Antihypertensive Effect of MK-421 in Rats
Role of the Renal Kallikrein-Kinin System

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SUMMARY To study the hypotensive mechanism of the new oral converting-enzyme inhibitor, MK-421, we evaluated the antihypertensive effect of MK-421 in rats with hypertension induced by chronic administration of norepinephrine (NE) or vasopressin and measured urinary kallikrein and kinin excretions as indices of the renal kallikrein-kinin system. When 6 mg/kg/day of MK-421 was administered simultaneously with 1.8 mg/kg/day of NE, the systolic blood pressure of conscious rats rose on Day 1 to only 122.6 ± 3.4 mm Hg compared with the rise to 146.3 ± 1.6 mm Hg when NE alone was infused (p < 0.001). Similarly, when the same dose of MK-421 was administered simultaneously with 7.2 U/kg/day of vasopressin, the systolic blood pressure of conscious rats rose on Day 1 to only 117.4 ± 3.8 mm Hg compared with the rise to 141.6 ± 3.4 mm Hg when vasopressin alone was infused (p < 0.01). The antihypertensive effect of MK-421 was sustained for 6 days in rats infused with NE or vasopressin. Infusion of NE alone resulted in a small but significant increase in urinary kallikrein excretion and no change in urinary kinin excretion. The combined administration of NE with MK-421 induced additional increases in urinary kallikrein and kinin excretions. Vasopressin alone resulted in marked decreases in urinary kallikrein and kinin excretions. The combined administration of vasopressin with MK-421 induced no additional changes in urinary kallikrein and kinin excretion. These results indicate that the hypotensive effect of MK-421 may depend on a reduced sensitivity of the peripheral arteries to vasoconstrictor substances. However, the dissimilar effect of MK-421 on the renal kallikrein-kinin system in NE- or vasopressin-induced hypertensive rats may reveal that the renal kallikrein-kinin system is not essential for the mechanism of the antihypertensive action of MK-421. (Hypertension 6:229-235, 1984)

KEY WORDS • angiotensin converting-enzyme inhibitor • MK-421 •
norepinephrine • vasopressin • renal kallikrein-kinin system

THE mechanism of action of angiotensin converting-enzyme inhibitors in lowering blood pressure has not been fully elucidated. In humans and animals, the hypotensive action of captopril, an angiotensin converting-enzyme inhibitor, has been reported in those with high, normal, and low levels of circulating renin. It is also known that angiotensin converting-enzyme inhibitors prevent the degradation of the depressor peptide bradykinin (BK) into inactive fragments. However, conflicting results have been obtained concerning the levels of circulating BK after the angiotensin converting-enzyme inhibition. These results suggest that the inhibitions of angiotensin II (AI) formation and of BK degradation may not be responsible for all of the hypotensive action of these drugs. Recent studies suggest that captopril attenuates the vasopressor action of norepinephrine (NE), and this may be an important mechanism in the hypotensive action of angiotensin converting-enzyme inhibitors.
The search for other mediators of the vascular response to the inhibition of angiotensin-converting enzyme has focused on the local kallikrein-kinin system. We have recently shown in the rat that captopril induced a local increase in the kallikrein-kinin system via the smooth muscle and kidney, which participated in water-sodium metabolism and blood pressure regulation.\(^1\)\(^2\) The interrelationship between the renal kallikrein-kinin system and the vasopressor substances, NE and vasopressin, has been largely overlooked.\(^3\)\(^4\)

A new, long-lasting, non-sulphydryl converting-enzyme inhibitor, N-(s)-1-(etoxycarbonyl)-3-phenylproplyl-L-alanyl-L-proline maleate (MK-421), has been developed. The hypotensive action of MK-421 has recently shown in the rat that captopril induced a local increase in the kallikrein-kinin system via the smooth muscle and kidney, which participated in water-sodium metabolism and blood pressure regulation.\(^1\)\(^2\) The interrelationship between the renal kallikrein-kinin system and the vasopressor substances, NE and vasopressin, has been largely overlooked.\(^3\)\(^4\)

To investigate the hypotensive mechanism of MK-421 further, we assessed its antihypertensive action in rats with hypertension induced by the chronic infusion of NE or vasopressin and studied the changes in the renal kallikrein-kinin system.

Methods

Male Sprague-Dawley rats weighing 150 to 250 g were housed in separate metabolic cages in a humidity- and temperature-controlled room and fed a regular diet (Oriental CFM, Oriental Yeast, Tokyo, Japan, 0.20 mEq of sodium/g, 0.27 mEq of potassium/g). They were infused with NE at a rate of 1.8 mg/kg/day alone or in combination with 6 mg/kg/day of MK-421. They were also infused with vasopressin at a rate of 7.2 U/kg/day alone or in combination with 6 mg/kg/day of MK-421 dissolved in physiologocal saline. The infusion was delivered for up to 6 days via osmotic mini-pump (Alza, Palo Alto, California) implanted intraperitoneally in rats under ether anesthesia. Control rats received the vehicle alone. In preliminary experiments, the vasopressor response to exogenous angiotensin I (AI) as a bolus was examined in anesthetized rats infused with MK-421 at a rate of 6 mg/kg/day for up to 6 days. The vasopressor response to 200 ng/kg of exogenous AI was inhibited by 95%, confirming the inhibition of conversion of AI to All by more than 90%.

MK-421 was supplied by Nippon Merck-Banyu Company, Tokyo. Norepinephrine bitartrate was obtained from Sigma Chemical Company, Sendai, Japan, and [Arg\(^{8}\)]-vasopressin and AI were purchased from Protein Research Foundation, Osaka, Japan.

Daily systolic blood pressure in the rats was recorded by the tail cuff method. The daily fluid intake, urine volume, urinary sodium and potassium excretions, and urinary kallikrein and kinin excretions were determined. Urine was collected into vessels containing 0.1 M o-phenanthroline at 4°C and was kept at -20°C until the assay. To check the degradation and formation of urinary kinin during the 24-hour urine collection, we examined the recovery rate in five samples into which 50 ng of synthetic BK had been added and allowed to stand for 24 hours under the same conditions. The recovery rate was 88.0% ± 2.8%.

Urinary kallikrein was measured as kininogenase activity by the method of Abe et al.\(^7\) The assay was done using low-molecular-weight bovine serum kininogen as the substrate. The generated kinin was measured by radioimmunoassay. Urinary kinin was measured by the method of Carretero et al.\(^8\) Urinary concentrations of sodium and potassium were measured with a flame photometer. All results were expressed as means ± SEM. The significance of differences between mean values was evaluated by Student's \(t\) test.

Results

Basal preinfusion data for each group of rats are summarized in Table 1. Body weight, systolic blood pressure, fluid intake, urine volume, urinary electrolytes excretion, urinary kallikrein and kinin excretions were not significantly different among the five groups prior to the infusions. MK-421 alone induced a small but significant reduction in systolic blood pressure and increases in urinary kallikrein and kinin excretions. In the NE study, the systolic blood pressure of the NE-alone group began to rise significantly on the 1st day of the infusion and remained high up to the 6th day, whereas that of the vehicle-alone group did not change. When 6 mg/kg/day of MK-421 was administered simultaneously with 1.8 mg/kg/day of NE, the

| Rat group | BW (g) | SBP (mm Hg) | WI (ml/day) | UV (ml/day) | U\(_{\text{Na}}\)V (meq/day) | U\(_{\text{K}}\)V (ng/20 min/day) | U\(_{\text{KK}}\)V (ng/day) |
|-----------|--------|------------|------------|------------|----------------|-----------------|----------------|}
| Vehicle (n = 7) | 229.1 ± 3.1 | 117.0 ± 4.2 | 26.0 ± 1.3 | 10.3 ± 1.1 | 1.10 ± 0.20 | 305.7 ± 31.6 | 5.3 ± 0.8 |
| MK (n = 7) | 218.4 ± 2.4 | 115.0 ± 1.8 | 26.7 ± 1.0 | 11.4 ± 0.7 | 1.31 ± 0.08 | 344.7 ± 32.2 | 4.6 ± 0.8 |
| NE (n = 7) | 209.0 ± 5.8 | 123.7 ± 4.3 | 26.9 ± 2.7 | 11.7 ± 0.8 | 1.04 ± 0.08 | 247.6 ± 24.8 | 4.0 ± 1.0 |
| NE + MK (n = 7) | 212.9 ± 8.8 | 121.3 ± 4.6 | 29.6 ± 2.1 | 11.0 ± 2.5 | 0.96 ± 0.08 | 304.2 ± 69.9 | 5.0 ± 1.2 |
| VP (n = 7) | 230.0 ± 2.4 | 121.1 ± 2.5 | 23.4 ± 1.0 | 10.3 ± 0.7 | 0.93 ± 0.06 | 239.0 ± 26.0 | 5.2 ± 0.5 |
| VP + MK (n = 7) | 244.0 ± 2.9 | 119.4 ± 4.3 | 26.6 ± 1.5 | 11.0 ± 1.3 | 0.97 ± 0.10 | 289.0 ± 44.2 | 4.1 ± 0.6 |

Results are means ± SEM. Abbreviations: BW = body weight; SBP = systolic blood pressure; WI = water intake; UV = urine volume; U\(_{\text{Na}}\)V = urinary sodium excretion; U\(_{\text{K}}\)V = urinary kinin excretion; MK = MK-421; NE = norepinephrine; VP = vasopressin.
TABLE 2. Effects of Norepinephrine (NE) Alone and in Combination with MK-421 in Rats

<table>
<thead>
<tr>
<th></th>
<th>Norepinephrine (n = 7)</th>
<th>Norepinephrine + MK-421 (n = 7)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Day 1</td>
<td>Day 3</td>
</tr>
<tr>
<td></td>
<td>146.3 ± 1.6</td>
<td>149.3 ± 4.0</td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td>122.6 ± 3.4‡</td>
<td>124.3 ± 3.1†</td>
</tr>
<tr>
<td>U_kkV (ng 20 mm day)</td>
<td>368.6 ± 24.1</td>
<td>309.4 ± 70.9</td>
</tr>
<tr>
<td></td>
<td>588.8 ± 59.7*</td>
<td>691.5 ± 77.2‡</td>
</tr>
<tr>
<td>U_kinV (ng day)</td>
<td>4.4 ± 0.8</td>
<td>6.0 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>11.8 ± 2.5*</td>
<td>17.4 ± 4.0*</td>
</tr>
</tbody>
</table>

Results are means ± SEM. SBP = systolic blood pressure; U_kkV = urinary kallikrein excretion; U_kinV = urinary kinin excretion.

* \( p < 0.05 \) compared with NE alone.
† \( p < 0.01 \) compared with NE alone.
‡ \( p < 0.001 \) compared with NE alone.

The tail systolic blood pressure of conscious rats rose only to 122.6 ± 3.4 mm Hg compared with 146.3 ± 1.6 mm Hg with NE alone on Day 1. The antihypertensive effect of MK-421 was sustained for 6 days in rats infused with NE. The blood pressure of the rats given NE with 6 mg/kg/day of MK-421 was not significantly different from that of the control rats given the vehicle (Figure 1, Table 2).

In the vasopressin study, the systolic blood pressure of the vasopressin-alone group began to rise significantly on the 1st day of the infusion and reached its maximum on the 3rd day. After that, it tended to decrease again, but still remained significantly higher than that of the control rats throughout the infusion period. When MK-421 was administered simultaneously with vasopressin, the tail systolic blood pressure of conscious rats rose only to 117.4 ± 3.8 mm Hg compared with 141.6 ± 3.4 mm Hg by vasopressin alone on Day 1. The antihypertensive effect of MK-421 was sustained for 6 days. The blood pressure of the rats administered vasopressin with MK-421 was not significantly different from that of the control rats given the vehicle (Figure 2, Table 3).

As to the 24-hour urinary excretion of kallikrein, the administration of NE induced a slight increase, whereas the administration of vasopressin induced a marked decrease. The rats treated with NE had 8% to 33% greater excretion of kallikrein than the controls, whereas the rats treated with vasopressin had 34% to 75% less excretion of kallikrein than the controls. The rats administered NE with MK-421 had an increased kallikrein excretion greater than those rats treated with NE alone. The rats administered vasopressin with MK-421 had a decreased kallikrein excretion similar to those rats treated with vasopressin alone (Figure 3, Tables 2 and 3).
As to the 24-hour urinary excretion of kinin, the administration of NE did not induce any change, whereas the administration of vasopressin induced a marked decrease. The rats treated with vasopressin alone had 71% to 89% less excretion of kinin than the controls. The rats administered NE with MK-421 had 28% to 106% greater excretion of kinin than those rats treated with NE alone. The rats administered vasopressin with MK-421 had a decreased kinin excretion similar to the rats treated with vasopressin alone (Figure 4, Tables 2 and 3).

Discussion

In the present study, we demonstrated that MK-421, an inhibitor of angiotensin-converting enzyme, attenuated the onset and development of hypertension induced by chronic administration of NE or vasopressin. Since the first report by Okuno et al. in 1979, several studies have revealed that captopril, an inhibitor of angiotensin-converting enzyme developed initially by Ondetti et al., had the properties of attenuating the vasopressor response to exogenous NE in vitro and in vivo and that this may be associated with the hypotensive effect of the drug. However, the precise mechanism still remains controversial. To our knowledge, the antihypertensive effect of MK-421 in rats made hypertensive by chronic infusion of NE or vasopressin has not been previously reported, whereas it has been well documented that in vitro and in vivo captopril attenuated acute vasopressor effects of exogenous NE.

The most likely mechanism for the hypotensive effect of angiotensin-converting-enzyme inhibitors is the blockade of the conversion of AI to All, thereby lowering plasma levels of All. Consistent with this interpretation are the recent reports by several investigators. Vasopressin has been reported to suppress plasma renin activity, which results in decreased plasma All levels. The effect of NE on renin release is

| Table 3. Effects of Vasopressin Alone and in Combination with MK-421 in Rats |
|-----------------------------------------------|-----------------------------------------------|
| Vasopressin (n = 7)                          | Vasopressin + MK-421 (n = 7)                   |
|                                              |                                               |
| Day 1                                        | Day 1                                        |
| Day 3                                        | Day 3                                        |
| Day 5                                        | Day 5                                        |
| SBP (mm Hg)                                  |                                               |
| 141.6 ± 3.4                                  | 117.4 ± 3.8†                                 |
| 155.9 ± 9.1                                  | 121.1 ± 4.6*                                 |
| 142.9 ± 4.0                                  | 124.3 ± 3.5*                                 |
| U_kkV (ng/20 min/day)                        |                                               |
| 107.0 ± 16.2                                 | 127.0 ± 25.7                                 |
| 77.0 ± 8.3                                   | 101.3 ± 16.9                                 |
| 92.5 ± 8.3                                   | 94.1 ± 14.7                                  |
| U_kmmV (ng/day)                              |                                               |
| 1.3 ± 0.2                                    | 1.8 ± 0.2                                    |
| 0.9 ± 1.1                                    | 1.5 ± 0.3                                    |
| 1.1 ± 0.2                                    | 3.1 ± 0.8                                    |

Results are means ± SEM. SBP = systolic blood pressure. U_kkV = urinary kallikrein excretion; U_kmmV = urinary kinin excretion.

†p < 0.01 compared with vasopressin alone.

‡p < 0.001 compared with vasopressin alone.
still controversial, however.\textsuperscript{24, 25} Vasopressin has also been reported to be incapable of sustaining its hypertensive effect owing to the activation of the baroreflex mechanism.\textsuperscript{26, 27} In our present experiments, three rats infused with vasopressin, after showing the maximum hypertension on the 3rd day, tended to show a decrease in blood pressure. However, the average systolic blood pressure on the 6th day of the infusion was still elevated. The hypertension induced by vasopressin may be associated with volume expansion,\textsuperscript{23} a condition in which the renin-angiotensin-aldosterone system could not take the principal role. Diz et al.\textsuperscript{28} also reported that plasma renin was not elevated in rats receiving NE in the same dose and manner as in our present experiments, but the systolic blood pressure was elevated. Thus, it is unlikely that the antihypertensive effect of MK-421 in the present experiments was exerted entirely by the inhibition of AII production.

Alternatively, the possibility that angiotensin converting-enzyme inhibitors exert their effect on arterial blood pressure via the kallikrein-kinin system must also be considered since angiotensin-converting enzyme is identical with kininase II, which inactivates potent vasodilator kinins.\textsuperscript{29} We have previously demonstrated\textsuperscript{38} that myometrial BK receptors in the rat decrease following the administration of captopril or BK, suggesting that captopril leads to a local increase in BK in its target tissues or organs. Clappison et al.\textsuperscript{30} reported that captopril also stimulates urinary kinin excretion in dogs, suggesting its contribution to the concomitant renal vasodilation. In our present experiments, we showed that the rats administered NE with MK-421 had increased kallikrein and kinin excretions greater than those of rats infused with NE alone, suggesting the role of an enhanced renal kallikrein-kinin system in the antihypertensive effect of MK-421. Furthermore, Malik and Nasjletti\textsuperscript{31} have reported that the polypeptide BK acts postsynaptically at the renal vascular neuroeffector function to inhibit the adrenergically induced vasoconstriction through a mechanism in-
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Collectively, the enhanced renal and vascular kallikrein-kinin system induced by MK-421 could have contributed to the antihypertensive effect on hypertension induced by NE.

However, the antihypertensive effect of MK-421 on hypertension induced by vasopressin could not be explained by enhanced activity in the renal kallikrein-kinin system, since vasopressin induced decreases in urinary kallikrein and kinin excretions, which were not affected by the administration of MK-421. Fejes-Toth et al.32 reported that vasopressin over its normal physiological range of concentration stimulates renal kallikrein in anesthetized rats. However, they made no reference to the chronic effect of larger doses of vasopressin in causing definite decreases in urinary kallikrein and kinin excretions. The reason for these decreases may be a reduction of renal blood flow induced by the vasoconstrictor action of a large dose of vasopressin in the renal vasculature. Another explanation for the decreases in urinary kallikrein and kinin might be a direct action of vasopressin on the renal kallikrein-kinin system. Unfortunately, we did not measure the renal functions, so we could not determine the mechanism of vasopressin action. We could not find any evidence to relate the antihypertensive effect of MK-421 to the changes in the renal kallikrein-kinin system in the vasopressin experiment. It may be possible that the activation of the kallikrein-kinin system in target tissues other than the kidney may in part contribute to the antihypertensive effect of MK-421 in rats with hypertension induced by NE and vasopressin, but we have no confirmation.

In conclusion, our present results show that the hypertensive effect of MK-421 may depend on a reduced sensitivity of the vasculature to vasoconstrictor substances. The dissimilar effect of MK-421 on the renal kallikrein-kinin system in NE- or vasopressin-induced hypertensive rats may reveal that the renal kallikrein-kinin system is not essential for the mechanism of the antihypertensive effect of MK-421.
Acknowledgments

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