Proc. Natl. Acad. Sci. USA
Vol. 88, pp. 4210–4214, May 1991
Physiology/Pharmacology

Mixed inhibitors of angiotensin-converting enzyme (EC 3.4.15.1) and enkephalinase (EC 3.4.24.11): Rational design, properties, and potential cardiovascular applications of glycopril and alatriopril

(atrial natriuretic factor/diuresis/natriuresis/hypertension)

CLAUDE GROS*, NADINE NOËL*, ANNY SOUQUE*, JEAN-CHARLES SCHWARTZ*†, DENIS DANVY‡, JEAN-CHRISTOPHE PLAQUEVENT‡, LUCETTE DUHAMEL‡, PIERRE DUHAMEL‡, JEANNE-MARIE LECOMTE§, AND JEAN BRALET¶

*Unité de Neurobiologie et Pharmacologie, Institut National de la Santé et de la Recherche Médicale, Centre Paul Broca, 2ter rue d'Alésia, 75014 Paris, France; ‡Laboratoire de Chimie Organique, Unité de Recherches Associée DO 464 Centre National de la Recherche Scientifique, Université de Rouen, Mont Saint Aignan, France; §Laboratoire Bioprojet, Paris, France; and ¶Laboratoire de Pharmacodynamie, Faculté de Pharmacie, Dijon, France

Communicated by E. E. Baulieu January 30, 1991 (received for review July 18, 1990)

ABSTRACT **Angiotensin-converting enzyme (ACE) and enkephalinase, two cell surface metallopeptidases, are responsible for angiotensin II formation and atrial natriuretic factor (ANF) degradation, respectively, and thereby play a critical role in the metabolism of hormonal peptides exerting essentially opposite actions in cardiovascular regulations. To affect simultaneously both hormonal systems by a single molecular structure, we have designed glycoprilat and alatrioprilat {(*S*)-*N*-[3-(3,4-methylenedioxyphenyl)-2-(mercaptomethyl)-1-oxopropyl]glycine and -alanine, respectively}. *In vitro* the two compounds inhibit both ACE and enkephalinase activities with similar, nanomolar potencies, and *in vivo*, glycopril and alatriopril, the corresponding diester prodrugs, occupy the two enzyme molecules in lung at similar low dosages (0.2–0.5 mg/kg of body weight, *per os*). The high potency of these compounds is attributable to interaction of the methylenedioxy group with the S_1 subsite of ACE and of the aromatic ring with the S_1' subsite of enkephalinase. In rodents, low doses of these mixed inhibitors exert typical actions of ACE inhibitors—i.e., prevention of angiotensin I-induced hypertension—as well as of enkephalinase inhibitors—i.e., protection from ^{125}I-ANF degradation or enhancement of diuresis and natriuresis following acute extracellular volume expansion. In view of the known counterbalanced physiological actions of the two hormonal peptides, whose metabolism is controlled by ACE and enkephalinase, mixed inhibitors of the two peptidases show promise for the treatment of various cardiovascular and salt-retention disorders.**

Two major hormonal peptide systems appear to play opposite roles in the regulation of electrolyte balance and blood pressure, so that their imbalance might be responsible for cardiovascular and salt-retention disorders affecting a large fraction of the population (1). The first one is the renin–angiotensin–aldosterone system, whose critical role in sodium retention and vasoconstriction was largely evidenced through the use of inhibitors of the membrane metallopeptidase angiotensin-converting enzyme (ACE; EC 3.4.15.1) (2, 3). These drugs, which prevent the formation of angiotensin II, a vasoactive, antidiuretic, and antinatriuretic peptide, and decrease blood pressure and aldosterone secretion, have gained wide application in the treatment of essential hypertension and congestive heart failure. The second hormonal system is constituted by the atrial natriuretic factor (ANF), a peptide secreted by the heart into the circulation to decrease blood pressure, raise the urinary excretion of water and sodium, and lower plasma renin and aldosterone levels (4). Recently, the role of another metallopeptidase, enkephalinase (EC 3.4.24.11, membrane metalloendopeptidase), in the inactivation of endogenous ANF was shown by the effects of inhibitors (reviewed in ref. 5). These drugs enhance the circulating level of the hormone in healthy volunteers as well as in patients with congestive heart failure or cirrhosis; induce diuresis, natriuresis, and urinary excretion of cGMP (6–10); and may exert antihypertensive activity (11).

Hence it appears that two cell surface, zinc-containing peptidases play a key role in the activation or inactivation pathways of the two hormonal systems. Although many compounds are highly selective inhibitors of either ACE or enkephalinase, some mercaptoalkyl inhibitors display limited but significant crossreactivity toward the two peptidases (12, 13). This partial overlap suggested that a careful analysis of structure–activity relationships would enable the design of a single molecular structure able to potently inhibit both enzymes *in vivo* and, thereby, lead to a class of potentially useful cardiovascular agents blocking both the generation of angiotensin II and the inactivation of ANF.

We have now designed such mixed inhibitors which interact with the two peptidases at nanomolar concentrations *in vitro* and at low dosage *per os*. These drugs elicit the characteristic actions of the two classes of enzyme inhibitors: prevention of angiotensin I-induced hypertension, protection of ANF, enhancement of diuresis and natriuresis, and increase in urinary cGMP excretion.

MATERIALS AND METHODS

Metallopeptidase Inhibitors. The preparation, purification, and optical resolution of compounds will be described elsewhere. See Table 1 for structures.

(*S*)-*N*-[3-(3,4-Methylenedioxyphenyl)-2-(acetylthio)methyl-1-oxopropyl]glycine benzyl ester, glycopril, 76% yield, $[\alpha]_D^{25}$ −15.8° (*c* 1.2 MeOH), m.p. 92°C.

(*S*)-*N*-[3-(3,4-Methylenedioxyphenyl)-2-(acetylthio)methyl-1-oxopropyl]-(*S*)-alanine benzyl ester, alatriopril, 77% yield, $[\alpha]_D^{25}$ −50.6° (*c* 1.3, MeOH), m.p. 104°C.

(*S*)-*N*-[3-(3,4-Methylenedioxyphenyl)-2-(mercaptomethyl)-1-oxopropyl]glycine, glycoprilat, 87% yield, $[\alpha]_D^{25}$ +54.4° (*c* 1.0, MeOH), m.p. 88°C.

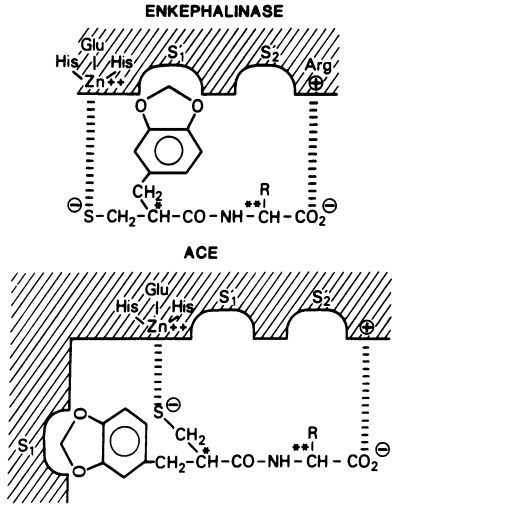
(*S*)-*N*-[3-(3,4-Methylenedioxyphenyl)-2-(mercaptomethyl)-1-oxopropyl]-(*S*)-alanine, alatrioprilat, 81% yield, $[\alpha]_D^{25}$ +12.9° (*c* 1.3, MeOH).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: ACE, angiotensin-converting enzyme; ANF, atrial natriuretic factor; rANF, rat ANF; hANF, human ANF; TCA, trichloroacetic acid.

†To whom reprint requests should be addressed.

Table 1. Glycoprilat and alatrioprilat, two mixed ACE/enkephalinase inhibitors: Structures, *in vitro* potencies, and modes of interaction with the two metallopeptidases



Compound	Configuration (*, **)	R	K_i, nM Enkephalinase	K_i, nM ACE
I (Glycoprilat)	*S*	H	5.6 ± 1.0	6.5 ± 1.3
II	*R*	H	12.0 ± 2.0	420 ± 100
III (Alatrioprilat)	*S,S*	CH_3	5.1 ± 0.8	9.8 ± 1.3
IV	*R,S*	CH_3	13.7 ± 0.9	215 ± 30

Spectral data (^1H NMR, ^{13}C NMR, IR) and microanalysis were in agreement with the proposed structures.

Preparation of ^{125}I-ANF and ^{125}I-MK351A. Human and rat ANF-(99–126) (hANF and rANF) from Bachem were iodinated using $Na^{125}I$ (2000 Ci/mmol, Amersham; 1 Ci = 37 GBq) and purified as described (8). The lisinopril derivative MK351A (14), a generous gift from Merck, was iodinated by the same method and purified by HPLC on a C_{18} μBondapak column in 10 mM ammonium acetate, pH 4.2/20% (vol/vol) acetonitrile. ^{125}I-MK351A was eluted with a linear gradient of 20–50% acetonitrile for 30 min at a rate of 1 ml/min with a retention time of 14 min.

Assay of Enkephalinase and ACE Activities. Enkephalinase activity of 1 ng of the recombinant human enzyme (15), a gift of B. Malfroy (Genentech), was evaluated with 25 μM succinyl-Ala-Ala-Phe-amidomethylcoumarin as the substrate (16). ACE activity from human kidney membranes was evaluated using 0.2 mM *o*-aminobenzoylglycyl-*p*-nitrophenylalanylproline as the substrate (17).

***In Vivo* Binding of [^{3}H]Acetorphan.** *In vivo* labeling of enkephalinase in mouse lung membranes was evaluated as described (18).

***In Vivo* Binding of ^{125}I-MK351A.** *In vivo* labeling of ACE in mouse lung membranes was evaluated 5 min after i.v. administration of 0.25 μCi of ^{125}I-MK351A. Lungs were homogenized in 4 ml of cold 50 mM Tris·HCl buffer, pH 7.0/75 mM NaCl with an Ultra Turrax (Kinematica, Littau, Switzerland), and an aliquot of the homogenate was immediately filtered over GF/B filters (Whatman). Membranes were washed three times with 3 ml of buffer and bound radioactivity was counted by γ-spectrometry. Specific binding, defined as that inhibited by oral administration of captopril (10 mg/kg of body weight), typically represented 43 ± 6 dpm/μl over a nonspecific binding of 1.7 ± 0.3 dpm/μl.

***In Vivo* Binding of ^{125}I-ANF.** *In vivo* labeling of ANF receptors in mouse lung membranes (19) was evaluated 5 min after i.v. administration of 0.5 μCi of ^{125}I-rANF. Lungs were homogenized in 4 ml of cold 50 mM Tris·HCl buffer (pH 7.4) containing 1% (wt/vol) bovine serum albumin and the homogenate was filtered over GF/B filters. Specifically bound radioactivity was defined as that inhibited by coadministration of nonradioactive rANF-(99–126) (150 μg/kg) and typically represented 2.5 ± 0.4 dpm/μl over a nonspecific binding of 0.6 ± 0.1 dpm/μl.

Recovery of ^{125}I-ANF Radioactivity in Kidney. Recovery of the intact hormone in kidney of mice receiving 0.5 μCi of ^{125}I-hANF-(99–126) i.v., 2 min before sacrifice, was evaluated by a trichloroacetic acid (TCA) precipitation test previously validated by HPLC analysis of the radioactivity (6, 20).

Angiotensin I-Induced Pressor Response. Male Wistar rats (300 g) anesthetized with pentobarbital (60 mg/kg, i.p.) were vagotomized and treated with mecamylamine (1.5 mg/kg, i.v.). The pressor response to angiotensin I (150 ng/kg, i.v.) was evaluated as described (21). The mean increase in pressure at the carotid artery level was initially evaluated after two injections of angiotensin I (given 10 min apart) and, then, 10 min after i.v. administration of inhibitors. The inhibition of pressor response elicited by these agents was expressed as a percentage of the mean initial response.

Urinary Excretion of Water, Na^+, K^+, and cGMP After Volume Expansion. Wistar rats (350 g) were anesthetized with pentobarbital (60 mg/kg, i.p.) and catheters were inserted into the bladder for urine collection, the femoral vein for infusions, and the carotid artery for blood pressure measurement. The rats were infused with a Ringer solution (120 mM NaCl/25 mM $NaHCO_3$/2.5 mM KCl/0.75 mM $CaCl_2$, warmed to 37°C) at a rate of 0.5 ml/hr per 100 g of body weight (22). After 30 min, a volume load was imposed by infusion of Ringer solution at 1 ml/min per 100 g for 2 min, followed by infusion at 0.5 ml/hr per 100 g for the duration of the experiment. Alatrioprilat (10 mg/kg) or saline was given i.v., 5 min before the load. Urine was collected every 5 min, the volume of fractions was measured, Na^+ and K^+ were assayed by flame photometry, and cGMP was radioimmunoassayed (Biosys, Compiègne, France).

RESULTS

Inhibition of Enkephalinase and ACE Activities. After a 15-min preincubation in the presence of recombinant enkephalinase, the hydrolysis of the model substrate was completely inhibited by glycoprilat, alatrioprilat, and their stereoisomers (compounds II and IV) with IC_{50} values of 6.7 ± 1.2, 6.1 ± 1.0, 14.3 ± 2.4, and 16.3 ± 1.1 nM, respectively (seven concentrations were tested). The hydrolysis of the model substrate by ACE from human kidney membranes (9.5 nmol/min per mg of protein) was inhibited by the same compounds with IC_{50} values of 13.0 ± 2.6, 19.6 ± 2.6, 840 ± 200, and 430 ± 60 nM, respectively. Corresponding K_i values are reported in Table 1.

Inhibition of *in Vivo* Binding of [^{3}H]Acetorphan and ^{125}I-MK351A. In control mice receiving [^{3}H]acetorphan the radioactivity bound to lung membranes was 45,000 ± 4000 dpm per animal. Oral administration of alatriopril or glycopril in increasing doses, 30 min before killing, progressively inhibited total binding by a maximum of 90% (Fig. 1*A*). In mice receiving ^{125}I-MK351A, the same compounds inhibited the specific binding of radioactivity to lung membranes (200,000 ± 20,000 dpm in controls) by a maximum of 75% and 85% for glycopril and alatriopril, respectively (Fig. 1*B*). Alatriopril was equipotent to glycopril as regards enkephalinase occupancy but twice as potent as regards ACE occupancy (Table 2).

Protection of ^{125}I-ANF Against Degradation *in Vivo*. Five minutes after i.v. injection of ^{125}I-rANF the total lung radioactivity of control mice was 19,200 ± 1000 dpm and the membrane-bound radioactivity was 10,500 ± 1000 dpm (means ± SEM of 14 experiments). Oral administration of

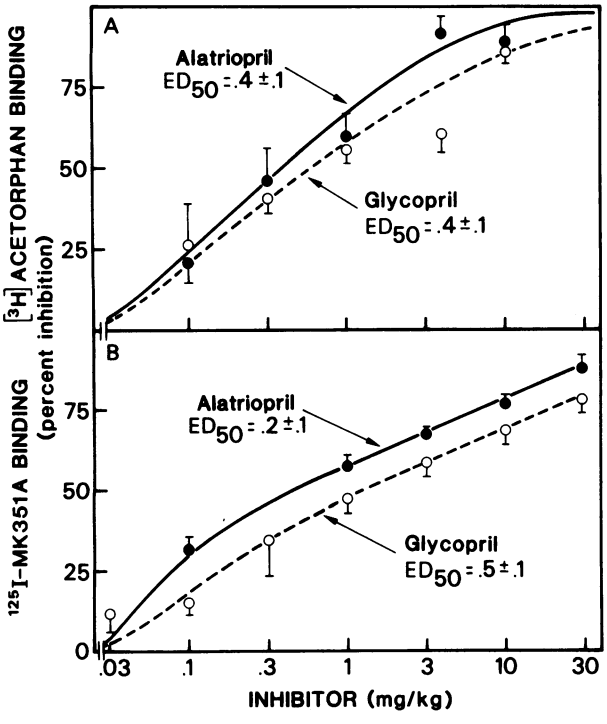


FIG. 1. Dose–response curves for the occupation of lung enkephalinase (*A*) and ACE (*B*) in mice treated with alatriopril (●) or glycopril (○). In both tests the peptidase inhibitors were administered orally 30 min before mice were killed. Occupation of enkephalinase and ACE was studied by evaluating the inhibition of specific binding of radioactivity to lung membranes 30 min after administration of [^{3}H]acetorphan (2.5 μCi, i.v.) or 5 min after administration of ^{125}I-MK351A (0.25 μCi, i.v.), respectively. Data are means ± SEM of 4–6 values.

glycopril or alatriopril in increasing doses, 30 min before killing, gradually enhanced binding (Fig. 2) by a maximum of 174 ± 24% and 194 ± 42%, respectively, and total radioactivity by a maximum of 84 ± 21% and 88 ± 41%, respectively. In mice receiving rANF in increasing dose together with ^{125}I-rANF, the binding to lung membranes was progressively inhibited by a maximum of 94% with an ID_{50} of 5.5 ± 1.0 μg/kg; binding was also inhibited by 96% in mice receiving SC 46,542 (1.5 mg/kg) (23), a gift of G. M. Olins (data not shown).

Protection of the natriuretic peptide was also assessed by evaluating the recovery of TCA-precipitable radioactivity from kidney 2 min after injection of ^{125}I-hANF (110,000 ± 4000 dpm in controls). Oral administration of glycopril or alatriopril, 30 min before killing, enhanced TCA-precipitable radioactivity by a maximum of 390% and 420%, respectively, with ED_{50} values reported in Table 2. Protection was also

Table 2. Potencies of glycopril and alatriopril in various tests reflecting enkephalinase or ACE inhibition *in vivo*

Test	ED_{50} or ID_{50}, mg/kg	
	Glycopril	Alatriopril
Enkephalinase occupation (mouse lung)	0.41 ± 0.15	0.42 ± 0.08
ACE occupation (mouse lung)	0.47 ± 0.09	0.19 ± 0.04
^{125}I-ANF protection (mouse kidney)	0.73 ± 0.09	0.23 ± 0.06
^{125}I-ANF protection (mouse lung)	3.7 ± 1.0	2.5 ± 1.2
Angiotensin I pressor response (rat)*	1.7 ± 0.2	0.9 ± 0.2

Values correspond to determinations performed 30 min after oral administration of glycopril or alatriopril.
*Glycoprilat and alatrioprilat were administered i.v. 5 min before angiotensin I.

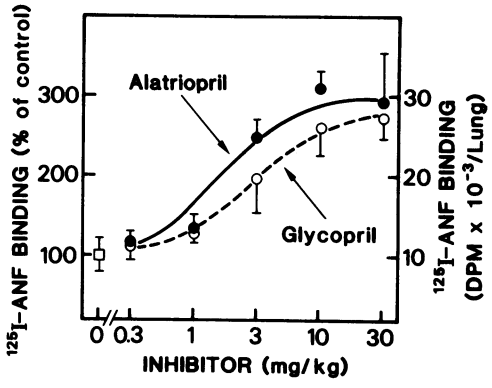


FIG. 2. Dose–response curves for the enhancement of ^{125}I-ANF binding to lung membranes in mice treated with alatriopril or glycopril. The specific binding of radioactivity to lung membranes was evaluated 30 min after oral administration of the peptidase inhibitors and 5 min after injection of ^{125}I-rANF (0.5 μCi, i.v.). Data are means ± SEM of 4–14 values.

confirmed by HPLC analysis (6) of the kidney radioactivity (data not shown).

Inhibition of Pressor Response to Angiotensin I. In control rats, i.v. infusion of angiotensin I at 150 ng/kg raised the mean arterial blood pressure by about 50 mmHg (1 mmHg = 133 Pa). Administration of alatrioprilat or glycoprilat in increasing doses i.v., 10 min before the test, progressively inhibited this pressor response by a maximum of 100% (Fig. 3), with ID_{50} values reported in Table 2.

Enhancement of Urinary Excretion of Water, Na^+, and cGMP After Volume Expansion. Baseline arterial pressure (120–130 mmHg) was not significantly modified at any time during the experimental period either in controls or in alatrioprilat-treated rats.

The urine excretion over the 30-min period following the volume load was increased by 54% (818 ± 66 μl instead of 530 ± 33 μl; $P < 0.001$) in rats receiving alatrioprilat (10 mg/kg, i.v.) 5 min before the load (data not shown). In the same animals alatrioprilat enhanced Na^+ excretion during the same period by 118% (152 ± 17 μmol instead of 70 ± 8 μmol; $P < 0.001$) (Fig. 4). Meanwhile, kaliuresis was not significantly enhanced by the inhibitor (117 ± 7 instead of 97 ± 8 μmol in 30 min). Hence, the Na^+/K^+ ratio was significantly ($P < 0.001$) enhanced by alatrioprilat: this ratio was 0.28 ± 0.07 before the load and became 1.58 ± 0.18 and 1.15 ± 0.11, respectively, 15 and 30 min after the load in alatrioprilat-treated rats, compared with 0.75 ± 0.12 and 0.42 ± 0.05 in

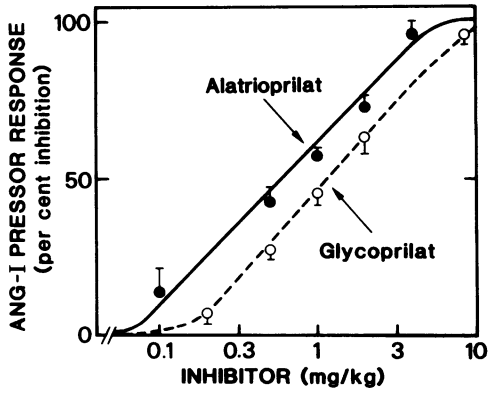


FIG. 3. Dose–response curves for inhibition of the angiotensin I (ANG-I)-induced hypertension in rats treated with alatrioprilat or glycoprilat. Data are means ± SEM of 5 values at each dose.

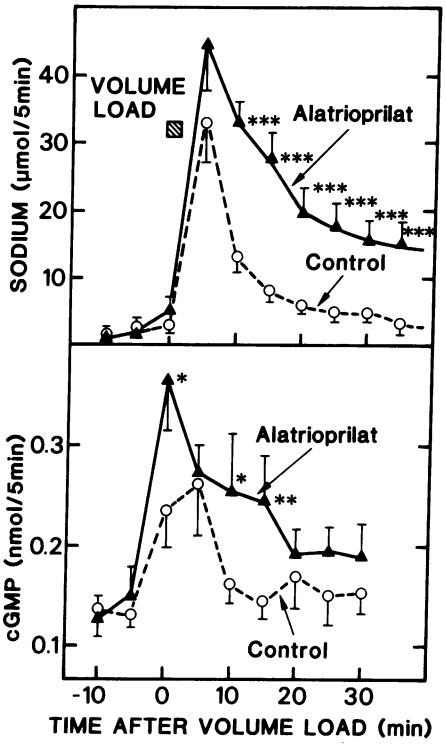


FIG. 4. Effects of alatrioprilat on urinary excretion of Na^+ and cGMP in rats subjected to volume expansion. Anesthetized rats received alatrioprilat (10 mg/kg, i.v.) or saline (control) 5 min before the i.v. infusion over 2 min of 2 ml of Ringer solution per 100 g of body weight. Urine fractions were collected every 5 min via an intravesical catheter. Data are means ± SEM of 10 values. Significance (two-tailed Student's *t* test): *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

controls (data not shown). Urinary excretion of cGMP was also significantly more enhanced after alatrioprilat than in controls (Fig. 3), representing 1.5 ± 0.2 instead of 1.1 ± 0.1 nmol in 30 min ($P < 0.05$).

DISCUSSION

A step-by-step modification of the structure of thiorphan (24) allowed us to design glycoprilat and alatrioprilat, both of which display nanomolar inhibitory potency toward enkephalinase and ACE.

Although ACE (25) and enkephalinase (15, 26, 27) display little overall amino acid sequence homology, they presumably share the same catalytic mechanism and their substrate specificities overlap somewhat, since they hydrolyze some peptides, such as enkephalins, bradykinin, or substance P, at the same amide bonds (28, 29). In addition, most inhibitors of the two peptidases incorporate essentially similar features—i.e., a zinc-chelating group borne by a modified dipeptide with a free C-terminal carboxylate interacting with a positively charged, presumably guanidinium residue (30, 31), identified as Arg^{95} in rabbit enkephalinase (32). However, the optimal dipeptide sequences binding to the S_1' and S_2' subsites (nomenclature according to ref. 33) differ in the two peptidases. For instance a P_1' aromatic residue is highly preferred in the case of enkephalinase but only tolerated in the case of ACE, and an unsubstituted CO-NH function is essential for binding to enkephalinase but not to ACE, most inhibitors of which have a C-terminal proline. As a consequence of these main features several compounds show crossreactivity *in vitro* (12, 13), and even some "selective" mercapto inhibitors of one peptidase display some inhibitory activity toward the other; e.g., the selectivity ratio is ≈100 for the enkephalinase inhibitor thiorphan (24) and ≈1000 for the ACE inhibitor captopril (34).

However, similarly high *in vivo* potency toward the two enzymes is obviously required for therapeutic applications of mixed inhibitors, their effective dosage being determined in the end by their affinity for the less readily inhibited enzyme.

The observation that the S_1' hydrophobic pocket of enkephalinase tolerates the phenyl ring of thiorphan in its two spatial orientations (16, 35, 36) suggested that this pocket was large enough to accommodate groups more bulky than a phenyl ring. By contrast, the high stereochemical preference of (*S*)-thiorphan for ACE suggested that the phenyl ring could interact differently with a subsite of this enzyme and that this putative interaction could be optimized by substitution of the ring. This hypothesis was verified with the synthesis of glycoprilat, in which the methylenedioxy substitution provides a 10-fold increase in affinity for ACE without significant loss in affinity for enkephalinase. Either glycoprilat or alatrioprilat, its methylated analogue, inhibits both enkephalinase and ACE at similar concentrations, in the nanomolar range, but with low and high stereospecificity ratios, respectively (Table 1). After analysis of molecular models (unpublished work), we propose that, in a discrete configuration of these compounds, their methylenedioxy substituent binds to the S_1 subsite of ACE in the same way as the phenethyl group of enalapril. Indeed, in the enalapril series of ACE inhibitors, the high affinity of compounds is attributable to the presence of *S*-configuration aromatic, aralkyl, or aliphatic groups able to bind to this S_1 subsite (4).

In vivo, glycopril and alatriopril, the corresponding diester prodrugs, showed high oral activity, with occupation of lung ACE (14) and enkephalinase (18) occurring at ED_{50} values of 0.2–0.5 mg/kg. Whereas alatrioprilat was less potent than glycoprilat as an ACE inhibitor *in vitro*, the converse situation, presumably due to differences in pharmacokinetics, was found for the corresponding prodrugs *in vivo*.

Interestingly, the mixed inhibitors displayed a combination of biological activities due to inhibition of either enkephalinase or ACE alone. For instance alatriopril enhanced the recovery of intact ^{125}I-hANF in kidney, a major target organ for the hormone, by about 5-fold—i.e., to the same extent as selective enkephalinase inhibitors—and with an ED_{50} lower than that of acetorphan (8). Protection of exogenous ANF after oral administration of alatriopril or glycopril was also evidenced by another test, i.e., by the enhanced binding of ^{125}I-rANF to lung membranes. This binding presumably occurs to the "clearance receptors" (37), since it was prevented by low doses of not only rANF but also an ANF analogue recognized by those receptors but inactive at biological receptors with a guanylate cyclase domain (23). In fact, both tests presumably reflect the enhanced half-life of ^{125}I-ANF in plasma of mice treated with enkephalinase inhibitors (38).

In turn, protection of endogenous ANF presumably accounts for the increased urinary excretion of water, Na^+, and cGMP in rats treated with alatrioprilat and subjected to extracellular volume expansion: this stimulus enhances ANF secretion (6, 7) and ANF antibodies, used as antagonists of the hormone, completely prevent these renal actions of enkephalinase inhibitors (39).

Finally, at doses in the low mg/kg range, alatrioprilat or glycoprilat completely prevented the hypertensive action of angiotensin I in rats, a typical effect of selective ACE inhibitors (3, 4), with ID_{50} values in the low mg/kg range.

Hence, our data suggest that mixed inhibitors trigger together the biological responses characteristic of each class of peptidase inhibitor. Coinhibition of the two peptidases may result in several potentially useful therapeutic effects that mainly reflect the counterbalance, at various physiological levels (1, 4), of the two functionally opposite hormonal sys-

tems these enzymes control. (*i*) Vascular smooth muscle and aldosterone secretion are affected in opposite manners by angiotensin II and ANF, so that inhibition of their formation and degradation, respectively, may have synergistic consequences. (*ii*) ANF (or enkephalinase inhibitors) reduce plasma renin activity (9, 10), an effect that may facilitate the prevention of angiotensin II formation through ACE inhibition, particularly during chronic treatments in which renin activity rises compensatorily (3, 4). (*iii*) Mixed inhibitors may facilitate the diuretic and natriuretic actions of intrarenal bradykinin, a peptide whose inactivation seems to depend on both ACE and enkephalinase (40). (*iv*) In view of their diuretic–natriuretic activity (without kaliuresis), via protection of endogenous ANF, mixed inhibitors may be useful in the treatment of essential hypertension: in this condition, various diuretics potentiate the effects of ACE inhibitors (3, 4).

Nevertheless, although ACE inhibitors did not blunt the biological responses to exogenous ANF in some studies (41, 42), this was not the case in others (43, 44), and coinhibition of the two peptidases may potentially lead to side effects. Hence additional, particularly clinical, studies are obviously required to assess the therapeutic utility of this class of drugs.

We thank C. Mossiat and C. Bouvier for excellent technical assistance.

1. Laragh, J. M. (1985) *N. Engl. J. Med.* **313,** 1330–1340.
2. Erdös, E. G. (1976) *Am. J. Med.* **60,** 749–759.
3. Wyvratt, M. J. & Patchett, A. A. (1985) *Med. Res. Rev.* **5,** 483–531.
4. Brenner, B. M., Ballermann, B. J., Gunning, M. E. & Zeidel, M. L. (1990) *Physiol. Rev.* **70,** 665–699.
5. Schwartz, J. C., Gros, C., Lecomte, J. M. & Bralet, J. (1990) *Life Sci.* **47,** 1279–1297.
6. Gros, C., Souque, A., Schwartz, J. C., Duchier, J., Cournot, A., Baumer, P. & Lecomte, J. M. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 7580–7584.
7. Northridge, D. B., Alabaster, C. T., Connell, C. T., Dilly, S. G., Lever, A. F., Jardine, A. G., Barclay, P. L., Dargie, H. J., Findlay, I. N. & Samuels, G. M. R. (1989) *Lancet* **ii,** 591–593.
8. Kahn, J. C., Patey, M., Dubois-Rande, J. L., Merlet, P., Castaigne, A., Lim-Alexandre, C., Duboc, D., Gros, C. & Schwartz, J. C. (1990) *Lancet* **335,** 118–119.
9. Lecomte, J. M., Baumer, P., Lim, C., Duchier, J., Cournot, A., Dussaule, J. C., Ardaillou, R., Gros, C., Chaignon, B., Souque, A. & Schwartz, J. C. (1990) *Eur. J. Pharmacol.* **179,** 65–73.
10. Dussaule, J. C., Granger, J. D., Wolff, J. P., Lecomte, J. M., Gros, C., Schwartz, J. C., Bodin, G. & Ardaillou, R. (1991) *J. Clin. Endocrinol. Metab.*, in press.
11. Lefrançois, P., Clerc, G., Duchier, J., Lim, C., Lecomte, J. M., Gros, C. & Schwartz, J. C. (1990) *Lancet* **336,** 307.
12. Gordon, E. M., Cushman, D. W., Tung, R., Cheung, H. S., Wang, F. L. & Delaney, N. G. (1983) *Life Sci.* **33,** Suppl. 1, 113–116.
13. Fournié-Zaluski, M. C., Lucas, E., Waksman, G. & Roques, B. P. (1984) *Eur. J. Biochem.* **139,** 267–274.
14. Chai, S. Y., Allen, A. M., Adam, W. R. & Mendelsohn, F. A. O. (1986) *J. Cardiovasc. Pharmacol.* **8,** Suppl. 10, 535–539.
15. Malfroy, B., Kuang, W. S., Seeburg, P. H., Mason, A. J. & Schofield, P. R. (1988) *FEBS Lett.* **229,** 206–210.
16. Giros, B., Gros, C., Schwartz, J. C., Danvy, D., Plaquevent, J. C., Duhamel, L., Duhamel, P., Vlaiculescu, A., Costentin, J. & Lecomte, J. M. (1987) *J. Pharmacol. Exp. Ther.* **243,** 666–673.
17. Carmel, A. & Yaron, A. (1978) *Eur. J. Biochem.* **87,** 265–273.
18. De la Baume, S., Brion, F., Tuong, M. D. T. & Schwartz, J. C. (1988) *J. Pharmacol. Exp. Ther.* **247,** 653–660.
19. Ou, L. C., Yen, S., Sardella, G. L. & Hill, N. S. (1989) *J. Appl. Physiol.* **67,** 1612–1616.
20. Gros, C., Souque, A. & Schwartz, J. C. (1990) *Eur. J. Pharmacol.* **179,** 45–56.
21. Laubie, M., Schiavi, P., Vincent, M. & Schmitt, H. (1984) *J. Cardiovasc. Pharmacol.* **6,** 1076–1082.
22. Hansell, P., Anden, N. E., Grabowska-Anden, M. & Ulfendahl, H. R. (1988) *Acta Physiol. Scand.* **134,** 421–428.
23. Koepke, J. P., Tyler, L. D., Trapani, A. J., Bovy, P. R., Spear, K. L., Olins, G. M. & Blaine, E. M. (1989) *J. Pharmacol. Exp. Ther.* **249,** 172–176.
24. Roques, B. P., Fournié-Zaluski, M. C., Soroca, E., Lecomte, J. M., Malfroy, B., Llorens-Cortes, C. & Schwartz, J. C. (1980) *Nature (London)* **288,** 286–288.
25. Soubrier, F., Alhenc-Gelas, F., Hubert, C., Allegrini, J., John, M., Tregear, G. & Corvol, P. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 9386–9390.
26. Malfroy, B., Schofield, P. R., Kuang, W., Seeburg, P., Mason, A. J. & Henzel, W. J. (1987) *Biochem. Biophys. Res. Commun.* **144,** 59–66.
27. Devault, A., Nault, C., Zollinger, M., Fournié-Zaluski, M. C., Roques, B. P., Crine, P. & Boileau, G. (1988) *J. Biol. Chem.* **263,** 4033–4040.
28. Erdös, E. G. & Skidgel, R. A. (1989) *FASEB J.* **3,** 145–151.
29. Schwartz, J. C. (1989) in *Design of Enzyme Inhibitors as Drugs*, Sandler, M. & Smith, H. J. (Oxford Univ. Press, New York), pp. 206–220.
30. Malfroy, B. & Schwartz, J. C. (1982) *Biochem. Biophys. Res. Commun.* **106,** 276–285.
31. Bunning, P., Holmquist, B. & Riordan, J. F. (1978) *Biochem. Biophys. Res. Commun.* **83,** 1442–1449.
32. Bateman, R. C., Jackson, D., Slaugher, C. A., Unnithan, S., Chai, Y. G., Moomaw, C. & Hersh, L. B. (1989) *J. Biol. Chem.* **264,** 6151–6157.
33. Schechter, I. & Berger, A. (1967) *Biochem. Biophys. Res. Commun.* **27,** 157–162.
34. Swerts, J. P., Perdrisot, R., Patey, G., De la Baume, S. & Schwartz, J. C. (1979) *Eur. J. Pharmacol.* **57,** 279–281.
35. Mendelsohn, L. G., Johnson, B. G., Scott, W. L. & Frederickson, R. C. A. (1985) *J. Pharmacol. Exp. Ther.* **234,** 386–390.
36. Fournié-Zaluski, M. C., Lucas-Soroca, E., Devin, J. & Roques, B. P. (1986) *J. Med. Chem.* **29,** 751–757.
37. Maack, T., Suzuki, M., Almeida, F. A., Nussenzveig, D., Scarborough, R. M., McEnroe, G. A. & Lewicki, J. A. (1987) *Science* **238,** 675–678.
38. Gros, C., Souque, A. & Schwartz, J. C. (1990) *Neuropeptides* **17,** 1–5.
39. Bralet, J., Mossiat, C., Lecomte, J. M., Charpentier, S., Gros, C. & Schwartz, J. C. (1990) *Eur. J. Pharmacol.* **179,** 57–64.
40. Ura, N., Carretero, O. A. & Erdös, E. G. (1987) *Kidney Int.* **32,** 507–513.
41. Hansell, P. & Ulfendahl, H. R. (1987) *Acta Physiol. Scand.* **130,** 393–399.
42. Richards, A. M., Rao, G., Espiner, E. A. & Yandle, T. (1989) *Hypertension* **13,** 193–199.
43. Hirata, Y., Ishii, M., Sugimoto, T., Matsuoka, M., Ishimitsu, T., Atarashi, K., Sugimoto, T., Miyata, A., Kangawa, K. & Matsuo, H. (1987) *Clin. Sci.* **72,** 165–170.
44. Wambach, G., Schittenhelm, U., Bönner, G. & Kaufmann, W. (1989) *Cardiology* **76,** 418–427.