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Human angiotensin I competes with two non-thiol converting enzyme inhibitors (CGS 13934 & MK-422) to inhibit the angiotensin-converting enzyme activity of human plasma

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A simple in-vitro enzyme system has been used to demonstrate that human angiotensin I interacts competitively with the two non-thiol converting enzyme inhibitors CGS 13934 and MK-422 to inhibit the hydrolysis of a synthetic substrate by human plasma ACE.

The role of the renin-angiotensin system in the pathogenesis of experimental and clinical hypertension has been extensively investigated during recent years (Brunner et al 1980). Inhibitors of angiotensin-converting enzyme (ACE) have been designed to compete with angiotensin I for ACE in order to reduce the formation of angiotensin II in plasma (Cushman et al 1977; Patchett et al 1980; Chen et al 1982; Jacot des Combes et al 1983). The present study was undertaken to assess whether the competitive interaction between human angiotensin I and ACE inhibitors such as MK-422 (Patchett et al 1980) and CGS 13934 (Chen et al 1982) indeed can be demonstrated in human plasma.

Methods

Heparinized human blood collected from 6 healthy volunteers (3 males and 3 females) was centrifuged at 4 °C for 15 min. The plasma was aspirated and kept frozen at −20 °C. 400 µl of pooled plasma were mixed with 50 µl of 2% $NaHCO_3$ and 50 µl of different concentrations of ACE inhibitors or angiotensin I at 4 °C. The effects of each ACE inhibitor or angiotensin I on plasma ACE activity were determined simultaneously in the same assay to minimize variations. At each concentration of ACE inhibitor or angiotensin I, the same Eppendorf pipette was used to introduce a constant amount of ACE inhibitor or angiotensin I into each plasma sample. Each plasma sample was kept frozen at −20 °C for ACE assay within 3–4 days. The binding activity between CGS 13934 or MK-422 and ACE did not change during a 2 month storage period at −20 °C (unpublished data).

ACE activity was determined by a radioenzymatic procedure (Ventrex Laboratories, Inc., Portland, ME) using [^{3}H]hippurylglycyl-glycine as substrate. At 4 °C, 50 µl of plasma samples were diluted 5 times with 0·05 M Hepes buffer (pH 8·0); 50 µl of these diluted samples were mixed with 50 µl of ACE substrate (16 mM) in glass tubes and incubated at 37 °C for 60 min. The reaction was stopped by adding 1·0 ml of 0·1 M HCl at room temperature (20 °C). Ethyl acetate (1·0 ml) was then added to extract radioactive hippuric acid liberated by ACE. All tubes were vortexed at the same time and centrifuged at 1000*g* at room temperature for 10 min. 300 µl of the upper ethyl acetate layer were then transferred to 10 ml of scintillation fluid and the [^{3}H]hippuric acid was counted by liquid scintillation spectrometer. CGS 13934, MK-422 or angiotensin I was dissolved in 2% $NaHCO_3$ and all assays were performed in duplicate. The same pooled human plasma (ACE activity: 86·6 ± 1·1 nmol hippuric acid min^{-1} ml^{-1}, mean ± s.e., n = 9) and stock solution of angiotensin I was used in all experiments. In most cases, a Dixon plot (Dixon & Webb 1964) was applied to obtain the slope of the concentration versus reciprocal ACE activity line for each compound and in combination with angiotensin I to test statistically for difference between each pair of concentration-response lines (Dixon & Massey 1969). Angiotensin I (human) was purchased from Senn Chemicals, Dielsdorf, Switzerland. CGS 13934 (*N*-(2,4-dimethylglutaryl)-2,3-1H-dihydro-indole 2-carboxylic acid) (Chen et al 1982) was supplied by CIBA-GEIGY Pharmaceuticals Division, Summit, New Jersey. MK-422 (*N*-(1-S-carboxy-3-phenylpropyl)-S-alanine-S-proline) (Patchett et al 1980) was donated by the Merck Institute for Therapeutic Research, West Point, Pennsylvania, USA.

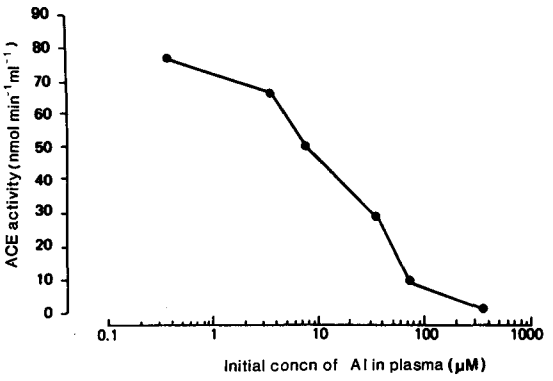

FIG. 1. Inhibition of human plasma ACE by human angiotensin I. Each point represents an average of two determinations.

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To estimate the amount of angiotensin I remaining in the incubation mixture in the face of uninhibited angiotensinases, angiotensin I concentrations were measured before and during the incubation procedure. Angiotensin I was added to pooled plasma containing 3·5 nM MK-422 to reach a final concentration of 24 μM. Aliquots of this mixture were then processed as for the determination of converting enzyme activity (see above). After 0, 15, 30 or 60 min of incubation time, proteins were precipitated with 2 ml of ethanol and angiotensin I concentration was measured by radioimmunoassay (Nussberger et al 1984).

Human angiotensin I (0·3–220 μM) inhibited the hydrolysis of synthetic substrate [^{3}H]hippuryl-glycyl-glycine by human plasma ACE activity in-vitro in a concentration-dependent manner (Fig. 1). A 10·1 μM concentration of angiotensin I inhibited plasma ACE activity by 50% while a 24 μM concentration inhibited plasma ACE activity by 60%. The higher concentration (24 μM) of angiotensin I was then used to study its competitive effect on inhibition of ACE by CGS 13934 and MK-422 in this in-vitro enzyme system. Like angiotensin I, CGS 13934 (4·5–1900 nM) and MK 422 (1·2–32 nM) inhibited ACE activity in a concentration-dependent manner. A 40 nM concentration of CGS 13934 or a 3·5 nM concentration of MK 422 produced approximately 50% inhibition of ACE activity.

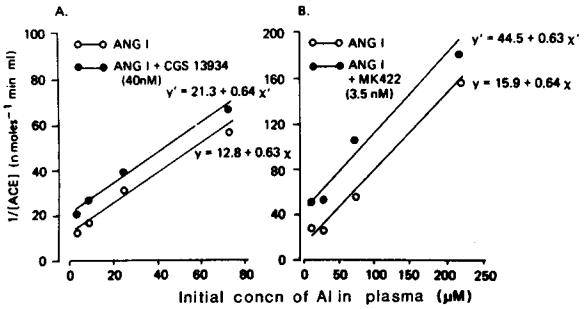

FIG. 2. Effects of added human angiotensin I on the inhibition by CGS 13934 (A) or MK-422 (B) of human plasma ACE. Each point represents an average of two determinations.

Using a Dixon plot, the concentration of CGS 13934 of MK-422 was shown to be positively correlated to the reciprocal of ACE activity (Fig. 2A, B) indicating that it follows a simple enzyme kinetics of the inhibition of ACE activity by CGS 13934 or MK-422. In the presence of angiotensin I, it caused a shift of the concentration-response line for CGS 13934 or MK-422 to the left. The difference between the two slopes of each set of concentration-response lines was not statistically significant which suggests no lack of parallelism between each pair of concentration-response lines. At time 0 of the incubation only 7·8 μM angiotensin I had remained in the mixture. At times 15, 30 and 60 min this concentration had decreased to 7·0, 6·4 and 5·3 μM respectively.

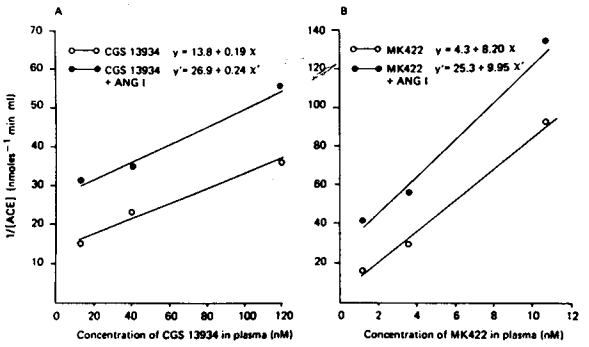

FIG. 3. Effects of CGS 13934 (A) or MK-422 (B) on the inhibition by human angiotensin I of human plasma ACE. Each point represents an average of two determinations.

Thus, before the incubation, 68% of the added angiotensin I was found to be metabolized. During incubation degradation of angiotensin I was much slower, possibly due to the dilution of the angiotensinases. These results reveal the competitiveness of angiotensin I with ACE inhibitors (CGS 13934 or MK-422) in blocking ACE. This competitive interaction between angiotensin I and ACE inhibitor was further evidenced as shown in Fig. 3A, B. In these two experiments, the presence of CGS 13934 (40 nM) or MK-422 (3·5 nM) likewise shifted the concentration-response line for angiotensin I to the left, with both paired slopes being almost identical and no lack of parallelism.

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REFERENCES

Brunner, H. R., Gavras, H., Waeber, B., Textor, S. C., Turini, G. A. (1980) Hypertension 2: 558–566

Chen, D. S., Dotson, R., Burrell, R., Aberg, G., Watkins, B. E. (1982) The Physiologist 25: 329

Cushman, D. W., Cheung, H. S., Sabo, E. F., Ondetti, M. A. (1977) Biochemistry 16: 5484–5491

Dixon, M., and Webb, E. C. (1964) Enzymes, Academic Press Inc., Publisher, New York, 328

Dixon, W. J., Massey, F. J., Jr. (1969) Introduction to statistical analysis, McGraw-Hill, 206

Jacot des Combes, B., Turini, G. A., Brunner, H. R., Porchet, M., Chen, D. S., Sen, S. B. (1983) J. Cardiovasc. Pharmacol. 5: 511–516

Nussberger, J., Brunner, D. B., Waeber, B., Brunner, H. R. (1984) J. Lab. Clin. Med. 103: 304–312

Patchett, A. A., Harris, E., Tristam, E. W. (1980) Nature 288: 280–283