Evolution of diuretics and ACE inhibitors, their renal and antihypertensive actions—parallels and contrasts

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1 The emergence of diuretic drugs and angiotensin converting enzyme (ACE) inhibitors ranks amongst the major therapeutic advances of modern medicine. The discovery of these drug groups arose largely by chance, yet each has dramatically influenced the treatment of congestive cardiac failure and arterial hypertension.

2 The central role which diuretics have had in the management of both oedema and hypertension hinges on their ability to induce a net renal excretion of solute and water by selective interference with either active or passive ion transport processes in different segments of the nephron. Irrespective of sites of action, the continued antihypertensive action of diuretics is characterized by a reduction in plasma volume and extracellular fluid (ECF) volume that lasts for as long as the diuretic is given. The mechanism of this effect remains unclear but may involve autoregulatory reactions that leave cardiac output unaltered but maintain a sustained reduction in total peripheral resistance.

3 ACE inhibitors also lower blood pressure by decreasing total peripheral resistance, leaving cardiac output, plasma volume and ECF volume unchanged. The detailed way these haemodynamic changes are achieved remains unknown but inhibition of converting enzyme present not only in the kidney but also in many extrarenal tissue sites, appears important. In both hypertension and cardiac failure, however, the kidney acts as a key target organ for ACE inhibitors. The increased renal vascular resistance and inappropriate renal salt excretion are reversed with enhanced renal blood flow and saluresis. Both angiotensin II (AII) and vasopressin-mediated contraction of glomerular mesangial cells is inhibited, making glomerular filtration more efficient. Reduced aldosterone secondary to blockade of AII formation contributes to saluresis whilst encouraging positive potassium balance. ACE inhibition also impairs breakdown of kinins which may contribute to intrarenal and peripheral vasodilation either on their own or via release of prostaglandins and other vasoactive substances.

4 The hypotensive actions of diuretics are potentiated by ACE inhibition primarily through blockade of AII formation and prevention of secondary aldosteronism. In combination, these drugs permit low doses to be used because of their synergistic effects.

5 Caution has to be exercised whenever ACE inhibition is used, without and especially with diuretics, in the management of renovascular hypertension and other low-perfusion states. In these circumstances, AII plays an important autoregulatory role in preserving glomerular filtration through an increase in post-glomerular resistance. Suppression of angiotensin formation with an ACE inhibitor can cause a critical reduction in glomerular

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perfusion. On the other hand, with careful titration, the dual actions of low-dose loop diuretics and an ACE inhibitor may be highly efficacious in treating severe heart failure and totally prevent such serious metabolic sequelae as azotaemia and hyponatraemia.

6 Unlike the experience with many other drugs where continued clinical usage has usually led to greater confidence in employing higher dosages with the passage of time, the opposite has occurred with both the thiazide diuretics and ACE inhibitors. This has come about by the appreciation, albeit at times tardily, of the significance of adverse events of a dose-dependent nature.

Keywords angiotensin converting enzyme inhibitors diuretics

Introduction

Few drugs can claim to have truly revolutionized the management of the conditions for which they were introduced as treatment. Orally-active diuretics and inhibitors of angiotensin-converting enzyme (ACE) can however justly claim to have achieved this status. Furthermore, both groups of agent have had dramatic impact not only on one disease state but on the same two cardiovascular disorders, heart failure and hypertension. In these conditions, they have separately, and together as concurrent therapy, offered major clinical benefits over a spectrum of severity of disease in a manner not previously possible. A spin-off that has accompanied these impressive therapeutic advances has been the remarkable opportunity afforded to basic scientists through availability of these drugs, to investigate the mechanisms underlying their actions. Barren areas of unexplored renal and cardiovascular physiology have suddenly opened up in a manner that could hardly have been conceived. The result is a medical literature that is now bulging with thousands of published papers describing in molecular terms, mechanisms of cellular and membrane action from all manner of tissues, that have only been made possible through the application of diuretics and ACE-inhibitors as molecular probes (Navar & Rosivall, 1984; Giebisch, 1985). The pace of these developments in producing major physiological and pathophysiological advances has been so extraordinarily rapid that it is more than appropriate to survey at this juncture some of the important parallels and contrasts in our acquired knowledge of the renal and cardiovascular actions of these two groups of drugs.

Discovery

Chance has played a key role in the discovery of both diuretics and ACE inhibitors. In 1919

Arnold Vogl was a third-year student in Vienna when he noticed the unexpected diuresis that followed the parenteral administration of an organic mercurial compound, merbaphen (Novasurol) to a non-oedematous emaciated young girl with congenital syphilis who weighed only 35 kg (Vogl, 1950). The use of toxic inorganic salts of mercury such as calomel to evoke a diuresis had been known for many years before. Indeed Stokes (1854), describing the then current management of congestive cardiac failure, reports: '... it happens again and again that the exhibition of mercury, will, as by enchantment, remove the anasarca, reduce the hepatic tumour, restore the heart to its ordinary, though not its normal condition, and for a period of time, more or less long, enable the patient to pursue the avocations of an active and laborious life'. However, it was Vogl's observations using a sample of new antisyphilitic agent, merbaphen, that established the therapeutic basis for organomercurial diuretics and stimulated the search for less toxic synthetic compounds such as, for example, mersalyl, introduced first in 1924, and still included in modern pharmacopoeias—though rarely used. Organomercurial diuretic therapy reigned supreme until the late nineteen fifties, its only rivals being the relatively weak and ineffective xanthines. A number of important and overlapping clinical and scientific observations were reported in this interim period with another unpredicted adverse drug effect featuring. Shortly after the introduction of sulphanilamide into clinical practice as an antibacterial it was noted that patients treated with this compound excreted an alkaline urine, displayed a mild diuresis and developed a metabolic acidosis (Southworth, 1937). Further study of these effects led to the conclusion that sulphanilamide was a specific inhibitor of carbonic anhydrase, an enzyme that had been recently

discovered to be important in maintaining renal acid-base balance. Schwartz (1949) then reported on the usefulness of sulphanilamide in the treatment of cardiac failure, but in view of the substantial toxicity of the drug, the search began for a safer molecule. The first success to emerge was a heterocyclic sulphonamide, acetazolamide, and then, in 1957, Novello & Sprague reported that, after having systematically studied a large number of aromatic sulphonamides with carbonic anhydrase-inhibitory activity, a series of molecules possessing a benzothiadiazine structure were capable of causing after oral administration, an effective saluresis without renal wastage of bicarbonate. Thus emerged for the first time a new type of diuretic, effective orally, and with so few adverse effects as to meet the requirements for safe chronic therapy. Within two years of the discovery of chlorothiazide, benzothiadiazine efficacy in the treatment of hypertension had been established. Many synthetic analogues followed quickly so that the 'thiazides' were soon to become one of the most widely used classes of drugs in medical practice. Thus the evolution of the modern diuretics can be seen to have followed a sequence of chance observations leading up to a highly organized synthetic programme aimed at designing an orally-effective inhibitor of a specific enzyme system, carbonic anhydrase. Interestingly, the same sequence underlies the evolution of the ACE-inhibitors since ACE, like carbonic anhydrase, is also a zinc-metalloprotein belonging to a large group of related enzymes that include carboxypeptidase A and alkaline phosphatase (Coleman, 1984). The trail that led to the discovery of the first ACE inhibitor began when a Brazilian post-graduate student, Sergio Ferreira, who had trained with Rocha e Silva (the discoverer of bradykinin), came in 1965 to work with Vane in London. This scientific group was working on the mechanisms whereby the venom of the poisonous Brazilian snake, *Bothrops jararaca* caused circulatory collapse. The venom was found to contain simple peptides that inhibited ACE and at the same time potentiated bradykinin. By chance, Vane happened to be acting as an external consultant to Squibb in Princeton, New Jersey, and in this capacity he suggested in 1967 to the company that they might examine the snake venom factor, isolate the active principles and test them in models of hypertension (Vane, 1985). The outcome was the isolation of a natural nonapeptide, teprotide (also known as bradykinin potentiating peptide, BPP_{9a} or SQ20,881). This material was found on injection intravenously to man to block the conversion of angiotensin I and II (Collier *et al.*, 1973) and also to lower blood pressure in

untreated essential hypertension (Gavras *et al.*, 1974). These observations were then followed by a remarkable sequence of planned molecular design based on the hypothesis that the active site of ACE might be similar to that of the Zn-metalloprotein, carboxypeptidase A, but that, instead of liberating single amino acids from the carboxylic acid end of the peptide chain as did the latter, it might release dipeptides. The systematic study of the structure-activity relationships of thousands of analogues of succinyl-L-proline led to the development of low molecular weight inhibitors of ACE that were orally active, culminating in the synthesis of captopril (Cushman & Ondetti, 1980). Shortly afterwards, a series of tripeptide analogues were synthesized which also bound strongly to the zinc atom of the active site of ACE but did not contain a sulphhydryl or mercapto-group (Patchett *et al.*, 1980). Enalapril was the first of the non-sulphhydryl containing ACE inhibitors and was unusual in being the esterified pro-drug of the active enzyme inhibitor, enalaprilat.

The developments of both the first orally-active diuretic and the first orally-active ACE inhibitor have thus followed a parallel path characterized by a combination of fortuitous accidents which, in each case led to substantial synthetic programmes within the pharmaceutical industry culminating in major chemical breakthrough that then served as a template for further molecular manipulation.

The chemical prototype and beyond

Whereas it took close on forty years from the discovery of the clinical efficacy of organo-mercurials as diuretics to the emergence of chlorothiazide, the hurdle of successfully making an orally-active ACE-inhibitor to succeed teprotide spanned a total period of only 5 years, between 1971 and 1976.

Chemical evolution of diuretics

A large number of orally-active diuretics is now available, covering a variety of chemical structures. The two most productive pathways for the synthetic evolution of these drugs have been quite distinct. On the one hand, the phenoxy-acetic acid structure of merbaphen offered a useful starting point. On the other hand, the sulphonamide nucleus of sulphanilamide served as a basis for generating several families of related but functionally separate diuretics. The evolution of these two pathways will be briefly outlined. A third and quite separate pathway which led to

the development of the antidiuretic diuretics originated from the successful synthesis of analogues of mineralocorticoid steroids such as aldosterone. Subsequent non-steroidal compounds were developed possessing nitrogen rich heterocycles such as pteridine or pyrazine nuclei; these were found to display potassium-sparing properties even in the absence of aldosterone. The chemical evolution of antidiuretics is dealt with in more detail by Feit (1979).

(i) *Phenoxyacetic acid diuretics* Organomercurials suffered from a number of important drawbacks. They were inactive orally, possessed substantial inherent toxicity, and when used clinically displayed a tendency to tachyphylaxis. However, organomercurials also exhibited a number of attractive pharmacological properties including a balanced saluretic effect of high-ceiling type, a relatively low degree of potassium wastage and a mild uricosuric effect. Realization that their mode of action depended on an interaction with sulphhydryl-containing molecules both *in vitro* and *in vivo* gave the impetus to use these compounds as drug models for further development of new diuretics (Weiner *et al.*,

1962). Both merbaphen and mersalyl are substituted phenoxyacetic acids (Figure 1). Following a series of extensive structure-activity studies carried out by Cragoe, Sprague and their co-workers (Cragoe, 1983), it was possible to show that molecular manipulation may not only induce quantitative changes in pharmacological response but also significant qualitative changes, some of which may be unique. Thus, for example, a high-ceiling, orally active acryloyl-phenoxyacetic acid derivative emerged in the form of ethacrynic acid in 1962. This was subsequently followed by other phenoxyacetic acid analogues which displayed the unusual combination of potent saluresis and uricosuria within the same molecule. One of these, tienilic acid (tricyclic) was extensively studied from both a pharmacological and clinical standpoint (Stote *et al.*, 1978), but, unfortunately, soon after its introduction on to the market in the United States in 1980, it was withdrawn because of hepatotoxicity. The drug remains in clinical use in France. Another uricosuric saluretic indacrinone remains an experimental drug of considerable interest mainly because of its unusual chemical and pharmacological characteristics. It exists in two enantiomeric

Figure 1 Evolution of the phenoxyacetic acid family of diuretics from the prototype, merbaphen, through ethacrynic acid to the uricosuric saluretics, tienilic acid and indacrinone. The common mono-or dichlorophenoxyacetic acid radical is encircled.

forms, each of which acts on anatomically and functionally distinct segments of the nephron (Brooks *et al.*, 1984).

(ii) *Sulphonamide diuretics* Evolution of the sulphonamide diuretics followed a different route largely through the chance observation that clinical use of sulphanilamide as an antimicrobial was associated with the occurrence of a mild alkaline diuresis. The association of this effect with inhibition of renal carbonic anhydrase led to the development of a hypothesis that sulphonamides could be produced that would produce a diuresis featuring sodium chloride rather than sodium bicarbonate (Beyer, 1977). This conceptual view ultimately bore fruit with the synthesis of the benzothiadiazines. What was

surprising was that further exploration of sulphonamide structure-activity relationships showed, just as it had done with phenoxyacetic derivatives, that chemical alteration of existing molecules could produce a dramatic change in the qualitative nature of the renal response (Figure 2). This was most strikingly revealed with the discovery of the first sulphamoylbenzoate diuretic, frusemide. The common presence of the sulphamoyl radical ($\text{SO}_2\text{-NH}_2$) in both classical carbonic anhydrase inhibitors, thiazides and the sulphamoylbenzoates, might have suggested that the renal receptor for all these compounds involved carbonic anhydrase. It is now clear that this is not so (Lant, 1985). Sulphamoylbenzoate diuretics interact with a Na, K, 2Cl-cotransporter within the luminal membrane

Figure 2 Evolution of the sulphonamide diuretics emphasizing the variety of both types of structure and qualities of diuretic effect, yet all examples contain at least one unsubstituted sulphamoyl ($\text{NH}_2\text{-SO}_2\text{-}$) radical. CTZ = chlorothiazide HCTZ = hydrochlorothiazide.

of the ascending limb of the loop of Henle. The renal receptor for thiazides remains unidentified.

Chemical evolution of ACE inhibitors

The history of ACE inhibitors is much shorter than that of diuretics and in terms of the pace of achievement, is all the more dramatic. The association of Ferreira with Vane in the mid-sixties led to the proposal that the same protein, angiotensin-converting enzyme, might be responsible for catalyzing both the deactivation of bradykinin and the activation of angiotensin I (Ferreira & Vane, 1967). Two years earlier, Ferreira had shown that a mixture of polypeptides isolated from the venom of *Bothrops jararaca* potentiated the actions of bradykinin (Ferreira, 1965) and Bahkle (1968) showed that this peptide mixture also inhibited the conversion of angiotensin I to angiotensin II by canine pulmonary tissue.

The first ACE inhibitor to be studied in man was the synthetic nonapeptide, teprotide (SQ 20881; BPP_{9a}) which was able to block the pressor response to infused angiotensin I and also lower blood pressure in patients with elevated plasma renin activity. Teprotide was only active parenterally, had a short duration of action and so its clinical utility was bound to be very limited from the outset.

Already in 1973, it was reported that D-2-benzyl-succinic acid was a potent competitive inhibitor of the Zn-metalloprotein, carboxypeptidase A (Byers & Wolfenden, 1973). The mechanism of inhibition was held to involve a simultaneous and stereospecific binding between the inhibitor and three substrate binding sites at the active site of the carboxypeptidase enzyme. Arguing that a similar stereospecificity might also characterize the active site of ACE, Cushman & Ondetti (1980) undertook a systematic study of peptide analogues of teprotide. They reasoned from the structure of the various snake venom peptides that the most tightly bound inhibitor might be one containing the amino acid proline with a methyl side chain analogous to alanine. By further replacing the carbonyl group which was postulated to interact with zinc at the active site, with a sulphhydryl group, there emerged the orally active ACE inhibitor, D-3-mercapto-2-methyl-propanoyl-L-proline (SQ14225; captopril). Early in its clinical use, captopril revealed an unusual cluster of adverse effects which included, rash, taste disturbance (dysgeusia) and proteinuria. These effects were reminiscent of the toxic profile of penicillamine and provided the incentive for synthesis of ACE inhibitors lacking the mercapto group. The loss of the mercapto group with its ready biotransformation

reactions *in vivo*, could also be expected to yield compounds with longer biological half-lives, and this was borne out by the findings of Patchett *et al.* (1980). In order to compensate for the lack of the mercapto zinc ligand, these workers found it necessary to add two more binding radicals, making seven binding sites in all, as in the drug enalaprilat (Figure 3). Enalapril is an esterified pro-drug that yields the free acid, enalaprilat, an active ACE inhibitor possessing an I_{50} for the enzyme of 0.001 μM , as compared with 0.023 μM for captopril and 330.0 μM for succinylproline (Patchett, 1984). Having in this way established the obligatory binding sites for effective inhibition of ACE, one can see clear analogies with the 'lock and key' hypothesis that formed the basis for the original development of organomercurial diuretics (Weiner *et al.*, 1962). The use of conformational analysis in respect of the hypothetical active site of ACE, by analogy with carboxypeptidase A and other Zn-containing peptidases, has provided an extremely versatile template with a boundless capacity for testing and producing new non-peptide inhibitors. A number of interesting variants have already emerged. By changing the amino acid backbone from proline to lysine, the orally active lisinopril (MK521) has been produced which is not a pro-drug; substitution of the carboxyl ligand of enalapril for a phosphoryl radical ($\text{HO}-\text{P}=\text{O}$) has yielded fosfenopril, an ACE inhibitor that undergoes hepatic as well as renal elimination; enalaprilat, which is formed from enalapril by hydrolysis in the liver is eliminated primarily via the kidney as is captopril together with its disulphide metabolites.

Intrarenal effects

Diuretics

(i) *Localization of tubular actions* By definition, diuretics are drugs that increase urinary flow. Their primary effect is the inhibition of salt and water reabsorption by the renal tubules which then results in increased intratubular osmolality and enhanced urine flow. Advances in the methodology of tubular micropuncture and the discovery of the Burg technique for perfusing isolated tubules (Burg *et al.*, 1973) made it possible to characterize the different mechanisms involved in NaCl reabsorption within the nephron. Using such methods, it is now clear that diuretics are most readily classified according to their maximal capacities to reject filtered sodium chloride. These capacities correlate most closely with the particular tubular site or

Figure 3 Diagrammatic representation of the binding of peptide inhibitors to the active site of angiotensin converting enzyme. S₁ and S₂ are subsites of the main active site of the enzyme that incorporates a number of positively charged or hydrogen-bonding loci, hydrophobic 'pockets' and a zinc ion. Striking increases in ACE inhibitory potency have been achieved by the successive introduction of the following molecular changes: a methyl group at C2; change of the succinyl carboxyl group into a mercapto (SH) function (as in captopril); extension of the peptide chain length with more hydrophobic groups to yield enalaprilat, in which there are seven binding loci, the last of which involves a phenylpropyl substituent that evidently fits into the enzyme's auxiliary binding site, S₁.

sites of action and offer a much more meaningful system of drug classification than methods based on either patterns of electrolyte excretion or what is known of the mode of action of diuretics at a cellular level.

High efficacy of 'loop' diuretics may cause the urinary rejection of more than 15% of filtered sodium chloride and act predominantly on the thick ascending limb of Henle's loop (TAL: site

II; Figure 4). This very specific localization to site II is of particular interest when one takes into account the surprising degree of structural heterogeneity that exists within this group of diuretics—ranging from the organomercurials through the sulphamoylbenzoates (typified by frusemide) to the laevoenantiomer of ozolinone or the strongly basic muzolimine (Lant, 1985). The saluretic effects of loop diuretics also involve

Figure 4 Diagrammatic representation of the four principal tubular sites of diuretic action showing the predominant localization of certain agents to specific loci within the nephron. The xanthines belong to the low efficacy group of diuretics and are the only agents whose primary effect is to increase glomerular blood flow; a lesser effect on proximal reabsorption of sodium chloride also occurs (reproduced with permission from Lant, 1986).

a significant contribution from induced changes intrarenal blood flow that are prostaglandin-mediated (Nies *et al.*, 1983).

The large family of benzothiadiazine or 'thiazide' and related diuretics at most cause the excretion of between 5 and 10% of filtered sodium chloride; their action is localized to the early portion of the distal tubule—the so-called 'cortical diluting segment'.

Low efficacy diuretics consist of a mixed group of diverse agents each only capable of rejecting a maximum of about 5% of the filtered NaCl load. Although the members of this group are insufficiently powerful to be useful on their own as major diuretics, some possess qualitatively unique features as adjunct diuretics, clinically useful when administered together with more powerful agents, acting more proximally in the nephron. Thus, for example, antikaluretics act in the late distal tubule and cortical collecting duct (Site IV) either by competitively antagonizing aldosterone (spironolactone; canrenoate) or by directly interfering with passive reabsorption of sodium chloride at the luminal membrane of these particular tubular segments (triamterene and amiloride).

(ii) *Molecular mechanisms* The first group of diuretics in which it proved possible to explain saluresis in molecular terms were the carbonic anhydrase inhibitors, typified by acetazolamide. In the original functional model of the proximal tubular cell, carbonic anhydrase was thought to be intracellular (Pitts, 1958). More recent evidence shows major carbonic anhydrase localization to both the luminal brush border and the peritubular cell membrane (Wistrand & Kinne, 1977). Though still extremely useful as molecular probes of cell transport mechanisms, carbonic anhydrase inhibitors have long since ceased to be useful diuretic drugs.

For a number of years, the enzyme system which attracted considerable attention as a potential tubular cell target for diuretics was the Na,K-ATPase or ubiquitous sodium pump, known to be located at the peritubular or basolateral membrane of cells (Epstein, 1973). Whilst undoubtedly inhibited in a highly specific way by cardiac glycosides, Na,K-ATPase does not, however, fulfil the criteria needed to serve as a primary receptor for diuretic drugs (Lant, 1985). There is general agreement that Na,K-ATPase in the basolateral membrane is the driving force

for Na^+ transport throughout the nephron. The coupled intake of Na^+ and Cl^- from the luminal surface can be viewed as a 'secondarily active' process sustained by the basolateral active pumping of Na^+ via Na,K-ATPase (Frizzell *et al.*, 1979).

Loop diuretics interact with a neutral Na^+-2Cl^- - K^+ -co-transporter localized within the luminal membrane of the TAL (Greger, 1985). The Tamm-Horsfall protein has been found also to localize to the luminal membrane of site II and so it has been proposed that this protein may be the transport carrier or an important component thereof (Greven, 1983).

The distal nephron and in particular, the collecting ducts contain specific peritubular receptors for binding mineralocorticoids (Stanton, 1985). These respond to the action of aldosterone by induction of RNA synthesis prior to the exchange of Na^+ with K^+ and protons. Aldosterone-induced changes also occur in the activity of peritubular Na,K-ATPase as well as in a number of mitochondrial enzymes involved in both ATP synthesis and the tricarboxylic acid cycle (Morel & Doucet, 1986). With the close chemical similarities of spironolactone and canrenoate to aldosterone, the mineralocorticoid receptor is the likely endogenous ligand for these antihypertensive diuretics (Feldman, 1978).

With respect to triamterene and amiloride, it appears that these agents primarily block the passive luminal entry step for sodium through Na^+ -conductive channels in the late distal tubule and collecting ducts. These channels normally transport sodium down its electrochemical gradient following which Na^+ is extruded actively by the Na,K-ATPase situated at the basolateral membrane. K^+ exchange then follows by passive diffusion out of the collecting duct cells via K^+ -conductive channels traversing the negatively depolarized luminal membrane (O'Neill & Boulpaep, 1979).

The unravelling of most, if not all, of these complex physiological processes of electrolyte transport within the nephron can thus be seen to be a direct consequence of having had available a range of remarkable chemicals possessing the common property of inducing a diuresis.

ACE inhibitors

(i) *Intrarenal haemodynamics* In a sense, the first successful approach at interfering intrarenally with the biological activity of the renin-angiotensin-aldosterone (RAA) system was the development of spironolactone compounds which competitively blocked the action of aldosterone at mineralocorticoid receptors of the distal nephron. Other strategies which were aimed at

inhibiting different stages of the RAA cascade have included the development of renin inhibitors (Haber, 1985) and substrate analogues of angiotensin II (Brunner *et al.*, 1973). However, it was only with the emergence of specifically designed inhibitors of ACE that it became possible for the first time to explore the local effects of angiotensin II (AII) on one of its key target tissues, the kidney. With their considerably shorter history of evolution, much less is known about the intrarenal effects of ACE inhibitors as compared with diuretics.

There are a number of major problems in defining the intrarenal effects of ACE inhibitors. First, although evidence can be obtained to show diminution by these drugs of angiotensin-dependent renal vascular resistance, it is difficult to determine how many of the observed changes are due to reduction in locally formed AII and how much to lowered delivery of circulating AII to the kidney or increased AI or kinin accumulation. A further difficulty relates to the fact that there is a significant compartmentalization of intrarenal AII not only within the interstitium but also at intravascular and intracellular sites.

Despite these many uncertainties, a number of clearcut effects of ACE inhibition emerge both in relation to induced changes in renal blood flow (RBF), glomerular filtration rate (GFR) and interference with tubular transport mechanisms. In both normal humans, on a normal or salt-depleted diet, ACE inhibition is associated with a pronounced increase in RBF (de Zeeuw *et al.*, 1983; McNabb *et al.*, 1986). Associated changes in GFR tend to be less consistent; in some instances there is a rise, in others no change and under other circumstances a fall (Hollenberg *et al.*, 1979; Aldigier *et al.*, 1982). Filtration fraction usually falls. The divergence of the reported changes in GFR may reflect differences in the sodium status of the subjects studied, since it is clear that the haemodynamic responses to ACE inhibition are accentuated by low sodium intake and blunted by salt loading. It is also apparent that the angiotensin-sensitive glomerular mesangial cell receptors which influence glomerular capillary patency are sensitive also to changes in salt balance (Ichikawa & Brenner, 1984).

Thus an important determinant of the overall haemodynamic response to ACE inhibition is the pre-existing influence exerted by the RAA system. Because the predominant effect of ACE inhibition is manifest on RBF with relatively smaller induced changes in GFR, it was assumed initially that AII was exerting its major effects on the efferent glomerular arterioles rather than the afferent arterioles (Imbs *et al.*, 1984). Recent

evidence indicates that ACE inhibition induces reductions in both pre- and post-glomerular arteriolar resistance (Navar *et al.*, 1986).

In essential hypertension, Hollenberg and his co-workers have noted that 40–50% of patients fail to increase renal blood flow in response to sodium loading because they appear to have a defect in renal and adrenal responsiveness to AII (Shoback *et al.*, 1983). These individuals have been termed 'non-modulators' and it is in this subset of hypertensives that ACE inhibition is effective both in reversing the failure of renal blood flow to respond to sodium loading and in causing a substantial blood pressure reduction (Redgrave *et al.*, 1985).

In the situation of renal artery stenosis, the intrarenal haemodynamic responses are even more complicated (Hollenberg, 1984; Wenting *et al.*, 1984). The autoregulatory responses to the fall in perfusion pressure distal to the stenosis result in an increase in afferent arteriolar dilatation and an increase in postglomerular resistance which help to maintain glomerular capillary pressure. Decrease in systemic arterial pressure either through hypovolaemia or ACE inhibition lowers pressure both proximal and especially distal to the stenosis. Whereas these evoked reductions in glomerular capillary pressure can be tolerated in a patient with unilateral renal artery stenosis and a healthy contralateral kidney, if the stenosis is bilateral or involves a solitary kidney, rapidly progressive acute renal failure may develop.

(ii) *Renal tubular responses* In comparison with the wealth of information available on diuretics effects on cellular transport systems within the nephron, little is known yet about the effects which ACE inhibition has on renal tubular functions. Presence of ACE and of angiotensin II receptors within the luminal brush border and basolateral cell membranes (Mendelsohn *et al.*,

1985) supports the view that ACE inhibition interferes directly with the tubular handling of water and electrolytes independently of any effects that may occur through changes in filtered solute load, local changes in intrarenal blood flow, peritubular hydrostatic forces or aldosterone secretion.

Natriuresis occurs with captopril and enalapril in normotensive and hypertensive subjects on both normal and low sodium intakes (MacGregor *et al.*, 1980; de Zeeuw *et al.*, 1983; Lant *et al.*, 1984). A concomitant rise in urinary phosphate and urate excretion (de Zeeuw *et al.*, 1983; Leary *et al.*, 1985; McNabb *et al.*, 1985) suggests that ACE inhibition may also be directly inhibiting proximal tubular transport of these ions.

The uricosuric effect of ACE inhibitors (Table 1) has important clinical application especially where these drugs are given in combination with a thiazide or loop diuretic which, on its own is known to cause a degree of hyperuricaemia in long term use. Thus, for example in chronic antihypertensive management, attenuation of the increase in plasma urate induced by hydrochlorothiazide has been reported when the patients have been treated with a combination of this drug with captopril (Weinberger, 1982).

Mechanism of the antihypertensive effect

Diuretics

Despite their extensive use as a first line form of therapy of mild to moderate hypertension, the precise mechanism of the antihypertensive action of diuretics remains controversial. It is clear, however, that the initial and long-term action of diuretics in lowering blood pressure differ. The early effects are directly related to the induced saluresis and include a consistent fall in plasma volume, a fall in cardiac output and a rise in

Table 1 Uricosuric effects of ACE inhibitors after oral administration in healthy volunteers on normal sodium intake. In the study by Leary *et al.* (1985), uricosuria occurred after monodosing with captopril whilst McNabb *et al.* (1985) noted significant increases in uric acid excretion after 8 days of therapy with enalapril

Drug	Urinary urate excretion (mmol 24 h ⁻¹)		n
	Placebo	Treatment (mean $\pm$ s.e. mean)	
Captopril 100 mg	4.49 $\pm$ 0.18	5.41* $\pm$ 0.26 (Leary <i>et al.</i> , 1985)	13
Enalapril 10 mg	2.98 $\pm$ 0.22	3.76** $\pm$ 0.16 (McNabb <i>et al.</i> , 1985)	10

* $P < 0.05$, ** $P < 0.01$, n = number of subjects

peripheral resistance (Shah *et al.*, 1978). The more sustained shrinkage of plasma volume that occurs with the longer-acting members of the thiazide group of diuretics explains the greater effectiveness of these compounds as antihypertensive agents when compared to loop diuretics such as frusemide (Anderson *et al.*, 1971).

Once the early phase of diuretic action is complete, cardiac output returns to pretreatment levels and thereafter there is a persistent fall in peripheral resistance (van Brummelen *et al.*, 1980). The underlying haemodynamic fault of essential hypertension is thus corrected but there is no generally accepted single hypothesis that has emerged to account for it. The long-term antihypertensive effect is seen with continued doses of diuretic that are below the threshold for effective saluresis. A number of possible mechanisms have been proposed and continue to be debated (Young *et al.*, 1985). These include adaptation of baroreflex sensitivity, local autoregulatory responses to the persistent fall in extracellular fluid volume, diminished vascular reactivity either due to induced changes in intrarterial wall sodium/calcium ion exchange or the enhanced production of endogenous vasodilator substances such as prostaglandins or kinins (Struyker-Boudier, 1983).

ACE inhibitors

Unlike diuretics, in whom antihypertensive efficacy was noted incidentally after the first thiazides had been introduced for treatment of cardiac failure, ACE inhibitors were specifically designed as antihypertensive agents in both conceptual and synthetic terms. One might therefore have assumed their mechanism of blood pressure lowering would be straightforward. Yet early in the clinical experience with ACE inhibitors, doubts were raised as to whether the blood pressure lowering effects of these agents was entirely due to inhibition of the plasma RAA system. First, there is no reflex tachycardia. Second, blood pressure does not return to baseline as soon as therapy with a short-acting ACE inhibitor such as captopril, is stopped, despite return of circulating ACE to pretreatment values. Third, the antihypertensive effect is seen in anephric patients (de Bruyn *et al.*, 1980). These features indicate that other mechanisms must be operative and as yet, they have not been fully defined. Since ACE is the same enzyme that degrades bradykinin, the suggestion has been raised that increased activity of dilator kinins and other peptides may contribute to the blood pressure reduction. Methodological difficulties have made it difficult to prove this point (Zusman,

1984). The ability of cyclo-oxygenase inhibitors to blunt the antihypertensive effects of ACE inhibitors lends support to the participation of prostaglandins in the mechanism of blood pressure reduction (Silberbauer *et al.*, 1982). Other mechanisms invoked include interference with sympathetic nervous systems function (Clough *et al.*, 1982) increased parasympathetic activity (Roman *et al.*, 1985) or altered baroreflex sensitivity (Vidt *et al.*, 1982).

Particular support for a major role of ACE inhibition within the kidney has come from observations which have shown that a sizeable proportion of patients with essential hypertension seem to have inappropriately increased production and/or activity of intrarenal AII. These patients respond to ACE inhibitors by restoring their renal vascular responsiveness and with this, an effective lowering of blood pressure (Williams & Hollenberg, 1985) (see page 36).

Attention is also currently being paid to the significance of inhibiting local tissue ACE at extra-renal sites (Ganten *et al.*, 1984). It is apparent that converting enzyme activity is present in many tissues such as lung, heart, brain and blood vessel wall. Appreciation of the potentially selective effects of different ACE inhibitors upon local tissue converting enzyme is likely to play an increasingly important role in characterizing the various mechanisms that contribute to the antihypertensive and other haemodynamic effects of these drugs.

Dosage considerations and toxic profile

Diuretics, and in particular thiazide and related compounds have been in use for 30 years as first line choices in management of hypertension either as monotherapy or as a baseline treatment when other antihypertensive drugs are also indicated. All the major clinical trials in mild to moderate hypertension worldwide have employed diuretics, and the results have shown significant benefit in terms of reduction in morbidity and mortality with this form of treatment that is both simple to use and of low cost (Moser, 1986; Wilcox *et al.*, 1986). Despite this, concerns have been raised in recent years about the potential long term adverse metabolic effects of diuretics, notably, drug-induced alterations in blood lipids, glucose and uric acid as well as reductions in circulating potassium and magnesium. A sustained clinical debate has centred on the question whether these biochemical abnormalities pose a real threat to health and could even be negating the beneficial effects achieved through lowering blood pressure

(Kaplan, 1983; Perez-Stable & Caralis, 1983; Hollifield, 1984). Yet underlying this controversy there has been a failure by physicians to appreciate that these adverse metabolic effects are largely an iatrogenic phenomenon (Lant, 1986).

Early in the history of thiazide usage in hypertension, studies of the dose-response relationship of the antihypertensive effects of these drugs revealed a flat-shaped curve. Cranston *et al.* (1963) concluded, after investigating the antihypertensive dose-response patterns for three thiazide-like substances, there was little benefit to be gained from using large doses of oral diuretics to reduce arterial pressure. Identical conclusions were reached subsequently by other workers (Tweeddale *et al.*, 1978; MacGregor *et al.*, 1983). Yet these observations were largely ignored and all the large-scale clinical trials of diuretics in hypertension of recent years have employed high-dose diuretic therapy (Lant, 1986). The exception is the EWPHE study (1985). The much-discussed MRFIT study (1982), for example, used 100 mg/day doses of chlorthalidone, or hydrochlorothiazide, whilst the UK MRC trial used 10 mg/day dosage of bendroflumazide (MRC study, 1985) as compared with a more appropriate dose range of 2.5–5.0 mg/day which would be generally regarded as the lowest effective antihypertensive dose for the latter drug.

One might have hoped that with this clear lesson of misunderstood clinical pharmacology of diuretics, when ACE inhibitors began to be applied to the treatment of hypertension, the principle of the lowest effective dose would have been applied. Alas, exactly the opposite occurred. By limiting the use of captopril, when it first appeared, to patients with complicated hypertension, the drug was made available, often on humanitarian grounds, to physicians looking after cases of previously resistant hypertension or hypertension associated with collagenoses. The result was that during the clinical development of the drug, doses often as high as 1 g/day were used. Drug efficacy was immediately obvious but a very misleading toxic profile emerged. Lack of appreciation that the kidney was a major route of captopril elimination was a further problem that led to drug accumulation in several early studies. In many instances, it became difficult to distinguish between drug toxicity and disease-related phenomena. With increasing clinical experience, it became clear that patients with mild to moderate hypertension can be controlled on captopril alone in low dosage and that when used in this way, the drug has outstanding tolerability. The arrival on the clinical scene of the first non-sulphydryl containing ACE inhibitor, enalapril, might have been expected to benefit

from the lessons of over-enthusiastic dosing with captopril, or for that matter the parallel story of thiazide abuse. Unfortunately, within 18 months of the introduction of enalapril into the U.K., an unduly high incidence of adverse effects directly attributable to ACE inhibition led to the issue of warning notices both by the manufacturers—Merck Sharp & Dohme, and the Committee on Safety of Medicines (Current Problems, 1986). The warnings centered around reports of abrupt decreases in blood pressure and functional renal insufficiency—both effects to be expected from the excessive pharmacological actions of the ACE inhibitor.

The unique cluster of adverse effects first reported with captopril and which consisted of rash, dysgeusia, nephrotoxicity and neutropenia, occurred under circumstances of excessive drug dosage in patients with severe renovascular hypertension. It is difficult therefore to judge the true difference between the incidence of these adverse effects for captopril and enalapril, mainly because the latter drug came through a totally different type of development programme. There is no doubt, however, that with the dramatic reduction in captopril dosage, an impressive fall has occurred in incidence of adverse effects; neutropenia, for example running at 0.04% (4849 cases, Stumpe *et al.*, 1984) or dysgeusia, 2.7% (6737 cases, Jenkins *et al.*, 1985). Loss of the mercapto function in enalapril appears to have virtually removed neutropenia and dysgeusia as adverse effects, making it likely that the sulphydryl group was indeed responsible (McFate-Smith *et al.*, 1984). The unheralded emergence of the rare effects of angio-oedema and dry cough, however emphasise the need for continued vigilance in the post-marketing clinical surveillance of ACE inhibitors, even when used at low dose.

Impressive efficacy with a defined toxic profile and the capacity to impart a feeling of well-being as reported by most patients treated with ACE inhibitors, makes it likely that these drugs will be increasingly used for treating less severe forms of hypertension and heart failure either on their own or in partnership with low-dose diuretic therapy. Before becoming unduly critical of either of these two groups of drugs because of unfortunate aspects of their toxic profiles that have been highlighted by overdosage, let us take note of the important messages which study of their clinical pharmacology gave us but which have not been adequately heeded to date. In this way, we may yet learn to apply sensibly and safely two of our most valuable acquisitions in cardiovascular drug therapy to the full benefit of the patient.

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