Vasodepressor Role of Endogenous Bradykinin Assessed by a Bradykinin Antagonist

ATHANASSIOS BENETOS, HARALAMBOS GAVRAS, JOHN M. STEWART, RAYMOND J. VAVREK, SIMON HATINOGLOU, AND IRENE GAVRAS

SUMMARY This study was designed to examine the contribution of bradykinin to the depressor effect of different antihypertensive drugs in two-kidney renovascular hypertensive rats, using a new specific antagonist of bradykinin. First, the inhibitory capacity of this peptide for exogenously injected bradykinin (75–200 ng) was tested. An inhibition of the vasodepressor action of bradykinin by over 50% was found when the bradykinin inhibitor was infused at a rate of 40 /xg/min, with little difference at higher rates of infusion. This inhibitor then was infused in three groups of renovascular hypertensive rats after their blood pressure had been decreased by pretreatment with the converting enzyme inhibitor enalapril (MK 421), saralasin, or sodium nitroprusside, respectively. Infusion of the inhibitor produced an immediate 30% increase in blood pressure only in the enalapril-treated group. These results indicate that bradykinin is involved in the decrease of blood pressure produced by converting enzyme inhibition in experimental renovascular hypertension. (Hypertension 8: 000-000, 1986)

KEY WORDS • experimental renovascular hypertension • converting enzyme inhibitor • saralasin • sodium nitroprusside

ALTHOUGH bradykinin (BK) is considered to be a powerful vasodilator,1 its contribution to blood pressure regulation remains obscure. Exogenous BK injected systemically causes a decrease in blood pressure,2 but it is rapidly inactivated after passage through the pulmonary vascular bed.3 Following the discovery of a BK-potentiating factor by Ferreira,4 it was demonstrated that the kininase II degrading BK in the lung and the angiotensin converting enzyme (ACE) transforming the inactive angiotensin I into the biologically active angiotensin II are in fact identical5 and that both effects are inhibited by the same factor.6 Theoretically, both actions should contribute to the hypotensive effect of various ACE inhibitors, yet only the elimination of angiotensin II has been proved beyond doubt. The actual accumulation of BK remains controversial,7,8 and its role is mostly based on indirect evidence.9-12

In several instances, the action of a vasoactive hormone has been demonstrated by the use of a specific competitive antagonist of that hormone at the vascular receptor level; examples include saralasin to determine the pressor effect of angiotensin II13 and V, antagonists to prove the pressor effect of vasopressin.14 In this study, we used a competitive antagonist of BK, the compound B4146 (synthesized by J.M.S. and R.J.V.), to prove that the depressor effect of ACE inhibition is indeed partly due to BK. The inhibitory capacity of this agent has been demonstrated in vitro on vascular, uterine, and ileum smooth muscle.15,16

Materials and Methods

The first part of the study consisted of a series of experiments designed to test the inhibitory capacity of the BK inhibitor B4146 (Arg-Pro-Hyp-Gly-Thi-Phe-Thi-Arg.TFA; Hyp = L^-hydroxyproline; Thi = 0-(2-thienyl)-l-alanine; TFA = trifluoroacetic acid). To this aim, nine male Wistar rats (Charles River Breeding Laboratories, Wilmington, MA, USA) weighing 250 to 350 g at the time of the study were used. One day before the experiment, the right external iliac artery and the right femoral vein were cannulated with PE-50 catheters for direct arterial pressure recording and drug infusions, respectively. On the day of the experiment, the unanesthetized, unrestrained rats were pretreated with the converting enzyme inhibitor ena-
pril (MK-421), 1 mg/kg i.v. Thirty to 60 minutes later, when a stable blood pressure was obtained, a series of intra-arterial injections of BK was given over a period of 20 minutes while blood pressure was continuously recorded on a Hewlett-Packard recorder (Model 77028; Lexington, MA, USA). Three different doses (75, 120, and 200 ng) were used, and each was given in triplicate at 5-minute intervals. The volume of each injection was 0.2 ml. The same procedure was repeated during a continuous infusion of the BK inhibitor (40 μg/min i.v. over 20 minutes). Thirty minutes after the end of the BK inhibitor infusion, the same series of BK injections were repeated for a total of 27 injections in each rat. The dose of 40 μg/min was chosen after pilot experiments showed that a dose of 20 μg/min produced inhibition consistently lower than 50% whereas a dose of 80 μg/min produced results essentially similar to those of the 40 μg/min dose in the same rats.

In the second part of the experiment, renovascular hypertensive Wistar rats (two-kidney, one clip) weighing 250 to 350 g were used. One day before the experiment, all rats were cannulated with three PE-50 catheters (iliac artery, femoral, and jugular veins). On the day of the experiment, the rats were maintained unanesthetized and unrestrained in a glass cage. Blood pressure was recorded for 30 to 60 minutes, and only rats showing a mean arterial pressure of greater than 150 mm Hg were included in the protocol.

All drugs used in this study were dissolved in 5% dextrose. They included enalapril (Merck Sharp & Dohme, West Point, PA, USA), 1 mg/ml; saralasin (Eaton Laboratories, Norwich, NY, USA), 200 μg/ml; sodium nitroprusside, 600 μg/ml; and B4146, the BK inhibitor, 200 μg/ml. This peptide was synthesized in a manner analogous to that described for other BK antagonists.

Group 1 rats (n = 19) were pretreated with enalapril, 1 mg/kg i.v. A stable blood pressure was obtained after 30 to 90 minutes. At that time, 13 rats received a continuous intravenous infusion of BK inhibitor (40 μg/min) over a period of 10 minutes and six received the same volume (0.2 ml/min) of 5% dextrose.

In Group 2 (n = 13), saralasin, 10 μg/kg/min i.v., was continuously infused. Twenty to 30 minutes after initiation of the infusion, when a stable blood pressure was obtained, the continuous infusion of BK inhibitor (n = 10) or 5% dextrose (n = 3) was started as in Group 1. Saralasin was infused continuously throughout the infusion of BK inhibitor or dextrose and for 30 minutes after the end of the latter infusion. The next day, seven of these rats received the same treatment as in Group 1 (enalapril followed by BK inhibitor).

Group 3 rats (n = 9) were pretreated with sodium nitroprusside (10 μg/min i.v., continuous infusion) to obtain the same decrease in blood pressure as in Group 1. After blood pressure stabilized, infusion of the BK inhibitor (n = 6) or 5% dextrose (n = 3) was started as in the other groups.

Animal handling and experimental procedures followed were in accordance with institutional regulations.

All results were expressed as means ± SEM. Statistical comparisons were made by one-way analysis of variance followed whenever F was significant by unpaired or paired t test with the Bonferroni correction. Correlations were calculated by linear regression analysis. A p value of less than 0.05 was considered significant.

Results

Table 1 shows the inhibitory effect of the BK inhibitor B4146, 40 μg/min, on the depressor effect of BK. The average degree of inhibition was over 50%. The specificity of BK inhibition by this compound was demonstrated by the lack of prevention of hypotension induced by other vasodilators (e.g., sodium nitroprusside).

Figure 1 summarizes the results of the second part of the study. Although baseline blood pressures tended to be somewhat lower in Group 1 rats, they were not significantly different from those in the other two groups. In Group 1, blood pressure fell by 41 ± 5 mm Hg after enalapril administration (from 168 ± 6 to 127 ± 5 mm Hg; p < 0.001). Infusion of the BK inhibitor caused an immediate 8 mm Hg increase in blood pressure in seven out of the 13 rats (mean, 12 ± 3 mm Hg), whereas no change in blood pressure was observed in the six remaining rats. For the group as a whole, this change after the BK inhibitor was statistically significant (p < 0.01). No correlation was found between the vasopressor effect of the BK inhibitor and the baseline blood pressure level or the decrease of the arterial pressure in response to enalapril.

In Group 2, the blood pressure decreased by 25 ± 6 mm Hg after the saralasin infusion (from 181 ± 6 to 156 ± 7 mm Hg; p < 0.001). This fall tended to be smaller than that produced by enalapril in Group 1, but the difference did not attain statistical significance. Infusion of the BK inhibitor caused no change in the arterial pressure. Seven of these rats were used again the next day, but this time received the same treatment as in Group 1. Administration of enalapril caused a fall in blood pressure similar to that observed in the first group (from 179 ± 8 to 139 ± 6 mm Hg; p < 0.001). Blood pressure increased in five out of seven of these animals after the infusion of the BK inhibitor. The

<table>
<thead>
<tr>
<th>Dose of bradykinin (μg)</th>
<th>Change in blood pressure (mm Hg)</th>
<th>% inhibition</th>
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<tbody>
<tr>
<td>With B4146</td>
<td>Without B4146</td>
<td></td>
</tr>
<tr>
<td>75</td>
<td>-14.0 ± 1.3</td>
<td>-7.9 ± 1.1*</td>
</tr>
<tr>
<td>120</td>
<td>-15.7 ± 1.3</td>
<td>-6.9 ± 0.9*</td>
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<tr>
<td>200</td>
<td>-18.1 ± 0.9</td>
<td>-8.2 ± 0.7*</td>
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Values are means ± SEM of 27 studies (i.e., rats injected in triplicate).
*p < 0.001, compared with values with no B4146 administered.
mean arterial pressure of the whole group increased from 138 ± 6 to 147 ± 6 mm Hg (p < 0.01). Again, no correlation was found between the response to the BK inhibitor and the baseline blood pressure level or the depressor effect of either saralasin or enalapril.

In Group 3, continuous infusion of sodium nitroprusside decreased blood pressure from 183 ± 7 to 128 ± 8 mm Hg (p < 0.001). None of these animals had a change in arterial blood pressure in response to the BK inhibitor. In the control rats of each group (i.e., those rats that, after the original antihypertensive treatment, received a continuous infusion of 5% dextrose instead of the BK inhibitor), there was no further change in blood pressure.

Discussion

It is now well established that the ACE is identical to kininase II, which is largely responsible for the inactivation of BK. As mentioned earlier, few studies have addressed the effects of ACE inhibition on BK, and their results are contradictory. For example, some studies reported an increase in BK levels with teprotide but not with captopril, while others reported an increase with captopril as well. Some of these discrepancies may be due to methodological differences in measuring BK levels. Moreover, it is possible that plasma levels of BK may not reflect concentration of this vasoactive peptide on vascular receptors. Studies using the potentiation of the depressor effect of exogenous BK as an end point have given more uniformly positive results, suggesting that the discrepancies between reports of plasma BK levels may indeed be due to methodological causes.

The key modification that converts BK agonists to BK antagonists appears to be the substitution of the proline at position 7 of BK with α-phenylalanine, whereas other modifications increase the antagonist’s potency. In this study, we had the opportunity to use a newly synthesized specific BK antagonist, designated here as B4146, which has been shown to be a potent antagonist of the activity of BK on smooth muscle. The ability of this compound to inhibit the myotropic activity of BK and two BK homologues (Met-Lys-BK and Lys-BK) has been tested on guinea pig ileum and compared with its effect on angiotensin II and substance P. In each case the BK antagonist inhibited the action of BK-related kinins but had no effect on angiotensin II or substance P. In addition, B4146 had a weak BK-like agonistic activity on the rat uterus and on the rat blood pressure (1–4% agonist potency relative to BK) with moderate resistance to the enzymatic breakdown of the kininas. In the preliminary studies, we determined that this compound achieved a 50 to 60% inhibition of the depressor effect of exogenous bradykinin when the dose ratio of BK inhibitor to BK was about 200. The action of this inhibitor was short and lasted for about 5 minutes after the end of a continuous infusion. Doubling the dose of the BK inhibitor did not produce a significantly greater degree of BK inhibition. In pilot studies, we have also determined that the BK inhibitor per se at these doses in normal rats with or without enalapril pretreatment had no vasopressor effect.

In the second part of the study, the BK inhibitor was used in rats with two-kidney, one clip renovascular hypertension. The BK system has been reported to be activated or suppressed in this type of experimental hypertension. Treatment with enalapril produced, as expected, a substantial decrease in blood pressure to normal or near-normal levels. Sodium nitroprusside was given at a dose calculated to produce the same degree of blood pressure lowering. Interestingly, treatment with saralasin tended to produce a smaller blood pressure fall. This finding is in agreement with earlier reports that attributed this difference to the presumed BK-potentiating action of the ACE inhibitor. Indeed, when the BK inhibitor was infused in the enalapril-pretreated rats, more than half of these rats showed an increase in blood pressure equal to about 30% of the initial drop induced by enalapril. This response can be considered the BK-dependent component of the blood pressure fall produced by ACE inhibition, the remaining 70% being attributable to the elimination of the vasoconstrictor action of angiotensin II. Conversely, in rats pretreated with saralasin or sodium nitroprusside, there was no rise in blood pressure following BK inhibition. The response to the BK inhibition was unrelated to the baseline blood pressure of these animals or to the magnitude of the blood pressure decrement following administration of enalapril. These findings are also in agreement with another study that used specific antibodies to BK.

Since the molecular structure of the BK inhibitor is very similar to that of BK itself, it is not surprising that this agent can be degraded to a certain extent by the same enzymes that inactivate BK. Accordingly, the various inhibitors of the ACE would be expected not
only to potentiate the effects of bradykinin, but also to prolong the half-life of its inhibitor and potentiate its action. It is therefore interesting that in normal rats the BK antagonist at this dose produced no change in blood pressure even after pretreatment with an ACE inhibitor. We therefore conclude that, under the conditions of the present experiments, BK appears to be partly responsible for the blood pressure-lowering effect of converting enzyme inhibition in renovascular hypertensive rats but does not contribute to the maintenance of blood pressure in normotensive animals.

References
Vasodepressor role of endogenous bradykinin assessed by a bradykinin antagonist.
A Benetos, H Gavras, J M Stewart, R J Vavrek, S Hatinoglou and I Gavras

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