Cardiovascular Effects of Brain Kinin Receptor Blockade in Spontaneously Hypertensive Rats

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Abstract
We studied the role of brain kinins in the regulation of cardiovascular function. Intracerebroventricular injection of 380 pmol bradykinin increased mean blood pressure by 20±2 mm Hg (P<.01) in normotensive Wistar-Kyoto (WKY) rats. Complete inhibition of this effect was achieved with intracerebroventricular administration of the newly synthesized, long-acting B2 receptor antagonist d-Arg,[Hyp3, Thr3, D-Tic7, Oic8]-bradykinin (Hoe 140). On a molar basis, Hoe 140 was two orders of magnitude more potent than antagonists of the first generation. Baroreceptor sensitivity, estimated as the heart rate response to blood pressure changes induced by intravenous injection of phenylephrine or sodium nitroprusside, was not altered by Hoe 140 in WKY rats. In spontaneously hypertensive rats (SHR), baroreceptor reflex sensitivity to increments in mean blood pressure was reduced by Hoe 140 (mean slope value: -0.47±0.07 versus -0.92±0.13 beats per minute per millimeter of mercury in controls, P<.05). Hoe 140 did not affect the tachycardic component of the baroreceptor reflex. Two-week intracerebroventricular infusion of Hoe 140 did not alter systolic blood pressure or heart rate in WKY rats. In SHR, systolic blood pressure increased (P<.01) similarly during the infusion of Hoe 140 or vehicle (from 174±6 to 220±5 mm Hg and 178±4 to 210±4 mm Hg at 2 weeks, respectively), whereas heart rate did not change. Failure of long-term blockade of B2 receptors to affect normal blood pressure of WKY rats and to blunt the progression of hypertension in SHR does not favor the hypothesis that brain kinins are involved in the regulation of blood pressure. On the other hand, the present study suggests modulation of baroreceptor reflex sensitivity by brain kinins in genetic hypertension.

Key Words • pressoreceptors • kinins • kallikrein • hypertension, essential

The brain kallikrein-kinin system may be involved in the central control of cardiovascular function. Intracerebroventricular (ICV) administration of bradykinin increases blood pressure, heart rate (HR), and baroreceptor sensitivity in normotensive rats.1-3 As ICV bradykinin antagonists reportedly fail to alter blood pressure and HR, circumventricular receptors might not modulate cardiovascular function in basal conditions.4,5 However, maneuvers that activate the brain kallikrein-kinin system are associated with hypertension and tachycardia, and these responses are prevented by blockade of brain bradykinin receptors6-8; thus, endogenous kinins may become important in stimulated conditions. The brain kallikrein-kinin system is hyperactive in spontaneously hypertensive rats (SHR), as suggested by the findings that in this strain the vasopressor response to ICV bradykinin is exaggerated9 and that the short-term blood pressure increase induced by ICV captopril, a kininase inhibitor,9 can be prevented by a bradykinin antagonist.9 In addition, short-term blockade of bradykinin receptors reduces the accelerated HR of 14-week-old SHR.10 Unfortunately, the low potency of first-generation bradykinin antagonists has limited their use to acute conditions. Because of their susceptibility to fast enzymatic degradation, these antagonists might be inactivated by endogenous kininases before reaching deep areas of the brain.11 These drawbacks have been overcome by the recent availability of the potent and long-acting B2 receptor antagonist d-Arg,[Hyp3, Thr3, D-Tic7, Oic8]-bradykinin (Hoe 140).12 In the present study, we tested the inhibitory potency of Hoe 140 on the vasopressor effect induced by ICV bradykinin. Then, we evaluated the effects of brain kinin receptor blockade by Hoe 140 on cardiovascular function in normotensive rats and SHR.

Methods
Male Wistar-Kyoto (WKY) rats and SHR (Charles River, Milan, Italy) weighing between 190 and 210 g were housed at constant room temperature with a 12-hour light/dark cycle and had free access to water and rat chow. The experimental protocol was approved by the local Animal Care and Use Committee. All procedures complied with the standards for the care and use of animal subjects as stated in Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, National Academy of Sciences, Bethesda, Md.). Surgical procedures (except cerebroventricular cannula implantation) were performed with rats under ether anesthesia using disappearance of corneal reflex to adjust the depth of anesthesia.

After 3 days of adaptation to the new environment, the rats were anesthetized with pentobarbital (50 mg/kg IP); a 22-gauge stainless steel cannula (15 mm long and bent at a 90° angle at midpoint) was implanted stereotaxically into each lateral cerebral ventricle (1.5 mm lateral and 1.0 mm posterior to the bregma, and 4.5 mm deep from the skull surface). The cannulas were anchored to the skull with screws embedded in dental acrylic cement. The free end of each cannula was attached to a silicone elastomer tube filled with sterile artificial cerebrospinal fluid (aCSF). The tube was tunneled under the skin, exteriorized between the scapulae, and occluded with a metal pin. After 5 days, the correct placement of the cannulas in the ventricles was tested by determining the dipsogenic response to angiotensin II.13

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Experiment 1: Inhibitory Potency of Kinin Antagonists

Six days after implantation of cannulas, a polyethylene catheter (PE-10 connected to a PE-50, Clay Adams, Parsippany, NJ) filled with heparinized saline was inserted into the left femoral artery and advanced into the abdominal aorta. The catheter was tunneled under the skin and exteriorized at the back of the neck. The following day, awake rats were placed in plastic restrainers. Mean blood pressure (MBP) was measured with a Statham transducer (Gould, Oxnard, Calif) connected to the femoral catheter and was recorded continuously on a Quartet recorder (Ugo Basile, Comerio, Italy). At the end of a 30-minute stabilization period, the vasopressor effect induced by ICV injection of 380 pmol bradykinin (Peninsula Laboratories, Belmont, Calif) in 5 μL aCSF was tested in WKY rats (n=6) given a simultaneous injection of 5 μL aCSF via the contralateral ICV cannula. The same dose of bradykinin was injected in WKY rats given a simultaneous ICV injection of 0.7 to 7000 pmol D-Arg1-Hyp5-Thr5,D-Phe7]-bradykinin (Bachem, Torrance, Calif), 0.7 to 7000 pmol Ac-D-Arg1-Hyp5,D-Phe7,Leu5]-bradykinin (Novabiochem, Laufelfingen, Switzerland), or 0.07 to 700 pmol Ac-D-Arg1[9-Hyp3,D-Phe7,Trp8,D-Tic9,Oic10]-bradykinin (Novabiochem, Torrance, Calif) via the contralateral cannula. Injections were made with a 50-μL syringe (Hamilton, Reno, Nev). Each group (n=6) received only one dose of antagonist.

Experiment 2: Baroreceptor Reflex Sensitivity

In addition to the above described surgical procedure, a PE-10 catheter was inserted into the right femoral vein and advanced into the cava. Twenty-four hours later, MBP and HR of awake SHR and WKY rats were recorded. HR was determined with a counter triggered by the arterial pressure pulse. After a 30-minute stabilization period, rats received an ICV bolus injection of 70 pmol Hoe 140 in 10 μL aCSF (n=7) or vehicle (n=7). Thirty minutes later, baroreceptor sensitivity was evaluated by determining the reflex changes of HR in response to changes in MBP induced by intravenous bolus injection of phenylephrine (from 0.12 to 5 nmol) or sodium nitroprusside (from 0.2 to 20 nmol) in 20 μL saline (0.15 mol/L NaCl). Doses of both drugs were given in a random order, and sufficient time was allowed (3 minutes) between injections for HR and MBP to return to control levels. Peak changes in HR occurring during the initial 5 seconds of the corresponding maximum change in MBP were considered.

Experiment 3: Effect of Chronic ICV Infusion of Hoe 140 on Blood Pressure and Heart Rate

Five days after implantation of cerebroventricular cannulas, chronic ICV infusion of Hoe 140 (70 pmol per 0.5 μL aCSF per hour) or vehicle (aCSF) was performed using Alzet osmotic pumps (Alza Corp, Palo Alto, Calif), which were attached to the ICV cannulas and implanted under the skin between the scapulae of WKY rats and SHR. Systolic blood pressure (SBP) and HR were measured before osmotic pump implantation and then every 2 days during the following 2 weeks by tail-cuff plethysmography using a W+1 recorder 8002 (Ugo Basile) in rats prewarmed for 15 minutes at 35°C. The inhibitory activity of Hoe 140 was tested at the end of the experiment by comparing the vasopressor effect of ICV bolus injection of bradykinin (380 pmol in 10 μL aCSF) in vehicle or Hoe 140 groups. Three hours later, the vasodepressor effect of an intra-arterial bolus of bradykinin (380 pmol in 100 μL saline) was tested in both groups. Each group consisted of 10 rats.

Statistical Analysis

All data are expressed as mean±SEM. Baroreceptor reflex sensitivity was determined by calculating the slope of the regression line (least-squares analysis) of HR and MBP changes induced by phenylephrine and nitroprusside. Multivariate repeated-measures analysis of variance was performed to test for interaction between time and grouping factor. Then, univariate analysis of variance was used to test for differences among groups and over time. Differences within or between groups were determined by paired or unpaired t tests with the Bonferroni multiple comparison adjustment.

Results

Experiment 1: Inhibitory Potency of Kinin Antagonists

ICV administration of 380 pmol bradykinin increased MBP by 20±2 mm Hg (from 102±2 to 122±2 mm Hg, P<.01) in the control group. ICV injection of 70 pmol Hoe 140 completely inhibited the vasopressor effect induced by bradykinin (from 102±4 to 102±3 mm Hg, P=NS).

As shown in Fig 1, on a molar basis Hoe 140 was two orders of magnitude more potent than D-Arg1[9-Hyp3,Thr5,D-Phe7]-bradykinin and Ac-D-Arg1[9-Hyp3,D-Phe7,Leu5]-bradykinin. In preliminary experiments we found that inhibition of the bradykinin-induced vasopressor effect was still complete 1 hour after the ICV administration of 70 pmol Hoe 140, whereas the vasopressor response to bradykinin was restored to control levels 10 minutes after the ICV injection of 700 pmol of D-Arg1[9-Hyp3,Thr5,D-Phe7]-bradykinin (21±2 mm Hg) or Ac-D-Arg1[9-Hyp3,D-Phe7,Leu5]-bradykinin (20±1 mm Hg).

Experiment 2: Baroreceptor Reflex Sensitivity

In groups of WKY rats, MBP and HR were similar 30 minutes after the ICV injection of 70 pmol Hoe 140 (MBP, 103±2 mm Hg; HR, 380±2 beats per minute) or aCSF (MBP, 103±3 mm Hg; HR, 378±2 beats per minute). Hoe 140 did not alter baroreceptor reflex sensitivity in WKY rats. Indeed, mean slopes of ΔHR/ΔMBP regression lines for phenylephrine-induced increases and nitroprusside-induced decreases of MBP were similar in the Hoe 140 group (phenylephrine, -1.60±0.28; nitroprusside, -2.77±0.28 beats per minute per millimeter of mercury) and vehicle group (phenylephrine, -1.32±0.15; nitroprusside, -3.60±0.59 beats per minute per millimeter of mercury).
In groups of SHR, MBP and HR were similar 30 minutes after the injection of Hoe 140 (MBP, 184±2 mm Hg; HR, 385±9 beats per minute) or aCSF (MBP, 182±6 mm Hg; HR, 398±10 beats per minute). As shown in Fig 2, the mean slope value of the regression line representing the relation between changes in HR and MBP induced by phenylephrine was significantly lower in SHR given ICV Hoe 140 compared with controls (−0.47±0.07 versus −0.92±0.13 beats per minute per millimeter of mercury, P<.05). By contrast, there was no significant difference between groups as far as reflex tachycardia induced by nitroprusside is concerned (−1.75±0.44 versus −1.60±0.40 beats per minute per millimeter of mercury in controls, P=NS).

**Discussion**

The present study indicates that Hoe 140 is considerably more potent than first-generation kinin antagonists in inhibiting the vasopressor effect induced by ICV administration of exogenous bradykinin. In addition, its inhibitory action is much longer in duration. High stability, due to resistance to kininasases, and high affinity to receptors, even greater than that of bradykinin itself, account for the potency of Hoe 140 compared with first-generation antagonists. The latter compounds show agonistic activity, ie, they increase blood pressure and HR as bradykinin does, when doses just fourfold higher than that needed to block kinins are injected into the cerebroventricular space. They also cause barrel rotation, an effect that is not related to kinin-blocking ability.\(^4\) By contrast, neither residual agonistic activity nor nonspecific effects, such as barrel rotation, were observed after ICV Hoe 140, even at a dose 10-fold higher than that able to block the vasopressor effect of 380 pmol bradykinin. Therefore, Hoe 140 proved to be a useful tool in evaluating the cardiovascular effects of endogenous kinins.

Gerken and Santos\(^3\) have shown that centrally infused bradykinin increases baroreceptor reflex sensitivity in response to graded doses of phenylephrine, whereas it does not alter the tachycardic reflex response to nitroprusside. They hypothesized that endogenous bradykinin, acting as a neurohormone, modulates the baroreceptor reflex control of HR. However, failure of Hoe 140 to affect the HR reflex response to short-term changes in blood pressure suggests that in normotensive WKY rats endogenous kinins do not play a major role in the regulation of baroreceptor reflex sensitivity.

Our findings confirm that a resetting of the baroreceptors occurs in SHR and that this phenomenon is associated with a depression of baroreceptor reflex gain. In SHR, Hoe 140 further reduced the sensitivity of the reflex for short-term increments in blood pressure. This suggests that brain kinins could partially counteract the tendency toward depression of baroreceptor reflex gain, which is a typical feature of genetic hypertension. The decrease in sensitivity cannot be attributed to changes in basal MBP and HR because Hoe 140 did not alter these parameters. In preliminary experiments, we found that intravenous administration of Hoe 140 does not alter the baroreceptor reflex in SHR, thus discounting the possibility that the effect of ICV Hoe 140 is determined by diffusion of the antagonist into the bloodstream leading to peripheral blockade of bradykinin receptors (P. Madeddu, unpublished observations). The difference between SHR and WKY rats regarding the effect of Hoe 140 on baroreceptor sensitivity is consistent with the hypothesis that the brain kallikrein-kinin system is hyperactive in SHR.\(^2\)

We found that the tachycardic reflex response to graded doses of nitroprusside was not altered by Hoe 140 in SHR, thus confirming the results of a previous study in which the first-generation antagonist D-Arg, [Hyp\(^+\),Thr\(^3\),D-Phe\(^1\)]-bradykinin was used.\(^6\) Selective al-
eratation of the bradycardic component of the baroreceptor reflex after administration of Bradykinin to hypertensive rats has already been reported. 3 Differential influence of Bradykinin on the multiple brain structures involved in the regulation of baroreceptor reflex control of HR 4 could account for such a selectivity. 3

The neural mechanisms and brain sites implicated in the modulatory action of kinins on the baroreceptor reflex were not investigated in the present study. Previous reports have proposed that parasympathetic mechanisms are responsible for the increase in baroreceptor reflex sensitivity induced by ICV bradykinin. The lateral septal area, the ventral portion of the third ventricle, and structures of the brain near the fourth ventricle have been identified as potential sites of the cardiovascular actions of bradykinin. 1,2,6 It has been suggested by Michelini and Lebrun 17 that bradykinin, acting at the solitari-vagal complex, can improve the bradycardic response to transient blood pressure increases in either normotensive rats or SHR. However, microinjection of Hoe 140 depressed the bradycardic response in normotensive rats only. 17 Recently, the cardiovascular effects induced by the injection of bradykinin into the nucleus tractus solitarii have been suggested to be mediated by B 1 rather than B 2 receptors. 18 Therefore, the nucleus tractus solitarii might not be implicated in the reduction of baroreceptor sensitivity that we found in SHR after ICV injection of the B 2 receptor antagonist Hoe 140.

Acute ICV injection of first-generation antagonists does not alter blood pressure of normotensive and hypertensive rats. 4,5 Privitera 19 reported that acute injection of d-Arg [Hyp 3 ,Thi 2 ,d-Phe 7 ]-bradykinin into the rostral ventrolateral medulla decreases blood pressure by 39% in SHR and 11% in normotensive Wistar rats. As far as we know, the present study is the first report indicating that chronic infusion of Hoe 140, at a dose able to completely block the vasopressor effect of ICV bradykinin, did not alter the normal blood pressure of WKY rats. Progression of hypertension in SHR was not affected as well, although a modest difference in SBP of vehicle and Hoe 140 groups was observed early during the experimental period. Diffusion of Hoe 140 from the cerebroventricular space to the periphery is discount by the finding that the vasodepressor effect induced by intra-arterial bradykinin in rats given chronic ICV Hoe 140 was similar to that observed in controls. These findings do not favor the hypothesis that brain kinins play a major role in long-term regulation of blood pressure.

In conclusion, brain kinins, acting as neurohormones, could modulate baroreceptor reflex sensitivity in SHR, whereas they may not be involved in the long-term progression of hypertension in this genetic model.

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