interconvert the left- and right-handed enantiomers. This result raises interesting possibilities concerning the existence of chiral structures for larger water clusters, such as the stable water clathrates recently studied by Castleman (*17, 18*). The interaction of such chiral water clusters with another molecule would break the left-right degeneracy and quench the interconversion tunneling, thereby yielding a complex having a well-defined handedness. In this situation, a racemic mixture of chemically equivalent right- and left-handed forms would result. It is also interesting to consider that the smallest repetitive unit of liquid water exhibits a diamond-like tetrahedral lattice (*19*). The asymmetry of the hydrogen bonding introduces the possibility of a local chirality existing in the liquid on a time scale shorter than the period of hydrogen-tunneling motions or fluctuations that rearrange the hydrogen bonding network (*20*). It seems that no previous work has considered either the existence of such transient local chiral structures in liquid water or the possible manifestations of this phenomenon in chemical dynamics.

## REFERENCES AND NOTES


1. F. Franks, *Water; A Comprehensive Treatise* (Plenum, New York, 1976).
2. R. C. Cohen and R. J. Saykally, *J. Phys. Chem.* **96**, 1024 (1992).
3. ———, *Annu. Rev. Phys. Chem.* **42**, 369 (1991).
4. J. M. Hutson, *Annu. Rev. Phys. Chem.* **41**, 123 (1990).
5. J. G. Loeser *et al.*, *J. Chem. Phys.*, in press.
6. N. Pugliano and R. J. Saykally, *ibid.* **96**, 1832 (1992).
7. For a recent review, see G. T. Fraser, *Int. Rev. Phys. Chem.* **10**, 189 (1991).
8. J. C. Owicki, L. L. Shipman, H. A. Scheraga, *J. Phys. Chem.* **79**, 1794 (1975).
9. E. Honeggar and S. Leutwyler, *J. Chem. Phys.* **88**, 2582 (1988).
10. K. Balasubramanian and T. R. Dyke, *J. Phys. Chem.* **88**, 4688 (1984).
11. G. A. Blake *et al.*, *Rev. Sci. Instrum.* **62**, 1701 (1991).
12. E. E. Haller, *Infrared Phys.* **25**, 257 (1985).
13. K. L. Busarow, thesis, University of California, Berkeley (1990).
14. P. R. Bunker, *Molecular Symmetry and Spectroscopy* (Academic Press, San Diego, CA, 1979).
15. G. Chalasinski, M. M. Szczesniak, P. Cieplak, S. Scheiner, *J. Chem. Phys.* **94**, 2873 (1991).
16. H. Saint-Martin, C. Medina-Llanos, I. Ortega-Blake, *ibid.* **93**, 6448 (1990).
17. S. Wei *et al.*, *ibid.* **94**, 3268 (1991).
18. X. Yang and A. W. Castleman, Jr., *J. Am. Chem. Soc.* **111**, 6845 (1989).
19. D. Eisenberg and W. Kauzmann, *The Structure and Properties of Water* (Oxford Univ. Press, New York, 1969).
20. F. Sciortino and S. L. Fornili, *J. Chem. Phys.* **90**, 2786 (1989).


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# Discovery of a Peptide-Based Renin Inhibitor with Oral Bioavailability and Efficacy


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**Peptidic renin inhibitors have been poorly absorbed across the intestine or rapidly eliminated by the liver and have been reported to have oral bioavailabilities of less than 2%. A peptide-based renin inhibitor, A-72517 (molecular mass of 706 daltons), was devised that has oral bioavailabilities of 8, 24, 32, and 53% in the monkey, rat, ferret, and dog, respectively. Dose-related reductions in blood pressure, plasma renin activity, and plasma angiotensin II in parallel with increased plasma drug concentrations were observed after oral administration of A-72517 to conscious, salt-depleted dogs. Thus, peptide-based molecules of sizable molecular mass can be absorbed intact into the systemic circulation of animals. These findings support the potential of peptide-based drugs for oral administration.**


Little is understood about the requirements necessary to allow a peptidic molecule to be transported intact across the intestine. The problem of devising an orally active renin inhibitor has served as a paradigm for the broader problem of designing peptide-based entities for oral administration (*1*). The physiologic role of the renin-angiotensin system (RAS) in regulating blood pressure and fluid balance has made the RAS a target for cardiovascular therapy. The wide degree of therapeutic applicability of angiotensin-converting enzyme (ACE) inhibitors in the treatment of diverse populations of hypertensives and individuals with congestive heart failure was unanticipated (*2*). However, the ability of ACE to interact with a host of substrates (for example, bradykinin, enkephalins, substance P, neurotensin, and luteinizing hormone–releasing hormone) in addition to angiotensin I (*3*) raises the possibility that the side effects associated with ACE inhibition may be unrelated to the RAS (*4*). An alternate approach to the interference of the RAS is through the direct inhibition of the enzyme renin, which catalyzes the first and rate-limiting step in the synthesis of the effector hormone angiotensin II (ANG II). Renin inhibitors offer a high degree of specificity because angiotensinogen is renin's only known natural substrate. The most successful design of renin inhibitors has been based on transition-state analogs of angiotensinogen that are peptidomimetics (*5*).


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The structure of A-72517, a transition-state analog inhibitor of renin with a dipeptide core, is shown in Fig. 1. This compound represents the second generation of dipeptide renin inhibitors discovered in our laboratory. A-72517 is a structural relative of A-64662 (enalkiren), a first-generation renin inhibitor that is intravenously efficacious and has been studied extensively in preclinical and clinical experiments and shown to lack oral bioavailability (*6*). These two compounds can be classified as peptidic renin inhibitors with comparable molecular masses of 706 and 657 daltons, respectively, and have in common a dipeptide-glycol fragment at the COOH-terminus (*7*), an alanine residue substituted with a heterocyclic moiety at the $P_2$-site, a replacement for the $P_3$-phenylalanine to impart stability to chymotrypsin degradation of the $P_2$-$P_3$ peptide bond (*8*), and a basic group at the $NH_2$-terminus to improve solubility.

However, they differ in three aspects. First, the $P_2$-site histidine and $NH_2$-terminal β-alanine residues of A-64662 are more basic than their counterparts in A-72517 and contain nitrogen-bound protons capable of forming hydrogen bonds. Consequently, A-72517 is the more lipophilic compound with a log P of 4.6 (in octanol-water, pH 7.4), as compared with a log P of 2.6 for A-64662, and the aqueous solubilities of the salts are 10 mg/ml versus 100 mg/ml, respectively. Second, the histidine and $NH_2$-terminal β-alanine residues of A-64662 are capable of forming conjugates; thus, these groups may play a role in the rapid clearance of A-64662. Finally, the $P_3$-site residue of A-72517 imparts proteolytic stability, and the sulfonamide moiety improves potency (*9*) tenfold as compared with A-64662.

To evaluate absorption from the intestine to the systemic circulation, we exposed several species to A-72517 by means of the intraduodenal (ID) or intravenous (IV) route (*10*) and obtained paired blood samples from the portal and systemic circulations. We used a renin-inhibition assay (*11*) to determine plasma drug concentrations because this method requires a small volume of plasma for the analysis and can detect concentrations on the order of 10 ng/ml (Table 1). The method is based on the inhibition of the human renin-angiotensinogen reaction of extracted plasma samples as compared to the activity of known standard concentrations of A-72517 in plasma. Plasma samples were taken at between five to nine time points during the first 2 hours of the experiments and hourly thereafter. Areas under the plasma drug concentration versus time curves (AUCs) were obtained by trapezoidal integration, and the percent bioavailability values were calculated as the dose-normalized ratio of the ID or orally administered (PO) AUC to the IV AUC. The ID bioavailability of A-72517 in the monkey was 5.1%. This low bioavailability could either reflect poor absorption from the intestine or efficient hepatic extraction. Because samples taken from the portal vein yielded drug concentrations that were significantly higher ($P < 0.05$) than the corresponding arterial concentrations, it was clear that A-72517 was well absorbed from the intestine but was subject to rapid hepatic elimination.

The situation was different in the other species examined. Both arterial plasma concentrations and bioavailability values after ID treatment in the dog, ferret, and rat were significantly higher ($P < 0.05$) than those from the monkey experiments. In contrast, overall AUC values and peak portal vein drug concentrations were indistinguishable among the four species. These results suggested that intestinal absorption was consistent across the species, whereas hepatic extraction was strongly species-dependent. Thus, contrary to dogma, dipeptide core renin inhibitors with molecular masses in the 700-dalton range are capable of being absorbed, and neither substitution of the peptide portion of the molecule nor reduction of molecular mass was necessary for intestinal transport.

Intraduodenal dosing eliminates the variable of passage through the stomach, but absorption may be affected in this protocol by anesthesia. Consequently, we administered A-72517 PO to the same four species in the conscious state (*10*) (Table 2). Under this protocol, blood samples were obtained only from the systemic circulation. The reduced number of blood samples as compared with the ID experiments allowed for larger blood volumes, sufficient for high-performance liquid chromatography (HPLC) analyses, to be withdrawn from the monkey and dog. In the monkey, bioavailability and plasma drug concentrations were similar to those observed in the ID experiments, and we assumed that the limited bioavailability was again primarily the result of hepatic extraction. There was also little deviation between the two routes of administration in the ferret, but plasma

**Fig. 1.** The chemical structure of A-72517 [(2S)-3-(4-methylpiperazin-1-yl)sulfonyl-2-(phenylmethyl)-propionyl]-*N*-[(1S,2R,3S)-1-(cyclohexylmethyl)-2,3-dihydroxy-5-methylhexyl]-L-[3-(thiazol-4-yl)alaninamide] and A-64662.

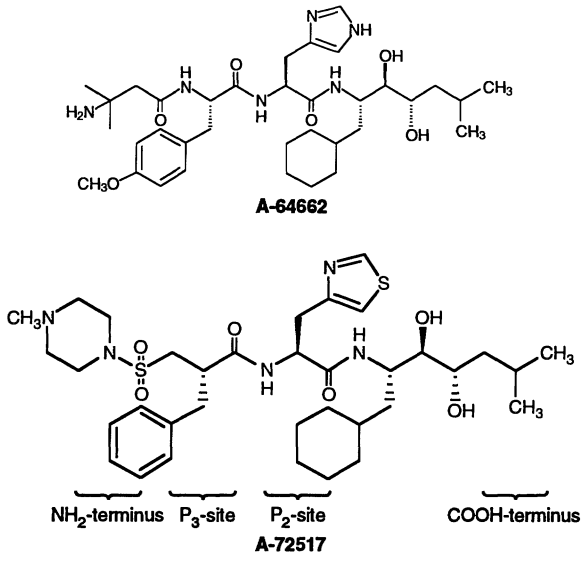


**Table 1.** Plasma drug concentrations and bioavailability of A-72517 given intraduodenally at 10 mg/kg in various species. Plasma drug concentrations are shown as mean ± SEM and were determined by a renin-inhibition assay; bioavailabilities are shown as mean ± SEM and were compared to the 1.0 mg/kg IV dose, except in the case of the ferret data, which was compared to the 0.3 mg/kg IV dose. AUCs are given in nanogram·hours per milliliter. Number of animals is indicated by *n*.

| Species | *n* | Hours | Portal | | Arterial | | Bioavailability (%) |
|---|---|---|---|---|---|---|---|
| | | | AUC | Peak (ng/ml) | AUC | Peak (ng/ml) | |
| Dog | 6 | 5 | 4700 ± 1400 | 2900 ± 700 | 2700 ± 1000 | 1000 ± 300 | 33 ± 12 |
| Ferret* | 6 | 2 | 2000 ± 600 | 1700 ± 400 | 1500 ± 600 | 940 ± 320 | 82 ± 32 |
| Monkey* | 5 | 5 | 3800 ± 1400 | 2400 ± 500 | 340 ± 140 | 130 ± 60 | 5.1 ± 2.2 |
| Rat | 5 | 2 | 1400 ± 700 | 1200 ± 600 | 500 ± 90 | 420 ± 60 | 35 ± 7 |

*Sodium depleted.

**Table 2.** Plasma drug concentrations and bioavailability of A-72517 in various species after oral exposure at 10 mg/kg in various species. Arterial drug concentrations were determined by HPLC (in the dog and monkey) or by a renin-inhibition assay (in the ferret and rat). Values for drug concentrations and bioavailabilities are shown as mean ± SEM.

| Species | *n* | Hours | Arterial | | Bioavailability (%) |
|---|---|---|---|---|---|
| | | | AUC | Peak (ng/ml) | |
| Dog | 6 | 6 | 8800 ± 1100 | 3100 ± 300 | 53 ± 8* |
| Ferret† | 5 | 5 | 1900 ± 500 | 1100 ± 200 | 32 ± 14‡ |
| Monkey | 3 | 10 | 500 ± 60 | 140 ± 10 | 8.1 ± 1.0§ |
| Rat | 4 | 6 | 270 ± 60 | 150 ± 20 | 24 ± 9‡ |

*Compared to the 10.0 mg/kg IV dose. †Sodium-depleted. ‡Compared to the 1.0 mg/kg IV dose. §Compared to the 3.0 mg/kg IV dose.

**Table 3.** In vitro inhibition by A-72517 of plasma renins of various species measured at pH 7.4.

| Species | $IC_{50}$ (nM) |
|---|---|
| Human | 1.1 |
| Monkey | 0.24 |
| Guinea pig | 9.4 |
| Hamster | 20 |
| Gerbil | 44 |
| Dog | 110 |
| Rabbit | 120 |
| Hog | 210 |
| Mouse | 210 |
| Ferret | 270 |
| Rat | 1400 |

drug concentrations and bioavailability tended to be higher in the dog and lower in the rat for the oral route.

A-72517 shows improved oral bioavailability, as compared with other peptide-based renin inhibitors. Three renin inhibitors (RO 425892, CGP 38560, and A-64662) have been tested in humans and were found to be <2% bioavailable (*12*). No other renin inhibitor has demonstrated consistent oral bioavailability based on plasma drug concentrations, even though they show oral activity through lowered blood pressure in animals (*5, 7, 13, 14*).

To examine potency, we tested A-72517 for inhibition of the endogenous renin found in the plasma of various species (*15*). A-72517 preferentially exerted its inhibitory activity against primate renin (Table 3). Traditionally, monkeys have served as efficacy models to test renin inhibitors. The combination of highly potent inhibitors of primate renin and the high degree of sensitivity of the nonhuman primate model was necessary to detect oral activity of early compounds with limited bioavailability. However, improved oral bioavailability of renin inhibitors has compensated for the lower activity against nonprimate renin, allowing alternate species such as the dog to be used as efficacy models. We estimated the oral bioavailability of A-72517 as parent drug in the dog to be 53% by HPLC (Table 2). This amount of oral bioavailability in a setting of enhanced basal activity of the RAS through robust salt-depletion offset the poor median inhibition concentration ($IC_{50}$) of 110 nM against dog plasma renin and afforded conditions conducive to demonstrate efficacy in this species, provided appropriately high doses of A-72517 were used.

Unrestrictive oral bioavailability is indicated by a consistent reproducible in vivo pharmacological performance. Figure 2 shows dose-related reductions in mean arterial pressure (MAP) affecting both systolic and diastolic pressures (*16*) in the conscious, salt-depleted dog. The changes were significantly different from baseline and vehicle control group values as indicated. The peak fall in blood pressure after 20 mg of drug per kilogram of body mass and 60 mg/kg was comparable, but the duration of the effect was prolonged at the 60 mg/kg dose. The recoveries from drug treatment were dose-dependent. No profound drops in blood pressure were noted, and the variability (SEM) of the blood pressure responses was small, as compared with those reported for other peptidic renin inhibitors (*5, 17*).

Heart rate was not affected by any dose of A-72517. Baseline values (mean ± SEM) were comparable among the groups and ranged between 106 ± 7 and 116 ± 4 beats per minute. Studies with ACE inhibitors (*18*), ANG II antagonists (*19*), and renin inhibitors collectively support the theory that inhibiting the RAS interrupts the normal, positive chronotropic reflex of the heart that is sympathetically mediated in response to episodes of hypotension (*18*).

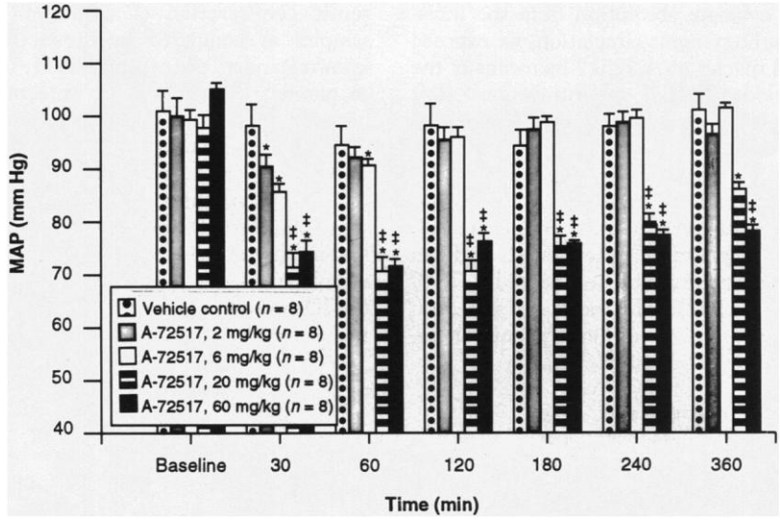


**Fig. 2.** Hemodynamic effect of A-72517. Forty male beagle dogs (8 to 12 kg) were trained to stand still for blood pressure measurements in restraining slings. Indwelling catheters were inserted into all animals under aseptic conditions 1 to 2 weeks before the experiment. During surgery, the animals were treated with 0.05 mg/kg intramuscular (IM) atropine sulfate, 10 mg/kg IM, acepromazine, and anesthesia with isoflurane. Studies were conducted after three consecutive days of treatment with a regimen consisting of a low-sodium diet (approximately 2 to 5 meq/day) and furosemide administration at a dose of 10 mg/kg IM. On the day of the experiment, each dog received one dose of A-72517·HCl (2, 6, 20, and 60 mg/kg PO, $n$ = 8 per group) dissolved in polyethylene glycol 400 or vehicle alone in a volume of 1 ml per kilogram of body mass. Each dog was used only once in this study. Blood pressure and heart rate were measured directly from the arterial catheter. MAP values (mean ± SEM) are shown. Arterial blood samples were collected from all dogs at given intervals for the determination of PRA, plasma ANG II concentrations, and plasma drug concentrations (Fig. 3). One-sample $t$ tests were used at each time point after drug administration to detect changes from baseline values in a group. Differences among groups were analyzed by one-way analysis of variance and Tukey's multiple comparison procedure. Significance was accepted at $P < 0.05$. Asterisk indicates a significant change from baseline values; double dagger indicates a significant change compared to the vehicle control group. All experimental procedures were performed after review and with the approval of the Abbott Institutional Animal Care and Use Committee.

Plasma renin activity (PRA) was determined at pH 7.4 by means of Clinical Assays Gamma coat $^{125}$I Plasma Renin Activity Radioimmunoassay (RIA) Kit (IncStar, Stillwater, Minnesota). Baseline PRA values determined for all dogs (mean ± SEM) were similar among the groups and ranged from 9.2 ± 0.8 to 13.2 ± 1.0 ng of ANG I per milliliter per hour. PRA was inhibited significantly ($P < 0.05$) in all treatment groups, as compared with baseline and vehicle control group values at all time points, starting with >95% inhibition detected in the first blood samples taken at 30 min. At 480 min after treatment, PRA was 48, 81, 89, and 98% suppressed below baseline in the 2, 6, 20, and 60 mg/kg–treated groups, respectively.

The conventional RIA we used to determine PRA is the most sensitive measure of inhibitory activity and, as in the present case, the results often do not correlate (*5, 20*) with the MAP response. The explanations of this effect are controversial and range from technical problems associated with the conventional PRA assay and the preference of some investigators to use an antibody-trapping method (*21*), to the idea that tissue, rather than plasma-related activity of renin inhibitors, may be the key site of action (*14*).

Plasma ANG II concentrations were determined as described (*22*) by a combination of HPLC and RIA procedures in randomly selected animals (three to five per group). Baseline values (mean + SEM) were 213 ± 45, 170 ± 27, 155 ± 17, and 169 ± 43 pg/ml in the 2, 6, 20, and 60 mg/kg–treated groups. At 90 min after treatment, plasma concentrations were suppressed to 107 ± 18, 92 ± 15*, 29 ± 3*, and 16 ± 2* pg/ml, respectively (asterisks indicate $P < 0.05$). At 360 min, the plasma ANG II concentrations in the 2, 6, and 20 mg/kg groups showed recovery to control, whereas 61% reduction persisted in the 60 mg/kg group. The acute suppression and

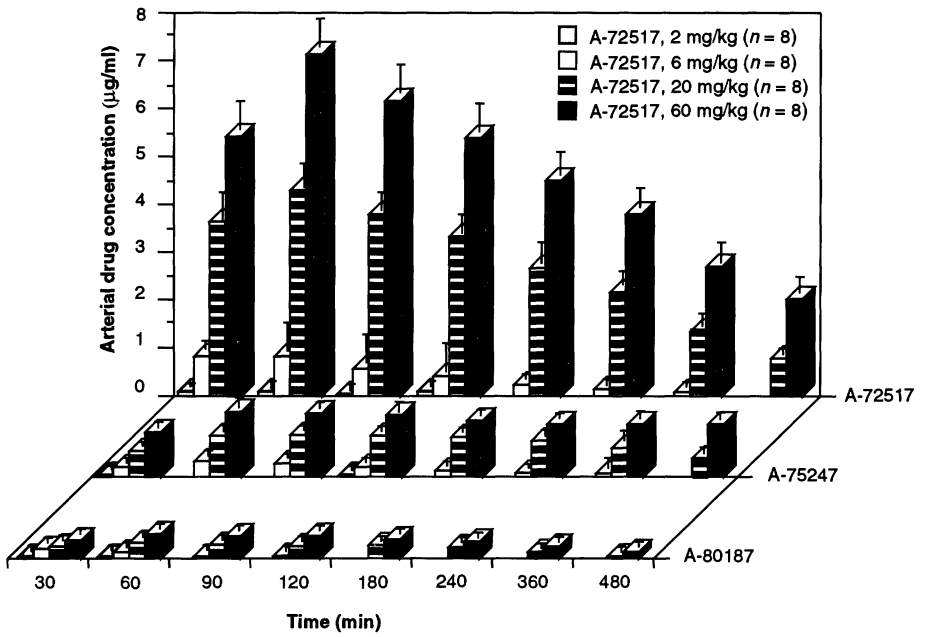


**Fig. 3.** Pharmacokinetic profile of A-72517 given orally to salt-depleted, conscious dogs. Arterial drug concentrations (mean ± SEM) of the parent compound and its two metabolites (des-methyl A-75247 and *N*-oxide A-80187) measured by HPLC are shown. Plasma was mixed with 10% $Na_2CO_3$ solution so that the final concentration of $Na_2CO_3$ was 2%. Compounds were extracted into ethyl acetate. An aliquot of the organic phase was evaporated to dryness and reconstituted in the mobile phase (40% acetonitrile in water containing 0.1% trifluoroacetic acid). An aliquot was removed and assayed by HPLC [μBondapak C18 reverse-phase column (Waters) with mobile phase at a flow rate of 1.5 ml/min and detection at 214 nm]. The sensitivity of detection for each compound was 100 ng/ml in plasma. The recovery ± SD of known concentrations added to plasma was 85 ± 16% for A-72517, 78 ± 11% for des-methyl A-75247, and 86 ± 17% for *N*-oxide A-80187.

dose-related recovery of plasma ANG II is a key marker, indicating that A-72517 is functioning by inhibiting the effector hormone of the RAS. The reduction or prevention of elevated baseline plasma ANG II concentrations associated with renin inhibition may be a therapeutic advantage, as compared to non-RAS–related drugs in treating hypertensive individuals because it has been suggested that high PRA or plasma ANG II may be associated with morbid cardiovascular events (*23*).

The pharmacokinetic and pharmacodynamic profiles were followed simultaneously in this study. A-72517 circulates primarily as the parent drug in the dog (Fig. 3). The metabolic fate of this drug was in agreement with predictions from the literature based on the reactivity of the *N*-methyl piperazine moiety (*24*). Two metabolites, the des-methyl A-75247 ($IC_{50}$ = 1.7 and 180 nM in human and dog plasma, respectively) and the *N*-oxide A-80187 ($IC_{50}$ = 2.4 and 180 nM in human and dog plasma, respectively) are formed from the $NH_2$-terminal methyl piperazine and have been identified in dog plasma but are considered to be of minor importance, contributing little to the pharmacological activity of A-72517. Based on AUC values, the metabolites constituted only 30% of circulating active renin inhibitor (Fig. 3). In general, the peak values and integrated AUCs for plasma drug concentrations were dose-dependent. However, our data do not define the details of the relation between circulating drug concentrations and pharmacodynamic responses.

We found that improved systemic availability of renin inhibitors can be achieved in a peptide-based series represented by A-72517. A-72517 is under clinical evaluation and data suggest that this compound is absorbed into the systemic circulation of human subjects. Definitive studies assessing oral bioavailability and antihypertensive activity of A-72517 are under way. Basic science research continues in an effort to learn more about the principles governing intestinal transport and hepatic uptake of peptide-based substances.

## REFERENCES AND NOTES


1. J. J. Plattner and D. W. Norbeck, in *Drug Discovery Technologies*, C. R. Clark and W. H. Moos, Eds. (Ellis Horwood, Chichester, United Kingdom, 1990), chap. 5.
2. H. R. Brunner *et al.*, *Ann. Intern. Med.* **90**, 19 (1979); R. J. Cody, *Am. J. Cardiol.* **55**, 36 (1985).
3. E. G. Erdos, *Hypertension* **16**, 363 (1990).
4. E. Slater *et al.*, *J. Am. Med. Assoc.* **260**, 967 (1988).
5. M. Szelke *et al.*, *Nature* **299**, 555 (1982); J. Boger *et al.*, *ibid.* **303**, 81 (1983); H. D. Kleinert, W. R. Baker, H. H. Stein, *Adv. Pharmacol.* **22**, 207 (1991).
6. H. D. Kleinert *et al.*, *Cardiovasc. Drug Rev.* **8**, 203 (1990); H. N. Glassman *et al.*, *J. Cardiovasc. Pharmacol.* **16** (suppl. 4), 76 (1990); G. W. Neuberg *et al.*, *Am. J. Cardiol.* **67**, 63 (1991).
7. J. R. Luly *et al.*, *J. Med. Chem.* **31**, 2264 (1988).
8. J. J. Plattner *et al.*, *ibid.*, p. 2277; I. Schechter and A. Berger, *Biochem. Biophys. Res. Commun.* **27**, 157 (1967).
9. P. Bühlmayer *et al.*, *J. Med. Chem.* **31**, 1839 (1988).
10. Plasma drug concentrations were quantitated after IV and ID dosing in fasted animals. Rats and ferrets [anesthetized with 100 to 200 mg/kg of Inactin (Abbott Laboratories Abbott Park, IL) intraperitoneally] and monkeys (sedated with 10 mg/kg ketamine hydrochloride) and dogs (anesthetized with 15 or 30 kg/mg, IV, of sodium pentobarbital) underwent catheterization of a peripheral artery, peripheral vein (IV only), and portal vein. Blood samples were collected simultaneously at given intervals from the portal vein and the peripheral artery for the estimation of plasma drug concentrations. Similarly, PO and IV dosing was conducted in conscious animals, but only peripheral blood samples were obtained. These protocols were approved by the Abbott Laboratories Animal Care and Use Committee.
11. S. H. Rosenberg *et al.*, *J. Med. Chem.* **32**, 1371 (1989).
12. E. Camenzind *et al.*, *J. Cardiovasc. Pharmacol.* **18**, 299 (1991); M. deGasparo *et al.*, *Br. J. Clin. Pharmacol.* **27**, 587 (1989); J. Cavanaugh *et al.*, *J. Clin. Pharmacol.* **29**, 861 (1989).
13. R. L. Panek *et al.*, *Clin. Exp. Hypertens. Theory Pract.* **A13**, 1395 (1991); D. T. Pals *et al.*, *Hypertension* **8**, 1105 (1986); B. D. Rush *et al.*, *Int. J. Pharmaceutics* **73**, 231 (1991); J. M. Wood *et al.*, *J. Cardiovasc. Pharmacol.* **14**, 221 (1989); F. Cumin *et al.*, *Drug Metab. Dispos.* **18**, 831 (1990).
14. W. Fischli *et al.*, *Hypertension* **18**, 22 (1991).
15. Studies were performed in EDTA-treated plasma at pH 7.4. The final incubation mixture (100 μl) contained Hepes buffer (0.100 M at pH 7.4), blood fraction (40 μl), EDTA (3.8 mM, except for the monkey reaction mixture, which had 3 mM), phenylmethyl sulfonyl fluoride (1.4 mM), dimethyl sulfoxide (1%), and 8-hydroxyquinoline (1.3 mM, except for the human reaction mixture, where none was present). At least three different concentrations of compound were added, and the solutions were incubated at 37°C for 1 to 4 hours, depending on the species. At the end of the incubation, the samples were placed in ice and an aliquot assayed for angiotensin I by means of a commercial kit (DuPont Biotechnology Systems). The percent inhibition of the reaction was determined, and the $IC_{50}$ calculated by regression analysis.
16. H. D. Kleinert *et al.*, unpublished data.
17. L. W. Schaffer *et al.*, *J. Hypertens.* **8**, 251 (1990); H. D. Kleinert *et al.*, *FEBS Lett.* **230**, 38 (1988).
18. B. Waeber, J. Nussberger, H. R. Brunner, in *Hypertension: Pathophysiology, Diagnosis and Management*, J. H. Laragh and B. M. Brenner, Eds. (Raven, New York, 1990), pp. 2209–2232; G. C. Scroop and R. D. Lowe, *Nature* **220**, 1331 (1968).
19. P. C. Wong *et al.*, *J. Pharmacol. Exp. Ther.* **252**, 719 (1990).
20. E. H. Blaine, T. W. Schorn, J. Boger, *Hypertension* **6** (suppl. I), I-111 (1984).
21. X. Jeunematre *et al.*, *Am. J. Hypertens.* **2**, 819 (1989).
22. K. M. Verburg *et al.*, *Hypertension* **13**, 262 (1989).
23. H. R. Brunner *et al.*, *N. Engl. J. Med.* **286**, 441 (1972); M. H. Alderman *et al.*, *ibid.* **324**, 1098 (1991).
24. J. Weissman *et al.*, *J. Med. Chem.* **28**, 997 (1985).


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