Protracted Effect of Converting-Enzyme Inhibition on the Rat’s Response to Intraarterial Bradykinin

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SUMMARY Intravenous infusion of the converting-enzyme (CE) inhibitor, MK422 (1 mg·kg⁻¹·hr⁻¹ for 30 minutes) in normotensive controls and two-kidney, one clip (2K1C) rats in the acute phase of renovascular hypertension had a significant hypotensive effect that persisted after 24 hours. In contrast to that prolonged effect, inhibition of the pressor responses to intraarterial or intravenous angiotensin I, and the potentiation of the depressor responses to intravenous bradykinin (BK), were evident during the hour following the infusion of MK422, but not 24 hours later. Potentiation of intraarterially administered BK, however, persisted for 24 hours after infusion of the CE inhibitor. It is concluded that at least the prolonged (24-hour) effect of the treatment with MK422 was due to inhibition of the CE activity in tissues other than the lung, and that increased levels of endogenous BK may be responsible for the inhibitor's hypotensive effect.

We observed that the blood pressure in control and two-kidney, one clip (2K1C) renal hypertensive rats is lowered prolongedly by a short intravenous infusion of MK422, the active metabolite of the pro-drug MK421. This led us to investigate the role of CE activity in the pulmonary and systemic circulations in the maintenance of blood pressure, by studying the effect of MK422 on the responses of control and 2K1C rats to intraarterial and intravenous injections of AI and BK.

Methods

MK422 (N-[(5)-1-carboxy-3-phenyl-propyl]-ala-pro) was kindly provided by Dr. E. H. Cordes, Merck Sharp & Dohme Research Laboratories, Rahway, New Jersey. The peptides AI ([5-isoleucine]-angiotensin I)¹⁸ and BK¹⁹ were synthetic products made in this laboratory. All drugs were dissolved in saline.

In 3-month-old male Wistar rats under ether anesthesia, two-kidney, one clip renovascular hypertension was induced by placing a silver clip with a 0.25 mm opening on the left renal artery through a loin incision. Twenty-six days after clipping, unselected 2K1C rats and age-matched controls were cannulated with polyethylene tubing (PE10 connected to PE50). Permanent cannulas were placed through the left femoral vessels in the abdominal aorta for measuring mean arterial pressure (MAP) and in the vena cava for intravenous infusions. In the groups of rats destined to receive intraarterial injections, a third cannula was placed in the ascending aorta through the left carotid...
Results

Effect of MK422 Infusion on Mean Blood Pressure

Figure 1 shows the effect of the infusion of MK422 on the blood pressure of control and 2K1C rats 4 weeks after artery clipping. The CE inhibitor induced, in both groups, a significant decrease in the MAP, which was more pronounced in the 2K1C rats. Twenty-four hours after the MK422 infusion (Day 3), the MAP was still significantly lowered in the two groups of animals.

Effect of MK422 on the Responses to Angiotensin I

The effect of MK422 on the responses to AI injected intravenously and intraarterially (fig. 2) was similar in the control and 2K1C rats. In both groups, the log dose-response curve had a lower slope for intraarterial than for intravenous AI, and the responses tended to be greater when the peptide was injected by the latter route. Thus, to elicit the same increase of 20 mm Hg in the MAP, the intraarterial/intravenous dose ratio was 1.6 in the control and 2.2 in the 2K1C group.

Discussion

Intravenous infusion of MK422 lowered the blood pressure in control and 2K1C rats for at least 24 hours after administration. In both groups of rats, the dose-response curves obtained by both routes of administration were markedly displaced to the right. The doses needed to produce the same response were increased about 10-fold by the intravenous route and 5-fold by the intraarterial route. These effects were not significantly different for the control and 2K1C groups. In both groups, also, the log dose-response curves obtained 24 hours after the MK422 infusion had shifted back to the preinfusion levels (fig. 2), indicating that the animals had regained their normal sensitivity to the pressor effect of intravenously or intraarterially injected AI.

Effect of MK422 on the Responses to Bradykinin

Figure 3 shows the log dose-response curves for intravenously and intraarterially injected BK in control and 2K1C rats. In both groups, larger doses were needed by the intravenous than by the intraarterial route to obtain the same effect. The intravenous/intraarterial dose ratio was 28 for the controls and 27 for the 2K1C group, indicating a similar rate of pulmonary inactivation of BK (96.5% and 96.3%, respectively).

After the infusion of MK422, the log dose-response curves obtained by the intravenous route were markedly displaced to the left. This potentiation of BK's effect was 30-fold in the control and 52-fold in the 2K1C group. By the intraarterial route, the potentiation was also significant, though less pronounced: 7-fold in the control and 5-fold in the 2K1C group.

Twenty-four hours after the treatment with MK422, a remarkable difference was found between the dose-response curves obtained by the two routes of administration. The responses to intravenously injected BK returned to normal levels, while the responses to the intraarterial injections, which remained consistently potentiated, were not significantly different from those obtained immediately after the MK422 infusion (fig. 3). This behavior was equally present in the control and 2K1C groups.
**CONTROL**

![Graph of control data](image-url)

**2 KIC**

![Graph of 2 KIC data](image-url)

**Figure 2.** Log dose-response curves for AII injected intravenously (i.v.) or intraarterially (i.a.) in control (left panel) and 2KIC (right panel) rats obtained before (○), immediately after (●) and 24 hours after (▲) infusion of MK422 at the rate of 1 mg·kg⁻¹·hr⁻¹ for 30 minutes. Each point represents the average of 8 to 10 experiments and the SEM are indicated.

**CONTROL**

![Graph of control data](image-url)

**2 KIC**

![Graph of 2 KIC data](image-url)

**Figure 3.** Log dose-response curves for BK injected intravenously (i.v.) or intraarterially (i.a.) in control (left panel) and 2KIC (right panel) rats. The curves were obtained before (○), immediately after (●) and 24 hours after (▲) the infusion of MK422 (1 mg·kg⁻¹·hr⁻¹) for 30 minutes. Each point represents the average of 7–10 experiments and the SEM is indicated.
Cohen and Kurz\textsuperscript{10} have shown in spontaneously hypertensive rats, that 24 hours after a single oral dose of captopril or MK421, when the hypotensive effect was still evident, the in vitro CE activity remained inhibited in the aorta and kidney, was partially recovered in the lung, and had returned to normal in the plasma, heart, and brain. In the lung, the CE activity was down to about 15\% of its normal value 1 hour after administration of the CE inhibitor, and 24 hours later was still inhibited to about 60\%. This partial recovery of the lung CE activity appears to be enough to restore the in vivo conversion of exogenous AI in that tissue to pre-treatment levels, since the pressor responses to intravenous AI were fully recovered 24 hours after the treatment with CE inhibitor. The lung is the main site of angiotensin conversion in vivo, one passage through the pulmonary circulation being enough to almost completely convert AI into All and other degradation products,\textsuperscript{8-10} and it is possible that this may continue to occur even when its CE activity is reduced to 60\%. Inhibition of the kidney CE activity cannot also be responsible for the prolonged hypotensive effect of the CE inhibitor, since the AI that escapes conversion in that organ is completely converted or metabolized during its passage through the lungs, before reaching the peripheral circulation, where All exerts its vasoconstrictor effect.

Intraarterial (but not intravenously) injected BK was still potentiated 24 hours after the MK422 infusion (fig. 3). Intravenously injected BK is mostly degraded in the lungs, while intraarterially administered BK exerts its depressor effect on the arterial smooth muscle before it reaches pulmonary circulation. Based on the responses observed, BK degradation in the arterial circulation was similar on the 1st and 24th hour after the infusion of CE inhibitor. This continued depression in BK degradation correlated to the observed effect on MAP and may be responsible for the hypotensive action of CE. Although the effect of a decreased conversion of physiological amounts of AI at the artery wall cannot be definitely discarded based on evidence obtained with exogenously administered drugs, we must conclude that the prolonged hypotensive effect of MK422 is associated with an increased concentration of endogenous BK due to the inhibition of endothelial kininase activity. This is in agreement with previous observations,\textsuperscript{11-13} which suggest that the antihypertensive effect of CE inhibitors may be at least partly due to changes in the kallikrein-BK system.

Previous studies with CE inhibitors usually emphasize their effect on different models of experimental and clinical hypertension, although a vasodepressor action of these compounds may also be present, in various degrees, in normotensive controls. Our results show that, after intravenous infusion of MK422, not only was there hypotensive action in the control animals but the effects on the responses to AI and to BK were similar in the control and 2K1C rats. This suggests that the same mechanism may be involved in the hypotensive activity of MK422 on the normal and in the 2K1C hypertensive animals.

References

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