Brain Kinins Are Responsible For the Pressor Effect of Intracerebroventricular Captopril in Spontaneously Hypertensive Rats

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The role of the brain kallikrein-kinin system in the regulation of arterial blood pressure of normotensive and spontaneously hypertensive rats was evaluated. Intracerebroventricular administration of the kinin antagonist [DArg°]Hyp³-Thi⁵'8'[DPhè⁷] bradykinin caused no change in mean blood pressure in Wistar-Kyoto, Sprague-Dawley, or spontaneously hypertensive rats. The antagonist proved to be very potent in blocking the pressor effect of intracerebroventricular bradykinin (32±3 vs. 3±1 mm Hg, p<0.01). It was specific, as the pressor effect induced by other unrelated peptides was similar during the infusion of either vehicle or kinin antagonist (angiotensin II, 25±4 vs. 26±2 mm Hg; prostaglandin E₂, 48±3 vs. 47±8 mm Hg; norepinephrine, 17±2 vs. 18±2 mm Hg; leucine-enkephaline, 15±2 vs. 16±1 mm Hg; neurotensin, 18±2 vs. 19±1 mm Hg; substance P, 19±2 vs. 19±2 mm Hg). Intracerebroventricular administration of 1 mg captopril, an inhibitor of kininase II (one of the enzymes responsible for kinin degradation), caused no change in mean blood pressure in normotensive rats, whereas it increased mean blood pressure by 44±9 mm Hg (p<0.01) in spontaneously hypertensive rats. This increase in mean blood pressure was blocked and then reversed into a hypotensive effect (22±6 mm Hg, p<0.05) during the infusion of kinin antagonist. Our data suggest that the pressor effect induced by intracerebroventricular captopril is due to a transient elevation in endogenous brain kinin levels, supporting the hypothesis that the brain kallikrein-kinin system plays a role in the central regulation of blood pressure in spontaneously hypertensive rats. (Hypertension 1990;15:407-412)

Several studies have suggested a role for the brain kallikrein-kinin system in the central regulation of cardiovascular function. Administration of bradykinin into the cerebral ventricles increases blood pressure and heart rate in normotensive and spontaneously hypertensive rats (SHR). Recently, Yang et al found that intracerebroventricular administration of an analogue of bradykinin, which has antagonistic properties, does not affect blood pressure of awake normotensive and hypertensive rats but decreases heart rate. Thus, kinins present in circumventricular structures of the brain might not be implicated in the regulation of blood pressure in basal conditions; however, they might be involved in maintenance of a high basal heart rate in SHR.

The brain kallikrein-kinin system might have a role in the regulation of cardiovascular function in stimulated conditions. Indeed, both cerebrospinal fluid kinin levels and mean arterial pressure are increased by the stimulation of the afferent fibers of the vagi and by intracerebroventricularly injected melittin, a substance able to activate membrane-bound kallikrein. Yang et al have shown that the increase in blood pressure induced by melittin is partially inhibited by premedication with an antagonist of bradykinin, strongly suggesting a cause-effect relation between the enhancement of brain kinin levels and the increase in mean blood pressure.

Endogenous kinin levels can be increased not only by the stimulation of their release but also by the inhibition of the enzyme systems responsible for their degradation. In SHR, intracerebroventricular administration of inhibitors of angiotensin converting enzyme (ACE), also called kininase II, exerts a long-lasting hypotension, an effect that was attributed to the blockade of angiotensin II formation. Unger et al have found that this hypotensive effect is preceded by an increase in blood pressure, when doses high enough to completely block ACE are...
used. They speculated that this hypertensive effect might be due to enhancement of endogenous kinin levels. However, because of the broad substrate specificity of ACE,26 the pressor effect of intracerebroventricularly administered ACE inhibitors might be attributable to the enhanced activity of other pressor peptides such as enkephalins, neurotensin, and substance P, which are potential substrates for ACE.

Our study was designed to expand and further detail the observation of the hypertensive effect induced by intracerebroventricularly administered captopril.21,22 We used a potent and specific antagonist of bradykinin to evaluate whether the effect induced by captopril is attributable to the enhancement of endogenous kinin levels.

Methods

Materials

Bradykinin, angiotensin II, prostaglandin E₂, nor-epinephrine, substance P, neurotensin, and leucine-enkephaline were purchased from Peninsula Laboratories (Merseyside, England); the kinin antagonist [d-Arg]Arg-Pro-Hyp-Gly-Thi-Ser[pPhe]Thr-Arg-TFA (Hyp, L-4-hydroxyproline; Thi, P-2-thienyl-L-alanine; TFA, trifluoroacetic acid) was purchased from Bachem (Torrance, California), and captopril was purchased from Squibb (Rome, Italy). These compounds were dissolved in artificial cerebrospinal fluid (Elliot's B solution).

Surgical Procedures

Male Sprague-Dawley (SD), Wistar-Kyoto (WKY) rats, and SHR (from Charles River, Milan, Italy) that weighed 300–320 g were housed at constant room temperature with a 12-hour light/dark cycle and had free access to water and rat chow. After 2 or 3 days of adjustment to the new environment, the rats were instrumented according to the following procedure. Under pentobarbital anesthesia (50 mg/kg i.p.), a 22 gauge stainless steel cannula (15 mm in length, bent at a 90° angle at midpoint) was implanted stereotaxically27 into each lateral cerebral ventricle (1.5 mm lateral, 1.0 mm posterior to bregma, and 4.5 mm deep from skull surface). The cannula was anchored to the skull with screws embedded in dental acrylic cement. The free end of the cannula was attached to a silastic tube filled with Elliot's B solution. The silastic tube was tunneled under the skin, exteriorized between the scapulae, and occluded with a metal pin. After 5 days, the correct placement of the cannulas in the ventricles was tested by determining the dipsogenic response to angiotensin II.28 On the morning of the following day, the rats were anesthetized with ether and a polyethylene catheter (PE-10 connected to a PE-50, Clay Adams, Parsippany, New Jersey) filled with heparinized saline was passed into the thoracic aorta via the left femoral artery; the catheter was then tunneled under the skin and brought out of the back of the neck.

Experiments were performed the following morning. Awake rats were placed in plastic restrainers. Infusions were made with a Harvard pump (Millis, Massachusetts). Mean blood pressure was continuously measured via the femoral catheter with a Statham transducer (Gould, Oxnard, California) and recorded on a S&W recorder (Medico Teknik, Albertslund, Denmark).

Experimental Protocol

Protocol 1: Specificity of the kinin antagonist. WKY rats were used for this experiment. Blood pressure was allowed to stabilize for at least 30 minutes. Bradykinin (0.5 µg), angiotensin II (40 ng), prostaglandin E₂ (0.5 µg), norepinephrine (1 µg), leucine-enkephaline (20 µg), neurotensin (10 µg), substance P (20 µg), or Elliot's B (n=6 in each group) were injected intracerebroventricularly (10 µl). The same protocol was performed in rats (n=6 in each group) receiving a 30-minute infusion of kinin antagonist (20 µg/hr). This dose of kinin antagonist was chosen because its effectiveness in blocking the cardiovascular effect of intracerebroventricular bradykinin was demonstrated by Yang et al14 previously.

Protocol 2: Effect of the kinin antagonist on mean blood pressure in normotensive and hypertensive rats. SD rats, WKY rats, and SHR were used. After stabilization for 30 minutes, the antagonist (20 µg/hr) or Elliot's B (20 µl) were intracerebroventricularly infused for 1 hour (n=5 in each group). To test the effectiveness of the antagonist, bradykinin (0.5 µg, 10 µl) was injected into the opposite lateral ventricle before halting the infusion of either Elliot's B or kinin antagonist.

Protocol 3: Effect of intracerebroventricular captopril on mean blood pressure in Wistar-Kyoto rats. After stabilization for 30 minutes, either Elliot's B (n=7) or captopril (10 µl) were intracerebroventricularly injected. Three doses of captopril (0.1, 0.5, and 1 mg; n=6, 7, and 7, respectively) were tested. To evaluate the ability of the different doses to inhibit kinin degradation, bradykinin (0.5 µg, 10 µl) was intracerebroventricularly injected 30 minutes after the administration of either captopril or Elliot's B.

Protocol 4: Effect of the kinin antagonist on the changes in mean blood pressure induced by captopril in Sprague-Dawley rats and SHR. After stabilization for 30 minutes, either Elliot's B (SD rats, n=7; SHR, n=7) or kinin antagonist (20 µg/hr) (SD, n=7; SHR, n=7) were intracerebroventricularly infused (20 µl). Captopril (1 mg, 10 µl) was injected into the opposite ventricle 5 minutes after the infusion was started. Another group (SD, n=4; SHR, n=4), which was infused with Elliot's B, was injected with captopril vehicle (adjusted to the same pH and osmolarity of captopril solution).

Statistical Analysis

The results were expressed as mean±SEM. Statistical analysis was done by analysis of variance with and without repeated measurements.
TABLE 1. Effect of Intracerebroventricular Captopril and Elliot's B on Mean Blood Pressure in Wistar-Kyoto Rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Time (min)</th>
<th>Time (min)</th>
<th>Time (min)</th>
<th>Time (min)</th>
<th>Time (min)</th>
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</thead>
<tbody>
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<td>0</td>
<td>2</td>
<td>4</td>
<td>8</td>
<td>16</td>
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<tr>
<td>Elliot's B</td>
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<td>118±5</td>
<td>118±6</td>
<td>112±6</td>
<td>113±5</td>
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<td>105±2</td>
</tr>
<tr>
<td></td>
<td>0.5 mg</td>
<td>111±4</td>
<td>111±5</td>
<td>107±6</td>
<td>102±6</td>
</tr>
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<td>1.0 mg</td>
<td>119±2</td>
<td>122±4</td>
<td>118±4</td>
<td>12±3</td>
</tr>
</tbody>
</table>

Results

Protocol 1: Specificity of Kinin Antagonist

The kinin antagonist was able to block the pressor effect of intracerebroventricularly injected bradykinin (Elliot's B, 32±3; kinin antagonist, 3±1 mm Hg; p<0.01). It was specific because the pressor effect of other unrelated peptides was similar during the infusion of Elliot's B or antagonist (angiotensin II, 25±4 vs. 26±2 mm Hg; proaspartaglandin E2, 48±3 vs. 47±8 mm Hg; norepinephrine, 17±2 vs. 18±2 mm Hg; leucine-enkephaline, 15±2 vs. 16±1 mm Hg; neurotensin, 18±2 vs. 19±1; substance P, 19±2 vs. 19±2 mm Hg).

Protocol 2: Effect of Kinin Antagonist on Mean Blood Pressure in Normotensive and Hypertensive Rats

Central infusion of the antagonist caused no change in mean blood pressure in WKY rats (from 112±3 to 113±2 mm Hg), SD rats (from 118±3 to 120±4 mm Hg), or SHR (from 165±2 to 166±3 mm Hg). The antagonist inhibited the pressor effect of intracerebroventricularly administered bradykinin by 95% in WKY rats, 93% in SD rats, and 96% in SHR (p<0.01). Intracerebroventricular infusion of Elliot's B had no effect on mean blood pressure.

Protocol 3: Effect of Intracerebroventricularly Administered Captopril on Mean Blood Pressure in Wistar-Kyoto Rats

Table 1 shows that neither captopril nor vehicle had any effect on mean blood pressure. Central injection of bradykinin (0.5 μg) induced a significant increase in mean blood pressure of rats given Elliot's B (28±3 mm Hg, p<0.01). This pressor effect was enhanced in rats receiving 0.1 and 0.5 mg captopril (40±2 and 45±3 mm Hg, p<0.05 as compared with controls) and even more in rats receiving 1.0 mg (60±2 mm Hg, p<0.05 as compared with 0.1 and 0.5 mg captopril).

Protocol 4: Effect of Kinin Antagonist on Changes in Mean Blood Pressure Induced By Captopril in Sprague-Dawley Rats and Spontaneously Hypertensive Rats

In SD rats, intracerebroventricular captopril caused no change in mean blood pressure when injected during the infusion of the antagonist (Figure 1). In SHR, captopril (1 mg) induced a significant increase in mean blood pressure, which peaked at the second minute (44±9 mm Hg, p<0.01) and lasted for 16 minutes (Figure 2). The pressor effect induced by captopril was prevented and then reversed by intracerebroventricular infusion of the kinin antagonist into a vasodepressor effect (22±6 mm Hg at the 16th minute, p<0.05). No change in mean blood pressure was observed after the injection of captopril vehicle, which had the same pH and osmolarity of captopril solution, either in SD rats or SHR.

Discussion

In the present study, we found that a newly synthesized kinin antagonist is very potent in inhibiting the pressor effect of intracerebroventricularly administered bradykinin. It also proved to be very specific, because it did not affect the pressor action of other unrelated peptides. The antagonist did not alter mean blood pressure in normotensive and hypertensive rats in basal condition. In contrast, it was able to block the pressor effect induced in SHR by captopril, an inhibitor of one of the enzymes responsible for kinin degradation.
The hypothesis of a functional brain kallikrein-kinin system is mainly derived from pharmacological studies in which bradykinin administration into the central nervous system affected behavior,29 thermoregulation,30 and renal11 and cardiovascular function.9-11 The recent synthesis of receptor antagonists of bradykinin has offered a new and more direct tool to study the physiological role of endogenous kinins.13-17 Intracerebroventricular administration of the antagonist [DArg]Arg-Pro-Hyp-Gly-Thi-Ser[DPhe]Thr-Arg-TFA, at a dose that significantly inhibited the pressor effect of exogenous bradykinin, did not cause any effect on mean blood pressure in normotensive and hypertensive rats.13 These results, which were confirmed in our study, imply that brain kinins located in circumventricular brain structures might not be involved in the regulation of blood pressure in basal conditions. On the other hand, the previously mentioned findings cannot rule out either a physiological role for kinins in brain regions not accessible to the antagonist or a possible influence of the brain kallikrein-kinin system on blood pressure in stimulated conditions. Indeed, either the stimulation of the afferent fibers of the vagi15 or intracerebroventricular infusion of melittin,16,17 a peptide that induces release of membrane-bound kallikrein from basolateral membranes isolated from kidneys,18 increases mean blood pressure and cerebrospinal fluid kinin levels in dogs and rats. Yang et al17 have suggested that there is a causal relation between the increase in endogenous kinin levels and the pressor effect of melittin, as the latter was blocked by the intracerebroventricular administration of a receptor antagonist of kinins. In theory, endogenous kinin activity can be enhanced not only by the release of membrane-bound kallikrein, the enzyme responsible for kinin formation, but also by the inhibition of kinin degradation. Several enzymes from neural tissue sources are capable of metabolizing kinins.7,8,19 Kininases I and II cleave the C terminal and an aminopeptidase removes the N terminal of metlys-bradykinin, lys-bradykinin, and bradykinin. The action of brain kininases is very rapid, as suggested by the relatively high pressor threshold dose and the short half-life of intracerebroventricularly administered bradykinin. Kinin degradation might be crucial in the regulation of the activity of this peptidergic system. Kininase inhibitors lessen the disappearance rate of intracerebroventricularly administered bradykinin,12 and they might also prolong the effect of endogenously produced kinins. Unger et al11 found that intracerebroventricularly administered captopril, at doses high enough to completely block ACE, increases blood pressure in SHR. This finding was confirmed in our study. The most attractive explanation is that the inhibition of kininase II leads to accumulation of kinins in the brain responsible for the increase in blood pressure. However, the possibility that this is a pharmacological high-dose effect without relevance to the drug’s mechanism of action had to be ruled out. Unspecific pressor effects, due to the acidity or osmolality of the drug solution injected, were already excluded.21 Intracerebroventricular administration of MK 422, a nonsulfhydryl kininase II, has a similar pressor effect in SHR;22 thus, the sulfhydrylic group is not responsible for the increase in mean blood pressure induced by captopril. It seems unlikely that 1 mg captopril induces hypertension as the result of its leakage from the cerebroventricular space into the blood because peripheral administration of a similar dose decreases blood pressure of SHR.21

In addition to being responsible for kinin degradation, kininase II cleaves several other peptides26 known to exert a hypertensive effect when administered by the intracerebroventricular route.32-34 Consequently, captopril might have induced an increase in blood pressure by enhancing the levels of peptides such as enkephalins, neuropeptide, and substance P. To exclude this possibility, we used an antagonist of bradykinin that proved very potent in blocking the pressor effect of intracerebroventricularly administered bradykinin. It was also specific as it caused no change in the hypertensive action of angiotensin II, prostaglandin E2, norepinephrine, substance P, neuropeptide, or leucine-enkephaline. This antagonist blocked the increase in blood pressure induced by captopril in SHR and then reversed it in a hypertensive effect, thus supporting the possibility that this pressor action was determined by the accumulation of endogenous kinins in the brain. It is unlikely that penetration of the kinin antagonist from cerebroventricular space into the circulation induced peripheral effects able to block the increase in mean blood pressure induced by intracerebroventricularly administered captopril. Indeed, intra-arterial infusion of the kinin antagonist, at doses much higher (30 μg/kg/min) than those used in the present experiment by the intracerebroventricular route, had no effect on
mean blood pressure in normotensive and hypertensive rats (personal observations).

The finding that a high dose of captopril is required to elicit pressor effects in SHR deserves some explanation. Inhibition of brain kininase II can be obtained with captopril even at a dose as low as 5 μg; however, this dose has no pressor effect in SHR. The inhibition of kininase II achieved with 5 μg is not complete because the pressor effect of intracerebroventricularly administered angiotensin I is inhibited by 50% 5 minutes after captopril injection and completely restored after 60 minutes. A higher dose of captopril (500 μg), able to completely inhibit the activity of kininase II, induced an increase in mean blood pressure of SHR. Thus, a complete inhibition of kininase II might be necessary to enhance endogenous kinins to levels high enough to exert pressor effects. This interpretation appears to be consistent with the finding that 1 mg captopril is more effective than lower doses in enhancing the pressor effect of intracerebroventricularly administered bradykinin. It has been reported that centrally administered bradykinin, when injected after captopril administration, can exert vasodepressor instead of pressor effects. The doses used in those studies were 10–20 times higher than those used by us. At high doses, the peptide may leak into the circulation, thus inducing vasodilation. This effect might have masked the central pressor action of bradykinin.

Our finding that captopril induces a pressor effect in SHR but not in WKY rats is only partially consistent with previous reports showing that kininase II inhibition increases mean blood pressure both in hypertensive and normotensive rats. This discrepancy might be because of strain differences, thus raising the question of whether WKY rats are really an appropriate control for SHR. In our study, SD rats, another normotensive strain, responded to intracerebroventricularly administered captopril similar to WKY rats. The different response to kininase II inhibition of genetic hypertensive compared with normotensive rats might be caused by a greater sensitivity to kinins, attributed to a dysfunction of the central mechanisms for cardiovascular regulation, or to a markedly reduced kininase activity. Consistently, inhibition of brain kinins is able to reduce the basal high heart rate of SHR but has no effect in WKY rats. A major difference of kallikrein-binding protein in SHR versus normotensive rats has been found in the brain, which might result in possible differences in modulation of kallikrein activity, delivery, and degradation. All these findings support the hypothesis that the brain kallikrein-kinin system might be involved in the biochemical alterations leading to high blood pressure and heart rate levels in SHR.

We found that the pressor effect induced by intracerebroventricularly administered captopril in SHR was not only blocked but reversed into a hypotensive effect by kinin antagonist. This is consistent with previous reports showing that inhibition of ACE induces long-lasting hypotension in SHR. Blockade of angiotensin II formation in the brain has been claimed as one of the possible mechanisms for the acute and chronic antihypertensive action of captopril and related drugs.

In conclusion, our data suggest that inhibition of brain kinin degradation can increase blood pressure in SHR and also support the hypothesis that both the renin-angiotensin and kallikrein-kinin systems might have a role in the central control of blood pressure in this hypertensive model.

References


**KEY WORDS** • kallikrein-kinin system • renin-angiotensin system • central nervous system • kinin antagonist • captopril
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