# ACE REVISITED: A NEW TARGET FOR STRUCTURE-BASED DRUG DESIGN

*K. Ravi Acharya*[*], *Edward D. Sturrock*[‡], *James F. Riordan*[§] *and Mario R. W. Ehlers*[||]

Current-generation angiotensin-converting enzyme (ACE) inhibitors are widely used for cardiovascular diseases, including high blood pressure, heart failure, heart attack and kidney failure, and have combined annual sales in excess of US $6 billion. However, the use of these ACE inhibitors, which were developed in the late 1970s and early 1980s, is hampered by common side effects. Moreover, we now know that ACE actually consists of two parts (called the N- and C-domains) that have different functions. Therefore, the design of specific domain-selective ACE inhibitors is expected to produce next-generation drugs that might be safer and more effective. Here we discuss the structural features of current inhibitors and outline how next-generation ACE inhibitors could be designed by using the three-dimensional molecular structure of human testis ACE. The ACE structure provides a unique opportunity for rational drug design, based on a combination of *in silico* modelling using existing inhibitors as scaffolds and iterative lead optimization to drive the synthetic chemistry.

*[*]Department of Biology and Biochemistry, University of Bath, Claverton Down, Bath BA2 7AY, UK.*
*[‡]Division of Medical Biochemistry and MRC/UCT Liver Research Centre, University of Cape Town, Observatory 7925, South Africa.*
*[§]Center for Biochemical and Biophysical Sciences and Medicine, Harvard Medical School, One Kendall Square, Cambridge, Massachusetts 02139, USA.*
*[||]Pacific Biometrics Inc., 220 W. Harrison Street, Seattle, Washington 98119, USA.*
*Correspondence to K.R.A.*
*e-mail: K.R.Acharya@bath.ac.uk.*
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Recent advances in molecular biology, high-throughput crystallization techniques, high-energy synchrotron sources, combinatorial and fragment-based chemistries, and bioinformatics have had a dramatic effect on the drug discovery process, and have provided a new impetus to undertake 'structure-based drug discovery'[1,2]. At present, there is enormous interest in identifying and validating 'druggable' targets in the human proteome and applying structure-based drug design to the development of novel therapeutics[1].

Angiotensin-converting enzyme (ACE) is a major target for cardiovascular therapies and ACE inhibitors have been on the market for more than 20 years. ACE is a central component of the renin–angiotensin system (RAS), which controls not only blood pressure and fluid and electrolyte homeostasis, but also renal and vascular function and myocardial remodelling[3]. As such, inhibitors of the RAS have found widespread application in cardiovascular disease, and current ACE inhibitors have a broad range of licensed indications, ranging from mild hypertension to post-myocardial infarction[4].

Although very successful, the design of current-generation ACE inhibitors was the result of serendipity combined with brilliant insights, and was achieved without knowledge of the sequence or three-dimensional structure of the enzyme. Only after the introduction of ACE inhibitors did it become evident that somatic ACE is a complex two-domain enzyme, comprising an N- and a C-domain, each containing an active site with similar but distinct substrate specificities and chloride-activation requirements[3]. Therefore, if domain-selective inhibitors can be designed, this could produce next-generation drugs with altered safety and efficacy profiles.

The recent breakthrough in determining the high-resolution crystal structure of human testis ACE (tACE) has offered new details at the molecular level[5]. The structure of tACE provides an opportunity for the design of N- and C-domain-selective inhibitors by structure-guided drug design. This would be an interesting example of revisiting an 'old' drug target that is well established in the clinic by applying modern drug discovery tools. Successful examples of this strategy are the selective cyclooxygenase (COX)-2 inhibitors (for example, rofecoxib), the selective peripheral histamine $H_1$-receptor antagonists (for example, loratadine), and the selective aldosterone receptor antagonist (eplerenone), all of which are 're-engineered' drugs active against well-understood targets[6–8].

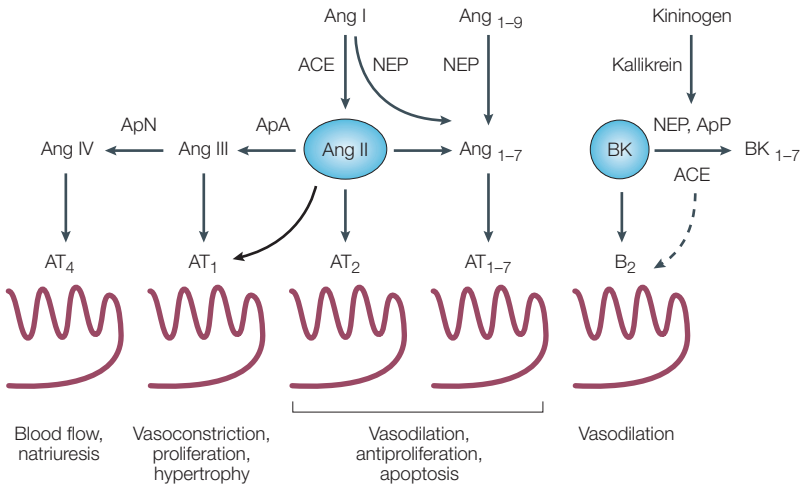


Figure 1 | **Metabolism of angiotensin peptides and bradykinin by ACE and other vasopeptidases.** Also shown are the principal angiotensin (Ang) and bradykinin (BK) receptors and their downstream effects. The broken arrow from angiotensin-converting enzyme (ACE) to $B_2$ BK receptor denotes evidence for crosstalk between the membrane-bound proteins. The conversion of Ang II to Ang $_{1-7}$ can be mediated by ACE2; see text and REFS 3,37. Ap, aminopeptidase; NEP, neutral endopeptidase.

Here, we provide an overview of ACE and the RAS, current ACE inhibitors and their clinical utility, insights from the tACE crystal structure, and the rationale and prospects for developing second-generation, domain-selective inhibitors by structure-guided design.

## ACE, the RAS and blood pressure regulation

The recognition that an elevated basal blood pressure can lead to a shortened life expectancy and higher morbidity (due to cardiovascular complications such as kidney failure, heart failure and stroke) only evolved during the first half of the twentieth century, when numerous clinical surveys demonstrated a normal distribution of blood pressures and a 10–20% prevalence of hypertension in an apparently healthy population[9]. Hypertension occurs in millions of people worldwide, the large majority of whom are unaware of their condition; a recent report indicated that prevalence rates are even higher than previously suspected, at 28% in North America and 44% in Europe[10]. Many long-term studies were required before the increased morbidity and mortality associated with hypertension became accepted and it was classified as a disease in need of treatment[9]. The underlying cause of hypertension in most cases was, and still is, unknown. Therapy was initially directed at reducing blood volume by the use of DIURETICS, SYMPATHETIC TONE by the use of adrenergic-blocking agents, or VASCULAR RESISTANCE by the use of vasodilators.

A major breakthrough in our understanding of blood pressure regulation came with the discovery of the RAS and ACE (reviewed in REF. 11). On the basis of a suspected relationship between kidney and blood pressure, Goldblatt, Houssay, Page and others identified the renal enzyme renin and the pressor substance angiotensin in the 1930s and 1940s[12–14]. In the mid-1950s, Skeggs and co-workers purified angiotensin and found that it existed in two forms: a decapeptide called angiotensin I (Ang I) and an octapeptide called angiotensin II (Ang II)[15]. Two years later a chloride-dependent, metalloenzyme that could convert Ang I to Ang II was purified from horse plasma[16], and was later referred to as ACE.

## Properties of ACE

ACE, also known as peptidyl-dipeptidase A (EC 3.4.15.1), belongs to the M2 family of the MA clan (a protein clan contains all the modern-day polypeptides that have arisen from a single evolutionary progenitor) of zinc metallopeptidases[17]. It is a dipeptidyl carboxypeptidase that catalyses the hydrolytic cleavage of dipeptides from the carboxyl terminus of a wide variety of oligopeptides *in vitro*. Its best known function is the *in vivo* conversion of Ang I (DRVYIHPFHL), which circulates in plasma, into the potent vasopressor Ang II by removal of the C-terminal His–Leu (FIG. 1). Ang I is generated primarily by the renin-catalysed hydrolysis of the Leu10–Val11 peptide bond of angiotensinogen, a liver-derived 55-kDa plasma protein. ACE also affects blood pressure by cleaving bradykinin (BK, RPPGFSPFR), thereby abolishing its vasodilating activity (because of this, ACE is sometimes referred to as kininase II (kininase I is carboxypeptidase N)). BK is generated from a kininogen precursor by the action of plasma kallikrein (a serine proteinase) in a process analogous to that of Ang I formation (FIG. 1).

Human ACE is a monomeric zinc metalloenzyme that is synthesized as a 1,306-amino-acid polypeptide, is processed to a 1,277-residue mature form, is heavily glycosylated (30% by weight), and is localized to the plasma membrane of endothelial and absorptive epithelial and neuroepithelial cells[18]. A sequence of 22 hydrophobic amino acids located near the carboxyl terminus of the protein serves as a transmembrane domain that anchors ACE to the cell surface. This creates a 28-residue cytosolic domain and a 1,227-residue glycosylated extracellular domain. Its cellular orientation defines ACE as a type I transmembrane ectoprotein and, in the case of endothelial cells, positions it optimally for interaction with its circulating substrates. The amino acid sequence of human endothelial ACE provides clear evidence for a gene duplication event in its evolutionary history. It consists of an N-terminal domain of about 612 amino acids, a 15-residue interdomain sequence and a 650-residue C-terminal domain. A 357-amino-acid segment of the N-domain has more than 60% sequence identity to the corresponding segment of the C-domain (FIG. 2).

Each domain contains a five-residue sequence of amino acids, HEMGH, which is characteristic of catalytic zinc sites found in a large family of neutral endoproteinases. Detailed kinetic and mutational analyses have established that both of the zinc sites have catalytic activity[19,20]. The physiological consequence of such tandem active sites in an enzyme is unknown. Some differences in catalytic properties have been observed for these two sites: the N-domain site is notably 50-times more active toward the haemoregulatory peptide *N*-acetyl-Ser-Asp-Lys-Pro (AcSDKP)[21], 1000-times

DIURETICS
Drugs that increase the net renal excretion of solute and water.

SYMPATHETIC TONE
The neural activity of the sympathetic nervous system, regulating (through adrenergic receptors) cardiac and vascular function.

VASCULAR RESISTANCE
The resistance to blood flow, which is directly proportional to the extent of vasoconstriction, is one of the primary determinants of blood pressure.

more sensitive to inhibition by the phosphinic peptide RXP407 (REF. 22), and more than 3000-times less sensitive to inhibition by RXPA380 (REF. 23) (TABLE 1). In addition, the activity of the C-domain is highly dependent on chloride concentration, whereas that of the N-domain is much less so[19,24]. However, given the relative constancy of plasma chloride concentration, the significance of this chloride dependency is not obvious.

Human endothelial ACE, also known as somatic ACE, is encoded by a single gene that consists of 26 exons,

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| sACE | N-domain | 1 | .....LD | PGLQPGNFSA | DEAGAQLFAQ | SYNSSAEQVL | FQSVAASWAH |
| sACE | C-domain | 602 | **PLPD** | **NYPEGIDLVT** | DEAEASKFVE | EYDRTSQVVW | NEYAEANWNY |
| tACE | | 20 | TTTHQATAHQ | TSAQSPNLVT | DEAEASKFVE | EYDRTSQVVW | NEYAEANWNY |
| sACE | N-domain | 43 | DTNITAENAR | RQEEAALLSQ | EFAEAWGQKA | KELYEPIWQN | FTDPQLRRII |
| sACE | C-domain | 646 | NTNITTETSK | ILLQKNMQIA | NHTLKYGTQA | RKFDVNQLQN | TT...IKRII |
| tACE | | 70 | NTNITTETSK | ILLQKNMQIA | NHTLKYGTQA | RKFDVNQLQN | TT...IKRII |
| sACE | N-domain | 93 | GAVRTLGSAN | LPLAKRQQYN | ALLSNMSRIY | STAKVCLPNK | TATCWSLDPD |
| sACE | C-domain | 693 | KKVQDLERAA | LPAQELEEYN | KILLDMETTY | SVATVCHPNG | ..SCLQLEPD |
| tACE | | 117 | KKVQDLERAA | LPAQELEEYN | KILLDMETTY | SVATVCHPNG | ..SCLQLEPD |
| sACE | N-domain | 143 | LTNILASSRS | YAMLLFAWEG | WHNAAGIPLK | PLYEDFTALS | NEAYKQDGFT |
| sACE | C-domain | 741 | LTNVMATSRK | YEDLLWAWEG | WRDKAGRAIL | QFYPKYVELI | NQAARLNGYV |
| tACE | | 165 | LTNVMATSRK | YEDLLWAWEG | WRDKAGRAIL | QFYPKYVELI | NQAARLNGYV |
| sACE | N-domain | 193 | DTGAYWRSWY | NSPTFEDDLE | HLYQQLEPLY | LNLHAFVRRA | LHRRYGDRYI |
| sACE | C-domain | 791 | DAGDSWRSMY | ETPSLEQDLE | RLFQELQPLY | LNLHAYVRRA | LHRHYGAQHI |
| tACE | | 215 | DAGDSWRSMY | ETPSLEQDLE | RLFQELQPLY | LNLHAYVRRA | LHRHYGAQHI |
| sACE | N-domain | 243 | NLRGPIPAHL | LGDMWAQSWE | NIYDMVVPFP | DKPNLDVTST | MLQQGWNATH |
| sACE | C-domain | 841 | NLEGPIPAHL | LGNMWAQTWS | NIYDLVVPFP | SAPSMDTTEA | MLKQGWTPRR |
| tACE | | 265 | NLEGPIPAHL | LGNMWAQTWS | NIYDLVVPFP | SAPSMDTTEA | MLKQGWTPRR |
| sACE | N-domain | 293 | MFRVAEEFFT | SLELSPMPPE | FWEGSMLEKP | ADGREVVCHA | SAWDFYNRKD |
| sACE | C-domain | 891 | MFKEADDFFT | SLGLLPVPPE | FWNKSMLEKP | TDGREVVCHA | SAWDFYNGKD |
| tACE | | 315 | MFKEADDFFT | SLGLLPVPPE | FWNKSMLEKP | TDGREVVCHA | SAWDFYNGKD |
| sACE | N-domain | 343 | FRIKQCTRVT | MDQLSTVHHE | MGHIQYYLQY | KDLPVSLRRG | ANPGFHEAIG |
| sACE | C-domain | 941 | FRIKQCTTVN | LEDLVVAHHE | MGHIQYFMQY | KDLPVALREG | ANPGFHEAIG |
| tACE | | 365 | FRIKQCTTVN | LEDLVVAHHE | MGHIQYFMQY | KDLPVALREG | ANPGFHEAIG |
| sACE | N-domain | 393 | DVLALSVSTP | EHLHKIGLLD | RVTNDTESDI | NYLLKMALEK | IAFLPFGYLV |
| sACE | C-domain | 991 | DVLALSVSTP | KHLHSLNLLS | SEGGSDEHDI | NFLMKMALDK | IAFIPFSYLV |
| tACE | | 415 | DVLALSVSTP | KHLHSLNLLS | SEGGSDEHDI | NFLMKMALDK | IAFIPFSYLV |
| sACE | N-domain | 443 | DQWRWGVFSG | RTPPSRYNFD | WWYLRTKYQG | ICPPVTRNET | HFDAGAKFHV |
| sACE | C-domain | 1041 | DQWRWRVFDG | SITKENYNQE | WWSLRLKYQG | LCPPVPRTQG | DFDPGAKFHI |
| tACE | | 465 | DQWRWRVFDG | SITKENYNQE | WWSLRLKYQG | LCPPVPRTQG | DFDPGAKFHI |
| sACE | N-domain | 493 | PNVTPYIRYF | VSFVLQFQFH | EALCKEAGYE | GPLHQCDIYR | STKAGAKLRK |
| sACE | C-domain | 1091 | PSSVPYIRYF | VSFIIQFQFH | EALCQAAGHT | GPLHKCDIYQ | SKEAGQRLAT |
| tACE | | 515 | PSSVPYIRYF | VSFIIQFQFH | EALCQAAGHT | GPLHKCDIYQ | SKEAGQRLAT |
| sACE | N-domain | 543 | VLQAGSSRPW | QEVLKDMVGL | DALDAQPLLK | YFQPVTQWLQ | EQNQQNGEVL |
| sACE | C-domain | 1141 | AMKLGFSRPW | PEAMQLITGQ | PNMSASAMLS | YFKPLLDWLR | TENELHGEKL |
| tACE | | 565 | AMKLGFSRPW | PEAMQLITGQ | PNMSASAMLS | YFKPLLDWLR | TENELHGEKL |
| sACE | N-domain | 593 | GWPEYQWHP. | .......... | .......... | .......... | .......... |
| sACE | C-domain | 1191 | GWPQYNWTPN | SARSEGPLPD | SGRVSFLGLD | LDAQQARVGQ | WLLLFLGIAL |
| tACE | | 615 | GWPQYNWTPN | SARSEGPLPD | SGRVSFLGLD | LDAQQARVGQ | WLLLFLGIAL |
| sACE | N-domain | | .......... | .......... | .......... | ....... | |
| sACE | C-domain | 1241 | LVATLGLSQR | LFSIRHRSLH | RHSHGPQFGS | EVELRHS | |
| tACE | | 665 | LVATLGLSQR | LFSIRHRSLH | RHSHGPQFGS | EVELRHS | |

Figure 2 | **Protein sequences of testis ACE and the N- and C-domains of somatic ACE.** The sequences are aligned and numbered according to REFS 18,26. The bridge region is in bold and the zinc-binding motif, as well as the third zinc ligand (Glu), are in purple. The C-domain N-glycosylation sites are in yellow, the chloride ligands in orange, and the active site residues depicted in FIG. 4 are in blue and green. The secondary structure elements for tACE[5] structure α-helices (α), β-strands (β) and $3_{10}$ helices are: α1(40–71); α2(74–100); H1(101–107); α3(109–120); H2(122–127); α4(128–149); β1(150–153); β2(157–161); α5(163–172); α6(174–211); α7(215–222); H3(223–225); α8(228–260); β3(270–272); H4(283–285); α9(286–291); α10(300–308); α11(311–326); α12(332–339); β4(355–359); β5(364–368); α13(374–394); H5(398–402); α14(406–430); α15(439–473); α16(480–494); β6(495–496); H6(506–511); α17(520–541); H7(546–550); α18(555–568); α19(573–583); α20(589–610). sACE, somatic angiotensin-converting enzyme; tACE, testis angiotensin-converting enzyme.

Table 1 | **N- and C-terminal selectivity of known (current) ACE inhibitors**

| Inhibitor | Inhibitor residues* $P_2$ $P_1$ $P_1'$ $P_2'$ | Domain inhibition (nM)‡ N-domain | C-domain |
|---|---|---|---|
| BPPa | -Gln-Ile-Pro-Pro | 100.0 | 1.0 |
| BPP-10c | -Gln-Ile-Pro-Pro | 200.0 | 0.5 |
| BPPb | -Lys-Ile-Pro-Pro | 10,000.0 | 30.0 |
| BPPc | -Pro-Ile-Pro-Pro | 80.0 | 80.0 |
| BPP-12b | -Pro-Ile-Pro-Pro | 5.0 | 150.0 |
| Ang $_{1-7}$ | -Tyr-Ile-His-Pro | 3,400.0 | 130.0 |
| Comp. II | Ac-Asp-Phe-Ala-Ala | 2.0 | 7.0 |
| RXP407 | Ac-Asp-Phe-Ala-Ala-$NH_2$ | 2.0 | 2,500.0 |
| Comp. IV | Ac-Ala-Phe-Ala-Ala-$NH_2$ | 5.0 | 200.0 |
| RXPA380 | Phe-Phe-Pro-Trp | 10,000.0 | 3.0 |
| Captopril | Ala-Pro | 8.9 | 14.0 |
| Enalaprilat | Phe-Ala-Pro | 26.0 | 6.3 |
| Lisinopril | Phe-Lys-Pro | 44.0 | 2.4 |
| Keto-ACE | Bnz-Phe-Gly-Pro | 1,500.0 | 40.0 |
| Trandolapril | Phe-Ala-Trp | 3.1 | 0.3 |

*C-terminal amino-acid residues of peptide inhibitors (BPP peptides and Ang $_{1-7}$), or amino-acid analogues in the phosphinic peptides (RXP407, Compounds II and IV, and RXPA380) and small-molecule angiotesin-converting enzyme (ACE) inhibitors (captopril, enalaprilat, lisinopril, keto-ACE and trandolapril). ‡Determined as $K_i$ in every case except keto-ACE ($IC_{50}$), at 150 mM NaCl (keto-ACE and Ang $_{1-7}$ (REF. 96)), 200 mM NaCl (BPP and phosphinic peptides[22,23,101]) or 300 mM NaCl (small-molecule inhibitors[24]).

all but one of which, exon 13, is transcribed into the corresponding messenger RNA[25]. A form of ACE found only in adult testis (tACE) is encoded by the same gene but its mRNA begins before exon 13 and continues through exon 26. It is transcribed by the interaction of a promoter with a site present in intron 12 that is active only in adult male germinal cells[25]. Translation of this mRNA results in a 701-amino-acid version of ACE that, except for the first 36 residues, is identical to the C-terminal domain of somatic ACE[26]. Testis or germinal ACE is found in developing sperm cells and mature sperm. Sperm lacking ACE are deficient in transport within the oviduct and in binding to the zonae pellucidae, and male $ACE^{-/-}$ mice have markedly diminished fertility[27].

A polymorphism involving a 287-base-pair insertion corresponding to an *Alu* repetitive sequence has been found in intron 16 of the human ACE gene[28]. The insertion is all or none: in one study the (I)/(D) frequency ratio was 44:56. The deletion (D) allele of the ACE gene lacks the sequence that is present in the insertion (I) allele. The D allele is associated with a higher plasma and tissue ACE activity. The DD genotype is positively correlated with plasma ACE activity and numerous attempts have been made to search for associations between the DD genotype and specific cardiovascular diseases, but the conclusions remain controversial[29]. The association of allele I with athletic performance is equally controversial. Some studies have shown that this polymorphism is associated with endurance performance, whereas others have shown the D allele to be associated with that of elite short-distance athletes[30,31].

ACE is released from the endothelial cell surface by the action of a so-called ACE-secretase, or sheddase, that cleaves the Arg1203–Ser1204 peptide bond near the transmembrane region[32,33]. Soluble ACE is a minor component of total ACE activity, and its physiological function is unknown. Activators of protein kinase C stimulate the release of ACE from endothelial and other cells in culture, and markedly elevated concentrations of plasma ACE are observed in certain diseases, particularly sarcoidosis.

Besides its role in blood pressure regulation, ACE also has been postulated to participate in 'local' or 'tissue' RASs. The Ang II arising from such systems is thought to act as a paracrine growth factor[4]. This activity has been implicated in the development of left ventricular dysfunction that can occur after a major heart attack, and seems to account for the beneficial effects of ACE inhibitor therapy in such situations.

## ACE homologues

The structural and functional conservation of the gene encoding ACE are indications of its widespread evolutionary importance as a key metallopeptidase involved in the metabolism of regulatory peptides[34]. The *Drosophila melanogaster* genome contains six genes (*Acer*, *Ance*, *Ance-2*, *Ance-3*, *Ance-4* and *Ance-5*) that encode ACE-like proteins. Two of these, angiotensin-converting enzyme (Ance) and angiotensin-converting enzyme-related (Acer), are enzymatically active and share 36% sequence identity with human ACE[35]. Ance displays properties very similar to those of the human C-domain, whereas Acer is inhibited by the N-domain-selective inhibitor RXP407 (REF. 36).

ACE2 (also known as ACEH) is an ACE homologue found in humans and rodents that functions as a carboxypeptidase with a preference for C-terminal hydrophobic or basic residues (reviewed in REFS 3,37). It is expressed mainly in the heart, kidney and testis and is important for the regulation of blood pressure and cardiac function. Interestingly, ACE2 is not inhibited by ACE inhibitors such as lisinopril, captopril and enalaprilat.

## Crystal structure of tACE

One of the main impediments to determining the structure of ACE by X-ray crystallography was the production of diffraction-quality crystals. The C-domain of ACE has seven potential N-linked glycosylation sites, six of which are located on the surface of the protein and the seventh in the juxtamembrane region. Two concurrent approaches succeeded in paving the way for the crystallization[38] and successful X-ray structure determination of tACE[5]. Five of the N-linked sites were disrupted by substituting glutamines for each of the asparagine residues in the glycosylation sequences and a truncated form was expressed in the presence of a glucosidase inhibitor yielding crystals suitable for X-ray diffraction. The latter protein was used for the subsequent three-dimensional structure determination of tACE. High-resolution crystal structures of the human tACE and its complex with the widely used inhibitor lisinopril at 2.0 Å resolution were recently reported[5].

*Drosophila* ACE (Ance) contains a single domain similar to tACE. However, it has only three potential N-linked glycosylation sites, which are not required for secretion and enzymatic activity[39]. Recently, the crystal structures of this homologue (which has considerable similarity to the tACE structure), bound to captopril and lisinopril, were reported[40]. The wild-type protein was expressed in a baculovirus expression system and the oligosaccharides did not hamper crystallization. However, it is unlikely that this will be the case with the ACE2 homologue, the ACE N-domain or the full-length somatic ACE structures — ACE structural milestones that are still eagerly awaited.

Prominent features of the tACE structure include an abundance of α-helices and a deep central cavity that divides the molecule into two halves, which pack together into an overall ellipsoid shape (FIG. 3). The active site, identified by the catalytic $Zn^{2+}$ ion bound to the HEXXH sequence (and to lisinopril in its complex with the enzyme), is located in the deep cavity, some 10 Å from its entrance. The amino-terminal helices (α1–3) form a lid-like extension that partially covers the active-site channel, which limits the access of substrates and inhibitors. In fact, the aperture of the channel opening is approximately 3 Å in diameter, which is too small for most peptide substrates, indicating that tACE must undergo some conformational change, possibly associated with its unique chloride ion activation, for the substrate to enter the channel. From the structure of the lisinopril–tACE complex (FIG. 4) it is evident that the phenyl ring of the inhibitor interacts with the $S_1$ sub-site in the active site, the lysine with the $S_1'$ sub-site, and the proline occupies the $S_2'$ sub-site. The carboxyl group, located between the phenylpropyl group and the lysine, binds to the $Zn^{2+}$ in the active site and also forms a hydrogen bond with the side-chain carboxylate of Glu384. Other key interactions occur between the side-chain amino group of the inhibitor lysine and Glu162 of tACE, and between the C-terminal proline carboxyl group with Lys511 and Tyr520. The binding of the inhibitor to the $S_1$, $S_1'$ and $S_2'$ pockets and its zinc coordination form the basis for the structure-guided design of improved domain-selective ACE inhibitors. In addition, two buried chloride ions that are important for the activation of the enzyme were identified in the crystal structure (outside the active site), both distant (20.7 Å and 10.4 Å, respectively) from the catalytic $Zn^{2+}$ ion. The first is bound to Arg186, Arg489 and Trp485, whereas the second is bound to Arg522 and Tyr224. The structure is indicative of an indirect mechanism for chloride activation, possibly through effects on active-site structure. This study also revealed that the structure of tACE (MA sub-clan of M clan of peptidases (M clan peptidases are metalloenzymes and the metal is involved in catalysis)) resembles that of rat neurolysin[41] and a newly identified carboxypeptidase from the hyperthermophilic archaeon *Pyrococcus furiosus*[42] — both of which are members of the MA clan — despite low sequence similarity.

Structure-based modelling of the N-domain of somatic ACE (using the three-dimensional structure of tACE[5], which has ~60% amino-acid sequence identity to somatic ACE) reveals some interesting features. First, notable differences in hydrophobicity and charge are observed in the lid-like structure comprising helices α1, α2 and α3 (using tACE nomenclature; FIG. 2). This part of the structure seems to affect the substrate specificity of the N- or C-domain, as tACE mutants which had this region replaced by the corresponding N-domain sequence showed a preference for N-domain substrates[43] (Z. L. Woodman *et al.*, unpublished data). Second, the $Zn^{2+}$-binding site with the canonical HEXXH motif is conserved. Third, it has been shown that the C-domain has greater chloride dependence than the N-domain, both in terms of substrate hydrolysis and inhibitor binding[19,24]. From the model we can predict that Arg186 (a key residue for binding one of the two chloride ions in tACE) is replaced by His164 in the N-domain. So, in the N-domain only one chloride-ion-binding pocket is plausible, involving Arg500 and Tyr202. Fourth, positioning of the lisinopril molecule in the active site of the N-domain model revealed that the full complement of structurally conserved residues was found as observed in the tACE structure, confirming that the N-domain of somatic ACE could also bind lisinopril with similar affinity, as previously reported[24,44]. Fifth, the $S_2$ sub-site is formed by Asn494 and Thr496, which replace a serine and a valine, respectively, in the C-domain. Furthermore, the asparagine occurs in an *N*-GLYCOSYLATION SEQUON (NVT) that is unique to the N-domain; the attachment of any glycan to this residue would occlude the $S_2$ pocket. This, in part, might explain how the bulky aromatic ring of the benzamido group in keto-ACE (an ACE inhibitor; see FIG. 5a) is accommodated by the $S_2$ site in the C-domain (tACE), making keto-ACE more C-domain selective. Sixth, the ACE inhibitor RXP407 has an *N*-acetyl group that confers a certain degree of N-domain specificity[22]. The model of RXP407 and the N-domain reveals two residues, Arg381 and Tyr369, that form van der Waals interactions with the *N*-acetyl carbonyl/methyl groups (FIG. 5d). However, a glutamate and phenylalanine are substituted for these two residues in the C-domain and might exert repulsive forces in the binding of the inhibitor to the C-domain active site.

Homology modelling of ACE2 using the atomic coordinates of the tACE structure revealed differences in the ligand-binding pockets of the two homologues that account for their substrate and inhibitor selectivity[105]. First, the accommodation of the $S_2'$ sub-site in tACE increases the substrate-binding cavity and permits the binding of an additional amino acid to the obligatory binding site; second, Gln281, which interacts with the carboxyl terminus of lisinopril in tACE, is replaced by an arginine in ACE2. This represents an elongation of the side chain of residue 281, which causes steric conflict with the $P_2'$ residue of lisinopril when it is docked in the active site of the ACE2 model. In addition, the substitution of the leucine and phenylalanine for the hydrogen-bonding Lys511 and Tyr520, respectively, and replacement of Thr282 in tACE by the more bulky Phe274 residue, probably account for the changes in substrate specificity. There are no striking differences in the large $S_1'$ sub-sites of tACE and ACE2 except for a proline in ACE2 which corresponds to Ala354 in tACE.

GLYCOSYLATION SEQUON
Consensus sequence Asn-X-Ser/Thr whose core glycosylation generally occurs at the Asn residue.

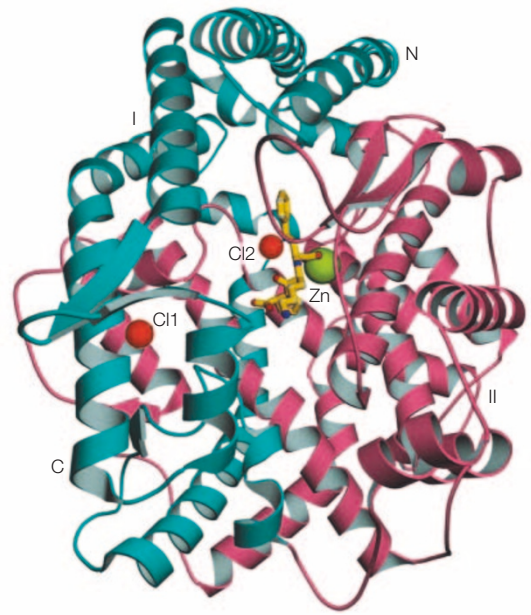


Figure 3 | **Overview of the tACE structure**[5]**.** The molecule can be divided into two halves, sub-domains I and II (shown in cyan and pink colour, respectively). The two bound chloride ions are shown in red. The catalytic site zinc ion and the inhibitor lisinopril molecule are shown in green and yellow, respectively. tACE, testis angiotensin-converting enzyme.

## First-generation ACE inhibitors

The story of the design and synthesis of the first orally active, potent inhibitors of ACE is one of the great success stories of modern medicinal chemistry. It has been described as one of the first examples of true 'rational drug design'[3], and although this might not be true in the sense that we understand that term today, the design of the ACE inhibitors in the late 1970s and early 1980s was certainly based on a series of brilliant insights that, together with a sprinkling of serendipity, constituted what might now be called 'rational intuition'.

It is not our intention to provide a comprehensive review of the events leading to the design and synthesis of captopril, enalaprilat and lisinopril, the original group of potent ACE inhibitors that formed the basis for all subsequent compounds of what can now be termed first-generation ACE inhibitors. Several excellent reviews of this history have been published during the past two decades, especially by the inventors themselves[45–50]. However, it is instructive to consider some of the key insights that led to these drugs, especially in the context of the crystal structure that is now available and the structure-guided drug design of second-generation ACE inhibitors that can now be undertaken.

A role for a metal in the catalytic mechanism had been suspected since the discovery of the enzyme[16] and was confirmed in the 1970s[51,52], leading to the proposal that ACE was mechanistically similar to carboxypeptidase A (CPA)[53,54]. We now know that ACE is unrelated to the CPA class of enzymes, but instead falls into the group of metallo-endopeptidases characterized by the HEXXH zinc-binding motif[55] (CPA possesses HXXE). Nevertheless, the use of CPA as a model led to key conceptual insights in inhibitor design, because CPA was much better understood and its crystal structure was known. Cushman and Ondetti reasoned that ACE was an exopeptidase like CPA, with the difference that ACE cleaved the penultimate peptide bond to release a dipeptide product.

The second major breakthrough in inhibitor design derived from an earlier observation that an extract from the South American pit viper *Bothrops jararaca*, known as bradykinin potentiating factor (BPF), could inhibit ACE[56–58]. BPF was a mixture of peptides[59], which were shown to be potent and specific inhibitors of ACE (TABLE 1), and structure–activity studies indicated that the optimal C-terminal inhibitory sequence was Phe-Ala-Pro[60]. This work led to the proposal that the venom peptides were substrate analogues that bound competitively to the obligatory substrate-binding sites in the ACE active site (FIG. 5a). What was needed were orally active, non-peptide analogues of BPF.

The third key insight derived from work by Byers and Wolfenden[61] describing a new design concept for inhibitors of CPA based on benzylsuccinic acid, referred to as by-product analogues. Cushman and Ondetti recognized that part of the binding affinity of benzylsuccinic acid derived from coordination of the active-site zinc by the carboxyl group, and predicted that a similar succinyl-amino acid derivative would inhibit ACE if its structure was analogous to the dipeptide product of ACE activity. On the basis of the Phe-Ala-Pro sequence derived from the BPF peptides, they synthesized methylsuccinyl-Pro (analogous to carboxy-Ala-Pro) and indeed found it to be a specific inhibitor, with an $IC_{50}$ of 22 μM[53]. Cushman and Ondetti then searched for a superior zinc-binding group and the potency breakthrough was achieved by replacing the carboxyl with a sulphydryl group, yielding captopril with an $IC_{50}$ of 23 nM[53,54] (FIG. 5a).

Captopril became the first ACE inhibitor in clinical use (first approved in 1981) and rapidly established itself as a powerful new therapeutic agent in the treatment of hypertension and heart failure. Reports of captopril-related side effects, such as loss of taste and skin rash, prompted Patchett and colleagues to focus on the design of non-sulphydryl ACE inhibitors, by reverting to carboxyl compounds and introducing additional functionalities that would complete the by-product design. Captopril did not make use of at least two potential binding sites: the $S_1$ binding site and a hydrogen-bonding site for the amide nitrogen of the (substrate) scissile bond (FIG. 5a). So, structure–activity studies were performed on a series of *N*-carboxyalkyl dipeptides of the general formula R-$CHCO_2$H-$A_1$-$A_2$. The R group, occupying the $S_1$ pocket, was best served by benzylmethylene, whereas $A_1$-$A_2$ was either Ala-Pro (as in the BPF peptides) or, surprisingly, Lys-Pro, leading to enalaprilat and lisinopril, respectively (FIG. 5a)[62], which show nanomolar inhibition constants.

Enalaprilat and lisinopril are essentially tripeptide analogues with a $Zn^{2+}$-coordinating carboxyl group substituting for the substrate scissile amide carbonyl. Enalaprilat closely resembles the Phe-Ala-Pro sequence

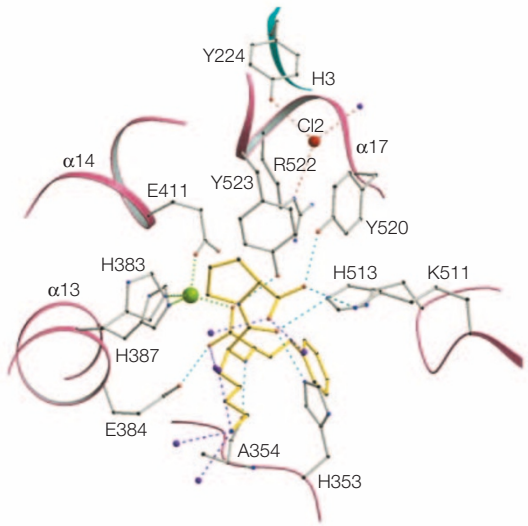


Figure 4 | **Ball-and-stick representation of the active site of tACE with the inhibitor molecule (lisinopril) in yellow[5].** The zinc atom is in green, chloride ion in red and water molecules in purple. tACE, testis angiotensin-converting enzyme.

that was found to be the optimal C-terminal sequence among the venom peptides. The structure of the ACE–lisinopril complex confirms that lisinopril makes extensive contacts with the active site, including occupation of the $S_1$, $S_1'$ and $S_2'$ pockets, binding of a lysine by the C-terminal carboxylate, a hydrogen bond involving the (substrate) scissile amide nitrogen, and coordination of the active-site $Zn^{2+}$ by the carboxyalkyl carboxylate[5] (FIG. 4). Lisinopril binds with twofold greater affinity than enalaprilat[63], probably because the lysine side chain makes better contacts with the deep $S_1'$ pocket, including a weak hydrogen bond between the lysyl ε-amine and Glu162 (REF. 5).

The design of captopril, enalaprilat and lisinopril was later extended by others, and a total of 17 ACE inhibitors have been approved for clinical use[4]. The later compounds are all variations on the original theme, with most of the differences residing in the functionalities that bind the active-site zinc and the $S_2'$ pocket[48]. The different types of zinc-coordinating groups are of interest, because these have also found application in the design of inhibitors for other metalloproteases, such as the matrix metallopeptidases. On the basis of work first described by Holmquist and Vallee[64], phosphonates have proven useful[22,23], as have hydroxamates[65], ketones[66] and silanediols[67].

### Clinical profile of ACE inhibitors

Since their introduction in 1981, ACE inhibitors have been studied extensively (for recent reviews, see REFS 4,68,69). ACE inhibitors are first-line therapy for hypertension, congestive heart failure, left ventricular systolic dysfunction and myocardial infarction, and are recommended for slowing the progression of diabetic and non-diabetic nephropathy[68,70]. More recently, ACE inhibitors have also been shown to slow the progression of atherosclerotic vascular disease. This vascular benefit has been attributed to a direct vascular protective and anti-atherogenic effect of ACE inhibitors, because it has been observed even in normotensive individuals[69].

A major debate concerns the mechanism by which cardiovascular benefits are conferred by ACE inhibitors. It is generally accepted that Ang II not only has direct pressor effects and stimulates salt and water retention (via aldosterone release), but also stimulates myocyte proliferation and exerts pro-atherogenic effects via the induction of oxidative stress, endothelial dysfunction and vascular inflammation[71,72]. However, there is also considerable evidence that some of the benefits of ACE inhibition derive from potentiation of BK signalling, which stimulates release of the vasodilator nitric oxide and of the fibrinolytic protein tissue plasminogen activator[72,73]. Indeed, co-administration of the specific BK-receptor antagonist icatibant significantly attenuated the hypotensive effect of captopril in both normotensive and hypertensive subjects[74].

Further complicating this debate is the recent appreciation that the RAS is more complex than originally thought. There are multiple Ang peptides and at least three or four Ang receptors, some of which have opposing activities (FIG. 1). For instance, the principal Ang II receptor, the $AT_1$ receptor, mediates the well-known effects of Ang II described above, but the $AT_2$ receptor, which has a more limited tissue distribution, mediates largely opposing effects. Similarly, Ang $_{1–7}$, which is derived from both Ang I and Ang II by the action of various peptidases including ACE and ACE2, opposes the actions of Ang II[3,37]. Moreover, as already discussed, ACE also acts on non-Ang peptides, including BK, substance P, luteinizing hormone-releasing hormone (LH-RH) and the haemoregulator AcSDKP; with the exception of BK, the importance of these peptides for the cardiovascular effects of ACE inhibitors is unknown.

Some insights might derive from comparisons of the efficacy and side-effect profiles of ACE inhibitors and $AT_1$-receptor blockers (ARBs). ACE inhibitors are generally well tolerated, but certain class-specific side effects have emerged, in particular cough and angioedema[70]. The incidence of cough has been estimated at 5–20% of patients and might result in the discontinuation of treatment[70,75]. Angioedema affects 0.1–0.5% of patients and can be life-threatening[76]. Both cough and angioedema have been attributed to alterations in levels of non-Ang peptides, especially raised BK concentration. Recently, an association has been found between ACE-inhibitor-related angioedema and low plasma levels of aminopeptidase P, an enzyme that is also involved in the metabolism of BK, indicating that these individuals are at risk for developing angioedema when treated with ACE inhibitors[76]. The potentiation of BK signalling by ACE inhibitors (FIG. 1) seems to result not only from reduced BK degradation but also from inhibition of desensitization of the $B_2$ BK receptor, possibly by inducing crosstalk between ACE and the $B_2$ receptor[77–79]. Therefore, both the benefits and side effects arising from increased BK signalling by ACE inhibitors seem to be mechanistically complex and have implications for the design of N- or C-selective inhibitors (see below).

### Alternatives to ACE inhibitors

The ARBs were introduced more recently but have also been studied extensively[4,80]. As is the case for ACE inhibitors, ARBs have been shown to be effective in the treatment of hypertension and heart failure, in reducing cardiovascular morbidity and mortality, and in slowing the progression of nephropathy. Studies in myocardial infarction and heart failure have indicated a trend towards lower mortality in patients treated with ACE inhibitors versus ARBs, leading to the recommendation that ACE inhibitors remain the first-line agents in these conditions[4,75,81,82]. This difference might be due to the additional effect of ACE inhibitors on BK, although this comes at the cost of increased side effects[75,81]. Interestingly, and contrary to earlier assumptions, angioedema has also been reported with ARB therapy[75,83], which might be related to unopposed activation of the $AT_2$ receptor leading to increased BK concentrations[73].

In light of the enormous importance of the RAS in cardiovascular pathophysiology, there is continued interest in novel compounds that target this system[4]. Eplerenone, the first selective aldosterone antagonist,

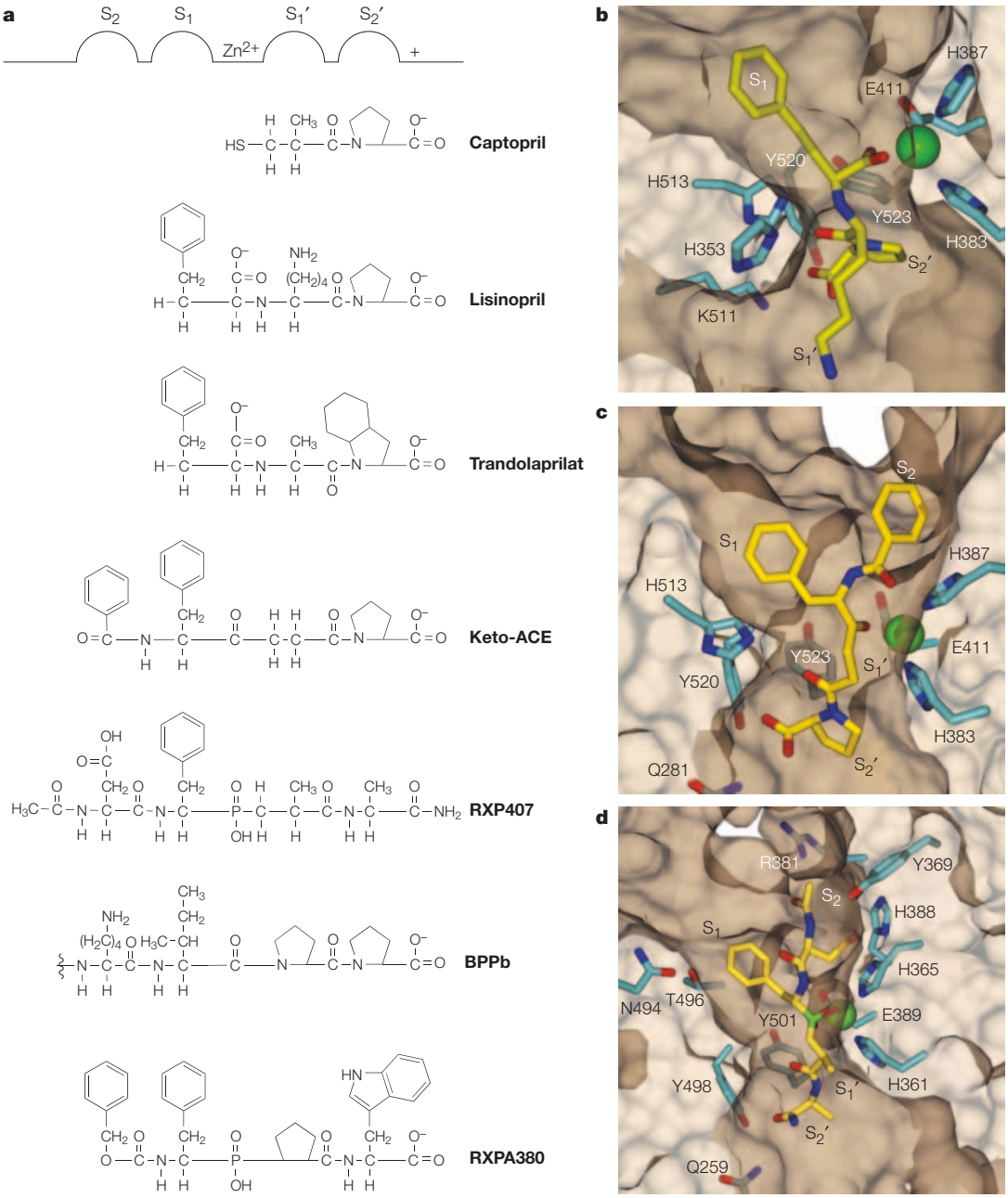


Figure 5 | **Models of interactions between inhibitors and the active sites of ACE. a** | Classical representation of inhibitor binding to the 'generic' ACE active site[53,62]. **b–d** | Three-dimensional structural representation of inhibitor-binding pockets in the tACE–lisinopril complex (**b**)[5], the modelled tACE–keto-ACE complex (**c**) and the modelled N-domain–RXP407 complex (**d**). The different sub-sites are marked as in FIG. 5a. ACE, angiotensin-converting enzyme; tACE, testis ACE.

was approved for the treatment of hypertension in September 2002 and will probably also find use in the treatment of severe heart failure[7]. An orally active renin inhibitor, aliskiren, is now in clinical development for hypertension[84].

Of particular interest are the vasopeptidase inhibitors, which are dual ACE–neutral endopeptidase (NEP) inhibitors, of which omapatrilat is the most advanced in clinical development. NEP, also a zinc-metallopeptidase, is the principal enzyme responsible for the degradation of natriuretic peptides, which are vasodilatory and diuretic peptides that reduce volume loading and are therefore beneficial in both hypertension and heart failure[85]. As expected, omapatrilat was equivalent or superior to ACE inhibitors in clinical trials, but was found to be associated with a significantly higher incidence of angioedema, which has delayed its approval[86]. The higher incidence of angioedema is probably related to the fact that NEP also inactivates BK (FIG. 1), and its inhibition might result in even higher plasma BK levels, which together with raised concentrations of natriuretic peptides (and potentially other vasodilatory peptides) might promote vasodilation-related adverse events[4,86]. NEP might also be required for the removal of the neurotoxic amyloid β-peptide, and so chronic NEP inhibition could lead to neurodegeneration[87].

The clinical results with omapatrilat have had a sobering effect on the field[88] and have indicated that broad inhibition of vasoregulatory peptides should be approached with caution[89]. Indeed, overly vigorous inhibition of neuro-hormonal activation during heart failure, by simultaneous treatment with an ACE inhibitor, an ARB and a β-blocker was associated with excess mortality in the Valsartan Heart Failure Trial (Val-HeFT)[90]. So, efforts to develop triple inhibitors of ACE, NEP and endothelin-converting enzyme-1 (ECE-1)[91] might need to be re-evaluated. ECE-1, which is structurally and functionally related to NEP, is the principal activating enzyme for the potent vasopressor peptides endothelin-1 and -3 (REF. 92), and so triple inhibitors might show even greater efficacy than single ACE or dual ACE/NEP inhibitors. However, there is clearly an even greater potential for adverse events such as angioedema.

## Second-generation ACE inhibitors

In light of these developments, highly specific, single-domain inhibitors of ACE offer an attractive alternative. As we have attempted to show in this review, our understanding of the RAS (and related vasoregulatory systems) has come a long way since the introduction of the first ACE inhibitors. The N- and C-domain sites of ACE hydrolyse Ang I and BK at comparable rates *in vitro*, but *in vivo* it seems that the C-domain is primarily responsible for regulating blood pressure[93,94]. This might indicate that a C-selective inhibitor would have a profile comparable to current mixed inhibitors, but this is not necessarily the case.

First, whereas Ang I is hydrolysed predominantly by the C-domain *in vivo*[94], BK is hydrolysed by both domains[23] and therefore selective inhibition of the C-domain site will allow some level of BK degradation to continue, catalysed by the N-domain. This could be sufficient to prevent the excessive BK accumulation that has been observed during attacks of angioedema[86]. Second, BK potentiation by $B_2$ receptor resensitization is maximal when both the N- and C-domains are inhibited[95], indicating that a pure C-selective inhibitor will have a lower propensity for excessive BK stimulation. Third, the multiple Ang and non-Ang peptides known to be vasoactive are not hydrolysed equally by the two domains[3,96], making it likely that the ratio of vasopressor to vasodilator peptides will differ between C-selective and mixed inhibitors. So, a highly selective C-domain inhibitor has the potential for effective blood pressure control with reduced vasodilator-related side effects.

In contrast to a C-selective inhibitor, an N-selective inhibitor might open up novel therapeutic areas. As discussed, the N-domain seems to play a minor role in blood pressure control *in vivo*. At least three physiologically important peptides are hydrolysed preferentially or exclusively by the N-domain: LH-RH, Ang $_{1–7}$ and AcSDKP[21,44,96]. The contribution of ACE to the metabolism of LH-RH and Ang $_{1–7}$ *in vivo* is unclear, but there is increasing evidence that ACE is the principal metabolizing enzyme for AcSDKP, a natural haemoregulatory hormone[97]. AcSDKP has antiproliferative and antifibrotic activities, and might have utility in protecting haematopoietic stem cells against chemotherapy-induced injury[97] and in limiting cardiac fibrosis[98]. Administration of ACE inhibitors results in a four to sixfold elevation of AcSDKP plasma levels[94,97]. This might be the basis for the observed association between ACE inhibitors and anaemia, and the effective treatment of altitude polycythaemia by the ACE inhibitor enalaprilat[99].

## Design of domain-selective ACE inhibitors

Current-generation ACE inhibitors in clinical use are essentially mixed N- and C-domain inhibitors[48]. Although a modest degree of domain selectivity can be observed in some cases, this is not likely to be clinically significant. Nevertheless, these differences might be instructive and can guide future attempts to develop highly domain-selective inhibitors. Captopril has been noted to be modestly N-selective, depending on $Cl^-$ concentration, whereas lisinopril and enalaprilat are more C-selective[24,44]. More recently, keto-ACE, originally described in 1980 (REF. 66), was found to exhibit a 40–50-fold C-selectivity[96]; one of the BPP peptides, BPPb, was shown to be 300-fold more C-selective[100]; and the phosphinic tetrapeptide RXPA380 is 3,000-fold more C-selective[23]. By contrast, BPP-12b is 30-fold, and the phosphinic tetrapeptide RXP407 1,000-fold, more N-selective[22,101] (TABLE 1).

Examination of these compounds reveals a number of features (TABLE 1 and FIG. 5a). Captopril is the smallest inhibitor and can be viewed as an *N*-thioalkyl derivative of the dipeptide Ala-Pro, whereas enalaprilat, lisinopril and keto-ACE are tripeptide analogues of Phe-Ala-Pro, Phe-Lys-Pro and Phe-Gly-Pro, respectively. This might indicate that a bulky $P_1$ group — present in enalaprilat, lisinopril and keto-ACE, but absent in

captopril — confers C-selectivity, and that a larger $P_1'$ side chain also promotes C-selectivity (FIG. 5b), because lisinopril is more C-selective than enalaprilat. Interestingly, trandolaprilat, although a potent inhibitor for both domains, was tenfold more C-selective[24] (TABLE 1). Trandolaprilat contains a C-terminal hexahydroindoline group, which also indicates that a bulky $P_2'$ group confers C-selectivity. This is confirmed by results from radioligand-binding studies, which indicated that both perindoprilat and quinaprilat, which contain hexahydroindoline and tetrahydroisoquinoline groups, respectively, in the $P_2'$ position, were 45–180-fold more C-selective[102]. Similarly, the highly C-selective tetrapeptide RXPA380 also contains a bulky methylindole group in the $P_2'$ position. These studies also indicated that lisinopril and 351A (a hydroxybenzamidine analogue of lisinopril) were 110–146-fold more C-selective, reinforcing the importance of a bulky $P_1'$ group[103].

On the other hand, structure–activity studies performed with a series of phosphinic tetrapeptides indicated that a phenylalanine in the $P_1$ position did not confer C-selectivity. Instead, the single most important determinant for N-selectivity was an amidated C-terminal carboxyl in the $P_2'$ position, followed by an acidic group in the $P_2$ position (FIG. 5d)[22]. The bradykinin potentiating peptides (BPPs) confirm the conclusion regarding the $P_2$ group: BPPa, BPPb, BPPc and BPP2 all end with the sequence Ile-Pro-Pro, yet only BPPc is unselective. BPPc has a proline in the $P_2$ position versus a lysine in the most C-selective peptide, BPPb[100]. Similarly, the C-selectivity of keto-ACE can probably be ascribed to a bulky $P_2$ benzyl group (FIG. 5c); the selectivity of RXPA380 is also consistent with the proposed importance of a phenyl $P_2$ group (FIG. 5a). Taken together, these data indicate that C-selectivity is conferred by bulky $P_1'$ and $P_2'$ groups and a large, neutral or basic $P_2$ group, whereas N-selectivity is conferred by a blocked C-terminal carboxyl and an acidic $P_2$ group.

The ACE C-domain crystal structure revealed that the $S_1'$ pocket was surprisingly deep, easily accommodating the lysyl group of lisinopril, with a hydrogen bond between Glu162 and the ε-amine (FIGS 4 and 5b)[5]. Additional modifications to the $P_1'$ group will potentially further enhance C-selectivity. Moreover, the C-terminal carboxylate of lisinopril was found to bind to Lys511, explaining the importance of a free C-terminal ($P_2'$) carboxyl for binding to the C-domain active site[22]. Binding to Lys511, instead of to an arginine (as originally predicted), might prompt investigation of functionalities other than carboxylates in this position. Since N-selectivity is conferred by a blocked $P_2'$ carboxylate, it can also be predicted that this sub-site is significantly different in the N-domain active site. Modelling of the $S_2$ sub-site in the N-domain has also revealed differences, as expected (see earlier), confirming its potential utility for conferring domain selectivity (FIG. 5c,d). These considerations can form the starting point for the structure-guided design of domain-selective inhibitors, which will be refined further once the N-domain structure becomes available.

An important caveat in considering the design and pharmacological utility of domain-selective ACE inhibitors is the potential for conformational effects that have not yet been observed in the tACE crystal structure. For instance, it is unknown whether chloride binding and dissociation result in significant movement of the N-terminal 'lid' formed by helices α1, α2 and α3, and thereby restrict substrate access[5]. Even more importantly, the physical orientation of the N- and C-domains in somatic ACE is unknown, as is whether there is any significant degree of domain interaction or cooperativity. Inhibitor titrations *in vitro*[44] and studies with domain-selective inhibitors *in vivo*[23] have provided indirect evidence for some form of domain interaction, which could have significant effects on the pharmacological profile of domain-selective inhibitors.

## Conclusion

The past decade has seen major advances in structure-based drug design approaches, including technologies such as mass spectrometry, X-ray crystallography and nuclear magnetic resonance. These are important tools in structural proteomics and to some extent have eliminated the scepticism about the feasibility and value of the structure-based approach. In particular, high-throughput structure-based drug design using protein crystallography has become a very attractive proposition for the pharmaceutical industry. Many examples exist today in which a combination of the three-dimensional structure of the target protein, computer-aided drug design (*in silico* or virtual screening), and a rational approach using high-throughput screening have produced important lead compounds that are now being evaluated in clinical trials (for recent reviews see REFS 1,2,103,104). In addition to mining the untapped riches of the human proteome, the application of modern structure-based drug design methods to existing drug targets will generate more selective compounds for known disease targets, such as ACE. We expect that next-generation, domain-selective ACE inhibitors will be a result of such endeavours.


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