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-Hyp²-Thi³-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 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 α-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 α-adrenergic receptors by excessive catecholamine release. 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-DPhe-Thi-Arg]trifluoroacetic acid, where Thi=(β-[2-Thienyl]-L-alanine). This compound was reported as the most potent antagonist in a series of bradykinin analogues tested by Beierwaltes et al without any 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 compound was dissolved in dextrose 5% in water with Na2CO3 added, and the pH was adjusted to 7.4 with HCl for a final concentration of 2 mg/ml. Prazosin was dissolved in 50:50 dextrose 5% in water and propylene glycol 1:1 solution to a concentration of 0.2 mg/ml.

Blood for catecholamines was centrifuged at 2,500 rpm for 20 minutes at 4°C, and the plasma was separated and stored at −80°C until processing by high-performance liquid chromatography with electrochemical detection. Normal values for intact rats under conditions similar to these experiments were 187±31 pg/ml for norepinephrine (NE) and 141±23 pg/ml (mean±SEM) for epinephrine.

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

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 α-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 1. Inhibition of the Vasodepressor Effect of Exogenous Bradykinin by Infusion of the Bradykinin Antagonist

<table>
<thead>
<tr>
<th>Dose of BKA (μg/min)</th>
<th>Before BKA (mm Hg)</th>
<th>During BKA (mm Hg)</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>50 (n=8)</td>
<td>187±1.1</td>
<td>170±1.4</td>
<td>49</td>
</tr>
<tr>
<td>100 (n=4)</td>
<td>243±1.1</td>
<td>224±1.4</td>
<td>67</td>
</tr>
<tr>
<td>200 (n=4)</td>
<td>198±1.1</td>
<td>190±1.4</td>
<td>66</td>
</tr>
</tbody>
</table>

BKA, bradykinin antagonist; MAP, mean arterial pressure; BK, bradykinin.

![Figure 1](https://example.com/figure1.png)

**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.

dent of sympathetic activation.

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was consistent and was not abolished by

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infusion in indomethacin-pretreated rats. Indeed,

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past2 produced increase in blood pressure that was

antagonist in rats with chemically blocked pros-

inhibition, yielded essentially the same response as

pressor component is counteracted in some other

from those of the classic B1 and B2 types. All of

addressed on the basis of the present data.

Other potential explanations of these findings include

effect only when prostaglandin generation has been

Table 2. Plasma Catecholamine Levels After Infusion of the
Bradykinin Antagonist

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

Discussion

In the present experiments we used the potent
decapeptide bradykinin antagonist [DArg2-Hyp1-Thi3-D-Phe7]bradykinin, which can produce a full
log dose shift in the dose–response curve of
bradykinin.9 Before the experiments, we confirmed
that it could inhibit the vasodepressor effect of
exogenous bradykinin by over 76%. This bradyki-
nin antagonist did not alter blood pressure when
infused in intact rats, despite a modest but signifi-
cant increase in plasma catecholamines (NE, two-
fold; epinephrine, threefold). The most likely expla-
nation 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 pros-

taglandin synthesis elicited a marked increase in
blood pressure, with a variable but not significant
change in plasma catecholamines. Blockade of α-
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 adren-
ergically mediated. Experiments with another brady-

kin analogue with antagonistic properties in the past2 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 antag-
onists 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 brady-

kinin by the bradykinin antagonist increases blood
pressure and that the pressor response is indepen-
dent of sympathetic activation.

The intricate relation between bradykinin and
prostanoids has been studied extensively in vitro.

This interaction occurs at several levels. Bradyki-
nin can activate phospholipase A2, which increases
the availability of arachidonic acid for the genera-
tion of several prostanoids.11 On the other hand,
prostaglandins, particularly PGE2 and PGH2, medi-
ate various effects of bradykinin.12,13 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.14,15
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 path-
ways and consequent reduction in prostaglandin
generation may well lead to an increase in bradyki-
nin 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 brady-
kinin antagonist becomes apparent only after pro-
taglandin inhibition.

Of course our results do not exclude the possibil-
ity that this bradykinin antagonist possesses an
action of its own, which may be independent of its
bradykinin-inhibiting effect. A recent study by Car-
bonell et al16 reported that a 4 mg/kg bolus of this
agent in intact rats produced a biphasic pressor-
depressor effect, of which only the depressor com-
ponent appeared to be prostaglandin mediated. If
so, prostaglandin inhibition would abolish the depres-
or 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 mani-
fest 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,17,18
or the possibility of an imbalance between action of
bradykinin and its analogues on the B1 and B2
bradykinin receptors.19 The possibility that some
kinin antagonists might exert their effects by inter-
ferring with other vasoactive peptides such as angio-
tensin or substance P was considered but rejected
by the authors of this last study.19 Finally, a recent
publication20 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 pros-
taglandins in the homeostasis of vascular tone, by
demonstrating that a bradykinin analogue with com-
petitive 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
Bradykinin antagonism and prostaglandins in blood pressure regulation.
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Hypertension. 1989;13:960-963
doi: 10.1161/01.HYP.13.6.960

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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World Wide Web at:
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