Biochemical Pharmacology, Vol. 30, pp. 683-685.

0006-2952/81/060683-03 $02.00/0

PRELIMINARY COMMUNICATION

EFFECT OF CAPTOPRIL ON PROTEINS AND PEPTIDE HORMONES

Rajko P. Igic, John T. Gafford and Ervin G. Erdös
Departments of Pharmacology and Internal Medicine,
University of Texas Health Science Center at Dallas,
Dallas, Texas

(Received 29 September 1980; accepted 22 December 1980)

Clinical use of administration of captopril (Capoten; SQ 14225) has generated widespread interest. This inhibitor of angiotensin I converting enzyme or kininase II (peptidyl dipeptidase; EC 3.4.15.1) has been tested in various forms of hypertension (1) and in congestive heart failure (1, 2). Although many investigators have reported very favorable results, chronic oral administration of the inhibitor caused a number of side effects such as skin rash, glomerulonephritis (1, 3) and proteinuria in humans (4, 5) and prolongation of gestation in rabbits (6). Neither the beneficial therapeutic effects nor the side effects of captopril can be entirely explained by inhibition of the enzymatic release of the vasopressor angiotensin II. Since converting enzyme inactivates kinins (7), the prolongation of the short half-life of the vasodepressor kinins by captopril has also been considered (7). It has long been known that many compounds, including penicillamine, which potentiate the hypotensive effects of bradykinin (8) or inhibit converting enzyme *in vitro* and *in situ* (e.g. glutathione; 9) have a free SH group. Since captopril is D-3-thio-2-methyl-propanoyl-proline (10) and its SH group appears to be quite stable in solutions, we investigated its ability *in vitro* to reduce S-S bridges in peptides and proteins which are sensitive to reducing agents. Specifically, we used vasopressin, oxytocin, gamma-globulin (IgG), and papain. Breaking S-S bridges by reducing agents inactivates the posterior pituitary hormones and cleaves gamma-globulin to light and heavy chains (11). Papain is activated *in vitro* by SH agents.

To explore the possibility that captopril acts as a reducing agent we carried out the following experiments.

1) In thin layer chromatography on silica gel plates, oxytocin and vasopressin have Rf values of 0.34 and 0.14 in an n-butanol, acetic acid and water solvent (4:1:5). After incubation of 0.1 mg oxytocin or vasopressin with 0.23 or 2.3 mg captopril, the Rf values changed to 0.48 and 0.21, respectively. Staining of the peptides on the plate with 5, 5'-dithio-bis-(2-nitrobenzoic acid) after incubation with captopril showed yellow coloring due to free SH groups. Using another stable reducing agent dithiothreitol, instead of captopril, caused identical changes in the Rf values.

2) The inactivation of vasopressin by captopril was tested on the blood pressure of male Sprague-Dawley rats in sodium pentobarbitol (50 mg/Kg) anesthesia pretreated with pentolinium tartarate (20 mg/Kg). Vasopressin was preincubated for 2 h at 37° with and without 0.1 mg of captopril and varying doses were injected into the animal i.v. Vasopressin (0.2 - 0.5 mU/100g) raised the systemic arterial blood pressure by 20-35 mm Hg. After incubation with captopril the dose-response curve was shifted to the right and the dose of vasopressin had to be increased approximately three-fold to obtain a comparable rise in blood pressure, indicating a 60 to 70% inactivation of vasopressin (n=3).

3) The possibility that captopril may act as an activator of SH dependent enzymes was explored with papain. Papain (6 mU) cleaves benzoyl-L-arginine ethylester (BAEE) only when cysteine is added to the incubation mixture. We measured BAEE (3mM) hydrolysis in a recording UV spectrophotometer at 253 nm in a 0.05 M phosphate buffer (pH 6.2). Papain was inactive in absence of an SH agent, but when cysteine (1.3 mM) was added the enzyme cleaved BAEE at a rate of 20.9 nmol per min. At 0.013 mM cysteine concentration the rate dropped to 1.4. Captopril proved to be a more efficient activator of papain than cysteine. The rate of hydrolysis in presence of varying concentrations of captopril was as follows: 1.3 mM captopril, 22.3 nmol; 0.33 mM, 13.9 nmol; 0.013 mM, 2.8 nmol; and 0.007 mM, 1.9 nmol per min.

4) The reduction of S-S bridges in proteins by captopril was shown in disc gel electrophoresis. IgG is held together by S-S bridges; if they are cleaved, the parent protein, with a molecular weight of about 150,000, dissociates into the less soluble heavy chain with a molecular weight of 50 - 60,000, and into the light chain with a molecular weight of 22,500 (11). IgG in 7.5% acrylamide gel (pH 8.9), in the presence of sodium dodecyl sulfate and urea, completely dissociated to yield the expected subunits, after it

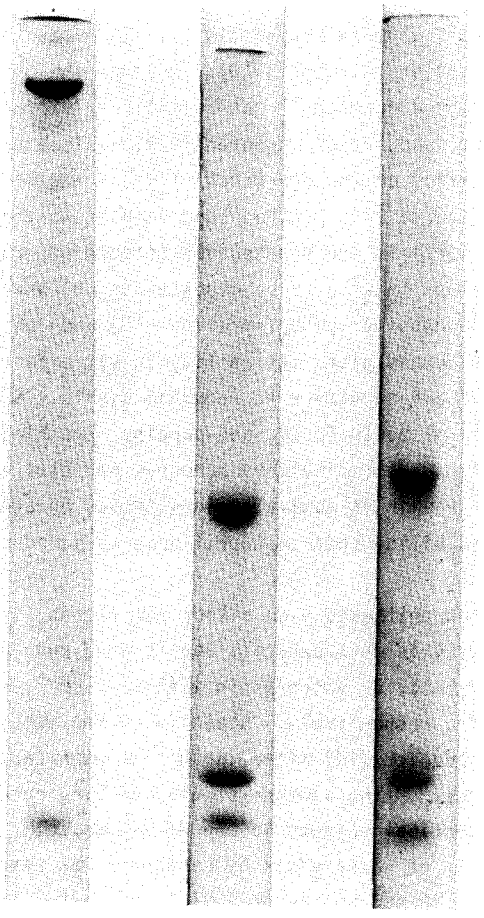

Fig. 1 Dissociation of human IgG to high and low molecular weight subunits by mercaptoethanol and by captopril in polyacrylamide SDS-urea electrophoresis. Gels from left to right: 1. Control (25 µg IgG) 2. IgG in presence of 1.3M mercaptoethanol, 3. IgG in presence of 1.3 mM captopril. Identical results were obtained in four different experiments at slightly different concentrations.

was exposed to 1.7 M mercaptoethanol. Replacing mercaptoethanol with 1.7 mM or 17 mM captopril gave identical results. IgG completely dissociated to the lower molecular weight subunits (Fig. 1).

Although captopril inhibits converting enzyme, which itself has a variety of functions such as cleaving angiotensin I, bradykinin or enkephalins (7), our experiments in vitro indicated that captopril has actions unrelated to inhibition of this enzyme. These include inactivation of oxytocin and vasopressin, dissociation of IgG into two subunits and activation of an SH dependent enzyme. In all of the experiments the results with captopril were identical with those obtained with reducing agents such as β-mercaptoethanol, dithiothreitol, or cysteine. The molar concentrations of captopril used were fairly high, but so were those of the peptide and protein substrates employed. Even if molar ratios of substrates to captopril were in the 1:100 to 1,000 range, after administration of a daily dose of 75 mg to 600 mg of captopril such ratios of endogenous proteins or peptides to captopril could very well occur at specific sites, such as the kidney.

These in vitro results may explain certain in vivo effects. The inactivation of oxytocin may interfere with parturition, as reported in rabbits (6), and inactivation of vasopressin can interfere with water metabolism. Captopril may also activate enzymes that require an SH agent. The low solubility of the heavy chain of IgG is well known (11) but in myeloma even the light chain may precipitate in the kidney. Dissociation of IgG by captopril may explain the occurrence of proteins in urine, since the lower molecular weight subunits may be filtered in the glomerulus, and a precipitation of these proteins in kidney may contribute to the immune complex glomerulopathy observed with captopril therapy (4).

Acknowledgement-The authors are grateful for the advice of Dr. D. Capra of UTHSC, Dallas, Texas.

References

1. B. A. Atkinson and J. I. S. Robertson, Lancet ii, 836 (1979).
2. H. Gavras, D. P. Faxon, J. Berkoben, H. R. Brunner and T. J. Ryan, Circulation 58, 770 (1978).
3. Editorial, Lancet ii, 129 (1980).
4. S. J. Hoorntje, J. J. Weening, H. T. Tjwan, C. G. M. Kallenberg, A. J. M. Donker, P. J. Hoedemaeker, Lancet i, 1212 (1980).
5. D. B. Case, S. A. Atlas, J. A. Mouradian, R. A. Fishman, R. L. Sherman, J. H. Laragh, JAMA 244, 346 (1980).
6. F. B. Pipkin, S. R. Turner, E. M. Symonds, Lancet i, 256 (1980).
7. E. G. Erdös, in Bradykinin, kallidin and kallikrein. Suppl. to Handbook of Experimental Pharmacol. 25, 427, Heidelberg, Springer (1979).
8. E. G. Erdös, J. R. Wohler, Biochem. Pharmacol. 12, 1193 (1963).
9. R. P. Igic, E. G. Erdös, H. S. J. Yeh, K. Sorrells, T. Nakajima, Circul. Res. 30-31, II51 (1972).
10. D. W. Cushman, H. S. Cheung, E. F. Sabo, B. Rubin, M. A. Ondetti, Fed. Proc. 38, 2778 (1979).
11. A. Nisonoff, J. Hopper, S. Spring, The Antibody Molecule, Academic Press New York p. 87, p. 241 (1975).