Centrally Infused Bradykinin Increases Baroreceptor Reflex Sensitivity

Viviani M.V. Gerken and Robson A.S. Santos

Kinins are present in the central nervous system, and central administration of bradykinin increases blood pressure and heart rate. In this study, we determined the effect of intracerebroventricular infusion of bradykinin on the baroreceptor reflex of conscious rats. Male Wistar rats were anesthetized with thiobarbital (40 mg/kg i.p.), and chronic intracerebroventricular cannulas (25 gauge) were implanted into the lateral ventricles. Baroreceptor control of heart rate was evaluated by recording reflex heart rate changes (beats per minute) in response to mean arterial pressure changes (mmHg) produced by bolus injection of phenylephrine (0.5–20 \( \mu \)g/kg i.v.) or sodium nitroprusside (0.5–25 \( \mu \)g/kg i.v.). The ratio beats per minute/mmHg or the mean slope of the individual regression lines of the relation between heart rate and mean arterial pressure changes for increases or decreases in arterial pressure was used as an index of baroreceptor reflex sensitivity. Baroreceptor control of heart rate was evaluated within 1 and 3 hours of intracerebroventricular infusion of bradykinin (7.5 \( \mu \)g/7 \( \mu \)l/hr) or saline (7 \( \mu \)l/hr). There was no change in basal mean arterial pressure or heart rate during central bradykinin infusion (112±2 mmHg and 402±18 beats per minute in the control period). After 1 hour of central bradykinin infusion, there was a significant increase of baroreceptor reflex sensitivity for increments in mean arterial pressure (-2.91±0.26 versus -1.5±0.24 beats per minute/mmHg in the control period; \( p<0.01 \), paired Student’s \( t \) test). In contrast, no significant changes were observed for the reflex tachycardia. Similar results were obtained with 3 hours of infusion. Intracerebroventricular infusion of saline or peripheral infusion of bradykinin at the same rate used for central infusion did not change baroreceptor reflex sensitivity. These results suggest a modulatory role for bradykinin, as a neurohormone, in the central control of the baroreceptor reflex. (Hypertension 1992;19[suppl II]:II-176–II-181)

There is increasing evidence that the kallikrein-kinin system participates in the central regulation of cardiovascular function.\(^1\)\(^–\)\(^3\) It has been shown that intracerebroventricular administration or microinjection of bradykinin in several brain sites alters blood pressure and heart rate (HR).\(^2\) In addition, components of the kallikrein-kinin system, necessary for the formation and metabolism of bradykinin and other related kinins, have been described in the brain\(^2\)\(^–\)\(^4\); maneuvers that increase the activity of the brain kallikrein-kinin system, such as central melittin administration, are associated with hypertension and tachycardia.\(^6\)\(^–\)\(^7\) These effects can be blocked by intracerebroventricular administration of a bradykinin antagonist.\(^7\) Pretreatment with a bradykinin antagonist also blocked the pressor effect produced by intracerebroventricular injection of high doses of captopril in spontaneously hypertensive rats.\(^8\)

The baroreceptor reflex control of HR participates in the moment-to-moment control of blood pressure, and it has been shown that this cardiovascular control loop is under peptidergic modulation, as demonstrated extensively for angiotensins.\(^9\)\(^–\)\(^11\) It has been reported recently that central administration of a bradykinin antagonist decreases HR in spontaneously hypertensive rats, suggesting a role for kinins in the central control of HR.\(^9\) There are no data, however, regarding the possible influence of brain or circulating kinins on the baroreceptor reflex control of HR. In this study, we investigated this possibility by determining the effect of intracerebroventricular and intravenous infusion of bradykinin on baroreceptor reflex sensitivity.

Methods

Animals

Experiments were performed in 34 normotensive, male Wistar rats weighing 230–270 g.
Intracerebroventricular Cannula Implantation

For intracerebroventricular cannula implantation, the animals were anesthetized with thiobarbital (40 mg/kg i.p.) and placed in a stereotaxic frame (David Kopf Instruments, Tujunga, Calif.) with the head in a horizontal position. Metallic cannulas were made with 25-gauge butterfly needles bent at right angles with one end connected to polyethylene tubing and the other end cut obliquely. The cannulas were inserted into the lateral ventricle (1.5 mm lateral, 1.0 mm posterior to the bregma and 4.5 mm below the skull) through a small hole drilled in the skull with a 22-gauge needle attached to the stereotaxic micro-manipulator. The cannulas were fixed to the skull with dental cement and a jeweler's screw. The polyethylene tubing, filled with sterile isotonic saline, was driven subcutaneously to the interscapular region and closed by a metallic pin. The total dead space of the cannula was measured before each experiment and averaged 2.5 μl. Postoperatively, the rats received a single dose of penicillin (60,000 IU i.m.; Pentobiótico Veterinario, Fontoura-Wyeth, São Paulo, Brazil). At the end of each experiment, proper cannula placement was verified by injection of Evans blue dye (5 μl).

Arterial Pressure Measurements

Twenty-four hours before the experiment, the animals were anesthetized with ether, and polyethylene catheters (PE-10 connected to a PE-50) were inserted into the abdominal aorta, through the femoral artery, and into the femoral vein. The catheters were tunneled under the skin to exit at the back of the animal. In some rats, an additional venous catheter was implanted in the other femoral vein for bradykinin infusion. Arterial pressure was monitored by a solid-state strain-gauge transducer (model TP-200T, Nihon Kohden, Tokyo), and HR was determined with a modified HR counter (model AT-601G, Nihon Kohden polygraph, São Paulo, Brazil). At the end of each experiment and averaged for statistical comparisons.

Baroreceptor Reflex Sensitivity

Five to seven days after intraventricular cannula implantation, baroreceptor reflex sensitivity was assessed using a technique similar to that described by Fletcher. To elicit reflex changes of HR in response to changes in mean arterial blood pressure (MAP), bolus intravenous injection of phenylephrine (0.5–20 μg/kg) or sodium nitroprusside (0.5–25 μg/kg) was given in 0.1 ml isotonic sodium chloride solution. Variable doses of each drug were given to produce changes in MAP ranging from 5 to 40 mm Hg. Sufficient time (1–2 minutes) was allowed between injections for HR and MAP to return to control levels. Peak changes in HR occurring during the initial 5 seconds of the corresponding maximum change in MAP were recorded. Baroreceptor reflex sensitivity was estimated in each rat by fitting a least-squares regression line for the relation between changes in HR (beats per minute) and MAP (mm Hg) for each data point obtained with graded injections of phenylephrine and nitroprusside. The slope of the line representing the relation (beats per minute/mm Hg) was used as an index of baroreceptor reflex sensitivity. In addition, values were expressed as the ΔHR/ΔMAP ratio. Individual measurements of reflex sensitivity were averaged for statistical comparisons.

Bradykinin Infusion

Bradykinin (Bachem Inc., Torrance, Calif.) was infused centrally at a rate of 7.5 μg/7 μl/hr (n=7). This infusion rate was chosen on the basis of preliminary experiments that showed that this was the minimum effective rate required to modify the baroreceptor reflex without a significant effect on basal MAP or HR. For peripheral infusion, the rates of 7.5 μg/0.7 ml/hr (n=8) and 50 μg/0.7 ml/hr (n=5) were used.

To determine the effect of bradykinin on baroreceptor reflex sensitivity, reflex HR responses were analyzed before and within 1 and 3 hours of central or peripheral infusion. In control groups, baroreceptor reflex sensitivity was evaluated before and within 1 and 3 hours of intracerebroventricular or within 1 hour of intravenous saline infusion.

Statistical Analysis

Results are expressed as mean±SEM. Data from baroreceptor reflex sensitivity were analyzed by paired Student’s t test (before versus 1-hour infusion) and two-way analysis of variance followed by Newman-Keuls test for differences among different time points. Data from MAP and HR measurements were analyzed by paired and unpaired t test. A value of p<0.05 was considered significant.

Results

Intracerebroventricular infusion of bradykinin (7.5 μg/7 μl/hr) did not change MAP or HR measured within 30 minutes and 3 hours of infusion (Table 1). The same was observed during intravenous infusion of the peptide or after intracerebroventricular or intravenous infusion of saline.

Table 2 shows the effect of intracerebroventricular infusion of bradykinin on baroreceptor reflex sensitivity. After 1 hour of infusion, there was a significant increase in baroreceptor reflex sensitivity for increases in MAP, represented by a significant shift to the left (p<0.05) in the regression line for the relation between changes in HR and MAP (Figure 1). As shown in Table 2, this change corresponded to a 90% increase in the mean slope of the individual regression lines for each animal (−2.9±0.26 versus −1.50±0.24 beats per minute/mm Hg before infusion; p<0.01). A similar increase in reflex HR responses to increases in MAP was observed after 3 hours of infusion. When the data were expressed as a ratio of changes in HR to changes in MAP, a similar increase was observed after 1 hour (−3.1±0.16 ver-
TABLE 1. Mean Arterial Pressure and Heart Rate in Rats During Intracerebroventricular or Intravenous Infusion of Bradykinin or Saline

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean arterial pressure (mm Hg)</th>
<th>Heart rate (bpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>30 min</td>
</tr>
<tr>
<td>ICV saline (n=8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.5 µg/hr</td>
<td>115±3</td>
<td>114±2</td>
</tr>
<tr>
<td>Intravenous saline (n=8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.5 µg/hr</td>
<td>112±2</td>
<td>116±4</td>
</tr>
<tr>
<td>50 µg/hr (n=5)</td>
<td>108±4</td>
<td>104±3</td>
</tr>
</tbody>
</table>

ICV, intracerebroventricular; bpm, beats per minute.

Sus -2.19±0.24 beats per minute/mm Hg before infusion; p<0.01 or 3 hours infusion (-2.88±0.22 beats per minute/mm Hg; p<0.01). In contrast, there was no significant change in reflex tachycardia evoked by administration of sodium nitroprusside when the data were expressed as a slope of the regression line (Table 2) or as a ratio of changes in HR to changes in MAP (-3.6±0.4 versus -2.93±0.25 and -3.43±0.22 beats per minute/mm Hg within 1 and 3 hours of bradykinin infusion, respectively). Intracerebroventricular infusion of saline did not change significantly either reflex bradycardia or tachycardia (Table 2).

In contrast to the results obtained with central infusion of bradykinin, peripheral administration of this peptide at the same rate (7.5 µg/hr) did not alter the slope of the ΔHR/ΔMAP regression for increments in MAP (-1.56±0.31 versus -1.42±0.2 beats per minute/mm Hg before infusion). The same tendency was observed when data were expressed as the ΔHR/ΔMAP ratio (-1.52±0.12 versus -1.61±0.12 beats per minute/mm Hg before infusion). Intravenous infusion of bradykinin also did not change the slope of the ΔHR/ΔMAP regression for decreases in MAP (Table 2). A similar result was obtained for the ΔHR/ΔMAP ratios (-3.48±0.45 versus -4.53±0.79 beats per minute/mm Hg before infusion). As observed for bradycardia, there was no change in reflex tachycardia after intravenous infusion of bradykinin at the lower rate (7.5 µg/hr, Table 2).

When bradykinin was infused intravenously at a rate sixfold higher than that used for intracerebroventricular infusion (50 µg/hr), there was a tendency toward an increase in the slope of the ΔHR/ΔMAP regression for increases in MAP (-2.84±1.0 versus -1.41±0.32 beats per minute/mm Hg before infusion). When the data were expressed as the ΔHR/ΔMAP ratio, there was a significant increase in reflex bradycardia (-2.51±0.19 versus -1.74±0.28 beats per minute/mm Hg before infusion; p<0.05). The higher dose of bradykinin also produced a significant reduction in the slope for decreases in MAP (-0.69±0.27 versus -4.42±1.59 beats per minute/mm Hg before infusion). However, when the data were expressed as the ΔHR/ΔMAP ratio, there was no change in reflex tachycardia (-3.98±0.3 versus -4.39±0.69 beats per minute/mm Hg before infusion). As shown in Table 2, intravenous infusion of saline did not change reflex bradycardia or reflex tachycardia.

TABLE 2. Mean Slopes of Individual Regression Lines for Phenylephrine-Induced Increases and Sodium Nitroprusside-Induced Decreases in Mean Arterial Pressure

<table>
<thead>
<tr>
<th>Treatment</th>
<th>PE-induced increases</th>
<th>NP-induced decreases</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>1 hr</td>
</tr>
<tr>
<td>Intracerebroventricular</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>-1.72±0.11</td>
<td>-2.4±0.41</td>
</tr>
<tr>
<td>Bradykinin (7.5 µg/hr)</td>
<td>-1.50±0.24</td>
<td>-2.91±0.26*</td>
</tr>
<tr>
<td>Intravenous</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>-1.26±0.19</td>
<td>-1.53±0.14</td>
</tr>
<tr>
<td>Bradykinin (7.5 µg/hr)</td>
<td>-1.42±0.20</td>
<td>-1.56±0.31</td>
</tr>
<tr>
<td>Bradykinin (30 µg/hr)</td>
<td>-1.41±0.32</td>
<td>-2.84±1.0</td>
</tr>
</tbody>
</table>

Values are mean±SEM and are shown as beats per minute per millimeter of mercury. PE, phenylephrine; NP, nitroprusside.

*p<0.01, t p<0.05.
Discussion

The major finding of our study is that intracerebroventricular infusion of bradykinin at a subpressor rate increased the sensitivity of the baroreceptor reflex control of HR for increments in MAP. In contrast, peripheral infusion of this peptide at the same rate used for intracerebroventricular administration did not change baroreceptor reflex sensitivity. These data provide further evidence that bradykinin acting as a neurohormone can participate in the central control of the cardiovascular system.

Several reports show that administration of bradykinin into the cerebral ventricles, as well as into discrete areas including hypothalamic nuclei, increases blood pressure and HR. It has been reported recently, however, that intracerebroventricular administration of a bradykinin antagonist does not change resting MAP in normotensive or hypertensive rats. These results have been interpreted as evidence that brain kinins present in circumventricular brain sites might not participate in the control mechanisms of blood pressure in basal conditions. Our present data, however, indicate that studies on the role of endogenous kinins in the control of blood pressure should include evaluation of the baroreceptor control of HR. In this regard, a possible role of brain kinins in the central control of HR was suggested by the observation that administration of a bradykinin antagonist into the cerebral ventricles reduced basal HR in spontaneously hypertensive rats.

It has been shown that intravenous infusion of phenylephrine can alter the arterial baroreceptor reflex control of renal nerves by a central effect. Thus, one might suggest that our data could be explained solely by a central action of phenylephrine rather than augmentation of baroreceptor reflex sensitivity by centrally infused bradykinin. There are at least two reasons why this is unlikely. First, the facilitatory effect of phenylephrine infusion is observed only with sustained (1–3-minute) increases in arterial pressure, whereas in our experiments using bolus injection of phenylephrine, only transient increases in arterial pressure were produced. Second, if a central facilitatory effect of phenylephrine were present, it should be noted in the saline-infused rats and in the animals infused intravenously with bradykinin. We observed, however, that there was no significant change in baroreceptor reflex sensitivity during central or peripheral saline infusion. In addition, in the rats infused intravenously with bradykinin, baroreceptor reflex sensitivity increased only in the group infused at a rate sixfold higher than that used for central infusion.

The increased sensitivity of the bradycardic component of the baroreceptor reflex after central bradykinin infusion cannot be attributed to changes in basal MAP or HR, because at the rate used, the nonapeptide did not change these parameters. Although the neural mechanisms by which bradykinin can modulate baroreceptor reflex sensitivity were not investigated in our study, several reports in the literature show that the central effects of bradykinin are complex and involve both the parasympathetic and sympathetic systems. Pharmacological studies have also indicated that the mechanism by which bradykinin alters blood pressure and HR differs with respect to the particular region in which it is administered. Bradykinin increases MAP and HR when microinjected into the posterior and dorsomedial hypothalamic nuclei mainly by inhibition of the parasympathetic system. In contrast, the increase in HR induced by microinjections of bradykinin into the anterior hypothalamic and medial preoptic nuclei is predominantly due to an increased activity of the cardiac sympathetic nerves. Increase in vagal tone leading to bradycardia occurs after bradykinin injection into the paraventricular nucleus. In our study, the reflex bradycardic responses were collected at time intervals (3–5 seconds after the peak in MAP change) corresponding mainly to an increased activity of the vagus nerve. Thus, it is likely that a parasympathetic mechanism was involved in the increased baroreceptor reflex sensitivity during intracerebroventricular infusion of bradykinin. Further studies are necessary to clarify this possibility.

We have observed that the reflex responses obtained with nitroprusside-induced decreases in MAP were not modified by central bradykinin infusion. Other authors have also described lack of alteration in reflex tachycardia after central or peripheral angiotensin II administration. A selective enhancement of the bradycardic component of the baroreceptor reflex was also reported after administration of angiotensin converting enzyme inhibitor to hypertensive rats. This selectivity may be related to the existence of multiple sites within the brain involved in regulating baroreceptor reflex control of parasympathetic outflow to the heart, as suggested by Reis.

![Graph](https://example.com/graph.png)
and Cuenod. It is possible that these sites can be reached or influenced differentially by bradykinin or angiotensins given by intraventricular or intravenous routes. It is also possible that the reflex responses to nitroprusside include excitation of chemoreceptors by cyanide produced from sodium nitroprusside. In addition, nitroprusside produces venodilatation, which influences the activity of low-pressure baroreceptors at the right atrium and ventricle. Thus, it is possible that the reflex response from chemoreceptors and low-pressure baroreceptors might mask the effect of bradykinin and angiotensins on the tachycardic component of the baroreceptor reflex.

The precise site of action for the facilitatory effect of bradykinin on the baroreceptor reflex after lateral ventricle infusion was not determined in our study. The pars ventralis of the lateral septal area, the ventral portion of the third ventricle, and sites in the brain stem close to the fourth ventricle have been identified as potential sites for the effects of bradykinin acting as a neurohormone. Further studies are necessary to determine whether these sites are also involved in the effects described in our study.

Studies using angiotensin converting enzyme inhibitors or stimuli such as melittin, vagal stimulation, and central vasopressin administration demonstrate that the endogenous brain kallikrein-kinin system can be activated and that kinins can reach sufficient concentration within the central nervous system to produce cardiovascular effects such as hypertension and tachycardia. Although we did not measure kinin levels in the cerebrospinal fluid, it is reasonable to suppose that during the infusion of a subpressor dose of bradykinin, the kinin concentration in the cerebrospinal fluid should be within the range achieved during activation of the brain kallikrein-kinin system or administration of angiotensin converting enzyme inhibitors.

There are several reports showing that angiotensin converting enzyme inhibitors increased baroreceptor reflex sensitivity in hypertensive animals and humans. These data have been interpreted as the result of diminished angiotensin II production in sites within the baroreceptor reflex arc. For example, we have shown that after a single intravenous dose of enalaprilat, there was a selective angiotensin converting enzyme inhibition in the nucleus tractus solitarii. Because kinins are a well-known substrate for angiotensin converting enzyme, our data can be taken as an alternative explanation for the beneficial effects of angiotensin converting enzyme inhibitors on baroreceptor reflex sensitivity. This intriguing possibility is currently under investigation in our laboratory.

In conclusion, our data show that bradykinin acting as a neurohormone can modulate baroreceptor reflex sensitivity. This observation suggests that in addition to the renin-angiotensin system, the brain kallikrein-kinin system participates in the central mechanisms involved in the control of the cardiovascular system.

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**Key Words** • bradykinin • kinins • baroreceptor reflex • kallikrein-kinin system • heart rate • hormones • baroreceptors • blood pressure
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