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SELECTIVE ACTIVATION OF THE CONVERTING ENZYME INHIBITOR MK 421 AND COMPARISON OF ITS ACTIVE DIACID FORM WITH CAPTOPRIL IN DIFFERENT TISSUES OF THE RAT

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Abstract—The hydrolysis of the new converting enzyme (CE) inhibitor MK 421 to its more active diacid form (MK-diacid) was studied by measuring CE inhibition in rat plasma and in some target organs of angiotensin. The activity of MK-diacid was compared with that of captopril *in vitro* and *in vivo*. Effective activation of MK 421 was found to occur in plasma and kidney, to a lesser degree in adrenals and in brain, and not at all in the lung. Based on the concentrations necessary for 50% enzyme inhibition (IC_{50}), MK-diacid revealed a 5–15 fold higher inhibitory potency against CE than captopril depending on the tissue tested. Following intravenous (i.v.) injections of 3, 15 and 300 μg/kg in conscious rats, MK-diacid was more active than captopril in reducing the pressor responses to i.v. angiotensin I (ANG I), and both MK 421 and MK-diacid produced a longer lasting CE inhibition than captopril. After intracerebroventricular (i.c.v.) injections of the same doses, MK-diacid and captopril produced similar reductions of the pressor responses to i.c.v. ANG I, but MK-diacid was again longer acting than captopril. MK 421 injected i.c.v. did not reduce the pressor responses to i.c.v. ANG I. However, at 300 μg/kg i.c.v., MK 421 was found, like MK-diacid and captopril, to inhibit the pressor responses to i.v. ANG I, which indicates that it passed from the cerebrospinal fluid to the systemic circulation and was activated peripherally. These data demonstrate that in rats MK-diacid is a more potent CE inhibitor than captopril *in vitro* and *in vivo*, peripherally and in the brain. The selective hydrolysis of MK 421 to its more active diacid form in different CE containing tissues may influence the bioavailability of the active inhibitor and its pharmacological activity in the target organs of angiotensin.

MK 421 represents a new class of nonsulfhydryl-containing orally active inhibitors of converting enzyme (CE, EC 3.4.15.1) and is undergoing clinical trials as an antihypertensive drug. It has been developed by Patchett *et al.* [1] and its pharmacology and antihypertensive activity in experimental hypertension have been described by Gross *et al.* [2] and Sweet *et al.* [3].

Unlike other known CE inhibitors, MK 421 is an ester that requires hydrolysis to its diacid form (MK-diacid) to be fully active [1]. Hydrolysis of MK 421 has been shown to occur in dog liver homogenates and in rat plasma [1], but whether the drug can be activated in other organs, particularly in those which contain high CE activity and which are target organs for angiotensin such as the lung, kidney, adrenals and brain, is not known.

Evidence has been growing that the antihypertensive mechanism of CE inhibitors cannot be satisfactorily explained by inhibition of the plasma renin angiotensin system (RAS) but appears to reside also in a local inhibition of the angiotensin II (ANG II) generation in tissues like arterial wall, kidney, adrenal gland and in the brain [4–6]. Thus, a local activation of MK 421 to the active diacid MK-diacid may constitute an important factor influencing the bioavailability and the antihypertensive action of MK 421 and similar prodrug CE inhibitors. We have, therefore, measured the conversion of MK 421 into MK-diacid in various CE containing tissues. In addition, *in vitro* and *in vivo* activity of MK 421 and its parent diacid MK-diacid was compared with captopril, the best studied orally active CE inhibitor. Particular attention has been paid to the activity of MK 421 and MK-diacid against brain CE *in vivo* and *in vitro*, since it has been shown previously that intracerebroventricular (i.c.v.) injections of captopril lowered arterial blood pressure in spontaneously hypertensive rats by a central action [7–9].

MATERIAL AND METHODS

In vitro *inhibition of converting enzyme*

Handling of blood and tissue samples. Male Wistar rats (250–300 g) were used. Blood was withdrawn from the abdominal aorta under ether anesthesia and transferred into chilled heparinized tubes, centrifuged at 10,000 *g* and 4° for 10 min, the plasma was separated and kept on ice. CE was measured immediately, since previous investigations have shown that in plasma samples from man and rat captopril loses its inhibitory activity upon cold storage due to oxidation of its mercapto group [10, 11].

Tissue (lung, brain, kidney, adrenals) was sampled after exsanguination into chilled tubes and hom-

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ogenized on ice in distilled water (1:5, w/v) containing 0.3% Triton X-100. Lung, kidney and adrenals were homogenized with a Polytron homogenizer (Polytron PTA 10-35, Kinematica GmbH, Lucerne, Switzerland) for 15 sec followed by sonification (Branson sonifier B-12, Sonic Power Corp. Connecticut) for 5 sec. Brain tissue was homogenized by sonification only. After centrifugation (15 min at 15,000 rpm at 4°), CE was assayed in the supernatant. Time between tissue removal and incubation was always less than 1 hr. Plasma and tissue pools from 3 or more animals were used for methodological studies.

Converting enzyme determination. Procedure. CE was assayed by a modified fluorometric method according to Depierre and Roth [12] using carbobenzoxy-phenyl-alanyl-histidyl-leucine (Z-Phe-His-Leu) as substrate. Plasma or tissue homogenate (50 μl) were diluted with cold 400 μl phosphate buffer (70 mM, pH 8.0 containing 300 mM/l sodium chloride). When CE inhibitors were added, an aliquot (100 μl) of the buffer was replaced by an equal volume of the inhibitor buffer solution. The reaction was started by adding 50 μl of a 10 mM substrate solution to the samples and incubation at 37°. At various time intervals the reaction was terminated by transferring 100 μl aliquots from the incubation into 1 ml of 0.1 N NaOH. All subsequent steps in the assay were continued in the dark: 25 μl 2% orthophthaldialdehyde solution in dimethylsulfoxide (DMSO) were added to the samples. After 30 min the reaction was terminated with 1 ml 0.8 N HCl, precipitates were spun down by a 3000 *g* centrifugation for 3 min and fluorescence (λ_{ex} 360 nm; λ_{em} 500 nm) was measured within 60 min (Jasco FP-550, Biotronics). Zero time blank values were subtracted from the corresponding test values. All measurements were done in triplicate. Protein content of the tissue homogenates was analysed according to Lowry *et al.* [13]. The results are expressed as nmole His-Leu/ml per min (plasma) or pmole His-Leu/mg protein per min (tissues).

Linearity of the assay with incubation time. The linearity of the assay with respect to substrate concentration and incubation time was established for plasma and each tissue separately. Plasma and lung were incubated at 13 different time points between 3 and 120 min; brain was incubated at 5 different time points between 30 and 180 min (Fig. 1); kidney and adrenals were incubated for 30 and 120 min. If not stated otherwise, the following incubation times were used: lung, 5 min; plasma, 30 min; brain, kidney, adrenals, 120 min at which time product formation was linear.

Stability of the inhibitors. Plasma and lung tissue homogenates were incubated for different periods of time between 3 and 120 min as described above. Captopril, MK-diacid and MK 421 were added to the incubation at final concentrations between 0.4 and 4×10^{-6} M. In the plasma, MK-diacid caused a constant inhibition for 120 min. The inhibitory potency of MK 421 increased with time, reaching a maximum between 60 and 75 min and decreasing slightly at 120 min. Captopril was stable for 24 min; incubations for longer than 60 min resulted in a precipitous decline of its inhibitory potency (Fig. 2). When incubated with lung extract, captopril showed stable inhibition for 6 min and MK-diacid for 24 min of incubation.

In vitro *activity of MK-diacid, MK 421 and captopril in plasma and tissue.* Plasma, lung, brain, kidney and adrenal were incubated as described. MK-diacid and MK 421 were added to the incubation at final concentrations between 4×10^{-9} and 4×10^{-5} M.

For comparison of the inhibitory potency of *captopril* and MK-diacid, incubation time was 5 min for lung and 25 min for plasma and the other tissues to avoid oxidation of captopril during prolonged incubation times. Both inhibitors were found to be stable in brain, kidney and adrenal homogenates during an incubation time of 25 min. MK-diacid and *captopril*, were added to give final concentrations between 4×10^{-10} and 4×10^{-5} M. The IC_{50} values were read from the semi logarithmic plots of per cent inhibition vs concentration.

In vivo *inhibition of converting enzyme*

Experimental animals and blood pressure recordings. All experiments were done in conscious chronically instrumented male Wistar rats (250–300 g). Details of the surgical procedures have been

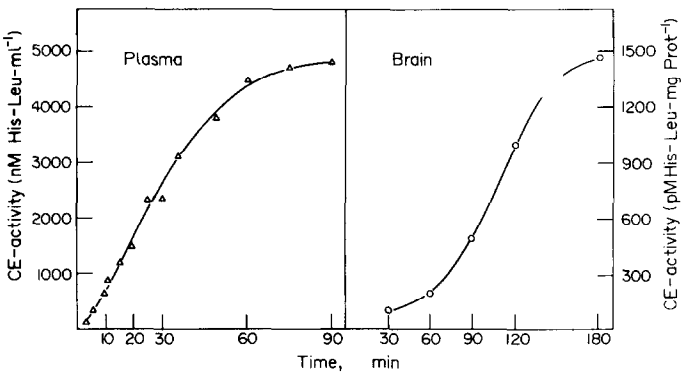

Fig. 1. Kinetics of converting enzyme measurement in plasma and in brain. Data are means of two experiments. Kidney and adrenals yielded curves similar to brain. In lung tissue, converting enzyme activity was highest between 3–6 min and declined thereafter (for details and substrate concentration see text).

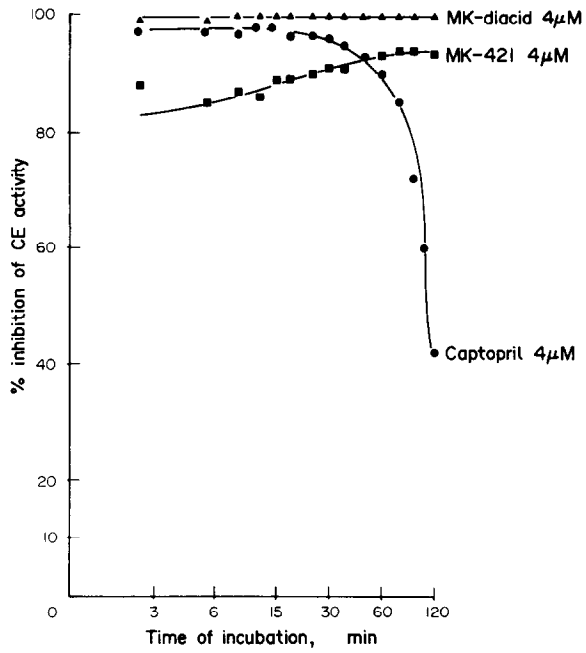

Fig. 2. Stability of the converting enzyme inhibitors MK 421, its active diacid form (MK-diacid) and captopril in rat plasma during incubation. Data are means of two experiments. Results with inhibitor concentrations of 4 μM are shown. Similar curves were obtained with inhibitor concentrations of 0.4 μM at which maximal CE inhibition by captopril was 81%. Note the instability of captopril at incubation times longer than 30 min and the increase with time of the MK 421 activity pointing to an activation process.

described elsewhere [9]. Injections of angiotensin I (ANG I) and the CE inhibitors into the lateral brain ventricles (i.c.v.) were made via chronically implanted cannulae (PP 20, Portex Corporation) and intravenous (i.v.) injections through catheters (PP 10) inserted into the femoral vein. Mean arterial blood pressure (BP) was recorded via a catheter (PP 10 in PP 50) placed in the femoral artery and connected to a Statham P 23 Db transducer, Gould Brush blood pressure computer and Gould Brush 2400 recorder.

Rats were divided into groups of at least 6 animals, each group receiving only one dose of the respective inhibitor, either i.v. or i.c.v. Each rat was challenged with 100 ng ANG I i.v. and i.c.v.

Peripheral administration of CE inhibitors. The CE inhibitors MK-diacid and MK 421 were dissolved in a 50 mM borate–phosphate buffer, pH 8.0. Captopril was dissolved in isotonic saline. Both vehicles had no effect on arterial blood pressure when given alone either i.v. or i.c.v. Rats were injected i.v. with 3 μg/kg, 15 μg/kg or 300 μg/kg of MK 421, MK-diacid and captopril. ANG I (100 ng) was injected i.v. 3, 10, 15, 30 and 60 min later and, at the high inhibitor doses, also after 120 and 180 min.

Control rats received the same injections but were treated i.v. with vehicle instead of the CE inhibitors.

Central administration of CE inhibitors. These experiments were performed to investigate an *in vivo* activation of MK 421 in the brain, further to compare the *in vivo* inhibition of brain CE by the different inhibitors, and finally to test the penetration of the CE inhibitors from the cerebrospinal fluid into the peripheral circulation. Rats were injected with 3 μg/kg, 15 μg/kg or 300 μg/kg of MK 421, MK-diacid and captopril, respectively. The injection volume was 10 μl of the inhibitor solution which was flushed with 5 μl isotonic saline. After 3 min, 100 ng ANG I was injected i.v. and when blood pressure had returned to control levels (2–3 min later) 100 ng ANG I was injected i.c.v. The i.v. and i.c.v. ANG I injections were repeated after 30 (i.v. only), 60, 120, 180, 240 and 300 min. Control rats received the same i.v. and i.c.v. injections of ANG I following i.c.v. injection of 15 μl isotonic saline instead of a CE inhibitor.

Drugs

Captopril was a gift from Z. P. Horovitz, The Squibb Institute, Princeton, NJ; MK 421 and its parent diacid were generously provided by C.S. Sweet, Merck Sharp & Dohme Research Laboratories, Rahway, NJ. The synthetic CE substrate Z-Phe-His-Leu was purchased from Bachem Biochemical Products, Bubendorf, Switzerland; ANG I was purchased from Beckman, Palo Alto, CA.

Statistics

The results of the *in vivo* studies are expressed as means ± S.E.M. Comparison between groups was performed using analysis of variance (ANOVA) followed by the Scheffé-test. A significance level of $P < 0.05$ was accepted.

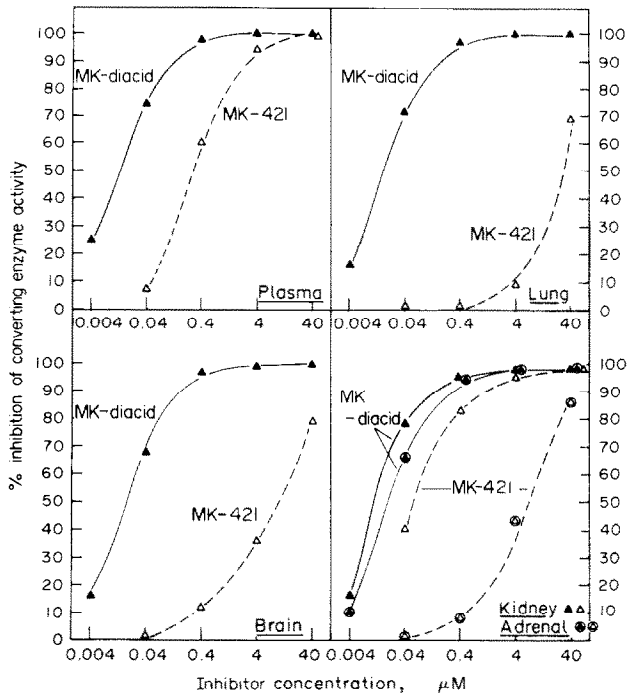

Fig. 3. Inhibition of converting enzyme activity by MK 421 and its active diacid (MK-diacid) in rat plasma and tissues. Note increased inhibition (ordinate) with increasing inhibitor concentrations (abscissa) and slow (brain, adrenal) or no (lung) activation of MK 421. For details see text.

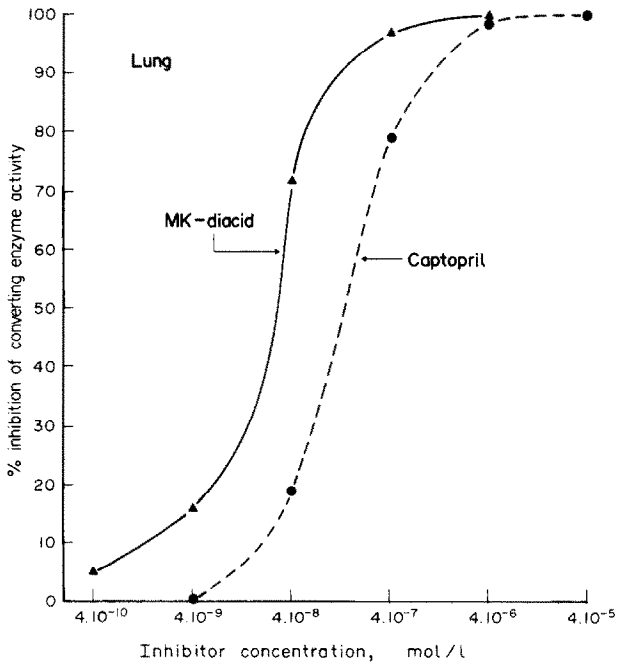

Fig. 4. Inhibition of converting enzyme activity in rat lung by MK-diacid and captopril. From this curve and similar curves obtained with plasma, kidney, adrenals and brain the IC_{50} values (Table 1) for both inhibitors were determined.

RESULTS

Experiments in vitro

Inhibition of plasma and tissue CE by MK 421 *and MK-diacid.* The inhibition of CE by MK 421 and MK-diacid in the plasma, lung, brain, kidney and adrenals is shown in Fig. 3. MK-diacid produced an almost complete inhibition of the enzyme at a concentration of 0.4 μM in the plasma and all tissues tested. Complete inhibition of CE activity was observed at 4 μM in the plasma and lung and at 40 μM in the brain, whereas in kidney and adrenals the maximal inhibition measured was approximately 98%.

MK 421 revealed an inhibitory potency close to that of MK-diacid in plasma and kidney, while being considerably less potent than MK-diacid in brain and adrenals. In the lung MK 421 did not inhibit CE except at the highest concentration.

In vitro *potencies of MK-diacid and captopril.* Inhibition of CE activity in the lung by the two CE inhibitors is shown in Fig. 4. Similar curves were obtained for the other tissues. The IC_{50} values for plasma, lung, brain, kidney and adrenals are summarized in Table 1. While MK-diacid was generally found to have a higher CE inhibitory potency than captopril *in vitro*, the differences between both CE inhibitors varied among the tissues investigated. Thus, MK-diacid was 15 times more active than captopril in the kidney, 10 times in the plasma, 8 times in the adrenal gland, 7 times in the brain and 5 times in the lung (Table 1).

Experiments in vivo

CE inhibition after intravenous inhibitor administration. At 3 μg/kg i.v., neither of the CE inhibitors reduced the pressor responses to 100 ng ANG I i.v. At 15 μg/kg i.v., MK 421 was still ineffective, while MK-diacid produced a significant inhibition of the ANG I pressor responses for 15 min with a maximum of 70% inhibition after 3 min. Captopril reduced the ANG I responses by 25% for less than 15 min only transiently (significant decrease only at the 3 min time point, Table 2). At 300 μg/kg i.v., MK 421 reduced the ANG I responses by 76% at 3 min; with maximal inhibition (80%) reached after 15 min and no significant inhibition any more after 120 min. MK-diacid produced an almost 100% inhibition after 3 min and was still active after 120 min (23% inhibition). Captopril was almost as potent as MK-diacid after 3 min (88% inhibition), but its action was much shorter, lasting less than 60 min (Fig. 5, Table 2).

CE inhibition after intracerebroventricular inhibitor administration. Similar to i.v. inhibitor administration, MK 421, MK-diacid and captopril did not

Table 1. Activities of converting enzyme inhibitors *in vitro*

Tissue	IC_{50} (nM) MK-diacid	Captopril
Plasma	12	120
Lung	28	140
Brain	30	175
Kidney	72	1050
Adrenal gland	29	240

Table 2. Pressor responses to 100 ng ANG I i.v. following intravenous injections of CE inhibitors in conscious rats

	Resting MAP	Control ANG I injection	Minutes after i.v. injection of the inhibitor 3	15	30	60	120	180
0.9% NaCl i.v.	97.2 ± 1.5	+52.5 ± 1.6	+47.1 ± 2.4	+47.8 ± 1.9	+48.2 ± 1.6	+49.5 ± 2.0	+50.4 ± 2.0	+49.0 ± 2.1
15 μg/kg i.v.								
MK 421	101.8 ± 2.3	+46.3 ± 2.9	+43.7 ± 4.2	+42.7 ± 4.8	+48.7 ± 4.8	+52.7 ± 3.2		
MK-diacid	99.2 ± 4.5	+52.0 ± 4.0	14.2 ± 3.9†	+33.4 ± 8.8*	+46.8 ± 4.6	+51.8 ± 3.7		
Captopril	100.3 ± 7.3	+52.3 ± 4.0	+35.5 ± 4.8*	+42.4 ± 2.9	+48.2 ± 2.7	+50.2 ± 3.0		
300 μg/kg i.v.								
MK 421	94.0 ± 9.1	+53.4 ± 5.9	+11.2 ± 2.8†	+ 9.8 ± 3.5†	+15.8 ± 3.1†	+20.2 ± 3.6†	+44.2 ± 3.9	+49.2 ± 6.0
MK-diacid	96.8 ± 2.7	+56.5 ± 2.2	+ 1.5 ± 1.0†	+ 6.5 ± 0.6†	+10.7 ± 2.3†	+18.3 ± 2.6†	+38.8 ± 5.5*	+46.0 ± 3.4
Captopril	98.0 ± 4.6	+51.7 ± 2.6	+ 5.6 ± 1.4†	+17.7 ± 1.7†	+34.3 ± 3.9*	+53.3 ± 2.8	+55.8 ± 2.1	+58.7 ± 2.1

* $P < 0.05$, † $P < 0.01$. MAP, mean arterial pressure.
Data were analysed statistically using an analysis of variance and a Scheffé-test with treatment means compared to saline-treated controls.

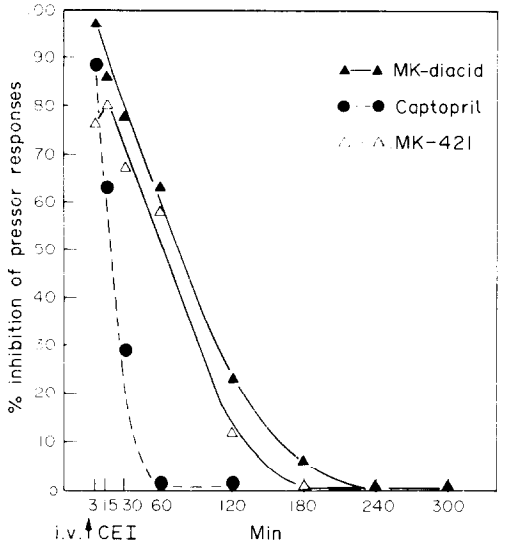

Fig. 5. Inhibition of the pressor responses to 100 ng ANG I i.v. following i.v. pretreatment with 300 μg/kg of the converting enzyme inhibitors MK 421, MK-diacid and captopril in conscious normotensive rats. Values represent means of 6 or more animals per group. The pressor responses in an untreated control group were used to calculate per cent inhibition in the treated groups.

reduce the pressor responses to 100 ng ANG I i.c.v. at a dose of 3 μg/kg i.c.v.

At 15 μg i.c.v., MK 421 was also ineffective, but MK-diacid inhibited the pressor responses to i.c.v. ANG I by more than 50% after 5 min and by 44% after 60 min. After 120 min this effect was not significant any more. Captopril also caused an inhibition

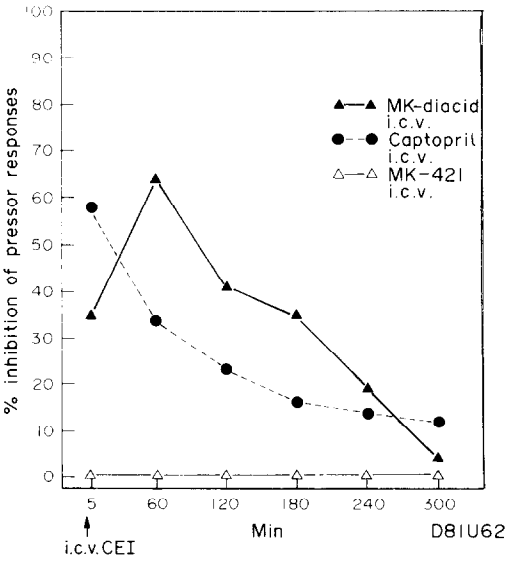

Fig. 6. Inhibition of the pressor responses to 100 ng ANG I injected into the lateral brain ventricle (i.c.v.) following i.c.v. pretreatment with 300 μg/kg of the converting enzyme inhibitors MK 421, MK-diacid and captopril in conscious normotensive rats. Percentage of inhibition was calculated from the ANG I pressor responses in untreated control rats. Note the slower mode of action of MK-diacid and lack of activation of MK 421 in the brain.

Table 3. Pressor responses to 100 ng ANG I i.c.v. following i.c.v. injections of CE inhibitors in conscious rats

	Resting MAP	Control ANG I injection	Minutes after i.c.v. injection of the inhibitor 5	60	120	180	240
0.9% NaCl i.c.v.	97.2 ± 1.5	+26.6 ± 1.8	+20.2 ± 1.6	+22.3 ± 1.8	+20.4 ± 1.5	+20.0 ± 1.6	+22.1 ± 1.4
15 μg/kg i.c.v.							
MK 421	98.7 ± 4.1	+26.7 ± 1.7	+22.2 ± 3.3	+22.8 ± 3.9	+26.2 ± 2.8		
MK-diacid	102.4 ± 3.7	+27.4 ± 2.6	+10.0 ± 1.8*	+12.5 ± 1.8*	+19.8 ± 1.9		
Captopril	98.5 ± 2.9	+26.2 ± 3.2	+12.0 ± 2.6*	+19.3 ± 2.5	+21.7 ± 2.3		
300 μg/kg i.c.v.							
MK 421	107.5 ± 3.9	+27.0 ± 2.1	+24.7 ± 1.3	+21.8 ± 1.7	+24.1 ± 1.9	+26.7 ± 5.0	+25.2 ± 4.2
MK-diacid	97.5 ± 3.9	+27.5 ± 1.3	+13.1 ± 2.3*	+ 8.0 ± 2.8†	+12.0 ± 2.6*	+13.0 ± 2.8*	+18.0 ± 1.1
Captopril	102.0 ± 3.9	+24.2 ± 1.4	+ 8.5 ± 1.8†	+14.8 ± 1.4*	+15.7 ± 1.3	+16.8 ± 1.8	+19.0 ± 2.0

* $P < 0.05$, † $P < 0.01$. MAP, mean arterial pressure.
Data were analysed statistically using an analysis of variance and a Scheffé-test with treatment means compared to saline-treated controls.

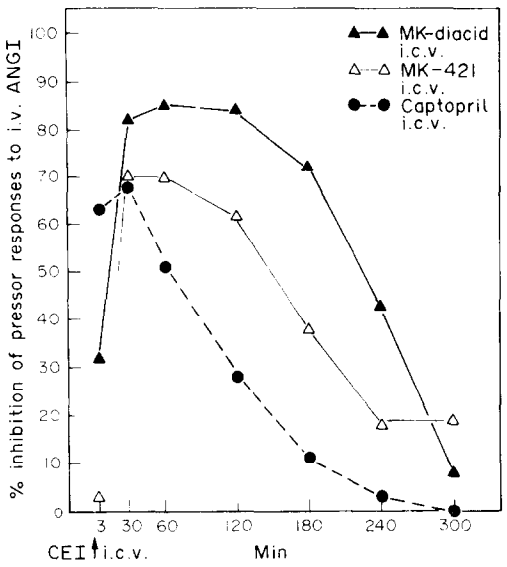

Fig. 7. Inhibition of the pressor responses to 100 ng ANG I i.v. following i.c.v. pretreatment with 300 μg/kg of the converting enzyme inhibitors MK 421, MK-diacid and captopril in conscious normotensive rats. Percentage of inhibition was calculated as above (Fig. 5). All three inhibitors passed from the cerebrospinal fluid into the peripheral circulation. Captopril penetrated faster than MK 421 and MK-diacid but caused the shortest peripheral converting enzyme inhibition. MK 421, while being completely inactive centrally, was activated in the periphery and produced a delayed but sustained peripheral converting enzyme inhibition.

of the central ANG I pressor responses, which lasted, however, less than 60 min (Table 3).

At the 300 μg/kg i.c.v. dose, MK 421 still failed to reduce the central ANG I responses, but caused a marked inhibition of CE in the periphery as indicated by the reduction of the pressor responses to i.v. ANG I (Fig. 6, Fig. 7).

In contrast to MK 421, i.c.v. injections of MK-diacid produced a marked inhibition of the *in vivo* CE activity centrally as well as peripherally of 3–4 h duration (Fig. 6, Fig. 7).

Captopril blocked the pressor responses to i.c.v. ANG I almost as effectively as MK-diacid in the beginning, but this inhibitory action lasted for 1 hr only. The central injections of captopril also produced an inhibition of the pressor responses to i.v. ANG I which was again of shorter duration than the corresponding effects of MK 421 and MK-diacid (Fig. 7).

DISCUSSION

MK 421 has been claimed to be a prodrug that has a 300–1000 times weaker inhibitory potency than the diacid MK-diacid form against hog plasma CE *in vitro* and it requires de-esterification to develop its full CE inhibitory activity [2].

The data presented here demonstrate considerable differences in potency of MK 421 *in vitro* depending on the organ investigated. These differences most likely reflect the ability of plasma and tissue hydrolases to convert the ester compound MK 421 into the active diacid MK-diacid.

In the dog, the liver has been claimed to be the primary site where this biotransformation takes place [1]. Since CE activity was unmeasurably low in rat liver, this possibility could not be tested. High potency of MK 421 was found against CE from plasma and kidney. This indicates that rat plasma and kidney contain sufficient hydrolase activity to de-esterify MK 421 to the more active diacid. In contrast, hydrolysis of MK 421 did not take place as easily — if at all — in brain tissue and adrenals. In these tissues marked differences in potency between MK 421 and the diacid MK-diacid were observed. In both tissues, the concentration required to produce a 50% inhibition of CE was more than 500 times higher for MK 421 than for MK-diacid.

In lung tissue homogenates, MK 421 was found to be inactive except for the highest inhibitor concentration used (4×10^{-5} M) while MK-diacid displayed an inhibitory potency similar to the one found in the other organs. The CE inhibition at high concentrations of MK 421 is most likely due to the inherent activity of MK 421 as a CE inhibitor, which was reported to have an IC_{50} of 1.2×10^{-6} M in an assay using purified hog plasma CE [2]. It appears, therefore, that the lung which contains most of the peripheral CE activity does not have any hydrolysing activity to de-esterify MK 421.

MK-diacid proved to be a more potent CE inhibitor than captopril in all tissues investigated, the difference between both CE inhibitors being three times greater in the kidney than in the lung. The IC_{50} values obtained for both CE inhibitors in the plasma were higher in our assay than those reported by Gross *et al.* [2] using purified hog plasma CE, but are still in a comparable order of magnitude. In the same study the authors reported MK-diacid to be 5 to 17 times more potent than captopril against hog plasma CE, depending on whether the more active isomer or the less active diastereomeric mixture of MK-diacid were used. Our finding of MK-diacid having a ten-fold higher inhibitory potency than captopril against CE in the plasma is in agreement with this report.

The *in vivo* inhibition of peripheral CE and of brain CE following i.v. and i.c.v. administration of MK 421, MK-diacid and captopril is in keeping with the *in vitro* results. The longer duration of action of MK 421 and MK-diacid has also been shown by Brunner *et al.* [14] in man and by Sweet *et al.* [3] in experimental animals. The fact that MK 421 needed 15 min to reach its inhibitory maximum, while both, MK-diacid and captopril, inhibited maximally within 3 min after i.v. injection, points again to the importance of the activation process for MK 421. Since activation is relatively slow, it is unlikely to take place in the plasma alone but may occur locally in tissues with esterase activity.

MK 421 did not reduce the ANG I pressor responses after 3 μg/kg i.v. and after 15 μg/kg i.v. This is at variance with the results reported by Gross *et al.* [2] who had calculated an ID_{50} of 14.0 μg/kg i.v. for MK 421. On the other hand, the ID_{50} values for MK-diacid and captopril reported by the same authors agree with our findings.

Following i.c.v. administration, MK-diacid and captopril inhibited the pressor responses to i.c.v. ANG I to a similar extent. However, MK-diacid was found to have a considerably longer duration of action than captopril, which corresponds to our findings following peripheral administration of the drugs. The maximal *in vivo* inhibition of brain CE by MK-diacid was not observed immediately as with captopril, but only after 60 min. The reason for this latency is not clear. One explanation could be that CE inhibition does not occur in the cerebrospinal fluid but also — and perhaps functionally more important — at periventricular sites such as the chorioid plexus which contains extremely high CE concentrations [15]. MK-diacid may need more time than captopril to penetrate from the CSF into these tissues.

MK 421, on the other hand, did not inhibit the pressor response to i.c.v. ANG I, even following a dose of 300 μg/kg i.c.v. This is in agreement with the *in vitro* results and provides *in vivo* evidence that the brain does not activate the prodrug MK 421 to the active diacid. However, at the dose of 300 μg/kg i.c.v. MK 421 produced a long lasting inhibition of the pressor responses to i.v. ANG I, indicating that the drug passed from the cerebrospinal fluid into the circulation and was activated peripherally. Similarly, the i.v. or orally administered MK 421 may have central effects after peripheral activation.

In conclusion, the results demonstrate that in rats MK-diacid is more active than captopril against CE in the periphery and in the brain. Activation of the prodrug MK 421 by hydrolysis takes place in plasma and kidney but to a much lesser extent in brain and adrenals, and not at all in the lung.

Activation of MK 421 appears to be relatively slow, which favours the participation of tissues in the activation process. Since a local inhibition of the ANG I conversion to ANG II in tissues has been postulated to be involved in the antihypertensive mechanisms of CE inhibitors, the selective activation of prodrug CE inhibitors in different tissues may be important for the action of these drugs. Further, this selectivity may provide a valuable tool to improve our understanding of the functional role of the tissue renin angiotensin systems.

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