Effects of a Kinin Antagonist on Mean Blood Pressure

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SUMMARY Administration of high doses of a kinin antagonist produces an increase in blood pressure. Thus, endogenous kinins may be involved in the regulation of blood pressure. Kinins can induce the release of vasoactive substances such as catecholamines, renin, vasopressin, histamine, and prostaglandins. To determine whether the blood pressure changes induced by high doses of kinin antagonist are due to agonistic activity mediated by these vasoactive substances, we studied the effect on blood pressure of a kinin antagonist (D-Arg*-Hyp*-Thi*-D-Phe*-bradykinin) administered to control, nephrectomized, and adrenalectomized rats, and to rats treated with vasopressin V₁-receptor antagonist, ganglionic and α- and β-adrenergic receptor blockers (either separately or combined), histamine H₁- and H₂-receptor blockers, and indomethacin, a prostaglandin synthesis inhibitor. Blood pressure changes were monitored on awake, restrained rats. In the control rat, the kinin antagonist injected as a bolus (4 mg/kg) into the ascending aorta produced a transient biphasic blood pressure response, first a pressor effect (ABP = 7 ± 1 mm Hg; p<0.05), then a depressor effect (ABP = -20 ± 6 mm Hg; p<0.05). The pressor response to the kinin antagonist was not affected by any of the treatments; however, the depressor effect of the kinin antagonist appeared to be caused by the release of vasodilator prostanoids from the kidney, since it was not observed in the nephrectomized rats or in those treated with indomethacin. The pressor effect induced by the kinin antagonist suggests that kinins may contribute to the regulation of blood pressure.

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KEY WORDS • bradykinin • kinin antagonist • blood pressure regulation • pressor • depressor

Kinins are potent vasodepressor peptides that appear to be involved as autacoids in the regulation of normal blood pressure. Recently, a family of bradykinin analogues having antagonistic properties was synthesized. High doses of one of these kinin antagonists produced both pressor and depressor effects in normotensive rats. However, kinin antagonists and bradykinin have similar structures, and possibly some of the effects of high doses of the antagonist were not only due to blocking endogenous kinins, but also related to some agonistic activity.

Kinins can stimulate renin secretion from kidneys and vasopressin from the central nervous system, as well as induce catecholamine release from the adrenal medulla and sympathetic ganglia. All these actions of kinins can increase blood pressure. On the other hand, both kinins and kinin antagonists can produce histamine release from rodent mast cells and skin mast cells, and kinins can induce prostaglandin release from several tissues. These effects of kinins and kinin antagonists could mediate the vasodepressor actions of high doses of kinin antagonist.

The purposes of this study were to 1) assess the blood pressure changes induced by the administration of a new kinin antagonist, D-Arg-Hyp-Thi-D-Phe-bradykinin, and 2) exclude the possibility that these blood pressure changes are due to mechanisms unrelated to blockade of endogenous kinins such as renin, vasopressin, catecholamine, histamine, or prostaglandin release.

Materials and Methods

Bradykinin was purchased from Peninsula Laboratories (Belmont, CA, USA). Kinin antagonist, D-Arg-Arg-Pro-Hyp-Gly-Thi-Ser-D-Phe-Thi-Arg-TFA (Hyp = L-4-hydroxyproline; Thi = β-2-thienyl-L-alanine; TFA = trifluoroacetic acid), was synthesized by J. Stewart (Denver, CO, USA) as described previously. Phenolamine (Regitine) was purchased from CIBA (Basel, Switzerland). Indomethacin, hexa-
methylene bromide, propranolol, and norepinephrine were purchased from Sigma Chemical (St. Louis, MO, USA). Isoproterenol was purchased from Elkins-Sinn (Cherry Hill, NJ, USA), and sodium nitroprusside from Abbott (Chicago, IL, USA). Arginine vasopressin and the vasopressin V1-receptor antagonist (β-mercapto-β-cyclopentamethylene-propionic acid, O-methyl-Tyr2, Arg*) vasopressin were purchased from Bachem (Torrance, CA, USA). The histamine H1-receptor blocker (chlorpheniramine maleate) was purchased from Schering (Kenilworth, NJ, USA), histamine H2-receptor blocker (cimetidine) was purchased from SK&F Laboratory (Carolina, Puerto Rico), and histamine from Eli Lilly (Indianapolis, IN, USA).

All drugs were dissolved with 0.9% NaCl, except indomethacin, which was dissolved in carbonate buffer, pH 7.4. The volume of each bolus injection was 0.1 ml, followed by 0.2 ml of 0.9% NaCl solution. The male Sprague-Dawley rats (230–260 g) used for these experiments were cared for according to the principles established in the Guide for the Care and Use of Laboratory Animals (U.S. Public Health Service). The rats were housed at a constant room temperature with a 12-hour light-dark cycle, and had free access to tap water and rat chow. The surgical maneuvers were performed with the rats under ether anesthesia. One day before the experiments, two catheters (PE-50, Clay Adams, Parsippany, NJ, USA) were inserted into the rats, one into the ascending aorta through the right carotid artery for bolus injections, and the other into the abdominal aorta by way of the left femoral artery for blood pressure measurement. The catheters were exteriorized in the scapular region as described previously. During the experiment, the rats were awake and kept semi-restrained in cylindrical plastic containers. Blood pressure was measured with a Statham transducer (Gould, Oxford, CA, USA) and recorded on a Brush recorder (Gould, Cleveland, OH, USA).

To determine the effectiveness of the bradykinin blocker, bradykinin (400 ng/kg) was injected both prior to and after the administration of the kinin antagonist (4 mg/kg). The 10 experimental groups were as follows: Group 1, six control rats with no previous treatment; Group 2, six 24-hour nephrectomized rats; Group 3, six rats treated with vasopressin antagonist (30 μg/kg); Group 4, six rats treated with a ganglionic blocker (hexamethonium, 25 mg/kg); Group 5, six 24-hour adrenalectomized rats; Group 6, six 24-hour adrenalectomized rats treated with hexamethonium (25 mg/kg); Group 7, six rats treated with α- and β-adrenergic receptor blockers (phentolamine, 4 mg/kg, and propranolol, 2 mg/kg); Group 8, six 24-hour nephrectomized and adrenalectomized rats treated with vasopressin antagonist (30 μg/kg), hexamethonium (25 mg/kg), phentolamine (4 mg/kg), and propranolol (2 mg/kg); Group 9, six rats treated with histamine H1- and H2-receptor blockers (chlorpheniramine, 240 μg/kg, and cimetidine, 16 mg/kg); and Group 10, six rats treated with indomethacin (7.5 mg/kg), a blocker of prostaglandin synthesis.

At the time of adrenalectomy, Groups 4 and 8 received injections of 0.2 mg of deoxycorticosterone acetate and 1.0 mg of hydrocortisone in oil suspension.

To test whether blood pressure changes from baseline induced by bradykinin and kinin antagonist were statistically significant, the data were analyzed by one-sample (paired) t test. Two-sample t tests were used to determine differences in baseline blood pressure due to the various treatments. A p value below 0.05 was considered significant.

**Results**

Hexamethonium inhibited the increase in heart rate produced by the hypotensive effect (30 mm Hg) of sodium nitroprusside (20 μg/kg) by 88% (80 ± 10 and 10 ± 5 beats/min before and after, respectively; n = 3). Phentolamine inhibited the hypertensive effect of norepinephrine (2 μg/kg) by 77% (48 ± 5 and 11 ± 1 mm Hg before and after, respectively; n = 5). Propranolol inhibited the increase in heart rate produced by isoproterenol (0.8 μg/kg) by 86% (105 ± 18 and 15 ± 2 beats/min before and after, respectively; n = 4). The vasopressin antagonist completely blocked the vasopressor effect of 10 μU of vasopressin (52 ± 2 mm Hg; n = 3). Chlorpheniramine and cimetidine inhibited the depressor effect of histamine (4 μg/kg) by 55% (18 ± 2 and 8 ± 2 mm Hg before and after, respectively; n = 4). The kinin antagonist at a dosage of 40 μg/kg/min blocked the vasodepressor effect of bradykinin (400 ng/kg) by 51 ± 4%. The vasodepressor effect of sodium nitroprusside (16 μg) was not altered (before, 29.6 ± 3 mm Hg; after, 29.2 ± 2 mm Hg; NS). The concentration of the kinin antagonist used in the present work was 100-fold more than that needed to partially block the hypotensive effect of injected kinins.

The basal mean blood pressure values of the different experimental groups are shown in Table 1. The nephrectomized rats (Group 2) had higher blood pressure measurements compared to the control group (Group 1). The data were analyzed using Student's t test, and a p value below 0.05 was considered significant.
pressure \((p < 0.001)\) than the control group (Group 1). The blood pressure of the rats treated with ganglionic blocker (Groups 4, 6, and 8) and with \(\alpha-\) and \(\beta-\) blockers (Group 7) were significantly lower \((p < 0.001)\) than that of the control rats.

In the control group (Figure 1), the bolus injection of kinin antagonist induced a small pressor effect followed by a depressor effect, whereas the bolus injection of bradykinin produced a decrease followed by a small increase in blood pressure. Both biphasic responses were transient, and blood pressure returned to baseline after 1 to 2 minutes. The pressor response induced by bradykinin was not present in the adrenalectomized rats from Group 5 (Figure 2) and did not reach statistical significance in the nephrectomized rats (Groups 2 and 8; see Figures 1, 2, and 3) or in the group treated with vasopressin V\(_1\)-receptor antagonist (Group 3; see Figure 2). However, the pressor responses induced by bolus injection of kinin antagonist were present in all experimental groups studied (see Figures 1, 2, and 3). Bradykinin induced a depressor effect in all groups, but in the nephrectomized and adrenalectomized rats treated with ganglionic and \(\alpha-\) and \(\beta-\) blockers, and with vasopressin antagonist (Group 8; see Figure 3), this effect did not reach statistical significance. The depressor effect induced by the kinin antagonist was blocked by nephrectomy (Groups 2 and 8; see Figures 1 and 3) and by indomethacin (Group 10; see Figure 3).

**Discussion**

Kinins are potent vasodepressor peptides that, by acting as autacoids, may regulate vascular resistance and consequently, normal blood pressure. In the last few years, many studies assessed the physiological role of kinins, but the approaches were indirect since no specific antagonists were available. Recently, a family of bradykinin analogues having antagonistic properties was synthesized. In this study, we used one of these kinin antagonists, \(\text{DArg}^1\text{Hyp}^3\text{Thr}^{2\text{A}}\text{dPhe}^7\text{bradykinin}\), to determine the role of endogenous kinins on normal blood pressure maintenance. Low doses of this kinin antagonist inhibit the vasodepressor effect of intra-aortically injected bradykinin by more than 50%. In normal rats, high doses of the antagonist produced a small but significant increase in blood pressure followed by a depressor effect. These results agree with those of Benetos et al., who showed that high doses of a similar kinin antagonist produced a pressor effect and also a depressor effect when injected into normal rats.

Administration of bradykinin produces a biphasic blood pressure effect: a decrease followed by an increase. An increase in blood pressure also was observed when bradykinin was administered into the carotid artery of the cat and dog. These findings suggest that, in addition to its peripheral vasodilator effect, bradykinin may exert peripheral and central effects tending to increase blood pressure, which could
be involved in the biphasic or pressor response described previously.

Kinins can stimulate renin secretion from the kidney⁴ and vasopressin from the central nervous system, as well as release catecholamines from the adrenal medulla⁴ and sympathetic ganglia.⁷ In our study, the pressor response induced by bradykinin was not present in adrenalectomized rats and was negligible in nephrectomized rats and in the group treated with vasopressin V₁-receptor antagonist. These results suggest that the vasopressor response to bradykinin is mediated by the release of vasopressor hormones from several sources. In contrast, the vasopressor action of the kinin antagonist cannot be explained by this mechanism, since it was present in all experimental groups. Thus, it is likely that the vasopressor actions of the kinin antagonist are due to inhibition of the vasodepressor activity of endogenous kinins, although a direct cardiovascular effect of high doses of the kinin antagonist cannot be completely ruled out.

The mechanisms mediating the vasodepressor actions of the kinin antagonist and of bradykinin appear to be different. The vasodepression induced by the kinin antagonist was not observed in nephrectomized rats or in indomethacin-treated rats. Thus, the hypotensive effects of the kinin antagonist appear to be due to the release of vasodilating prostanoids from the kidney. Kinins can release prostaglandins from a variety of tissues, including the kidney.¹⁰ It could be that at the dose administered, the kinin antagonist induced prostaglandin synthesis by a kinin-like agonistic activity on renal receptors. It cannot be ruled out that the hypotensive response induced by the kinin antagonist may be due to an effect related to impurities present in the preparation when administered at a high dose. We are not aware, however, of the presence of contaminants in the synthetic kinin antagonist preparation.

The results show that bradykinin causes a vasodepressor effect by a direct action on peripheral vessels, which may be mediated by the release of endothelial-derived relaxing factor.¹⁴ At the dose of bradykinin used, release of prostanoids from the kidneys did not appear to contribute to its vasodepressor activity.

We conclude that the depressor effects of the kinin antagonist may be due to agonistic action. The pressor effect induced by kinin antagonist suggests that endogenous kinins may contribute to the maintenance of normal blood pressure.

References


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