Hypertensive Effect of a Bradykinin Antagonist in Normotensive Rats

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SUMMARY The purpose of these experiments was to study the possible contribution of bradykinin to normal blood pressure maintenance. The bradykinin analogue B4146, a competitive antagonist-partial agonist of bradykinin, was used in three groups of normotensive unanesthetized Wistar rats. Two intra-aortic injections of B4146 (1 mg in 0.2 ml of dextrose) were given 5 minutes apart (i.e., well after return of blood pressure to baseline, which occurred within 68 ± 19 seconds). One group had been pretreated with the angiotensin converting enzyme inhibitor HOE 498, 1 mg/kg (Hoechst), and one received only dextrose as the first injection to serve as controls. The bradykinin antagonist produced an average increase in mean arterial pressure of approximately 13 mm Hg for all groups. In five animals, however, the first injection of B4146 produced a hypotensive effect, whereas the second one consistently produced a rise in blood pressure. Pretreatment with the angiotensin converting enzyme inhibitor did not affect the magnitude of the subsequent blood pressure increase in response to B4146.

Since smaller doses of B4146, sufficient to block exogenous bradykinin, do not cause changes in normal blood pressure, we conclude that endogenous bradykinin does contribute to normal blood pressure maintenance, but its effect can be demonstrated only if very high doses of its antagonist are injected, maybe because a high concentration of the compound is necessary to displace not only circulating but possibly tissue receptor-bound bradykinin as well. (Hypertension 8: 1089-1092, 1986)

KEY WORDS • bradykinin antagonist • endogenous bradykinin • blood pressure regulation • angiotensin converting enzyme inhibition

THE physiological role of bradykinin, a vasoactive nonapeptide, in the cardiovascular homeostasis has long been debated. Despite its potent effect in pharmacological doses, the contribution of endogenous bradykinin to the regulation of arterial pressure remains obscure.

Several investigators have attempted to show changes in blood, urine, or tissue levels of kinins in different types of experimental or human hypertension, but these results are far from conclusive. On the other hand, the application of bradykinin in vitro on isolated vessels has various effects, depending on the species, the type of vessel, and the bradykinin concentrations, producing contraction, dilation, or biphasic responses — results that indicate the system's variability and complexity.

The action of other vasoactive systems (i.e., renin-angiotensin, vasopressin) was revealed by the use of a specific competitive antagonist of the hormone at the vascular receptor level. In this study, we used a competitive antagonist of bradykinin, the compound B4146. The key modification that converts bradykinin to an antagonist is the substitution of D-phenylalanine for proline at position 7; other modifications increase the potency of the antagonist. The inhibitory capacity of this compound had previously been tested in vitro and in vivo. In the present experiments, we studied the effect of B4146 in normotensive rats with or without pretreatment with an angiotensin converting enzyme (ACE) inhibitor to investigate the participation of bradykinin in normal blood pressure maintenance.

Materials and Methods
Intact male Wistar rats (Charles River Breeding Laboratories, Boston, MA, USA) weighing 225 to 275 g were used for the following protocol. One day before the experiment, all rats were cannulated under light ether anesthesia with two PE-50 catheters; one was
inserted into the iliac artery for blood pressure measurements, and the other inserted into the ascending aorta through the right carotid artery for drug injection. On the day of the experiment, the rats were maintained unanesthetized and unrestrained in plastic cages. All procedures followed were in accordance with institutional guidelines. Blood pressure was monitored for 30 to 60 minutes before the beginning of the experiment.

All drugs used in this study were dissolved in 5% dextrose and consisted of the ACE inhibitor HOE 498 (Hoechst-Roussel Pharmaceuticals, Somerville NJ, USA), 1 mg/ml, and the bradykinin analogue competitive antagonist Arg-Pro-Hyp-Gly-Thi-Ser-DPhe-Thi-Arg-TFA (formula B4146), 5 mg/ml, synthesized by Dr. John M. Stewart, School of Medicine, University of Colorado (Denver, CO, USA). The efficacy of this antagonist has been determined in previous pilot experiments, where the depressor effect of exogenous bradykinin was inhibited by over 50%.

The rats were divided into three groups. Group 1 (n = 10) received two 1-mg injections of the bradykinin antagonist B4146 (0.2 ml) 5 minutes apart through the aortic catheter. In Group 2 (n = 6), the first injection was replaced by 0.2 ml of the vehicle alone (i.e., dextrose 5%). Five minutes later the animals received the B4146 injection as in Group 1. The rats of Group 3 (n = 10) were pretreated with the ACE inhibitor HOE 498, 1 mg/kg, followed 30 to 45 minutes later by the same procedure as in Group 1.

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. Correlations were calculated by the Spearman rank correlation method. Results were considered significant if p was less than 0.05.

**Results**

In Group 1 (n = 10) the first injection of B4146 produced two different responses (Figure 1). Blood pressure increased by 12 ± 3 mm Hg (range, 7–25 mm Hg) in six out of 10 rats from a baseline of 117 ± 4 mm Hg (p < 0.01). This increase began a few seconds after the end of the injection and lasted for 68 ± 19 seconds (range, 25–150 seconds). In two of these rats a period of slight hypotension, which lasted 2 to 3 minutes before blood pressure returned to baseline. The second injection of B4146 produced a significant hypertensive response: blood pressure increased by 29 ± 9 mm Hg (range, 7–25 mm Hg) in six out of 10 rats from a baseline of 117 ± 7 mm Hg (p < 0.002). This response was highly significant (p < 0.01).

In three rats the rise in blood pressure was followed by a period of slight hypotension, which lasted 2 to 3 minutes before returning to baseline. The second injection produced a significant increase in blood pressure from this new baseline in three of these animals and no change in the fourth animal. For Group 1 as a whole, the increase in mean blood pressure after the second injection was highly significant (p < 0.001).

Group 2 (n = 6) received a first injection of vehicle only (0.2 ml of 5% dextrose), which did not produce any blood pressure change. When B4146 was given 5 minutes later, a significant hypertensive effect was observed in all but one rat; mean blood pressure rose by 16 ± 2 mm Hg (range, 10–20 mm Hg) from a baseline of 117 ± 7 mm Hg (p < 0.002). This response to B4146 was significantly different from the response to the vehicle (p < 0.02; see Figure 1).

In Group 3 (n = 10), pretreatment with the ACE inhibitor HOE 498 decreased blood pressure by 13 ± 5 mm Hg from a baseline of 120 ± 5 mm Hg (p < 0.01). The first injection of the bradykinin antagonist subsequently increased the blood pressure by 11 ± 3 (p < 0.02 for the group). Blood pressure increased in seven rats from this group, decreased in one, and did not change in the remaining two. The duration of these changes was identical to those of the previous groups. In three rats the rise in blood pressure was followed by a period of slight hypotension, which lasted 2 to 3 minutes before returning to baseline. The second injection of B4146 produced a hypertensive action in nine out of 10 rats (average increase, 17 ± 4 mm Hg; p < 0.01). There was no significant difference between the response to the first and the second injection. There was no correlation between the preintervention blood pressure levels or the fall in blood pressure induced by the ACE inhibitor and the subsequent pressor response to B4146.

The heart rate remained essentially unchanged throughout the experimental procedure in all three groups.
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Discussion

The objective of these experiments was to evaluate the contribution of bradykinin to normal blood pressure maintenance by assessing the pressor response to a bradykinin antagonist, the compound designated as B4146. The intra-aortic injection of this antagonist produced an average rise in blood pressure of approximately 13 mm Hg for all groups. However, in five individual animals (four in the first group and one in the third group) the first injection of B4146 produced a hypotensive effect whereas the second injection of B4146 had no depressor effect in any of these animals.

Many authors have suggested a role for bradykinin in blood pressure regulation under various conditions and an interaction of this system with other vasoregulatory systems. However, the lack of specific antagonists of bradykinin and the problems of accurately estimating the plasma and tissue levels of bradykinin have seriously hampered these efforts.

In this study we used a bradykinin analogue competitive antagonist of bradykinin, which has been shown to have a specific inhibitory action for bradykinin but no effect for other vasoactive compounds. In vitro studies have also shown a weak agonistic activity of this peptide on the rat uterus (1-4% agonist potency relative to bradykinin), with moderate resistance to the enzymatic breakdown of kininases. This partial agonist effect could explain the few instances of hypotension observed in this study. However, it is difficult to explain why this was produced only in some animals and after the first, but not after the second, injection.

The doses of B4146 used for this study were much higher than those needed to inhibit the action of exogenously injected pharmacological doses of bradykinin. Several authors have shown the importance of the circulating plasma bradykinin as well as the tissue receptor-bound and locally generated kinins. Other studies have demonstrated the presence of kinin-forming enzymes in the vascular tissue. The kinins released by the arterial wall resemble bradykinin in their chemical and pharmacological properties, and they are inhibited by antibodies specific to bradykinin. It is therefore possible that bradykinin generated and bound locally may have an important role in the regulation of the peripheral vascular tone, either through a direct effect on the arterial wall or through interactions with other vasoactive systems, especially the prostanoids. According to the high concentrations of a competitive bradykinin antagonist may be required to displace not only circulating but possibly tissue-bound hormone as well. Interestingly, the rate of degradation of tissue-bound bradykinin seems to be much slower than that of the circulating hormone.

Despite the known bradykinin-potentiating effect of ACE inhibition, pretreatment with an ACE inhibitor did not affect the magnitude of the pressor response to B4146. There was no correlation between either the baseline blood pressure or the depressor response to ACE inhibition and subsequent pressor response to bradykinin inhibition in these animals.

In this study we provide the first evidence (to our knowledge) that bradykinin is involved in the maintenance of normal blood pressure in normotensive experimental animals and could be a major counteractive mechanism to other vasoconstrictor systems. Consequently, a deficiency in the bradykinin system may contribute to the development of certain types of hypertension. For example, the finding that spontaneously hypertensive rats are especially sensitive to bradykinin could be suggestive of up-regulation of bradykinin receptors secondary to bradykinin deficiency. Although this interpretation is highly speculative, our data suggest that if bradykinin participates in blood pressure regulation in the normotensive state, it may play a role in the development of certain hypertensive states as well.

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