Rostral Ventrolateral Medulla as a Site for the Central Hypertensive Action of Kinins

Philip J. Privitera, Harold Thibodeaux, Phillip Yates

Abstract In the present study, we focused on the rostral ventrolateral medulla as a possible site of action for kinins because of its established importance in the central regulation of the cardiovascular system. Unilateral microinjections of 100 pmol to 4 nmol bradykinin into the rostral ventrolateral medulla produced dose-dependent increases in mean arterial pressure in Sprague-Dawley (SD) rats, Wistar-Kyoto (WKY) rats, and spontaneously hypertensive rats (SHR). The dose-response curves for the hypertensive responses to bradykinin in SD and WKY rats were essentially the same, whereas the hypertensive effect of bradykinin was significantly greater in SHR than in either SD or WKY rats. The kinin B2 receptor antagonists D-Arg°,Hyp°,Thi°,D-Phe7-bradykinin and Hoe 140 inhibited the hypertensive responses to bradykinin in both SHR and WKY rats. The hypertensive effect of 500 pmol bradykinin was reduced 65±5% after 4 nmol of D-Arg°,Hyp°,Thi°,D-Phe7-bradykinin in SHR and 50±16% in WKY rats.

Increasing evidence suggests the presence of an endogenous tissue kallikrein-kinin system within the brain. A tissue kallikrein indistinguishable from rat urinary kallikrein has been isolated and characterized in rat brain, and its synthesis is directed in cell-free translation systems by brain mRNA. Tissue kallikrein has been localized immunocytochemically in hypothalamic cell bodies and brain ventricular epithelium and quantitated in several other sites by radioimmunoassay techniques. Bradykinin-immunoreactive neuronal systems also have been localized immunocytochemically within the brain, and bradykinin has been found in mammalian brain tissue. In addition, the presence of immunoreactive kinin in canine cerebral spinal fluid also has been demonstrated. Kini-nogen has been detected in human cerebrospinal fluid and recently has been localized immunohistochemically in neurons of the hypothalamus of the adult rat. Lastly, the cDNA for the rat bradykinin B2 receptor was recently reported, and an mRNA encoding the bradykinin B2 receptor was found to be present in the rat brain.

The function or functions of the brain kallikrein-kinin system remain to be defined, although a role for kinins in central cardiovascular regulation has been suggested by pharmacologic studies in which the administration of bradykinin into the central nervous system caused increases in blood pressure and heart rate. Intracerebroventricular administration of melittin, an activator of membrane-bound kallikrein, also was found to increase blood pressure and heart rate in the dog, and these effects occurred in association with elevated levels of immunoreactive kinins measured in the cerebrospinal fluid. In that study, concomitant administration of the kallikrein inhibitor aprotinin along with melittin reduced the pressor response to melittin and abolished the tachycardia. In addition, the melitin-induced pressor response in the rat was significantly attenuated by simultaneous intracerebroventricular infusion of a bradykinin receptor antagonist. Intracerebroventricular injection of kallikrein also increases blood pressure and brain kinin levels, and these responses are prevented by concomitant coadministration of aprotinin to block the activity of kallikrein. Moreover, the pressor response to afferent vagal stimulation is accompanied by increased kinin levels in cerebrospinal fluid.

Collectively, these findings suggest a possible linkage between endogenous brain kinin and the central regulation of the cardiovascular system.

The precise sites within the brain involved in the central cardiovascular actions of kinins are still to be defined. Both the lateral septal area and certain hypothalamic nuclei have been demonstrated to be sites at which kinins can act to produce changes in cardiovascular function. However, there is no information regarding the effects of bradykinin in the medullary regions of the brain, which are known to be important in the tonic and reflex control of arterial pressure. Consequently, in the present study we determined whether microinjection of bradykinin into the area of the rostral ventrolateral medulla (RVLM) containing the Cl group...
of epinephrine-containing cell bodies causes changes in blood pressure and heart rate and whether these bradykinin-induced cardiovascular responses can be blocked by the selective bradykinin B2 receptor antagonists D-Arg\(^6\), Hyp\(^7\), Thr\(^8\), D-Phe\(^9\)-bradykinin\(^{26}\) and D-Arg\(^6\) [Hyp\(^7\), Thr\(^8\), D-Tic\(^9\), Oic\(^{10}\)]-bradykinin (Hoe 140).\(^{27}\) The cardiovascular effects of D-Arg\(^6\) [Hyp\(^7\), Thr\(^8\), D-Phe\(^9\)]-bradykinin and Hoe 140 administered into the RVLM were also examined.

**Methods**

**Surgical Procedures**

Experiments were performed on 16- to 18-week-old male Sprague-Dawley (SD) rats, Wistar-Kyoto (WKY) rats, and spontaneously hypertensive rats (SHR) (Charles River Laboratories, Wilmington, Mass) weighing 250 to 350 g that were anesthetized with thiobutabarbital (Inactin, 120 mg/kg IP; BYK-Gulden, Konstanz, Germany). All rats were housed in facilities accredited by the American Association for Accreditation of Laboratory Animal Care, and all animal studies were approved by the Institutional Animal Care and Use Committee (David Kopf Instruments, Tujunga, Calif), with the bite bar set 12 mm below the interaural line. An occipital craniotomy was performed, and the atlanto-occipital membrane was cut to insert a femoral artery for recording of arterial pressure and a venous outflow for intravenous administration of drugs. The trachea was cannulated, and the animals were ventilated artificially on a Harvard respirator pump with 100% oxygen (90 breaths per minute, 2.5 to 3.0 mL per breath). The animals were then paralyzed with tubocurarine chloride (0.4 mg/kg SC) (ER Squibb, Princeton, NJ) and observed an increase in arterial pressure and tachycardia, and injecting 0.3 nmol clonidine, an a2-adrenergic receptor agonist, was selected because microinjection of the compound into the Cl area of the RVLM but not affecting fibers of passage, was selected because microinjection of the compound into the Cl area of the RVLM but not surrounding areas consistently elicits a pressor response and heart rate were recorded continuously, and body temperature was maintained between 37.0° and 37.5°C. Bradykinin B2 Receptor Antagonists Into the RVLM The head of the rat was mounted in a stereotaxic apparatus (David Kopf Instruments, Tujunga, Calif), with the bite bar set 12 mm below the interaural line. An occipital craniotomy was performed, and the atlanto-occipital membrane was cut to expose the most caudal portion of the fourth ventricle. The coordinates for the Cl area of the RVLM used in these experiments were 1.9 mm lateral to the midline, 2.9 to 3.1 mm below the floor of the fourth ventricle and were the same as those identified in previous studies. Animals were allowed to stabilize at least 30 minutes before the experiment was begun. At the end of each experiment, the injection site was verified pharmacologically to be in the Cl area of the RVLM. After a 30-minute stabilization period, 1 to 4 nmol of D-Arg\(^6\) [Hyp\(^7\), Thr\(^8\), D-Phe\(^9\)]-bradykinin (Stewart Ant, generously provided by Dr John Stewart, University of Colorado, Denver) or 1 nmol Hoe 140 (a gift from Hoechst AG, Frankfurt, Germany) was administered into the RVLM also was examined. Bradykinin and Clonidine Into the RVLM

**Experimental Protocols**

**Cardiovascular Effects of Microinjections of Bradykinin and Clonidine Into the RVLM**

SD and WKY rats and SHR were used for these experiments. After stabilization for 30 minutes, 50 pmol to 4 nmol bradykinin (Sigma) was injected unilaterally into the RVLM of the rat with not more than four doses being administered in any given experiment. Successive injections were given on alternate sides, with a 20-minute recovery time between injections. This protocol was used because it was found in pilot experiments that tachyphylaxis begins to occur when more than four doses are administered to the same rat. In a separate series of experiments, a single dose of 300 pmol clonidine was injected unilaterally into the RVLM of WKY rats (n=3) and SHR (n=3).

**Cardiovascular Effects of Microinjections of Selective Bradykinin B2 Receptor Antagonists Into the RVLM**

WKY rats and SHR were used in these experiments. After a 30-minute stabilization period, 1 to 4 nmol of D-Arg\(^6\) [Hyp\(^7\), Thr\(^8\), D-Phe\(^9\)]-bradykinin (Stewart Ant, generously provided by Dr John Stewart, University of Colorado, Denver) or 1 nmol Hoe 140 (a gift from Hoechst AG, Frankfurt, Germany) was administered unilaterally into the RVLM over a 2-minute period, and the cardiovascular effects of the drug were monitored for 60 minutes. Only one dose of each agent was studied in each animal.

**Effect of Selective Bradykinin B2 Receptor Antagonists on the Hypertensive Response to Bradykinin**

After a 30-minute stabilization period, Stewart Ant (2 to 4 nmol) or Hoe 140 (1 nmol) was injected unilaterally into the RVLM of an SHR or WKY rat. Ten minutes later, 500 pmol bradykinin was injected on the opposite side twice, with a 20-minute recovery period between injections. To test the effectiveness of the antagonist, we compared the hypertensive response to bradykinin injected on the same side of the antagonist with the response seen to the second dose of bradykinin on the opposite side.

**Data Analysis**

Statistical analysis of the data was done using Student's t test or an analysis of variance followed by post hoc comparisons using Scheffé's method or Bonferroni's t test. Differences were considered to be significant at a value of P≤0.05. Data are expressed as mean±SEM.

**Results**

**Cardiovascular Effects of Microinjections of Bradykinin, Glutamate, and Clonidine into the RVLM**

Initial mean arterial pressures in anesthetized SD rats (n=12), WKY rats (n=6), and SHR (n=9) were 99±4,
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117±6, and 188±3 mm Hg, respectively. Initial heart rates were 305±10 beats per minute in the SD rats, 298±12 beats per minute in WKY rats, and 314±15 beats per minute in SHR. Administration of vehicle (5% dextrose solution) alone into the RVLM did not significantly alter mean arterial pressure in SHR (−2±1 mm Hg) or WKY rats (2±2 mm Hg). Heart rate also was unaffected by injection of the vehicle. Unilateral microinjections of bradykinin into the RVLM in doses of 100 pmol to 4 nmol produced dose-dependent increases in mean arterial pressure in each of the groups tested (Fig 1). The dose-response curves for the hypertensive responses to bradykinin in the SD and WKY rats were essentially the same. In comparison, the hypertensive effect of bradykinin was significantly greater in the SHR than in either the SD or WKY rats (/>.001). The maximal response, however, was not significantly different among the groups (+21±3 in SD rats, +20±1 in WKY rats, and +23±2 mm Hg in SHR). Bradykinin was approximately four times more potent in the SHR than in the WKY or SD rats. The ED50 for the hypertensive effect was 0.95 and 0.82 nmol in SD and WKY rats, respectively, whereas the ED50 in SHR was 0.18 nmol. The dose-response curves for the heart rate responses to bradykinin administration were similar in all three rat groups (Fig 2).

In SHR, comparison of the pressor responses to 500 pmol bradykinin injected into the left versus right RVLM of the same animal demonstrated no difference in the magnitude of the pressor response (15±2 versus 17±4 mm Hg). Likewise, injection of a second dose of bradykinin into the RVLM on the same side produced the same pressor response to that seen with the first dose (17±5 versus 14±4 mm Hg).

Unilateral glutamate injection (1 nmol) into the RVLM at the conclusion of each experiment decreased mean arterial pressure in the WKY rats and SHR (15±2 versus 37±4 mm Hg, respectively, P<.001). Heart rate increased 14±1 and 21±4 beats per minute in WKY rats and SHR, respectively.

Unilateral injection of 300 pmol clonidine into the RVLM of WKY rats (n=3) decreased mean arterial pressure 15±6 mm Hg and reduced heart rate 13±4 beats per minute. In SHR (n=3), the same dose of clonidine produced a significantly greater decrease in blood pressure (−52±8 mm Hg, P<.02), whereas the bradycardic response (−24±4 beats per minute) was not significantly different (P>.1) from that observed in WKY rats.

Cardiovascular Effects of Microinjections of Selective Bradykinin B2 Antagonists into the RVLM

The effects of Stewart Ant or Hoe 140 administered into the RVLM on mean arterial pressure and heart rate were determined in both SHR and WKY rats. Unilateral microinjection of Stewart Ant produced dose-dependent decreases in mean arterial pressure and heart rate (Table). After 4 nmol of Stewart Ant, blood pressure decreased fairly rapidly, with the nadir being reached within 10 minutes of drug injection in both SHR and WKY rats. Heart rate decreased with a time course similar to that seen for blood pressure. The 4-nmol dose of Stewart Ant caused a maximal decrease in mean arterial pressure of 70±8 mm Hg (P<.001) in SHR.

Cardiovascular Responses to D-Arg°,Hyp1,Thl4,-β-Phe7-Bradykinin Microinjected Into the Rostral Ventrolateral Medulla of Study Rats

<table>
<thead>
<tr>
<th>Dose, nmol</th>
<th>SHR</th>
<th>WKY</th>
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<tbody>
<tr>
<td>1 (n=5)</td>
<td>−21±6*</td>
<td>−21±4*</td>
</tr>
<tr>
<td>2 (n=7)</td>
<td>−35±8*</td>
<td>−30±11</td>
</tr>
<tr>
<td>4 (n=10)</td>
<td>−70±8*</td>
<td>−49±9*</td>
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<tr>
<th>Dose, nmol</th>
<th>SHR</th>
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<tr>
<td>4 (n=6)</td>
<td>−12±4*</td>
<td>−30±12</td>
</tr>
</tbody>
</table>

MAP indicates mean arterial pressure; HR, heart rate; SHR, spontaneously hypertensive rats; and WKY, Wistar-Kyoto rats. Values are mean±SEM.

*P<.05.
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**FIG 3.** Plots show effects on mean arterial pressure and heart rate of microinjection of 4 nmol of the bradykinin antagonist D-Arg°.Hyp3,Thl8B,r>Phe7-bradykinin into the rostral ventrolateral medulla of spontaneously hypertensive rats (SHR, n=10) and Wistar-Kyoto (WKY, n=6) rats. *P*<.05 vs WKY rats. bpm indicates beats per minute.

SHR (n=10) and only 12±4 mm Hg (*P*<.05) in WKY rats (n=6). Heart rate decreased 49±9 beats per minute (*P*<.001) in SHR and 30±12 beats per minute (*P*<.05) in WKY rats. Before injection of 4 nmol of Stewart Ant, basal blood pressure was significantly higher in the SHR than in the WKY rats (183±3 versus 110±5 mm Hg, *P*<.05), whereas during maximal response to this kinin antagonist, pressure was not different between the two groups (112±9 versus 98±4 mm Hg in SHR and WKY rats, respectively) (Fig 3). Similarly, heart rate was significantly higher (*P*<.05) in the SHR (326±11 beats per minute) before administration of Stewart Ant, whereas heart rate at the height of the drug response was not significantly different between the two groups (277±11 versus 254±8 beats per minute in SHR and WKY rats, respectively). Sixty minutes after drug administration, blood pressure was still decreased in the SHR (*P*<.05) but not in the WKY rats.

Microinjection of the new selective bradykinin antagonist Hoe 140 (1 nmol) into the RVLM produced a rapid and prolonged decrease in blood pressure and heart rate (Fig 4). As observed with the Stewart compound, the hypotensive response to Hoe 140 was significantly greater in SHR (n=9) than WKY rats (n=9, *P*<.05). Mean arterial pressure decreased 51±7 (P<.001) and 17±3 mm Hg (P<.001) in SHR and WKY rats, respectively. Similarly, heart rate decreased 25±4 (P<.001) in SHR and 18±4 beats per minute (P<.001) in WKY rats.

**Effect of Selective Bradykinin B2 Antagonists on the Hypertensive Response to Bradykinin**

The microinjection of Stewart Ant in both WKY rats and SHR 10 minutes before the administration of bradykinin inhibited the hypertensive response to bradykinin. The hypertensive effect of 500 pmol bradykinin was reduced 65±5% (*P*<.001) after 4 nmol of Stewart Ant in SHR (n=9) and 50±16% (*P*<.05) in WKY rats (n=6, Fig 5). Lower doses of Stewart Ant were ineffective in inhibiting the pressor response to bradykinin.

Stewart Ant did not significantly alter the hypertensive response to angiotensin II. Unilateral injection of 500 pmol angiotensin II into the RVLM of SHR in-
The present study demonstrates that kinins can act in the RVLM to produce a centrally mediated hypertensive effect. Microinjection of bradykinin into the RVLM produced dose-dependent increases in mean arterial pressure in SD and WKY rats and SHR. In addition, the hypertensive responses to bradykinin were significantly reduced by prior treatment of the RVLM with the selective bradykinin B2 receptor antagonists D-Arg9,Hyp3,Thi5,8,D-Phe7-bradykinin19 and the newer, more potent Hoe 140 and Stewart Ant or Hoe 140 into the RVLM caused significantly greater reductions in mean arterial pressure and heart rate in SHR. Although the cardiovascular response to Stewart Ant was greater in SHR, the absolute levels of blood pressure and heart rate observed at the peak of the response to this antagonist in SHR were similar to those seen in the WKY rats. An increase in vascular responsiveness does not appear to be responsible for the observed enhanced blood pressure responses in SHR because pressor responses to intravenous norepinephrine and the α1-adrenergic agonist phenylephrine in SHR have been demonstrated to be similar in magnitude to those in WKY rats.33,35 On the other hand, enhanced cardiovascular responses to administration of the excitatory amino acid L-glutamate and α2-agonist clonidine in the RVLM also were observed in SHR. In addition, others have reported that microinjection of the cholinergic agents nicotine and
physostigmine into the RVLM also produces a more pronounced increase in blood pressure in SHR than WKY rats.37,38 Thus, there appears to be an increased responsiveness of neurons in the RVLM of SHR to a number of substances that act through different receptor mechanisms.

The mechanism for the increased sensitivity in the RVLM of SHR to the hypertensive action of bradykinin is not clear but might be related to reduced brain kininase activity39-40 or a dysfunction in central cardiovascular regulatory mechanisms in SHR.33 Heightened tonic sympathetic activity of central origin has been implicated as a major contributing factor in the initiation and maintenance of elevated blood pressure in SHR.38 The RVLM is a major integration site for sympathetic nervous activity in the central nervous system and is involved in tonic and reflex control of arterial pressure.39 This vasomotor area receives input from the nucleus of the solitary tract, the major site of termination of baroreceptor and cardiopulmonary afferent neurons, and neurons originating in this area project directly to the intermediolateral cell columns of the thoracic spinal cord where they innervate preganglionic sympathetic neurons.40-41 Electrical stimulation or pharmacologic activation of neurons in the RVLM with L-glutamate increases blood pressure, heart rate, and peripheral sympathetic nerve activity.40 In contrast, bilateral lesions of this area abolish the vasodepressor components of baroreceptor and cardiopulmonary reflexes and reduce blood pressure to levels comparable to those seen after spinal cord transection.42 The cardiovascular responses to bradykinin and bradykinin B2 antagonists administered locally into the RVLM suggest that activation of bradykinin B2 receptors in this area leads to excitation of neurons, resulting in an increased blood pressure and heart rate presumably due to increased central sympathetic outflow. In addition, the hypotension and bradycardia seen after Stewart Ant and Hoe 140 administration imply that there is tonic activation of bradykinin receptors by kinins in the RVLM, which are involved in the maintenance of blood pressure in the SHR.

The findings in the present study strongly suggest that a hyperactive kallikrein-kinin system in this brain region contributes to the maintenance of elevated blood pressure in the SHR. Madeddu et al43 reached a similar conclusion in their studies in which intracerebroventricular administration of Stewart Ant caused no changes in mean arterial pressure in normotensive SD, WKY, or Wistar rats43-45 or in SHR.43,44 However, heart rate of the SHR was reduced by acute cerebroventricular administration of Stewart Ant.44 These studies suggest that kinins present in circumventricular brain structures may not play a role in the regulation of blood pressure. It is possible that the lack of effect on blood pressure and heart rate of cerebroventriculically administered Stewart Ant may have been due to the inability of the compound to gain sufficient access to brain regions such as the RVLM in high enough concentrations to produce effective kinin receptor blockade. In our study, this problem was circumvented by the direct administration of the kinin antagonist into the RVLM. It is also conceivable that bradykinin B2 receptor blockade in the RVLM decreased blood pressure and heart rate in the present study because of a local overproduction of kinins in the region due to activation of plasma kallikrein caused by the glass micropipette used to administer drugs.47 Activation of the plasma kallikrein-kinin system by contact with glass usually leads to a maximal increase in plasma kinin levels within 2 to 5 minutes.47 However, placement of the tip of the glass micropipette in the RVLM was not associated with any cardiovascular changes, so it appears unlikely that this phenomenon played a role in the cardiovascular responses to RVLM administration of Stewart Ant and Hoe 140.

In conclusion, our data indicate that the RVLM is a site where kinins act to influence blood pressure and heart rate. The results support the hypothesis that there is a hyperactive kallikrein-kinin system in this brain region of the SHR that is important in the central regulation of blood pressure in this hypertensive model.

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