Vascular and Sympathoadrenal Responses to Bradykinin and a Bradykinin Analogue

ROGERIO MULINARI, ATHANASSIOS BENETOS, IRENE GAVRAS, AND HARALAMBOS GAVRAS

SUMMARY These experiments were designed to assess the interaction of bradykinin and its antagonist (Arg-Pro-Hyp-Gly-Phe-Ser-DPhe-Phe-Arg-trifluoroacetic acid) with the sympathoadrenal system. Three groups of male Wistar rats received 5-minute intra-arterial infusions of either dextrose (Group 1, n = 6), bradykinin, 250 μg/min (Group 2, n = 5), or bradykinin, 25 μg/min (Group 3, n = 4). Six other groups received a similar infusion of the bradykinin antagonist at 250 μg/min. They were either intact rats (Group 4, n = 10) or rats previously submitted to chemical sympathectomy (Group 5, n = 17), to adrenal enucleation (Group 6, n = 8), to combined α-adrenergic and β-adrenergic blockade (Group 7, n = 7), to α₁-adrenergic receptor blockade (Group 8, n = 8), or to α₂-adrenergic receptor blockade (Group 9, n = 8). Bradykinin infusion produced a sustained fall in mean arterial pressure (MAP) in Groups 2 and 3 (by -48 ± 3 and -36 ± 7 mm Hg, respectively) associated with similar increases in plasma epinephrine levels (100-fold), and norepinephrine (sevenfold) as compared with Group 1. The bradykinin antagonist infusion in intact rats produced a 23 ± 4 mm Hg rise in MAP associated with a sixfold increase in epinephrine and a twofold increase in norepinephrine. Group 5 rats with lower baseline catecholamine levels had an even larger MAP rise (30 ± 6 mm Hg) accompanied by a rise in epinephrine and norepinephrine proportionally similar to that of intact animals. Groups 6 to 8, which lacked either epinephrine or available α₁-adrenergic receptors, had no increase in MAP. We conclude that, while bradykinin produces a MAP fall with sharp stimulation of epinephrine release, the bradykinin antagonist raises MAP and also produces a direct adrenomedullary stimulation; its hypertensive response apparently depends on the α₁-agonistic properties of epinephrine. (Hypertension 11: 754–757, 1988)

KEY WORDS • bradykinin • bradykinin analogues • vasoconstriction • catecholamines • chemical sympathectomy • adrenal enucleation

THE kallikrein-kinin system is known to have considerable links with the sympathetic nervous system. It also interacts closely with the adrenal glands, where kinins potentiate the release of catecholamines, in particular, epinephrine (E), from the adrenal medulla. Since bradykinin (BK) is a potent vasodilator, it is unclear how much of its catecholamine stimulating effect is due to the vasodilation and resultant hypotension and how much is due to direct stimulation of adrenomedullary tissues.

The recent development of a series of BK analogues with antagonistic properties promises to enhance our knowledge of the effects of BK and its interaction with other vasoactive systems. Indeed, administering one of these analogues in relatively small doses to renovascular hypertensive animals whose blood pressure had been normalized by treatment with enalapril produced a rise in blood pressure. This response confirmed that BK participates in the hypotensive action of angiotensin converting enzyme inhibitors, as had been suggested with the use of BK antibodies. A pressor response was also observed when larger doses of a BK antagonist were infused to normotensive Wistar rats, suggesting a possible role of BK even in maintaining normal blood pressure.

As the use of BK antagonists in the investigation of BK’s cardiovascular effects is becoming more widespread, it is important to better define the properties of these antagonists. We designed this study to further assess the actions of a BK analogue (Arg-Pro-Hyp-Gly-Phe-Ser-DPhe-Phe-Arg-trifluoroacetic acid) by
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comparing vascular and sympathoadrenal responses to exogenous BK with those elicited by this BK analogue. Its efficacy as an antagonist of the vasodepressor effect of BK has been established elsewhere. 7

Materials and Methods

Male Wistar rats weighing 200 to 300 g (Charles River Laboratories, Kingston, NY, USA) were used in these experiments. The animals had catheters inserted in the carotid and iliac arteries (PE-50, Intramedic, Becton-Dickinson, Parsippany, NJ, USA) under light ether anesthesia on the day preceding the experiment. The carotid catheter, through which test drugs were infused, was advanced to the ascending aorta. Blood pressure and heart rate were monitored through a Gould-Statham P23ID pressure transducer (Gould, Cleveland, OH, USA) connected to the iliac catheter and recorded on a Gould 2200S paper chart recorder.

On the day of the experiment, the animals were conscious and unrestrained in plastic cages, with continuous monitoring of blood pressure and heart rate. A 1-hour stabilizing period was observed before the actual initiation of the experiments. Rats were allocated to one of nine different groups and received a 6-minute i.a. infusion (vehicle only for 1 minute and active drug for 5 minutes). Group 1 (n = 6) was composed of rats that received only vehicle infusion (5% dextrose in water, D5W) at 100 μl/min throughout the 6-minute infusion period. Group 2 (n = 5) comprised animals subjected to infusion of a BK solution (Sigma Chemical, St. Louis, MO, USA) at 250 μg/min for the latter 5 minutes of the infusion period. Group 3 (n = 4) also received a BK infusion for the latter 5 minutes of the infusion period, but at a rate of 25 μg/min. Group 4 (n = 10) included intact animals that received an infusion of the BK antagonist at a rate of 250 μg/min for 5 minutes. Group 5 (n = 17) comprised animals submitted to chemical sympathectomy starting 48 hours before the short-term experiment. Sympathectomy was achieved by i.p. injections of reserpine (Sigma), 1.0 mg/kg body weight in two doses (44 and 20 hours before the experiment), and metyrosine (Sigma), 25 mg/kg i.p. in five doses (48, 40, 24, 16, and 4 hours before the experiment). 8 Eight of these rats subsequently received an infusion of BK antagonist, and nine served as controls, receiving the vehicle only (designated as Group 5a). Group 6 (n = 8) was subjected to bilateral adrenal enucleation 15 days before the experiment and maintained on 1% saline as drinking fluid for 10 days and on tap water thereafter. Group 7 (n = 7) was pretreated with the α-adrenergic antagonist phentolamine (Regitine, Ciba), 10 mg/kg i.a., at −30 minutes, followed by the β-adrenergic antagonist propranolol (Inderal, Merck Sharp & Dohme), 2 mg/kg i.a., at −20 minutes. Animals in Group 8 (n = 8) were pretreated with the α-adrenergic receptor antagonist prazosin (Minipress, Pfizer), 0.2 mg/kg s.c., at −30 minutes. Finally, rats in Group 9 (n = 8) received the α-adrenergic receptor antagonist yohimbine (Sigma), 0.125 mg/kg i.a., at −20 minutes. Immediately on completion of the infusion, a 1-ml sample of blood was drawn for catecholamines in Groups 1 through 6.

BK solutions were prepared in D5W to final concentrations of 1.6 and 0.16 mmol/L. A 1.6 mmol/L solution of the BK antagonist in D5W was infused. Reserpine was dissolved in a 14% adipic acid solution in water. Metyrosine was dissolved in D5W to a concentration of 25 mg/ml. Phentolamine and propranolol were given in their commercial solutions. Prazosin was brought to a suspension (0.2 mg/ml in 50/50 D5W and propylene glycol). Yohimbine was dissolved in D5W to a final concentration of 0.125 mg/ml.

Blood for catecholamines was centrifuged at 2500 rpm for 20 minutes, and the plasma was separated and stored at −80°C until processing by radioenzymatic assay. 9

Statistical analysis was performed by analysis of variance for repeated measures. Differences within groups were analyzed by means of a Dunnett test. Comparison of the magnitude of changes between groups was assessed by a Mann-Whitney test. The analysis of independent data was accomplished with Student’s t test. Results are presented as means ± SEM.

Results

The infusion of vehicle (D5W) to Group 1 did not alter mean arterial pressure (MAP) or heart rate. The administration of BK at 250 μg/min (Group 2) induced a marked fall in MAP, averaging 48 ± 3 mm Hg at Minute 5 of infusion (p<0.01 vs baseline, down from a baseline of 112 ± 3 mm Hg). Heart rate rose from 438 ± 26 to 582 ± 29 beats/min (p<0.05). Rats in Group 3, receiving BK, 25 μg/min, displayed similar results. Blood pressure decreased by 36 ± 7 mm Hg (p<0.01), and heart rate increased significantly from 410 ± 23 to 555 ± 36 beats/min (p<0.01). Plasma norepinephrine (NE) and E levels rose sharply during the infusion of either dose (Table 1).

The infusion of BK antagonist at a rate of 250 μg/min to Group 4 (n = 10) induced a significant increase in MAP, averaging 23 ± 4 mm Hg at Minute 5 of

<table>
<thead>
<tr>
<th>Group</th>
<th>Norepinephrine (pg/ml)</th>
<th>Epinephrine (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>332 ± 66</td>
<td>445 ± 125</td>
</tr>
<tr>
<td>2</td>
<td>2498 ± 522*</td>
<td>47,933 ± 8354*</td>
</tr>
<tr>
<td>3</td>
<td>1670 ± 292*</td>
<td>33,301 ± 9654*</td>
</tr>
<tr>
<td>4</td>
<td>616 ± 110†</td>
<td>2673 ± 672†</td>
</tr>
<tr>
<td>5a</td>
<td>125 ± 37‡</td>
<td>158 ± 21†</td>
</tr>
<tr>
<td>5</td>
<td>378 ± 68§</td>
<td>974 ± 246‡§‡‡‡‡‡</td>
</tr>
<tr>
<td>6</td>
<td>567 ± 187</td>
<td>66 ± 20†§</td>
</tr>
</tbody>
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Values are means ± SEM. *p<0.01, compared with values in Groups 1 and 4. †p<0.05, compared with values in Group 1. ‡p<0.02, compared with values in Group 1. §p<0.05, compared with values in Group 5A. ‡‡‡‡‡‡p<0.05, compared with values in Group 4.
infusion ($p<0.01$), up from a baseline of 105 ± 4 mm Hg (Figure 1). The increase in blood pressure was immediate and sustained throughout the infusion period. At the end of the infusion, blood pressure slowly returned to baseline. Blood pressure was unchanged during the first minute of infusion, when vehicle was being given. Plasma NE and E in Group 4 were significantly increased when compared with Group 1 values (see Table 1).

Rats in Group 5 had significantly lower baseline MAP after chemical sympathectomy when compared with Group 4 (89 ± 5 vs 105 ± 4 mm Hg; $p<0.05$). However, they showed a marked increase in MAP during the infusion of BK antagonist, up by 30 ± 6 mm Hg at Minute 5 ($p<0.01$, see Figure 1), which was actually larger than that seen in Group 4 ($p<0.05$). Catecholamines were depressed in the vehicle-treated Group 5a when compared with levels in the intact rats of Group 1. At the end of the infusion of BK antagonist in Group 5, catecholamines rose significantly but were still much lower than in the BK antagonist–infused intact rats of Group 4.

In sharp contrast to the two previous groups, the rats subjected to adrenal enucleation exhibited only a small and transient, though significant, increase in MAP by the 30 first seconds of active infusion (8.9 ± 2.8 mm Hg; $p<0.05$; see Figure 1). The magnitude of this rise was comparable to that seen in intact animals within the same time frame. However, this MAP change subsided within 1 minute, remaining similar to baseline during the rest of the period. NE rose to a comparable extent as in Group 4, while E remained very low, bordering the sensitivity of the method.

The results of BK antagonist in animals pretreated with α-adrenergic and β-adrenergic blockade are shown in Figure 2. Combined α-blockade and β-blockade in Group 7, while producing no significant alteration in baseline MAP (99 ± 1 mm Hg), prevented any change when BK antagonist was administered. On the other hand, pretreatment with the α1-adrenergic receptor antagonist (Group 8), which slightly lowered the baseline MAP to 93 ± 3 mm Hg, when followed by infusion of BK antagonist resulted in a further decrease in MAP by 7.3 ± 4.3 mm Hg at 5 minutes ($p<0.05$). In animals pretreated with the α2-antagonist (Group 9), BK antagonist infusion increased MAP by 17 ± 2 mm Hg at 30 seconds ($p<0.01$) from a baseline of 110 ± 3 mm Hg. This initial response was similar to that of intact animals. From then on a slight, but significant, reduction in the magnitude of change in MAP was recorded, so that at 5 minutes it was 9.6 ± 3.4 mm Hg ($p<0.05$ vs baseline; $p<0.05$ vs Group 4). Heart rate showed no consistent or statistically significant changes during infusion of BK antagonist in Groups 4 through 9.

**Discussion**

The extent of the influence of kinins in general and BK in particular on cardiovascular regulation has yet to be established. Recent availability of potent antagonists of its vasodepressor action has suggested that BK could be playing an important role in the hypotensive effects of some drugs as well as in blood pressure maintenance in normotensive animals.

As analogues, the compounds used as BK antagonists in such experiments are very similar in amino acid sequence to BK. The only differences between the BK antagonist used in the present study and BK itself are the peptides 8-D-phenylalanine (which confers the vascular antagonistic properties) and 3-hydroxyproline. Therefore, despite the well-established vascular antagonistic properties of this compound, it could still act as an agonist to BK receptors of other tissues.

Our experiments in intact rats confirm that infusion of BK is associated with a fall in blood pressure and a sharp rise in plasma catecholamine levels. In the present studies, NE increased sevenfold and E increased 100-fold compared with levels in dextrose-infused rats. In contrast, a similar infusion of BK antagonist increased blood pressure consistently, yet it was also associated with a marked release of catecholamines, suggesting that this effect was due to direct stimulation of the adrenal medulla and was not a reaction to hypotension. Chemical sympathectomy, despite lowering the baseline levels of NE and E, did not decrease
the pressor response elicited by the BK antagonist; on the contrary, the BK antagonist produced a pressor response of significantly greater magnitude in these rats than in the intact rats. Again, this response was associated with a marked increase in circulating catecholamines, although the levels attained were one half to one third of those obtained in sympathetically intact rats. However, if the adrenal medulla was removed 15 days before the infusion of the BK antagonist, no sustained blood pressure response was observed. In this instance, NE increased to levels similar to those in intact animals, but E remained low, often close to undetectable.

Blockade of both α-adrenergic and β-adrenergic receptors revealed that, during impaired neural transmission, infusion of BK antagonist did not significantly alter blood pressure in otherwise normal rats. Selective blockade of α1-adrenergic or α2-adrenergic receptors produced markedly different results. Prior α1-blockade with prazosin produced reversal of blood pressure response (i.e., a significant decrease in MAP). In contrast, α2-blockade with yohimbine did not substantially alter the pressor response to BK antagonist but only attenuated its magnitude.

Taken together, these experiments suggest that the pressor effect elicited by the BK antagonist infusion in the adrenergically intact rats was due not to inhibition of the vascular dilative action of BK but rather to an excessive sympathetically mediated vasopressor effect. Interestingly, in chemically sympathectomized animals, even though catecholamine synthesis was to some extent impaired, the relative increase in levels was similar to that of intact rats after BK antagonist administration. Furthermore, although the absolute catecholamine levels were lower in the sympathectomized animals, the rise in MAP was more pronounced than in the intact ones, possibly due to a homologous up-regulation of adrenergic receptors in the former group.10

The ratios of increase in catecholamines in all animals favored E over NE after both BK and BK antagonist infusions. BK is known to release E from the adrenal medulla through direct action, independent of sympathetic integrity.3 After adrenal enucleation, the administration of BK antagonist elicited an increase in NE levels, while E remained low. This response was accompanied by a lack of sustained elevation of MAP. These results point to the release of E as the cause of blood pressure elevation during BK antagonist administration to intact rats. This hormone has both α-adrenergic and β-adrenergic agonistic effects.11 However, at physiological levels, the latter effect usually predominates; thus, BK is generally believed to cause peripheral vasodilation, although in the long run it can lead to an increase in arterial pressure, mainly systolic, secondary to its inotropic and chronotropic actions. However, in the presence of such excessive amounts of E as those released after infusion of these BK analogues, it appears that α1-adrenergic receptors of the vessel wall are also stimulated to cause a blood pressure rise. This explanation is supported further by the reversal of the blood pressure response in prazosin-pretreated rats, where in the absence of available α1-adrenergic receptors, only a β-adrenergic receptor-mediated effect was seen, namely, vasodilation. The finding that, after combined α-adrenergic and β-adrenergic receptor blockade, there was no change in blood pressure in response to the BK antagonist confirmed previous reports that this antagonist possesses no in vivo vasoactive effect of its own, either pressor or depressor.3

The results from these experiments pertain to this particular antagonist of BK and cannot be extrapolated and applied to other analogues, which may have various combinations of antagonistic and partial agonistic properties in different tissue receptors. These findings emphasize the need to properly evaluate the characteristics of a specific analogue before its use in physiological studies in order to avoid pitfalls in the interpretation of experimental data.

References

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