Converting Enzyme Inhibition in Kinin-Deficient Brown Norway Rats

Lars Danckwardt, Ikuko Shimizu, Gerd Bönner, Rainer Rettig, and Thomas Unger

The contribution of endogenous kinins to the acute antihypertensive actions of the converting enzyme inhibitor ramipril was investigated in kinin-deficient Brown Norway rats and in Brown Norway-Hannover rats and Wistar rats as controls. In Brown Norway rats, urinary kinin excretion was measurable but extremely low when compared with control strains. The depressor responses to intra-arterial bradykinin injections 1) were not different between Brown Norway and Brown Norway-Hannover rats, 2) were potentiated by intravenous ramipril (60 \mu g), and 3) were attenuated by intra-arterial infusion of the bradykinin antagonist B4146 (40 \mu g/kg/min) to a similar extent in both strains. In renal hypertensive (two-kidney, one clip) Brown Norway rats, the blood pressure reductions to intravenous bolus injections of ramipril (100 \mu g) were significantly reduced both in extent and duration when compared with hypertensive Brown Norway-Hannover and Wistar rats. Intra-arterial infusion of B4146 (40 \mu g/kg/min) attenuated the depressor response to ramipril in Wistar and Brown Norway-Hannover rats but had no effect in Brown Norway rats. In contrast, all three groups showed similar depressor responses to intravenous infusions of the angiotensin II receptor antagonist saralasin. These responses were not influenced by the bradykinin antagonist. Our data support the hypothesis that kinins are important for the acute antihypertensive actions of converting enzyme inhibitors. (Hypertension 1990;16:429-435)

A potentiation of the vasodilator and natriuretic actions of endogenous kinins has been implicated in the antihypertensive mechanisms of converting enzyme (CE) inhibitors, as CE is identical with the kinin-degrading enzyme kininase II. However, attempts to elucidate the contribution of endogenous kinins to the various cardiovascular actions of CE inhibitors have yielded equivocal results (for review, see Reference 2). The apparent discrepancies in the literature may be partly due to methodological difficulties. For instance, until recently kinin concentrations in blood and urine could not be measured reliably, and tools to antagonize the actions of kinins, such as bradykinin (BK) receptor antagonists, were lacking. This situation has now improved considerably with the introduction of reliable kinin assays as well as highly specific and reasonably potent BK antagonists. Additional help to study the actions of endogenous kinins is the Brown Norway (BN) rat (May/Pfd/f), a kinin-deficient animal that has recently become available for research.

BN rats completely lack one of the kinin precursors, the high molecular weight kininogen, and are further deficient in low molecular weight kininogen and plasma prekallikrein. These various defects of the kallikrein-kinin system are associated with impaired blood coagulation and reduced inflammatory responses.

In the present study, we used the BN rat to investigate the contribution of endogenous kinins to the acute antihypertensive actions of a CE inhibitor in experimental renal hypertension. We hypothesized that if kinins were important, kinin-deficient animals would generally respond less to the CE inhibitor than their normal-kinin controls. We assumed further that in rats with an intact kallikrein-kinin system, the contribution of kinins would be reflected by an attenuation of the antihypertensive effects of the CE inhibitor by a BK antagonist, whereas in kinin-deficient animals the BK antagonist would be without effect.

Methods

Animals

Male kinin-deficient BN rats of the May/Pfd/f "catholic" strain were obtained from the Katholische Universiteit of Leuven, Heverlee, Belgium.* BN-

*As of January 1990, this strain is bred at the Department of Pharmacology of the University of Heidelberg.
Hannover (BN-HA) control rats were obtained from the Zentralinstitut für Versuchstierkunde, Hannover, FRG. Wistar control rats were purchased from Dr. K. Thomae, GmbH, Biberach, FRG. Animals were housed under conditions of constant temperature with a 12-hour light/dark cycle and had free access to tap water and rat chow.

**Surgery, Catheters, Renal Artery Clipping**

Constant infusions were made by an infusion pump (Braun AG, Melsungen, FRG) that delivered 20 μl/min peptide or vehicle solutions. Blood pressure was measured via an arterial line with a Statham P23Db pressure transducer, Gould Brush pressure computer, and Gould Brush 2400 recorder (all Gould Inc., Oxnard, Calif.). During the experiments, rats were conscious and unrestrained in their home cages. For surgery, rats were initially anesthetized with ether followed by intravenous injections of thiohexi-nal (10 mg/kg) when required.

One day before the experiment, all rats were instrumented with catheters placed in the abdominal aorta (PP-10 in PP-50, Portex Corp., Hythe, Kent, UK) and inferior vena cava (PP-25) via the right femoral artery and vein for monitoring arterial pressure and for intravenous administration of drugs. Two additional catheters (PP-10 in PP-50) were introduced into the left carotid artery for administration of BK and the BK antagonist, respectively. Catheters were brought to the exterior at the nape of the neck.

Renal hypertension was induced by placing a solid silver clip (0.2 mm i.d.) on the left renal artery (two-kidney, one clip hypertension). Male BN, BN-HA, and Wistar rats weighing between 70 and 90 g at the time of surgery were used. Rats were anesthetized and an incision 2 cm in length was made in the left side. The renal artery was stretched by means of a retractor placed between kidney and muscle layer, separated from the renal vein, and cleaned of connective tissue. Clips were applied on the renal artery as close to the aorta as possible. The contralateral kidney was left untouched. Systolic blood pressure (tail-cuff plethysmography under light ether anesthesia) and body weight were determined before and at weekly intervals after surgery. Before and 6 weeks after induction of hypertension, blood (1 ml) was taken from the retro-orbital plexus under light ether anesthesia for measurement of plasma renin concentration (PRC). After 6 to 8 weeks, when blood pressure had risen to levels of about 180 mm Hg, the acute experiments were performed.

**Experiment 1: Effects of Bradykinin Receptor Blockade and Converting Enzyme Inhibition on Depressor Responses to Exogenous Bradykinin in Brown Norway and Brown Norway–Hannover Rats**

BN (n=7) and BN-HA (n=7) rats weighing about 250 g were used. On the day of the experiment, the rats were attached to the monitoring equipment. After 60 minutes of stabilization, intra-arterial bolus injections of BK (100, 200, 400, and 800 ng/kg) were given via a carotid catheter at 5-minute intervals. Thirty minutes later, an infusion with the BK antagonist B4146 (40 μg/kg/min) was started via the second carotid catheter, and the intra-arterial BK bolus injections were repeated. Two hours later, the rats were pretreated with 60 μg/kg ramipril intravenously, and the BK injections were repeated. The dose of the BK antagonist was selected on the basis of previous reports using the same antagonist.5,15

**Experiment 2: Influence of Bradykinin Receptor Blockade on Depressor Response to Converting Enzyme Inhibition in Renal Hypertensive Rats**

One day before the experiment, all rats were catheterized as described with the exception of the second carotid catheter. Rats of each strain (n=8 per group) were randomly allocated to two groups (n=4 each) for a 3-day protocol in which each rat served as its own control.

On day 1, group 1 of each strain received an intravenous bolus injection of the CE inhibitor ramipril (100 μg/kg) during an intra-arterial infusion of vehicle (0.9% saline). On day 3, the ramipril injection was repeated but this time during an intra-arterial infusion of the BK antagonist (40 μg/kg/min) instead of vehicle. The intra-arterial infusions were commenced 5 minutes before and lasted for 45 minutes after the ramipril injection. Group 2 of each strain underwent the same protocol but in reverse order with respect to day 1 and day 3. Because statistical analysis of the preinjection baseline parameters did not reveal any differences between group 1 and 2 of each strain, the data from both subgroups were pooled for further calculations.

**Experiment 3: Influence of Bradykinin Receptor Blockade on Depressor Responses to an Angiotensin II Receptor Antagonist in Renal Hypertensive Rats**

Induction of hypertension, preparation of rats, subgroup allocation, and 3-day protocol was as in experiment 2. BN (n=7), BN-HA (n=7), and Wistar (n=7) rats received a 50-minute intra-arterial infusion of either 0.9% saline or the BK antagonist. Five minutes after commencing the vehicle/BK antagonist infusion, an intravenous infusion of the angiotensin II (Ang II) receptor antagonist saralasin (0.15 μg/kg/min) was given for 45 minutes. The dose of saralasin used had been shown in previous experiments to completely prevent the pressor responses to an intravenous injection of 10 ng Ang II and, further, to decrease blood pressure in renally hypertensive Wistar rats to a similar extent as the dose of ramipril (100 μg i.v.) used. It was not intended in these experiments to compare the maximal antihypertensive effects of Ang II receptor antagonism with those of CE inhibition.

**Experiment 4: Urinary Kinin Excretion in Brown Norway, Brown Norway–Hannover, and Wistar Rats**

Urinary kinin excretion was determined in BN (n=8), BN-HA (n=8), and Wistar (n=7) rats.
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TABLE 1. Depressor Response to Exogenous Bradykinin

<table>
<thead>
<tr>
<th>Strain</th>
<th>Treatment</th>
<th>Basal MAP (mm Hg)</th>
<th>Change of MAP (mm Hg) to BK (ng/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BN-HA (n=7)</td>
<td>Vehicle</td>
<td>112.5±4.0</td>
<td>-14.4±3.2</td>
</tr>
<tr>
<td></td>
<td>BKA</td>
<td>119.5±5.1</td>
<td>-20.7±2.0</td>
</tr>
<tr>
<td></td>
<td>CEI</td>
<td>110.9±4.3</td>
<td>-17.7±4.7</td>
</tr>
<tr>
<td>BN (n=7)</td>
<td>Vehicle</td>
<td>117.5±4.0</td>
<td>-10.2±1.1</td>
</tr>
<tr>
<td></td>
<td>BKA</td>
<td>118.6±3.2</td>
<td>-8.3±2.5</td>
</tr>
<tr>
<td></td>
<td>CEI</td>
<td>111.6±3.9</td>
<td>-28.0±4.7</td>
</tr>
</tbody>
</table>

Reported data are mean±SEM. In both groups the BKA significantly (p<0.01) attenuated, and ramipril potentiated (p<0.01), the BK-induced depressor responses (analysis of variance of entire curves, see Methods). There was no difference between strains. MAP, mean arterial pressure; BK, bradykinin; BN-HA, Brown Norway-Hannover rats; BN, Brown Norway rats; BKA, bradykinin antagonist (B4146); CEI, converting enzyme inhibitor (ramipril).

rats had been unilaterally nephrectomized 2 months before the experiment.

A silicone catheter was placed in the left ureter under anesthesia and exteriorized at the nape of the neck. The implantation techniques have been described previously. In the present experiment, a modified ureter catheter without flushing tube was used. After 2 days of recovery, urine samples were collected in the morning at 9:00 AM (two collection periods) and in the evening at 5:00 PM (two collection periods) into ice-cooled Eppendorf tubes. Each collection period lasted for 20 minutes. Urinary kinin content was determined with a direct radioimmunoassay after ethanol extraction. Individual animal data of the four sampling periods were averaged to account for the intraindividual fluctuations in urinary flow.

Statistics

All data are expressed as mean±SEM. Statistical comparisons were made by analysis of variance for basal values and by multivariate repeated-measure analysis of variance with drug as within-subject factor and strain as between-subject factor followed by Bonferroni’s tests and Student’s t tests as post hoc tests when appropriate. Statistical significance was accepted when p<0.05.

TABLE 2. Depressor Responses to Ramipril (100 µg/kg i.v.) During Vehicle or Bradykinin Antagonist Infusion (40 µg/kg/min i.a.) in Rats with Renal Hypertension

<table>
<thead>
<tr>
<th>Strain</th>
<th>Treatment</th>
<th>Basal MAP (mm Hg)</th>
<th>Max MAP change (mm Hg)</th>
<th>Duration of response (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wistar (n=7)</td>
<td>CEI+vehicle</td>
<td>183.3±5.1</td>
<td>-47.7±6.6</td>
<td>&gt;90</td>
</tr>
<tr>
<td></td>
<td>CEI+BKA</td>
<td>181.1±4.4</td>
<td>-17.8±3.0</td>
<td>&gt;90</td>
</tr>
<tr>
<td>BN-HA (n=7)</td>
<td>CEI+vehicle</td>
<td>180.0±5.6</td>
<td>-36.8±1.4</td>
<td>&gt;90</td>
</tr>
<tr>
<td></td>
<td>CEI+BKA</td>
<td>175.6±3.3</td>
<td>-17.8±3.2</td>
<td>42.0±5.4</td>
</tr>
<tr>
<td>BN (n=7)</td>
<td>CEI+vehicle</td>
<td>174.3±4.0</td>
<td>-31.8±2.9</td>
<td>28.0±2.6</td>
</tr>
<tr>
<td></td>
<td>CEI+BKA</td>
<td>174.9±4.1</td>
<td>-29.9±2.3</td>
<td>30.9±6.8</td>
</tr>
</tbody>
</table>

Reported data are mean±SEM. Basal MAP was not different between strains. Changes in MAP under CEI+vehicle were significantly different (p<0.05–0.01) between strains (Wistar versus BN-HA and BN rats; BN-HA versus BN rats). Differences between strains with respect to duration of the response were obvious. MAP, mean arterial pressure; CEI, converting enzyme inhibitor (ramipril); BKA, bradykinin antagonist (B4146); BN-HA, Brown Norway-Hannover rats; BN, Brown Norway rats.

*p<0.05; **p<0.01, differences within strains.

Drugs

BK, Ang II, and saralasin were purchased from Serva Laboratories, Heidelberg, FRG; ramipril and the BK receptor antagonist B4146= K864321 (DArg[HyP1,Thi3,8,DPhe7]bradykinin) were generously provided by Dr. B.A. Scholkens, Hoechst AG, Frankfurt, FRG. Thiocarboxil (Brevalit) was purchased from Eli Lilly, Gießen, FRG. All drugs were dissolved in 0.9% NaCl before the experiment.

Results

Experiment 1

Basal mean arterial pressure (MAP) was not different, and the depressor responses to BK injections showed no significant difference between BN and BN-HA rats (Table 1). In both strains, the hypotensive responses to BK were attenuated under a BK antagonist infusion with no difference between BN and BN-HA rats. After intravenous injection of the CE inhibitor ramipril, the responses to BK were potentiated in a similar fashion in both strains (Table 1). Ramipril by itself did not affect MAP in this experiment.

Experiment 2

Six to eight weeks after clipping of the renal artery, MAP ranged between 174.3 and 183.3 mm Hg and
was not significantly different between groups (Table 2). PRC was significantly increased in all strains at this time point when compared with the prehypertensive state (Table 3). Infusion of the BK antagonist 5 minutes before the CE inhibitor did not significantly alter MAP values.

During vehicle infusion, ramipril lowered MAP within 10 minutes in all strains. However, the antihypertensive effect was significantly different between strains with respect to both maximal effect and duration (Table 2).

In Wistar rats, the depressor effect of ramipril was greatest and lasted longer than the 90-minute observation period (Figure 1). The antihypertensive effect of ramipril was significantly attenuated by the infusion of the BK antagonist (Figure 1, top panel; Table 2). After the BK antagonist infusion was stopped, blood pressure did not return to baseline values but tended to decrease further.

In BN-HA rats, the depressor response to ramipril was also significantly attenuated and shortened by infusion of the BK antagonist (Figure 1, middle panel; Table 2).

In BN rats, the antihypertensive effect of ramipril was least expressed and shortest. Intra-arterial infusion of the BK antagonist did not affect the depressor response to the CE inhibitor (Figure 1, bottom panel; Table 2).

**Experiment 3**

During vehicle infusion, intravenous saralasin reduced MAP within 10 minutes in all strains (Table 4). In contrast to the depressor effects of ramipril, the responses to saralasin were not different between strains, and the antihypertensive effects of saralasin were not attenuated by the infusion of the BK antagonist (Table 4).

In all strains, blood pressure returned to preinjection levels within 45 minutes after the saralasin infusion was stopped.

**Experiment 4**

Measurements of urinary kinin excretion in Wistar rats revealed more than seven times higher kinin values than in BN rats and more than four times higher kinin values in BN-HA rats than in BN rats. Between the two control groups, urinary kinin excretion was significantly higher in Wistar rats than in BN-HA rats (Figure 2).

**Discussion**

The kinin-deficient BN rat proved to be a valuable animal model to elucidate the participation of endogenous kinins in the acute antihypertensive effects of CE inhibitors. In accordance with previous reports, our findings demonstrate that these animals, despite the complete lack of high molecular weight kininogen as well as reduced low molecular weight kininogen and prekallikrein levels, are not completely deficient in the kallikrein-kinin system as they excreted small amounts of kinins in the urine. This finding could be explained by the fact that T-kininogen, a trypsin-sensitive kinin precursor,19 is expressed in the kidney of BN rats20 and may give rise to urinary kinins in these animals.

BN rats reacted to exogenous BK with depressor responses similar to those observed in their BN-HA controls. In addition, pretreatment with the BK antagonist engendered an attenuation, and pretreatment with the CE inhibitor a potentiation, of the depressor responses to BK similar to the respective
changes in BN-HA rats. Thus, there was no evidence for an alteration of vascular BK receptors in BN rats.

We chose the two-kidney, one clip model of hypertension for our study for two reasons. First, consistently high blood pressure levels can be produced with relatively little initial traumatization; second, this type of hypertension responds well to acute antihypertensive treatment with CE inhibitors (see Reference 2 for review). Theoretically, the renin dependency of this model could have shifted the antihypertensive mechanism of the CE inhibitor away from kinin potentiation toward reducing elevated circulating Ang II levels. However, together with those of Carbonell et al., our results do not provide any evidence that such renin-dependent models of hypertension are not suited to study the role of kinins for the acute antihypertensive actions of CE inhibitors. Moreover, it has been reported that BK antagonist infusions increased blood pressure preferentially under conditions of elevated circulating Ang II such as salt depletion in spontaneously hypertensive rats, or barbiturate anesthesia, or intravenous infusions of pressor substances such as Ang II in normotensive rats. These findings suggest that in the presence of elevated Ang II levels, endogenous kinins may become important for blood pressure control to antagonize the actions of the circulating pressor agent.

In hypertensive animals, intravenous bolus injections of ramipril lowered blood pressure in all strains investigated. However, with respect to magnitude and duration, these effects were remarkably different between strains. In Wistar rats, the antihypertensive response was greatest and outlasted the observation period of 90 minutes. Intravenous infusion of the BK antagonist drastically reduced the depressor responses. When the BK antagonist infusion was stopped after 45 minutes, blood pressure tended to decrease further. In BH-HA rats, the depressor response to ramipril was less than in Wistar rats both in extent and duration. The BK antagonist significantly attenuated the response but not as drastically as in Wistar rats. Interestingly, in BN-HA rats, blood pressure did not show any tendency to decrease further after the BK antagonist infusion was stopped.

In BN rats, ramipril was markedly less effective than in the two other strains with respect to both extent and duration of the depressor response. In these rats, blood pressure values were back to pretreatment levels within 30 minutes after the injection of ramipril. With respect to hypertensive Wistar and BN-HA rats, our data conform with those obtained by Carretero et al. using captopril and antibodies to kinins and with those reported by Benetos et al. who used enalapril and the same BK antagonist in renovascular hypertension. Similar findings were also obtained in a recent study by Carbonell et al. who used enalapril and the same BK antagonist in severely hypertensive rats with aortic ligation between both renal arteries, and by Seino et al. who used captopril and the BK antagonist B4147 in anesthetized normotensive rats. Although it cannot be excluded that a stimulation of catecholamine release from the adrenal gland contributes to the attenuation of the CE inhibitor–induced depressor effects by BK antagonist, these findings clearly support the hypothesis that endogenous kinins are important for the acute antihypertensive effects of CE inhibitors.

A question that remains to be answered in view of the present and previous findings is the site of kinin potentiation by CE inhibitors. Plasma BK was not measured here, but Carbonell et al. did not observe increased plasma BK levels after CE inhibition in their study of comparable design. It is possible that a CE inhibitor–induced increase of tissue kinins rather
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than plasma BK is involved in the antihypertensive action of these drugs. However, unless tissue kinins can be measured reliably, this idea remains speculative.

One might argue that our findings in BN rats, both with respect to the antihypertensive action of ramipril and the failure of the BK antagonist to influence the response to the CE inhibitor, merely reflect the fact that hypertensive BN rats, in general, react less to antihypertensive agents than their controls. One could also speculate that, in BN rats, an upregulation of vascular BK receptors could give rise to the apparent resistance of these animals to the BK antagonist. However, these two possibilities appear unlikely in view of our control experiments: hypertensive BN rats responded to intravenous infusions of the Ang II receptor antagonist saralasin with blood pressure decreases similar to those seen in hypertensive BN-HA rats and Wistar rats. Further, BN rats reacted to the BK antagonist and to ramipril with inhibition and potentiation of the BK-induced depressor response in a fashion not different from their BN-HA control rats.

We observed two major differences between Wistar rats and BN-HA rats, the two control strains. In BN-HA rats the depressor responses to ramipril were less in magnitude and duration than in Wistar rats, and on cessation of the BK antagonist infusion, blood pressure returned quickly to the elevated baseline levels. At present, we have no stringent explanation for these differences, except that BN-HA rats may carry some minor yet unidentified defects in the kallikrein-kinin system. This assumption is supported by our observation that BN-HA rats excreted significantly less kinins in the urine than Wistar rats.

The acute antihypertensive effects of CE inhibitors are not the only cardiovascular actions of these drugs that are closely related to the kinin system. Recently, Schöllkens et al.\(^2\) and Linz et al.\(^2\) reported that in isolated ischemic rat hearts, the beneficial cardiodynamic and metabolic effects of ramipril could be mimicked by BK at extremely low concentrations and could be abolished by a BK antagonist.

Thus, it appears that with the introduction of more specific tools to study the kallikrein-kinin system, the participation of the kinins in the various cardiovascular effects of CE inhibitors becomes more and more apparent. Moreover, these studies on CE inhibitors may prove to be helpful in our endeavour to further elucidate the role of kinins in cardiovascular regulation.

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