Chronic Kinin Receptor Blockade Attenuates the Antihypertensive Effect of Ramipril

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The contribution of endogenous kinins to the chronic antihypertensive effect of angiotensin converting enzyme inhibitors was investigated in two-kidney, one clip hypertensive Wistar rats, using the new bradykinin B1-receptor antagonist HOE 140 (D-Arg, [Hyp3, Thi5, D-Tic7, Oic8]-bradykinin). In a first protocol, rats were pretreated orally with the angiotensin converting enzyme inhibitor ramipril (1 mg/kg per day), for 4 weeks. Acute blockade of bradykinin receptors by intravenous injections of HOE 140 at doses of 8.4 and 100 μg/kg, which inhibited the depressor responses to exogenous bradykinin, did not affect the antihypertensive effect of ramipril in these animals. Bradykinin receptors were then blocked chronically by subcutaneous infusion of HOE 140 (500 μg/kg per day) via osmotic minipumps for 6 weeks, while ramipril treatment was continued. HOE 140 partially reversed the antihypertensive effect of ramipril from 115.3±4.6 to 123.8±3.7 mm Hg (mean arterial blood pressure) after 3 weeks and to 121.3±2.9 mm Hg after 6 weeks. In contrast, in controls (ramipril plus subcutaneous vehicle infusion) mean arterial blood pressure decreased further from 112.0±6.0 to 110.3±4.9 mm Hg after 3 weeks and to 103.7±5.0 mm Hg after 6 weeks (p<0.05 and p<0.01, HOE 140 versus controls). Plasma catecholamines were not significantly different between the two groups at the end of the experiment, indicating that the partial reversal of the antihypertensive effect was not due to a bradykinin-like agonistic effect on catecholamine release. In a second protocol, HOE 140 (500 μg/kg per day s.c) was coadministered with ramipril (1 mg/kg per day p.o.) in two-kidney, one clip hypertensive Wistar rats for 6 weeks. Again, HOE 140 attenuated the antihypertensive effects of ramipril (mean arterial blood pressure 131.8±2.6 versus 115.7±4.5 mm Hg after 3 weeks and 124.4±4.2 versus 105.4±4.8 mm Hg after 6 weeks; p<0.05, HOE 140 versus controls). Our results demonstrate that potentiation of endogenous bradykinin contributes to the chronic antihypertensive action of an angiotensin converting enzyme inhibitor in renovascular hypertension.

Key Words • bradykinin • blood pressure • antihypertensive therapy • angiotensin converting enzyme inhibitors • catecholamines • Goldblatt hypertension • rat studies

Angiotensin converting enzyme (ACE), also known as kininase II, catabolizes bradykinin (BK) to inactive fragments. Therefore, a potentiation of endogenous BK has been implicated in the antihypertensive action of ACE inhibitors. However, due to the lack of specific BK antagonists, conclusions from earlier studies about the role of BK in the antihypertensive action of ACE inhibitors were largely based on indirect evidence.

The development of the first BK B1-receptor antagonist by Vavrek and Stewart4 in 1985 provided a tool to investigate the role of BK in the acute antihypertensive effects of ACE inhibitors. Results from studies using this and related BK antagonists were rather controversial. Some authors5-8 have demonstrated that the acute antihypertensive effect of ACE inhibitors could be attenuated by BK antagonists; however, others could not confirm these results.5,10 To further complicate the issue, Mulnari et al11 suggested that the pressor effect by one of the BK antagonists, B3852 ([Hyp3, d-Phe7]-BK), was due to a BK-like agonistic action on catecholamine release from the adrenal gland.

All these studies were limited by the fact that the BK antagonists used had a low potency and were found to be relatively unspecific. In addition, chronic studies were prohibited by the fact that these compounds had a very short half-life in vivo and could only be administered intravasally.

Recently, a highly potent, specific and long-acting BK B1-receptor antagonist, HOE 140 (D-Arg, [Hyp3, Thi5, d-Tic7, Oic8]-BK), has been developed.12,13 This compound now offers a much better opportunity to elucidate the effects of chronic blockade of BK receptors on the antihypertensive action of ACE inhibitors.

In the present study, we first evaluated the potency and duration of action of the new BK antagonist, HOE 140, after subcutaneous administration and then investigated the effect of chronic subcutaneous administration of this compound on the chronic antihypertensive action of the ACE inhibitor ramipril in two-kidney, one clip (2K1C) Goldblatt hypertensive rats. Our results, demonstrating that chronic blockade of BK receptors attenuates the chronic antihypertensive action of ramipril, support the hypothesis that potentiation of endog-
enous kinins can contribute to the antihypertensive actions of ACE inhibitors.

Methods

Animals

Male Wistar rats (Dr. K. Thomae GmbH, Biberach, FRG) were used for the experiments. 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. Rats weighing 300±25 g were used for evaluation of the BK antagonist HOE 140. For other experiments, 2K1C hypertensive rats were prepared as follows: rats weighing 90–100 g were anesthetized with ether. An incision 2 cm in length was made on the back, 1 cm lateral along the vertebral column. The left renal artery was carefully separated from the vein, and a solid silver clip of 0.2 mm i.d. was placed on the artery as close to the aorta as possible. After 4–6 weeks, 70–80% of the rats developed hypertension. Systolic blood pressure (SBP) was measured by tail plethysmography. Only rats with a SBP of more than 190 mm Hg were used for further experiments. All experiments were preapproved by a governmental committee on animal welfare.

Blood Pressure Measurement

SBP was measured by tail plethysmography under light ether anesthesia. Mean arterial blood pressure (MAP) and heart rate (HR) were measured directly by use of a Statham P23Db pressure transducer, amplified by a Gould Brush pressure processor (both Gould Inc., Oxnard, Calif.), and recorded on-line by a PC-AT computer with specifically developed software that was also used for data calculation.14 Measurements of both SBP and MAP were always performed 2 hours after oral ramipril treatment.

Drugs and Drug Administration

BK (Serva GmbH, Heidelberg, FRG) was dissolved in physiological saline and was administered intravenously (0.1 ml). The ACE inhibitor ramipril (Hoechst AG, Frankfurt/M, FRG) was dissolved in distilled water and administered daily between 8 and 9 AM by gavage. B4146 (D-Arg-[Hyp 3, Thi 4, D-Phe 7]-BK, Hoechst AG, Frankfurt/M, FRG) was dissolved in physiological saline and was administered intravenously (0.1 ml). HOE 140 (Hoechst AG) was dissolved in physiological saline and administered intravenously (0.1 ml), injected subcutaneously (1 ml), or infused subcutaneously (0.49 µl/hr) via osmotic minipumps. Osmotic minipumps (model 2002, Alza Corp., Palo Alto, Calif.) were filled with HOE 140 or physiological saline and implanted subcutaneously for 2 weeks.

Evaluation of the Inhibitory Potency of the Bradykinin Receptor Antagonist HOE 140

One day before the experiments, rats were cannulated under ether anesthesia with catheters (PP-10 or PP-50 for arterial catheters, PP-25 for venous catheters, Portex Corp., Hythe, UK) in the right femoral artery for direct recording of MAP and HR and in the left carotid artery as well as in the right femoral vein for intraarterial and intravenous drug applications. One day after the surgery, the femoral artery catheter was connected to the transducer, which was attached to the monitoring computer system. MAP and HR were recorded continuously on-line by the computer system in conscious and unrestrained animals.

Experiment 1. Twelve Wistar rats were randomly divided into two groups. After a 60-minute stabilization period, BK (250 ng, 0.1 ml) was injected twice within 5 minutes via the carotid artery catheter for the determination of basal depressor responses. Thereafter, group 1 (n=6) received a bolus subcutaneous injection of HOE 140 at a dose of 200 µg/kg, and group 2 (n=6) received physiological saline. Measurement of the depressor responses to BK was repeated at 30 minutes as well as 1, 2, 3, 4, and 5 hours after the subcutaneous injection of HOE 140 or saline.

Experiment 2. Osmotic minipumps filled with either HOE 140 (500 µg/kg per day; n=5, group 1) or physiological saline (n=5, group 2) were implanted subcutaneously in the backs of the animals under ether anesthesia. MAP and depressor responses to BK were measured before as well as 24 hours and 14 days after the implantation as described in experiment 1.

Acute Blockade of Bradykinin Receptors in Ramipril-Pretreated 2K1C Hypertensive Rats

The ACE inhibitor ramipril, at a dose of 1 mg/kg per day for 4 weeks, was administered orally by gavage to 2K1C hypertensive rats (n=8) with a SBP of 215±13 mm Hg. At the end of the treatment period, rats were cannulated with femoral artery and vein catheters. One day after surgery, rats were attached to the monitoring system. After a 60-minute stabilization period, physiological saline, B4146 (4 mg/kg), and HOE 140 (8.4 µg/kg, 100 µg/kg, and 4 mg/kg) were consecutively injected intravenously at 15-minute intervals. The average values of MAP during a period of 2 minutes before and during the MAP responses to the intravenous injections were calculated by the computer.

Chronic Blockade of Bradykinin Receptors in Ramipril-Pretreated 2K1C Hypertensive Rats

Ramipril was administered orally as described above for 4 weeks in 2K1C hypertensive rats (n=26) with a SBP of 213±5 mm Hg. SBP was measured weekly by tail plethysmography under light ether anesthesia. At the end of this pretreatment period, rats were cannulated with a femoral artery catheter. One day after surgery, after a 60-minute stabilization period, MAP and HR were recorded in conscious rats over a 60-minute period via the arterial catheter. The average values of MAP and HR during this time were calculated by the computer. Rats were then divided randomly into two groups. Group 1 (MAP 115.3±4.6 mm Hg, n=14) was treated with HOE 140 (500 µg/kg per day); group 2 (MAP 112.0±6.5 mm Hg, n=12) with physiological saline. The drugs were applied subcutaneously via osmotic minipumps for 6 weeks. Osmotic minipumps were changed every 2 weeks. During this time ramipril treatment was continued, and SBP was determined weekly by tail plethysmography. Direct measurements of MAP and HR in conscious rats were repeated 3 and 6 weeks after the start of subcutaneous infusions. At the end of the experiment, 1 ml blood was collected from the arterial catheter for catecholamine determination.
FIGURE 1. Bar graph shows inhibitory effect of the bradykinin (BK) antagonist HOE 140 on depressor responses to BK. Normotensive Wistar rats were treated subcutaneously with either HOE 140 (200 µg/kg, n=6, black columns) or physiological saline (n=6, white columns). The depressor responses to BK (250 ng i.a.) were measured before, as well as 30 minutes, 1, 2, 3, 4, and 5 hours after the subcutaneous injections. MAP, mean arterial blood pressure. *p<0.05, **p<0.01.

Chronic Blockade of Bradykinin Receptors in Non-Pretreated 2K1C Hypertensive Rats

A femoral artery catheter was used to cannulate 2K1C hypertensive rats (n=21) with a SBP of 219±5 mm Hg. One day after surgery, MAP and HR were measured as described above. Rats were then divided randomly into two groups. Group 1 (MAP 180.3±4.1 mm Hg, n=11) was treated with ramipril (1 mg/kg per day) per os plus HOE 140 (500 µg/kg per day s.c. via osmotic minipumps) for 6 weeks; group 2 (MAP 180.1±8.1 mm Hg, n=10) with ramipril plus physiological saline as control. SBP was measured weekly; MAP and HR 3 and 6 weeks after the start of treatment.

Plasma Catecholamine Determination

One milliliter blood was collected from conscious rats via the arterial catheter into heparinized plastic tubes. Blood samples were immediately centrifuged at 2,500 rpm and 4°C for 20 minutes. Plasma was separated and stored at —80°C. Plasma epinephrine and norepinephrine were measured by high-performance liquid chromatography (HPLC) technique with electrochemical detection according to Eriksson and Persson.15

Statistics

Mean±SEM are reported. Data were subjected to analysis of variance (ANOVA) followed by multiple pairwise comparisons (Bonferroni). Differences were considered significant at p<0.05.

Results

In Vivo Evaluation of the Antagonistic Potency of the Bradykinin Receptor Antagonist HOE 140

In normotensive rats, the depressor responses to BK (250 ng i.a.) were completely inhibited 30 minutes after a subcutaneous bolus injection of 200 µg/kg HOE 140 (0.2±1.2 mm Hg versus 38.9±4.5 mm Hg in controls) and were still inhibited by 51.4% at 5 hours (17.6±2.2 mm Hg versus 36.2±3.4 mm Hg in controls), reflecting an in vivo half-life of subcutaneously injected HOE 140 of about 5 hours (Figure 1). Chronic subcutaneous infusion of HOE 140 (500 µg/kg per day) inhibited the depressor responses to BK by more than 90%, 24 hours after implantation of osmotic minipumps and by 86% after 14 days (Table 1). Basal MAP was not significantly changed by HOE 140 treatment.

Acute Blockade of Bradykinin Receptors in Ramipril-Pretreated 2K1C Hypertensive Rats

Oral pretreatment (4 weeks) with ramipril normalized blood pressure in 2K1C hypertensive rats (SBP from 215±13 before to 136±5 mm Hg after). Acute bolus intravenous injection of the BK antagonist B4146 (4 mg/kg) increased MAP from 116.0±1.5 to 127.6±1.6 mm Hg (p<0.05) for 1–2 minutes. HOE 140 at an equipotent dose of 8.4 µg/kg as well as a more than 10-fold higher dose of 100 µg/kg had no effect on MAP (115.0±1.6 versus 116.0±3.3 mm Hg and 114.0±1.5 versus 114.0±1.5 mm Hg, respectively). A relatively high dose of 4 mg/kg HOE 140 increased MAP from 114.0±1.2 to 125.3±3.2 mm Hg (Figure 2, p<0.05). This pressor effect of HOE 140 was more prolonged (5–30 minutes) than the one observed after B4146 injection.

Chronic Blockade of Bradykinin Receptors in Ramipril-Pretreated 2K1C Hypertensive Rats

Chronic blockade of BK B2-receptors by subcutaneous infusion of the BK antagonist HOE 140 for 6 weeks partially reversed the antihypertensive effect of the ACE inhibitor in ramipril-pretreated 2K1C hypertensive rats (Figure 3). The differences in SBP between the HOE 140– and the vehicle-treated group became significant 2 weeks after the start of the subcutaneous infusion (145±3 versus 124±10 mm Hg, p<0.05) and remained significant until the end of the experiment (Figure 3). These findings were confirmed by direct

| Table 1. Inhibitory Effect of Subcutaneous Infusion of the Bradykinin Antagonist HOE 140 (500 µg/kg per day) on Depressor Responses to Bradykinin (250 ng i.a.) in Normotensive Rats |
|---|---|---|---|
| Time | Group (n=5) | MAP (mm Hg) | Max MAP change (mm Hg) | Inhibition (%) |
| **Before** | Vehicle | 101.8±4.5 | —39.8±3.7 | no difference |
| | HOE 140 | 105.3±7.3 | —43.9±1.0 | |
| 24 hours | Vehicle | 98.0±6.5 | —34.3±2.6 | |
| | HOE 140 | 100.5±6.9 | —2.9±2.1* | 92 |
| 14 days | Vehicle | 101.1±5.2 | —35.4±4.4 | |
| | HOE 140 | 106.1±4.1 | —5.1±1.3* | 86 |

Mean±SEM are reported. Max, maximum; MAP, mean arterial blood pressure. *p<0.01.
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intra-arterial measurements of MAP. MAP in the HOE 140–treated group increased from 115.3±4.6 to 123.8±3.3 mm Hg and to 121.3±2.9 mm Hg 3 weeks and 6 weeks after the start of HOE 140 subcutaneous infusion, respectively, whereas MAP values in the vehicle-treated group decreased further from 112.0±0.5 to 103.7±5.0 mm Hg, 3 and 6 weeks after the start of saline subcutaneous infusion. HR was not changed throughout the experiment.

Chronic Blockade of Bradykinin Receptors in Non-Pretreated 2K1C Hypertensive Rats

In 2K1C hypertensive rats, SBP was lowered from 218±5 to 128±8 mm Hg 1 week after the start of oral ramipril plus subcutaneous saline treatment. After coadministration of ramipril and HOE 140, the reduction in SBP was attenuated (from 219±4 to 149±4 mmHg, \( p<0.05 \), Figure 4). The difference between the two groups remained significant during the 6-week treatment period. These results were confirmed by direct intra-arterial measurements of MAP after 3 and 6 weeks of treatment. MAP in the ramipril plus saline–treated group decreased from 180.1±8.1 to 115.7±4.5 mm Hg 3 weeks and to 105.4±4.8 mm Hg 6 weeks after the start of treatment, whereas MAP in the HOE 140 plus ramipril–treated group decreased only to 131.8±2.6 and 124.4±4.2 mm Hg, respectively. HR was not significantly different between the HOE 140– and vehicle-treated groups.

Plasma Catecholamines

Plasma catecholamines measured 6 weeks after subcutaneous infusion of HOE 140 or physiological saline are shown in Table 2. Plasma epinephrine and norepinephrine were not significantly different between HOE 140– and vehicle-treated groups.

Discussion

In the present study, we demonstrate for the first time that the chronic antihypertensive action of an ACE inhibitor can be attenuated by chronic blockade of BK \( \beta \)-receptors. The partial reversal of the antihypertensive effect of the ACE inhibitor became significant 1 week (non-pretreated rats) or 2 weeks (ramipril-pretreated rats) after the start of the subcutaneous HOE 140 infusion and remained significant throughout the remaining treatment period. These results support the

**TABLE 2. Plasma Catecholamine Levels at the End of the 6-Week Treatment Period With the Bradykinin Antagonist HOE 140 or Physiological Saline in Ramipril-Treated Two-Kidney, One Clip Hypertensive Rats**

<table>
<thead>
<tr>
<th>Group</th>
<th>Epinephrine (pg/ml)</th>
<th>Norepinephrine (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle (n=12)</td>
<td>131.3±14.2</td>
<td>71.5±5.5</td>
</tr>
<tr>
<td>HOE 140 (n=14)</td>
<td>151.4±9.0</td>
<td>102.6±20.8</td>
</tr>
</tbody>
</table>

Values are mean±SEM. There is no significant difference between the two groups.
hypothesis that BK potentiation plays a role in the chronic antihypertensive action of ACE inhibitors.

One of the BK antagonists, B3852, was reported to stimulate catecholamine release from the adrenal gland when given at high doses, although in other studies with related BK antagonists, including B4146, this effect was not observed. To exclude the possibility that a release of catecholamines from the adrenal gland might account for the effect of HOE 140 on blood pressure, we measured plasma catecholamine levels at the end of the treatment period. Plasma epinephrine and norepinephrine, though showing a tendency to increase, were not significantly elevated after 6 weeks of HOE 140 treatment as compared with vehicle treatment. Therefore, it appears unlikely that the partial reversal of blood pressure in HOE 140-treated rats was due to an agonistic action of the BK antagonist on catecholamine release.

In normotensive Wistar rats, chronic 2-week subcutaneous infusion of the BK antagonist HOE 140 at the dose used in the main experiment effectively blocked the depressor responses to exogenously applied BK. Thus, this compound infused subcutaneously proved to be suitable for chronic blockade of BK receptors in animal experiments. MAP did not change in these animals during chronic infusion of the BK antagonist, suggesting that endogenous BK does not play a major role in maintaining basal blood pressure in normotensive rats. On the other hand, our results in 2K1C hypertensive rats indicate that kinins may become more important under certain conditions, such as renovascular hypertension or after potentiation of endogenous kinins by ACE inhibitors, and may then participate in blood pressure regulation.

Several studies have pointed to a role of endogenous BK in the acute effect of ACE inhibitors in normotensive8,16 and in hypertensive rats.5,6,7 In these studies the acute antihypertensive effect of an ACE inhibitor could be attenuated or partially reversed by injection or infusion of BK antagonists. In contrast, in the present study, short-term blockade of BK receptors by intravenous injection of HOE 140 at doses of 8.4 and 100 μg/kg, although effectively inhibiting the depressor responses to exogenous BK,17 did not raise blood pressure in 2K1C hypertensive rats pretreated chronically with ramipril. A partial reversal of blood pressure was only observed after acute intravenous injection of a relatively high dose of 4 mg/kg HOE 140 as well as after 4 mg/kg B4146 in animals pretreated chronically with ramipril. This discrepancy between our present and previous findings may be due to different study designs, since in our present study the animals were pretreated chronically with the ACE inhibitor, whereas in the above cited studies the ACE inhibitors were applied acutely. An additional explanation could be that high concentrations of BK antagonists were required to penetrate into tissues to antagonize tissue-bound kinins.16 However, against this possibility speaks the fact that only B4146 evoked a pressor response under these experimental conditions, whereas HOE 140 at a dose of 8.4 μg/kg that had been shown to be equipotent as 4 mg/kg B4146 in blocking BK receptors in vivo17 did not.

From our study and the majority of those published previously, it is clear that the antihypertensive effects of ACE inhibitors can be attenuated by blockade of BK receptors. Interestingly, as demonstrated here, BK antagonists seem to be particularly effective in renovascular models of hypertension associated with a stimulated renin-angiotensin system4,7 but less effective in genetic hypertension with normal or suppressed plasma renin (G. Bao, unpublished results), although one might have expected the opposite, i.e., that kinin potentiation by ACE inhibitors would be more effective in non-renin-dependent hypertension. At this point we can only speculate on the reasons for this phenomenon. Endogenous kinins may gain importance for blood pressure regulation in cases where elevated blood pressure is maintained by circulatory pressor agents such as angiotensin II.10 Under these conditions, BK could contribute to endothelial production of relaxing factors such as nitric oxide to counteract the pressor actions of angiotensin II. ACE inhibitors could then potentiate the nitric oxide generating vasodilating capacity of BK as has most recently been demonstrated in vitro.18 The question as to whether the potentiation of endogenous kinins also contributes to the antihypertensive actions of ACE inhibitors in non-renin-dependent hypertension remains to be answered.

References


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