Bradykinin Antagonism and Prostaglandins in Blood Pressure Regulation

Rogerio Mulinari, Irene Gavras, Roberto Franco, and Haralambos Gavras

These experiments were designed to analyze the interaction of a bradykinin antagonist with prostaglandins in blood pressure regulation of normotensive rats. Male Wistar rats, divided into three groups, received a 5-minute intra-arterial infusion of the bradykinin antagonist ([dArg²-Hyp⁴-Thi⁵-dPhe⁷]BK-TFA) at 250 µg/min. Groups were either intact rats (group I, n=5), pretreated with indomethacin (group II, n=10), or pretreated with both indomethacin and prazosin (group III, n=8). The bradykinin antagonist infusion, which was shown to inhibit exogenous bradykinin by greater than 76% in intact animals, did not alter mean arterial pressure in group I rats despite a twofold increase in norepinephrine and a threefold increase in epinephrine. Group II rats presented a progressive increase in mean arterial pressure during the bradykinin antagonist infusion (14±3 mm Hg), with no statistically significant change in plasma catecholamines. Group III, with lower baseline mean arterial pressure due to α₁-adrenergic blockade, had an increase in mean arterial pressure comparable with group II during bradykinin antagonist infusion (22±5 mm Hg), confirming that this response was not sympathetically mediated. We conclude that in normotensive rats bradykinin plays a role in blood pressure regulation that is closely linked to that of prostaglandins and that points to a balance between these systems. (Hypertension 1989; 13:960-963)

The assessment of the physiological role of bradykinin in blood pressure regulation has always been complicated by its interaction with other vasoactive systems, such as the sympathetic nervous system, the renin-angiotensin, and the prostaglandin-endoperoxides systems. Prostaglandins are involved in several biological actions of bradykinin, functioning either as modulators or as mediators of these actions. Both prostaglandin E₂ (PGE₂) and prostacyclin (PGI₂) are important effectors of vascular bradykinin receptor stimulation under certain experimental conditions, although the pattern of this involvement depends on the particular vascular bed and the species being studied. The development of analogues of bradykinin with competitive antagonistic properties represented a turning point in the exploration of this hormone and whose previous investigation depended on plasma measurements of arguable validity.

In the present experiments we attempted to analyze the interaction of one of the bradykinin antagonists with prostaglandins by comparing blood pressure and catecholamine responses before and after cyclooxygenase inhibition.

Materials and Methods

In these experiments we used 23 male Wistar rats weighing 200–250 g (Charles River Labs., Inc., Kingston, New York). The rats had catheters inserted into the right carotid and right iliac arteries (PE-50, Intramedic, Becton-Dickinson, Parsippany, New Jersey) under light ether anesthesia on the day preceding the experiment. The carotid catheter, through which drugs were infused, was advanced to the ascending aorta. Blood pressure and heart rate were monitored through a Gould Statham P23ID pressure transducer (Gould Inc., Cleveland, Ohio) connected to the iliac catheter and recorded on a Gould 3200 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 stabilization period was observed before the initiation of the experiments. Rats were allocated to one of three different groups and received a 6-minute intra-aortic (i.a.) infusion at 100 µl/min. During the first minute rats were infused with the vehicle only (dextrose 5% in water), and during the subsequent 5 minutes they received the bradykinin antagonist at 250 µg/min. Group I (n=5)
was composed of intact rats. Group II (n=10) comprised animals pretreated with the cyclooxygenase inhibitor indomethacin (Sigma Chemical Co., St. Louis, Missouri), 10 mg/kg i.a., 60 minutes before infusion of the bradykinin antagonist. Group III (n=8) included rats pretreated with indomethacin as above, in addition to an α₁-adrenergic receptor antagonist prazosin (Minipress, Pfizer, New York, New York), 0.2 mg/kg, given subcutaneously 30 minutes before administration of the bradykinin antagonist. The reason for this was that the pressor reactions to another bradykinin antagonist in the past was found to be due to stimulation of α₁-adrenergic receptors by excessive catecholamine release.2 Immediately on completion of the infusion, a 1-ml sample of blood was drawn for catecholamines in groups I and II.

The bradykinin antagonist used in this study was the analogue [dArg-Arg-Pro-Hyp-Gly-Thi-Ser-Pro-Hyp-Tyr-Ala]trifluoroacetic acid, where Thi=β-[2-Thienyl]-L-alanine.7 This compound was reported as the most potent antagonist in a series of bradykinin analogues tested by Beierwaltes et al with no intrinsic effect on systemic blood pressure. Its efficacy as an antagonist to the vasodepressor effect of exogenous bradykinin was also confirmed by us in preliminary work with eight other rats. The pressor reactions to another bradykinin antagonist in the past was found to be due to stimulation of α₁-adrenergic receptors by excessive catecholamine release.2

Results

The antagonistic property of the bradykinin antagonist was ascertained by testing its inhibitory capacity against the vasodepressor action of exogenous bradykinin. Doses of 50 μg/min, 100 μg/min, and 200 μg/min of the bradykinin antagonist could inhibit the blood pressure (BP)-lowering effect of a 250-ng bolus of bradykinin by 49%, 67%, and 76%, respectively (Table I).

Figure 1 illustrates the BP response observed in the various experimental groups. The infusion of the bradykinin antagonist at 250 μg/min for 5 minutes in group I did not alter mean arterial pressure (MAP) (from 113±1.2 to 116±1.9 mm Hg) or heart rate (from 420±14 to 442±17 beats/min). Plasma NE and epinephrine were twofold and threefold, respectively, higher than normal (Table II).

Pretreatment of rats in group II with the cyclooxygenase inhibitor indomethacin did not affect baseline MAP (from 108±4.6 to 107±4.8 mm Hg) or heart rate (from 423±13 to 405±14 beats/min). Infusion of the bradykinin antagonist in these rats induced a progressive increase in MAP, from 107±4.8 to 122±6.6 mm Hg at 5 minutes (p<0.01) with no change in heart rate. MAP remained stable after the second minute of active infusion and returned gradually to baseline when infusion ended. Plasma catecholamines showed wide individual variability but overall were not statistically different from those of either normal rats or of group I, although the average value of epinephrine was still three times larger than in normal rats.

Rats in group III had a significantly lower baseline MAP after pretreatment with the cyclooxygenase inhibitor and the α₁-adrenergic receptor antagonist (from 105±3.7 to 76±4.2 mm Hg, p<0.01), but heart rate was unaltered. Infusion of the bradykinin antagonist increased MAP from 76±4.2 to 98±3.8 mm Hg by 5 minutes with no change in heart rate. The magnitude of increase in MAP was comparable with that observed in group II. Blood

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>MAP (mm Hg)</th>
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<tbody>
<tr>
<td>1</td>
<td>70</td>
</tr>
<tr>
<td>2</td>
<td>80</td>
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<td>3</td>
<td>90</td>
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<td>4</td>
<td>100</td>
</tr>
<tr>
<td>5</td>
<td>110</td>
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</table>

**Figure 1.** Blood pressure change during infusion of bradykinin antagonist in intact rats, rats pretreated with indomethacin, and rats pretreated with both indomethacin and prazosin. *p<0.05 vs. baseline; **p<0.01 vs. baseline.
prostanoids has been studied extensively in vitro. The pressor response is independent of sympathetic activation. The intricate relation between bradykinin and prostanoids has been studied extensively in vitro.

**TABLE 2. Plasma Catecholamine Levels After Infusion of the Bradykinin Antagonist**

<table>
<thead>
<tr>
<th>Group</th>
<th>Norepinephrine (pg/ml)</th>
<th>Epinephrine (pg/ml)</th>
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<tbody>
<tr>
<td>I</td>
<td>329±48*</td>
<td>435±97*</td>
</tr>
<tr>
<td>II</td>
<td>215±60</td>
<td>426±147</td>
</tr>
<tr>
<td>Normal</td>
<td>187±31</td>
<td>141±23</td>
</tr>
</tbody>
</table>

Group I, rats infused with bradykinin antagonist; Group II, rats pretreated with indomethacin and infused with bradykinin antagonist.

*p<0.05 vs. normal.

pressure gradually returned to baseline within 2 minutes after discontinuation of the infusion.

**Discussion**

In the present experiments we used the potent decapeptide bradykinin antagonist [DArg²-Hyp⁵-Thr³-DPhe⁷]bradykinin, which can produce a full log dose shift in the dose–response curve of bradykinin. Before the experiments, we confirmed that it could inhibit the vasodepressor effect of exogenous bradykinin by over 76%. This bradykinin antagonist did not alter blood pressure when infused in intact rats, despite a modest but significant increase in plasma catecholamines (NE, two-fold; epinephrine, threefold). The most likely explanation for this is that simultaneous stimulation of vascular α- and β-adrenergic receptors by the two hormones produced no appreciable overall change. In contrast, a similar infusion of this bradykinin antagonist in rats with chemically blocked prostaglandin synthesis elicited a marked increase in blood pressure, with a variable but not significant change in plasma catecholamines. Blockade of α₁-adrenergic receptors, in addition to cyclooxygenase inhibition, yielded essentially the same response as when the adrenergic receptors were available, which indicates that the pressor response was not adrenergically mediated. Experiments with another bradykinin analogue with antagonistic properties in the past produced increase in blood pressure that was attributable not to bradykinin inhibition but rather to excessive stimulation of adrenal catecholamines, as this increase was completely abolished by α₁-adrenergic receptor blockade. In the present studies the increase in plasma catecholamines was only modest in intact rats receiving the bradykinin antagonist and was certainly not responsible for the change in pressure after the bradykinin antagonist infusion in indomethacin-pretreated rats. Indeed, inconsistent changes in catecholamine levels were observed in the latter group, but the pressor response was consistent and was not abolished by α₁-adrenergic blockade. The experiments suggest that, in the absence of prostaglandins, blockade of bradykinin by the bradykinin antagonist increases blood pressure and that the pressor response is independent of sympathetic activation.

This interaction occurs at several levels. Bradykinin can activate phospholipase A₂, which increases the availability of arachidonic acid for the generation of several prostanoids. On the other hand, prostaglandins, particularly PGE₂ and PGH₂, mediate various effects of bradykinin. Taking the available in vitro data on the interaction between the two systems as a whole, it seems that the inhibition of cyclooxygenase tends to blunt the response to bradykinin at both known receptor sites, B₁ and B₂, in certain experimental settings. However, in vivo there is usually a balance between vasoactive systems so that when one is impaired there is a compensatory activation of the others. Accordingly, blockade of the cyclooxygenase pathways and consequent reduction in prostaglandin generation may well lead to an increase in bradykinin release and hence to its influence on vascular regulation through alternative pathways (i.e., enhancement of its effects on other vasoactive substances not mediated via prostaglandins). Such a sequence of events, although highly speculative, might explain why the pressor response to a bradykinin antagonist becomes apparent only after prostaglandin inhibition.

Of course our results do not exclude the possibility that this bradykinin antagonist possesses an action of its own, which may be independent of its bradykinin-inhibiting effect. A recent study by Carbonell et al. reported that a 4 mg/kg bolus of this agent in intact rats produced a biphasic pressor-depressor effect, of which only the depressor component appeared to be prostaglandin mediated. If so, prostaglandin inhibition would abolish the depressor action and leave unopposed the pressor effect. Alternatively, our results would suggest that the pressor component is counteracted in some other way by prostaglandins, since it can become manifest only in the absence of prostaglandin generation. Other potential explanations of these findings include the possibility of a prostanoid-mediated effect on other substances linked with bradykinin functions, such as the endothelium-derived relaxing factor, or the possibility of an imbalance between action of bradykinin and its analogues on the B₁ and B₂ bradykinin receptors. The possibility that some kinin agonists might exert their effects by interfering with other vasoactive peptides such as angiotensin or substance P was considered but rejected by the authors of this last study. Finally, a recent publication raised the possibility of a third type of bradykinin receptor, with characteristics different from those of the classic B₁ and B₂ types. All of these explanations are speculative and cannot be addressed on the basis of the present data.

In conclusion, our data highlight a new aspect of the intricate interrelations of bradykinin and prostaglandins in the homeostasis of vascular tone, by demonstrating that a bradykinin analogue with competitive antagonistic properties produces a pressor effect only when prostaglandin generation has been
inhibited. The data suggest that bradykinin plays a role in blood pressure regulation in normotensive rats, but this role is closely linked to that of prostaglandins and points to a balance between these two vasoactive systems.

References


KEY WORDS • bradykinin • prostaglandins • blood pressure
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