Bradykinin B₂-Receptor Blockade Facilitates Deoxycorticosterone-Salt Hypertension

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The contribution of endogenous kinins to the regulation of blood pressure, urinary volume, and renal sodium excretion was evaluated in Wistar rats on high sodium intake by using the new bradykinin receptor antagonist Hoe 140 (D-Arg, Hyp³, Thi⁴, D-Tic⁷, Oic⁸)-bradykinin). Neither Hoe 140 (3 nmol/hr s.c. for 4 weeks) nor its vehicle altered systolic blood pressure (tail-cuff plethysmography) or renal function in rats given saline solution (0.15 mol/L NaCl) to drink ad libitum. Four-week administration of deoxycorticosterone (DOC), combined with high sodium intake and uninephrectomy, increased systolic blood pressure from 127±3 to 160±3 mm Hg (p<0.01). When long-term infusion of Hoe 140 was combined with DOC, high sodium intake, and uninephrectomy, systolic blood pressure rose from 127±3 to 175±3 mm Hg (p<0.01). The hypertensive effect was greater in the Hoe 140 group (48±4 versus 33±3 mm Hg in controls, p<0.05). This difference was confirmed by direct measurement of mean blood pressure (Hoe 140 group, 154±4 mm Hg; vehicle group, 139±4 mm Hg; p<0.05). The antagonist blunted the increase in urinary volume induced by salt load and DOC in uninephrectomized rats, whereas it did not alter the increase in urinary sodium excretion. These results suggest that endogenous kinins do not play a major role in the regulation of normal blood pressure in sodium-loaded rats, whereas they may attenuate the hypertensive effect induced by long-term administration of mineralocorticoids and salt in uninephrectomized rats. (Hypertension 1993;21:980-984)

KEY WORDS • kinins • bradykinin • kallikrein • blood pressure • mineralocorticoids

Kinins are endogenous vasodilators that act as local hormones by activating the release of endothelium-derived relaxing factors and prostaglandins. In addition, they could participate in the regulation of blood pressure by modulating the renal excretion of sodium and water. In turn, changes in sodium and water metabolism can alter kallikrein synthesis, kinin release, kininase activity, and kinin receptor expression. These alterations may contribute to the maintenance of normal blood pressure and renal function.

At least two distinct kinin receptor subtypes, namely, B₁ and B₂, have been identified. Most cardiovascular and renal effects of bradykinin are mediated by B₂-receptors, whereas the B₁-subtype may become important in particular conditions, such as inflammation and tissue damage. Studies on the effects of long-term kinin receptor blockade have been precluded by the shortcomings of first-generation kinin receptor antagonists, namely, residual agonistic activity and short biological half-life. These drawbacks have been overcome by the recent synthesis of a potent, long-acting, specific, and selective bradykinin B₂-receptor antagonist, D-Arg, Hyp³, Thi⁴, D-Tic⁷, Oic⁸)-bradykinin (Hoe 140). We found that administration of Hoe 140 for 6 weeks causes hypertension in deoxycorticosterone (DOC)-treated rats on normal sodium intake, an experimental model in which the kallikrein-kinin system is activated.

The present study was designed to evaluate the action of endogenous kinins during long-term alterations in sodium balance. Therefore, we studied the effect of long-term blockade of bradykinin B₂-receptors by Hoe 140 on blood pressure and renal function in 1) rats given saline solution to drink ad libitum and 2) uninephrectomized rats administered DOC combined with high salt intake.

Methods

Male Wistar rats (Morini, Como, Italy) weighing between 220 and 240 g were housed at a constant room temperature with a 12-hour light/dark cycle and had free access to 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 animals as stated in Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, National Academy of Science, Bethesda, Md.). All surgical procedures (catheter and osmotic pump implantation and uninephrectomy) were performed with rats under ether anesthesia using disappearance of the corneal reflex to adjust the depth of anesthesia.

Experiment 1: Effects of Hoe 140 in Rats With High Sodium Intake

Systolic blood pressure, heart rate (tail-cuff plethysmography method, W+W BP Recorder 8002, Ugo...
vascular effect of an intra-aortic bolus injection of 
Rats With High Sodium Intake were deprived of food.
Experiment 2: Effects of Hoe 140 in 
Uninephrectomized Deoxycorticosterone-Treated 
Rats With High Sodium Intake
The experimental protocol was similar to experiment 1 except that, after basal blood pressure measurements and urine collection, rats of both the vehicle (n=10) and Hoe 140 (n=10) groups underwent unilateral nephrectomy (at the same time as osmotic pump implantation) and were injected subcutaneously with DOC enantate (Shering, Milan, Italy) at a dose of 75 nmol in 100 µL saline per kilogram body weight once a week. In preliminary experiments, we found that, 1 week after this dose, plasma DOC concentration exceeded normal levels by sixfold (18.2±0.7 versus 3.2±0.3 nmol/L in controls). During the experimental period, rats were given saline solution (0.15 mol/L NaCl) to drink ad libitum instead of tap water.

Analytical Procedures
Urinary volume was determined gravimetrically. Urinary sodium was determined with flame photometry. Urinary creatinine was measured with an automatic analyzer (Hitachi 704). Kallikrein activity in urine was measured by using the synthetic substrate H-D-Val-Leu-Arg-paranitroanilide (S2266, Kabi, Stockholm, Sweden) in the presence of soybean trypsin inhibitor (Sigma Chemical Co., St. Louis, Mo.) and was expressed in nanokatal (1 nkat represents the enzyme activity able to cleave 1 nmol p-nitroaniline per second from substrate).

Results
Body weight gain during the study was similar among groups. As shown in the left panels of Figure 1, Hoe 140 did not alter systolic blood pressure and heart rate of rats on high sodium intake. As shown in the right panels of Figure 1, systolic blood pressure was increased by DOC and high salt intake in uninephrectomized rats (vehicle group, from 127±3 to 160±3 mm Hg, p<0.01; Hoe 140 group, from 127±3 to 175±3 mm Hg, p<0.01). The hypertensive effect was greater in the Hoe 140 group (48±4 versus 33±3 mm Hg in controls, p<0.05). This difference was confirmed by direct measurement of mean blood pressure at the end of the study (Hoe 140 group, 154±4 mm Hg; vehicle group, 139±4 mm Hg; p<0.05). The vasodepressor effect of bradykinin was inhibited by Hoe 140 in rats with (0±1 versus 28±3 mm Hg in controls) or without (0±2 versus 19±3 mm Hg in controls) DOC treatment.

As shown in Figure 2, Hoe 140 blunted the increase in urinary volume induced by long-term sodium load and DOC in uninephrectomized rats, whereas it did not alter the increase in urinary sodium excretion. Urinary creatinine excretion remained unchanged in both groups of experiment 1, whereas it increased similarly in DOC-treated rats given vehicle or Hoe 140 (Table 1). Urinary kallikrein excretion (measured weekly) increased (p<0.01) in rats on high sodium intake (vehicle: from 11.9±0.9 to 11.8±0.8, 11.3±1.2, 14.4±0.8, and 16.5±0.8 nkat per 24 hours; Hoe 140: from 13.3±1.1 to 13.6±0.8, 12.0±1.2, 14.4±0.8, and 16.7±1.2 nkat per 24 hours). An initial decrease was observed after uninephrectomy (p<0.01), then urinary kallikrein excretion increased (p<0.01) during the following weeks of DOC administration (vehicle: from 9.0±1.0 to 3.0±0.7, 4.1±0.6, 4.4±0.3, and 4.6±0.3 nkat per 24 hours; Hoe 140: from 8.7±0.6 to 2.5±0.3, 3.3±0.2, 4.0±0.5, and 4.3±0.2 nkat per 24 hours).

Discussion
The finding that long-term administration of Hoe 140, at a dose that abolishes the vasodepressor effect of exogenous bradykinin, did not alter blood pressure, urinary volume, and renal sodium excretion in normotensive salt-loaded rats is consistent with previous studies performed in rats on normal sodium intake. In addition, Hoe 140 did not alter blood pressure in uninephrectomized rats on normal sodium intake (P. Madeddu, unpublished observations). Collectively,
these results suggest that endogenous kinins do not play a major role in the regulation of blood pressure and renal function in normotensive rats during alterations in sodium balance.

Long-term administration of DOC, a mineralocorticoid that enhances renal kallikrein activity and excretion,\(^5,7\) does not normally induce sustained hypertension unless combined with sodium loading and uninephrectomy. In previous studies, we demonstrated that long-term blockade of bradykinin B\(_2\)-receptors by Hoe 140 causes hypertension in DOC-treated rats on normal sodium intake.\(^3\) We speculated that activation of the renal kallikrein-kinin system by DOC could represent an important compensatory response to counteract the vasoconstrictive and salt-retaining effect of this mineralocorticoid.

As expected, long-term administration of DOC combined with high sodium intake caused a sustained increase in blood pressure in uninephrectomized rats. The decrease in urinary kallikrein excretion found 1 week after nephrectomy is clearly the consequence of the reduction in renal mass combined with salt load, whereas the subsequent increase may reflect stimulation of renal kallikrein synthesis, release, or both by long-term mineralocorticoid administration. The finding that inhibition of bradykinin B\(_2\)-receptors by Hoe 140 enhanced the hypertensive effect of DOC suggests that the kallikrein-kinin system can partially counteract mineralocorticoid hypertension. After completion of the present study, further experiments were performed in which the delivery of Hoe 140 was maintained for 2 additional weeks by replacing osmotic pumps 28 days after implantation. At 6 weeks, a group difference in systolic blood pressure was still observed (vehicle group, 170±4 mm Hg; Hoe 140 group, 186±4 mm Hg; \(p<0.05\)) (P. Madeddu, unpublished observations).

Recently, Majima et al\(^9\) reported that Brown Norway Katholiek rats, which have a congenitally deficient kallikrein-kinin system, exhibit a more rapid early increase in systolic blood pressure during DOC-salt treatment compared with normal rats of the same strain (Brown Norway Kitasato rats). The difference in systolic blood pressure between the two groups averaged 25 mm Hg at the fourth week of DOC treatment, whereas in our study Hoe 140 enhanced the hypertensive effect of DOC by 15 mm Hg only. The antagonist, even at a dose that completely inhibits the vasodepressor effect of exogenous bradykinin, might not be able to reproduce the condition of Brown Norway Katholiek rats, which are deficient in kininogen in plasma and devoid of kinin release in the urine. This possibility appears unlikely because, due to high affinity for receptors and resistance to kininases, Hoe 140 has been shown to exert a very potent and long-lasting inhibition of endogenous kinins within the vasculature as well as at the interstitial and tubular side of the nephron.\(^4,5\) In addition, the increase in systolic blood pressure observed by Majima in Brown Norway Katholiek rats was similar to that found in our DOC-treated rats given Hoe 140 (170 versus 175 mm Hg), whereas the difference between the two studies consisted mainly in a milder hypertensive effect induced by DOC in normal Brown Norway Kita-
sato rats compared with that observed in rats given Hoe 140 vehicle (145 versus 160 mm Hg). This difference might be explained by the finding that activation of the kallikrein-kinin system by DOC was more pronounced in Majima’s study.

Despite the reduced renal mass, urinary volume and sodium excretion of DOC-treated rats increased to levels similar to those found in rats on high salt intake. An increase in glomerular filtration rate by the untouched kidney might have occurred (as suggested by the increase in urinary creatinine excretion), thus contributing to the escape from the sodium-retaining effect of DOC.

Hoe 140 blunted the increase in urinary volume observed in DOC-treated rats on high salt intake, whereas it did not alter the changes in urinary sodium excretion. A dissociation in the effects of kinin blockade on urinary volume and sodium excretion has been reported in DOC- and salt-treated rats infused for a short time with a kinin antagonist of the first generation.10 This finding was interpreted as a consequence of more effective blockade of kinins in the vascular interstitial space of the kidney. However, effectiveness of Hoe 140 in the intratubular part of the nephron is documented by its excretion with urine as intact compound.5 Because in the present study sodium/water balance was not determined, it is difficult to say, on the basis of the difference in urinary volume between vehicle- and Hoe 140-treated groups, whether any renal mechanism was implicated in accelerating DOC-induced hypertension in rats given the antagonist. An intriguing possibility is that the increase in blood pressure was enhanced by the blockade of kinins generated within the arterial vasculature,11 leading to predominance of vasopressor systems.

In conclusion, our results indicate that the kallikrein-kinin system does not play an important role in the regulation of normal blood pressure in rats on high salt intake. On the other hand, they suggest that endogenous kinins counteract, at least in part, hypertension induced by an excess of mineralocorticoids and salt in uninephrectomized rats.

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