marked decrease in AP, reducing AP to the same extent as autonomic blockade, at least in anesthetized animals.

Despite the importance of the tonic activity of RVLM neurons to the maintenance of baseline AP, the mechanisms maintaining the activity of RVLM vasomotor neurons are incompletely understood. Injections of excitatory amino acid (EAA) receptor antagonists into the RVLM fail to alter baseline AP,2–4 suggesting that inputs to the RVLM using EAA neurotransmitters are not involved in generating the tonic activity of RVLM vasomotor neurons. However, on the basis of our observation that after the removal of inhibitory inputs to the RVLM, injection of the EAA receptor antagonist kynurenic acid (KYN) into the RVLM greatly reduces AP,4 we suggested that tonically active EAA inputs to the RVLM do contribute to the activity of RVLM vasomotor neurons, although this excitatory influence is normally balanced by EAA-mediated inputs that drive an inhibitory influence on RVLM vasomotor neurons.

On the basis of our model of tonically active EAA-mediated excitatory and inhibitory influences on RVLM vasomotor neurons, we proposed that shifts in the balance of these excitatory and inhibitory influences would affect baseline AP and that a shift toward excitation could result in increased activity of RVLM neurons and a sustained increase in AP. In support of this notion, we recently reported that injecting KYN into the RVLM of spontaneously hypertensive rats restores AP to normotensive levels.5 Similarly, KYN injected into the RVLM reportedly decreases AP in renal hypertensive rats.6 Thus, hypertension in these animal models may result from an imbalance of the EAA-driven excitatory and inhibitory influences on RVLM vasomotor neurons, causing relatively more excitation than inhibition.

High dietary salt may also alter the regulation of RVLM vasomotor neurons. Pawloski-Dahm and Gordon7 demonstrated that rats that had an increased consumption of NaCl showed exaggerated pressor responses to the injection of the EAA glutamate into the RVLM. We confirmed this observation and also showed that other excitatory substances injected into the RVLM result in an enhanced pressor response in rats fed a diet containing 8% NaCl compared with 1% NaCl.8 Despite the increased pressor responses elicited by the excitation of the RVLM, increased dietary salt intake does not increase AP in otherwise normal rats. We suggested that this lack of an effect of NaCl on AP results from potentiated inhibitory and excitatory influences on the RVLM.8 Consistent with this notion, we observed enhanced depressor re-

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responses in response to the injection of γ-aminobutyric acid into the RVLM in rats fed a diet containing 8% NaCl (Madden and Sved, unpublished observations, 2000).

On the basis of the hypotheses that hypertension can result from an imbalance of excitatory and inhibitory influences on the RVLM driven tonically by EAA-mediated inputs to the RVLM and that elevated dietary salt intake enhances both excitatory and inhibitory influences on RVLM vasomotor neurons, we further hypothesize that salt-sensitive hypertension may be due to unequal potentiation of these excitatory and inhibitory influences on the RVLM. The present study begins to test this hypothesis using the Dahl model of salt-sensitive hypertension. This hypothesis predicts that the injection of KYN into the RVLM would not alter AP in Dahl salt-resistant (DR) rats, whereas it would decrease AP in Dahl salt-sensitive (DS) rats on a high-salt diet.

Methods
Six-week-old male DS rats and DR rats were obtained from Seac Yoshitomi Ltd (Fukuoka, Japan). Animals were housed in groups of 2 or 3 in hanging wire mesh cages in temperature-controlled rooms with a fixed 12-hour light/dark cycle for 4 weeks before experiments. Food (Oriental Yeast Co) and tap water were available ad libitum. All rats were initially fed a diet containing 0.3% NaCl for 1 week. Then, rats of each strain were divided into 2 groups, with one group continuing to receive the 0.3% NaCl diet, whereas the other group received a diet containing 8% NaCl (high-salt diet). Experiments were conducted 3 weeks later, when rats were 10 weeks of age.

Rats were anesthetized with halothane and prepared for measuring AP, mean AP (MAP), and heart rate (HR) during injections of substances into the brain stem, as described previously. Briefly, cannulae were inserted into a femoral artery and a femoral vein. The trachea was cannulated, and the rat was connected to a ventilator. The rat was placed into a stereotaxic instrument, and the dorsal surface of the medulla was surgically exposed. After all surgery was completed, the rat was injected with α-chloralose (60 mg/kg IV), and the halothane was terminated. Additional chloralose (20 mg/kg IV) was administered hourly. Rats were injected with tubocurarine (0.5 mg/kg, supplemented hourly with 0.2 mg/kg) and ventilated with 100% oxygen for the remainder of the experiment. Injections of solutions into the brain stem were made using single-barrel glass micropipettes. All injections were in a volume of 100 nL of artificial cerebrospinal fluid vehicle administered during 3 to 7 seconds using a PicoPump (WPI). KYN was initially dissolved in 200 mmol/L sodium bicarbonate and then diluted in artificial cerebrospinal fluid. Bilateral injections were made one side at a time, with ~30 seconds separating the 2 injections. Coordinates for injections into the RVLM were, with the pipette tip angled 20 degrees rostrally, 1.8 mm rostral to the caudal tip of the area postrema, 1.8 mm lateral to the midline, and 2.9 mm below the dorsal surface of the brain stem. In each rat, initial test injections of 1 nmol of L-glutamate in 100 nL were made into the RVLM on each side to confirm that the coordinates identified a functional pressor site. After identification of functional pressor sites in the RVLM bilaterally, baseline MAP and HR were recorded for at least 30 minutes, and then KYN (2.7 nmol) was injected bilaterally into the RVLM.

At the conclusion of the experiment in DS rats, hexamethonium (20 mg/kg) was injected intravenously to determine the effect of total autonomic blockade on MAP. In both DS and DR rats, ~20 nL of 1% Fast green dye was injected into the RVLM for histological verification of the center of the microinjection site. The brain stem was removed, frozen, and cut in the transverse plane into 30-μm sections. Sections were mounted onto microscope slides and examined by light microscopy. All RVLM injection sites were located in the rostral medulla, just ventral to the compact portion of nucleus ambiguus, similar to the injection sites that we have published previously.

Results
Effects of KYN Injection into RVLM
DR rats, anesthetized with α-chloralose, had similar MAP whether they were fed a diet containing 0.3% NaCl or 8% NaCl (Table). Injection of KYN (2.7 nmol) bilaterally into the RVLM had little effect on MAP in DR rats fed either diet (Figure 1), in agreement with previous studies in which KYN was injected into the RVLM of normotensive rats. DS rats had increased MAP compared with the DR rats, and this difference was markedly exaggerated by consumption of the high-salt diet (Table). In contrast to what was observed with the DR rats, injection of KYN into the RVLM of DS rats resulted in a decrease in MAP (Figures 1 and 2). Furthermore, the KYN-evoked decrease in MAP was considerably greater in DS rats consuming the 8% NaCl diet compared with the 0.3% NaCl diet (Figures 1 and 2). The KYN-evoked decrease in MAP in DS rats began rapidly after the injection of KYN (Figure 2) and persisted for many minutes. In DS rats fed the 8% NaCl diet, MAP began to decrease within 15 seconds of the bilateral injection of KYN, although the latency to the maximal decrease in MAP was 13 ± 4 minutes; the duration of the response (defined as the time it took for MAP to return to preinjection baseline) was 48 ± 3 minutes. The effects of injection of KYN into the RVLM on HR showed a similar pattern of responses across the 4 groups of rats (Figure 2 legend).

So that the KYN-evoked decrease in MAP in DS rats could be analyzed relative to the total extent to which the autonomic nervous system supports MAP, at the end of the experiment, DS rats were injected intravenously with hexamethonium (20 mg/kg). In DS rats consuming the 0.3% salt diet, hexamethonium decreased MAP by 58 ± 4 mm Hg, whereas MAP decreased by 99 ± 4 mm Hg in DS rats consuming the 8.0% NaCl diet. Thus, KYN reduced MAP by 27 ± 4% of the decrease in MAP elicited by autonomic blockade in DS rats on the 0.3% NaCl diet, compared with a 39 ± 3 decrease in DS rats on the high-salt diet (P < 0.05).

Baseline Variables in DS and DR Rats

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Body Weight, g</th>
<th>MAP, mm Hg</th>
<th>HR, bpm</th>
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<tr>
<td>DR rats</td>
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<tr>
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<td>7</td>
<td>330 ± 4</td>
<td>108 ± 3</td>
<td>350 ± 15</td>
</tr>
<tr>
<td>8.0% NaCl diet</td>
<td>7</td>
<td>324 ± 3</td>
<td>111 ± 4</td>
<td>395 ± 19*</td>
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<tr>
<td>DS rats</td>
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<tr>
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<td>336 ± 6</td>
<td>123 ± 4†</td>
<td>435 ± 15†</td>
</tr>
<tr>
<td>8.0% NaCl diet</td>
<td>15</td>
<td>313 ± 5*</td>
<td>177 ± 3†</td>
<td>465 ± 16†</td>
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</tbody>
</table>
*Significantly different from the 0.3% NaCl diet group of the same strain, P < 0.05.
†DS rats significantly different from DR rats fed the same diet, P < 0.05.

KYN, L-glutamate, and hexamethonium were purchased from Sigma Chemical Co; other drugs and chemicals were obtained from standard commercial suppliers.

Data are expressed as mean ± SEM. Responses in DS and DR rats fed with a high- or low-salt diet were compared by 2-way ANOVA (strain X diet) with post-hoc t tests using commercially available software (Statistica).
Effects of Glutamate Injection into RVLM

Glutamate (1 nmol) injected unilaterally into the RVLM increased MAP in both DS and DR rats. However, the magnitude of the response varied depending on the rat strain and the salt content of the diet (Figure 3). In DR rats, the increase in MAP was approximately 100% greater in rats fed a high-salt diet compared with rats fed a low-salt diet (Figure 2), although baseline MAP did not differ between these groups. The glutamate-evoked increase in MAP was greater in DS rats than in DR rats on either diet, although the salt content of the diet did not influence the response in DS rats. Injection of glutamate into the RVLM produced a decrease in HR that was larger in DS rats than in DR rats, and it was enhanced by increased dietary salt intake in only DR rats (Figure 3).

Discussion

The key finding of the present study is that the injection of the EAA receptor antagonist KYN into the RVLM decreases MAP in DS rats, and this response is exaggerated in association with salt-induced hypertension in this strain. Furthermore, the pressor response evoked by the injection of glutamate into the RVLM is greater in DS rats compared with DR rats. Although this response is increased by elevated dietary salt intake in DR rats, the response in DS rats is not influenced by dietary salt content. These data are consistent with the hypothesis that salt-sensitive hypertension may result from a potentiation of an underlying imbalance between tonically active EAA-mediated excitation of RVLM vasomotor neurons and tonic inhibition of these neurons driven indirectly by EAA inputs to the RVLM.
Differences in the Response to Glutamate Injected into the RVLM in DS and DR Rats

In comparing the pressor response to injection of glutamate injected into the RVLM in DS and DR rats, 2 differences were noted. First, the magnitude of the pressor response was greater in DS rats compared with DR rats. Although simply comparing evoked pressor responses between DR and DS rats is complicated by differing baseline MAP, the difference in baseline MAP does not seem to explain an increased pressor response to glutamate injected into the RVLM in the DS rats. Baseline MAP are not very different between DS and DR rats consuming the 0.3% NaCl diet, whereas the pressor responses are quite different. Additionally, the potentiated response in DS rats is still apparent when data are expressed as percent change from baseline. Another factor complicating interpretation of the larger glutamate-evoked pressor responses observed in the DS rats is the differing vascular sensitivity to pressor substances that has been reported in DS rats. However, this factor does not seem to fully account for the current results.

Although previous reports noted greater pressor responses to the intravenous injection of vasoconstrictor substances in DS compared with DR rats, the magnitude of this enhanced response is typically in the range of 50% to 100%, whereas in the present study the difference between DS and DR rats was substantially larger (eg, >300% with the 0.3% NaCl diet). Furthermore, in previous studies, the difference in pressor responses to vasoconstrictor substances between DS and DR rats is essentially eliminated by expressing evoked changes in MAP as a percent of baseline MAP, whereas in the present study, the increased pressor response to glutamate injected into the RVLM was still noted when data are expressed in this manner. Thus, it seems that the greater pressor response in DS rats compared with DR rats evoked by the injection of 1 nmol of glutamate into the RVLM reflects a difference in the neural control of the circulation, although the present data do not address whether this difference results from differences at the level of the RVLM or distal to it. Also, since the present study used only a single dose of glutamate, these data do not address whether the difference reflects an increased sensitivity to glutamate or enhanced maximal responsiveness (ie, whether in DS rats the dose-response curve is shifted to the left or rather shifted up).

Another complicating factor in comparing the responses evoked by the injection of glutamate into the RVLM of these 2 rat strains is the precise localization of the injection site. Although the microinjection coordinates were similar across all groups in the present study and the histological location of injection sites appeared similar, the anatomical location of pressor sites in the RVLM has not been extensively mapped in these 2 distinct rat strains. However, the use of a rather large injection volume (100 nl) and a rather large dose of glutamate (1 nmol) should obscure any slight difference in the anatomical location of RVLM pressor sites between DS and DR rats. Even so, it should be noted that the maximal increase in MAP with the injection of 1 nmol of glutamate into the RVLM in DR rats is less than that in other strains of normotensive rats.

A second difference in the pressor response to glutamate injected into the RVLM between DS and DR rats relates to the effects of dietary salt on this response. In DR rats, the increase in MAP evoked by the injection of glutamate into the RVLM was potentiated by the high-salt diet. We observed this previously with normotensive Sprague-Dawley rats; indeed, the magnitude of the effect of the high-salt diet is similar to what we reported previously in Sprague-Dawley rats. In contrast, increased dietary salt intake in DS rats did not alter the glutamate-evoked pressor response. Tsuchihashi et al previously reported similar data in DS rats, additionally noting that pressor responses were also similar in DS rats fed diets containing 0.3% and 8.0% NaCl when tested with smaller doses of glutamate and other EAA, indicating that our results cannot be explained simply by us having reached a maximum of how high MAP can increase. In addition, Tsuchihashi et al demonstrated that these similar pressor responses were accompanied by similar increases in sympathetic nerve activity, suggesting that the similar increases in MAP were not confounded by the large differences in baseline vasoconstrictor tone. However, this interpretation of the data is complicated by potential effects of dietary salt intake on baseline sympathetic nerve activity in DS rats.

Taken together, these results suggest that the stimulation of EAA receptors in the RVLM elicits a larger pressor response in DS rats compared with DR rats and that, unlike DR rats and other normotensive Sprague-Dawley rat strains, this response is not enhanced by increased dietary salt intake in DS rats. However, it must be noted that the pressor response to glutamate injected into the RVLM is based on direct excitation of RVLM vasoconstrictor neurons and the excitation of indirectly-mediated inhibitory influences. The importance of this indirect inhibitory influence to the overall response to glutamate injected into the RVLM is supported by our previous observation that after unilateral inhibition of the caudal ventrolateral medulla, the site of most inhibitory input to the RVLM, the response to glutamate injected into the RVLM was substantially enhanced. Because increased dietary salt intake potentiates the depressor effects of -glutamatic acid injected into the RVLM (Madden and Sved, unpublished observations, 2000) as well as the pressor effects of glutamate injected into the RVLM, the overall responsiveness to glutamate injected into the RVLM of DS rats fed a high-salt diet reflects the net effect of increased dietary salt on potentiating both excitatory and inhibitory influences.

Differences in the Response to KYN Injected into the RVLM in DS and DR Rats

KYN injected bilaterally into the RVLM of anesthetized normotensive rats has been previously reported to have little effect on MAP and, in this regard, DR rats are no different. In contrast, KYN significantly decreased MAP in DS rats, even when they were fed a low-salt diet so their MAP was in a normotensive range. However, it needs to be noted that even on a low-salt diet, baseline MAP in DS rats is higher than that in DR rats, as amply documented in the literature. The decrease in MAP caused by KYN injection into the RVLM of DS rats was markedly greater when dietary salt
intake was increased, making them hypertensive. Even when expressed as a percent of baseline MAP or a percent of maximal decrease in MAP produced by autonomic blockade, the KYN-induced decrease in MAP was greater in DS rats consuming the 8.0% NaCl diet compared with DS rats consuming the 0.3% NaCl diet. The observation that KYN injected into the RVLM of DS rats decreased MAP suggests that tonically active EAA inputs to the RVLM act to support MAP in this strain. This is different than what has been reported in normotensive rat strains but similar to what has been reported in 2 experimental models of hypertension in rats (spontaneously hypertensive rats\(^5\) and renal hypertensive rats\(^6\)).

**Incorporation of These Results in Dahl Rats into a Model of RVLM Control of Blood Pressure**

In DR rats, like in other normotensive rats, injection of KYN into the RVLM has little effect on MAP. We previously suggested that this is due to a precise balance between tonically active EAA-mediated inputs to the RVLM that directly excite and those that indirectly inhibit RVLM vasomotor outflow. Placing normotensive rats on diet with a very high salt content does not increase MAP, although it does increase responsiveness to excitatory and inhibitory influences on the RVLM. Apparently, the excitatory and inhibitory influences on the RVLM remain in balance, and the lack of an effect of KYN in DR rats, despite an increase in glutamate-evoked pressor responses, provides evidence to support this notion.

In contrast, in 3 different models of experimental hypertension (DS rats, spontaneously hypertensive rats, and renal hypertensive rats), KYN injected into the RVLM decreases MAP.\(^5\)\(^6\) This could be explained by the balance of excitatory and inhibitory influences in the RVLM driven by tonically active EAA inputs to this region being shifted toward excitation. The present data suggest that in the case of the DS rat consuming a diet with a relatively low salt content, resulting in rather normotensive levels of MAP, there is an underlying imbalance between these influences. Placing DS rats on a high-salt diet magnifies this imbalance; therefore, baseline MAP is increased and falls to a greater extent when KYN is injected into the RVLM.

Interestingly, increasing the salt content of the diet does not increase the pressor response evoked by EAA injected into the RVLM in DS rats as it does in other rat strains. Thus, the hypertension observed in DS rats fed a high-salt diet cannot simply be explained by salt-evoked increased responsiveness to EAA in the RVLM. Possibly, an altered potentiation of EAA-mediated inhibition and excitation of the RVLM could explain these data; additional studies are needed to examine this issue.

This model would also predict that in other models of experimental hypertension in which there is an underlying imbalance between excitatory and inhibitory influences in the RVLM, the hypertension should be exaggerated by increased dietary salt intake. Interestingly, the 2 other experimental models of hypertension in which KYN injected into the RVLM has been found to decrease MAP are known to be exacerbated by increased dietary salt.\(^15\)

**Conclusions**

In summary, the present data demonstrate an abnormal regulation of MAP in DS rats mediated by EAA in the RVLM. The data are consistent with the hypothesis that hypertension in the DS rat is due to an imbalance of the excitatory and inhibitory influences of EAA-mediated inputs to the RVLM, and this imbalance is magnified by increased dietary salt.

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**References**

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