by quantities of trypsin that have no observable effect on actin and tropomyosin. The purified 120000-M₉ protein binds tightly to actin and tropomyosin-actin. It has very little effect on the activity of a skeletal myosin/VM actin system, but when VSM tropomyosin is present it is a potent inhibitor of the ATPase. Inhibition of up to 90% has been obtained with stoichiometries as low as one 120000-M₉ protein per 20-30 VSM actin monomers (Fig. 2c). Thus the 120000-M₉ protein can account for the inhibitory part of the regulatory mechanism; it is, however, usually Ca²⁺ insensitive. A number of smooth-muscle inhibitory proteins have been reported from other sources, though they are of lower molecular weight and may represent fragments of a 120000-M₉ protein (reviewed in Marston, 1983).

In the intact VSM thin-filament regulatory system there is a Ca²⁺-dependent release of inhibition (Fig. 2a). In skeletal muscle this function is performed by tropomin C. VSM thin-filament preparations contain rather small quantities of proteins resembling tropomin C or calmodulin and it is conceivable that such a protein has been largely lost during preparation. The 120000-M₉ protein binds to a calmodulin affinity column in Ca²⁺ and is released by EGTA so it has the capacity for interaction with a calcium-binding protein. Affinity-column methods are being used to try and recover calcium-binding proteins from VSM preparations.

We have been able to reconstitute a functional Ca²⁺ regulated thin filament using just four proteins, skeletal muscle actin, VSM tropomyosin, VSM 120000-M₉ protein and brain calmodulin, as Ca²⁺-binding proteins (Fig. 2d). This demonstrates in principle the mechanism of Ca²⁺-dependent regulation of the VSM thin filament. The 120000-M₉ protein inhibits actin, tropomyosin propagates the effect over a large number of actin monomers and Ca²⁺-calcium-binding protein complexes bind to the 120000-M₉ protein, relieving the inhibition. There are as yet practical difficulties in demonstrating a calcium-sensitive system using all VSM proteins. Under our experimental conditions the 120000-M₉ protein binds VSM actin+ tropomyosin very tightly indeed (K = 10⁸-10⁹ M⁻¹) and inhibition cannot be relieved by brain calmodulin. It is likely that a change of experimental conditions or a native Ca²⁺-binding protein is required. A similar regulatory mechanism has been proposed for gizzard thin filaments (Kakiuchi & Sobue, 1983).

The Renin–Angiotensin Converting Enzyme Cascade

**Renin and blood pressure**

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**Renin, a blood-borne hormone**

In 1898 Tigerstedt & Bergman extracted a potent, heat labile, non-diffusible, pressor material from the renal cortex of the rabbit. On intravenous infusion it raised blood pressure in test rabbits. They named the material 'renin' and speculated that it was formed in the kidney, then released into blood where as a chemical messenger it produced an effect at a distance constricting peripheral vessels and thereby raising blood pressure. Their speculation is the subject of this review.

The enzymic nature of renin was not recognized until 1939 (see Pickenberg, 1968). It is an aspartyl protease formed in the juxtaglomerular apparatus of the renin cortex, mainly in the afferent glomerular arteriole. Immunoperoxidase techniques stain positively both storage granules and endoplasmic reticulum in the juxtaglomerular cells, suggesting that renin is synthesized as well as stored in these cells (Lindop et al., 1983). Renal renin exists in active and
inactive forms. The review by Inagami et al. (1984) in this Colloquium deals with the chemistry of renin.

Active and inactive forms of the enzyme are released from the kidney into blood. In man active renin cleaves a Leu-Val bond in the N-terminal sequence of a plasma protein substrate to produce the decapeptide, angiotensin I (Tewskbury et al., 1981). The scissile bond in the corresponding substrates for horse pig and rat renin is Leu-Leu (Skeggs et al., 1957). Angiotensin I is largely inactive biologically, but undergoes rapid enzymic conversion to the active octapeptide, angiotensin II. All components of this mechanism, renin, renin-substrate, angiotensin I, converting enzyme and angiotensin II, circulate in plasma (Brown et al., 1983).

**Renin, angiotensin II and blood pressure**

Apart from its ability to raise blood pressure, angiotensin II has a very wide range of pharmacological effects (see Leckie & Semple, 1983; Brown et al., 1979): on the kidney, on the central and peripheral nervous systems and on the secretion of corticosteroids, catecholamines, vasopressin and probably corticotrophin. The existence of these pharmacological effects is not in question. Doubt does arise, however, about their importance as physiological or pathological processes. The great difficulty of interpretation arises from the complex inter-relation of mechanisms by which angiotensin might influence arterial pressure. There are at least six of these. As a blood-borne hormone, angiotensin II may raise arterial pressure rapidly by direct vasoconstriction; also, at lower plasma concentration, it may raise blood pressure gradually by a different mechanism, probably involving the autonomic nervous system (Brown et al., 1981). Another possibility is that blood-borne renin may enter the vessel wall producing angiotensin II locally in an amount sufficient to cause vasoconstriction (Swales, 1979). Renin is also synthesized in blood vessels other than the afferent glomerular arteriole (endoplasmic reticulum stains by immunoperoxidase in some such vessels (Lindop et al., 1983)) and here again it might generate angiotensin II locally (Swales, 1979). Aldosterone excess raises blood pressure and as angiotensin II stimulates aldosterone secretion an increased circulating concentration of angiotensin II may increase blood pressure via an effect on aldosterone. It locally (Swales, 1979). Aldosterone excess raises blood pressure to influence urinary sodium excretion (pressure natriuresis) is important in the long-term regulation of arterial pressure (Guyton et al., 1972). Angiotensin in low dose alters pressure-natriuresis. Finally, there is a large but controversial literature on the possible pressor effects of angiotensin II generated locally within the brain.

Studies in which angiotensin II is infused in normal subjects suggest that the plasma concentration of endogenous peptide is close to a range in which it influences arterial pressure as a blood-borne hormone (Brown et al., 1979). In patients with renin-secreting tumours, high values are found and it is certain that these are responsible for the elevation of arterial pressure. Increased plasma angiotensin II concentration can cause persistent hypertension, therefore. There is also an elevation of plasma angiotensin II concentration in patients with renal artery stenosis, but the elevation is less consistent and the evidence implicating renin and angiotensin in pathogenesis is less certain (Brown et al., 1979).

**Renin inhibitors and blood pressure**

Each step in the sequence renin → angiotensin I → angiotensin II → receptor can be inhibited competitively. We are concerned here with inhibitors of renin. As is described in more detail by Veber et al. (1984) in this Colloquium there are now several highly potent inhibitors of renin. Szkel et al., (1982a, 1983) have synthesized a series of inhibitors which are analogues of the transition state of renin-substrate (Table 1). The affinity of a protease for the transition state of its substrate is greater than its affinity for the unmodified substrate (Hofmann, 1974). Analogues of renin-substrate were therefore designed to imitate this transition state. With a reduced isostere, statine, or a hydroxy isostere in the cleavage site large increases of inhibitory potency were produced (Table 1). The most potent inhibitor so far is the hydroxy isostere (Table 1).

Renin inhibitors are enzyme-specific. For example, they are much less potent inhibitors of closely related aspartyl proteases such as cathepsin D and pepsin (M. Szkel, M. Tree, B. Leckie, B. Atrash, S. Beattie, B. Donovan, A. Hallet, M., Hughes, M. D. Jones, A. F. Lever, J. J. Morton & J. Sueiras, unpublished work). They are also species specific: an inhibitor of human renin is relatively ineffective against dog renin (Table 1) and vice versa (Szkel et al., 1983). Hydroxy isosteres and analogues with statine substituted in the scissile bond are particularly enzyme-specific while the reduced isostere is enzyme-specific and even more species-specific (M. Szkel, M. Tree, B. Leckie, B. Atrash, S. Beattie, B. Donovan, A. Hallet, M. Hughes, M. D. Jones, A. F. Lever, J. J. Morton & J. Sueiras, unpublished work). The concentration of angiotensin II in plasma and arterial pressure also fell markedly (Fig. 1). The fall of

| Table 1. Five inhibitors of renin based on the N-terminal sequence of human renin substrate |
|---------------------------------|---------------------------------|
| H.112                          | 6    7    9    9    10  11  12  13 | Human renin |
|                                | 313000 > 1000000  |
| H.113                          | 190  155000  | Dog renin |
| H.176                          | 17    220  | |
| H.194                          | 2.7    20  | |
| H.261                          | 0.7    20  | |

Vol. 12
Changes of plasma angiotensin II concentration and mean arterial pressure in six anaesthetized baboons before, during and after infusion of H.261 at 0.1 and 1.0 μmol/kg per h. Comparison by paired t test from the first sample: *, P < 0.05; **, P < 0.01; ***, P < 0.001. Abbreviations: ANG, angiotensin; MAP, mean arterial pressure. From M. Szellke, M. Tree, B. Leckie, B. Atrash, S. Beattie, B. Donovan, A. Hallet, M. Hughes, M. D. Jones, A. F. Lever, J. J. Morton & J. Sueiras (unpublished work).

Blood pressure was related to the fall of plasma angiotensin II concentration (r = 0.73, P < 0.001). This experiment suggests strongly that angiotensin II contributes to the maintenance of arterial pressure in the salt-deplete anaesthetized baboon. The anaesthetic was probably not important in producing this response since similar changes occur in the conscious state in dogs (Szellke et al., 1982a), monkeys (Burton et al., 1980) and man (Fig. 2 and Webb et al., 1983).

Conclusions

Our main conclusions from these and earlier studies (Brown et al., 1979) are that angiotensin II circulates in plasma at a concentration capable of influencing arterial pressure, partly by direct vasoconstriction and probably also by a slower-developing mechanism. Renin is also present in blood vessels and in the brain, but these tissues are less accessible than blood for analysis which may be the reason why knowledge of the role of extravascular renin is less well advanced. Meanwhile, we conclude that Tigerstedt & Bergman (1898) were correct: renin is released from the kidney into blood and it is a blood-borne chemical messenger producing an effect at a distance by acting (indirectly via angiotensin II) on blood vessels. Although correct theirs is unlikely to be the whole story.


Biochemistry of renin

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Purification

Lack of a pure preparation of renin was one of the major obstacles to the progress of research on the renin–angiotensin system and high blood pressure. Successful purification of renin initiated a spectacular development of renin research. The purification of renin in the early stage was attained by the application of an affinity chromatographic technique employing pepstatin as an affinity ligand. Pepstatin had been found to inhibit acid proteases strongly and renin with a moderate K value. This moderate affinity of pepstatin to renin turned out to be ideal for the affinity chromatography. Use of protease inhibitors was essential in obtaining stable preparations of renin from kidneys (Murakami et al., 1975; Yokosawa et al., 1979). Renin obtained by conventional chromatographic methods from mouse submandibular gland which is rich in renin (Cohen et al., 1972; Misono et al., 1982). Renin from this gland was found to immunologically cross-react with mouse kidney renin. Renin was found in other tissues as well. Hirose et al. (1982) obtained it in a homogeneous state from mouse submandibular gland further confirmed the close structural similarity of renin and acid proteases. This renin was found to consist of one heavy chain with 288 amino acid residues and one arginyl residue in the active site of renin. These results indicated a close similarity of renin with acid proteases in its active site structure.

Amino acid sequence

Determination of the amino acid sequence of mouse submandibular gland renin further confirmed the close structural similarity of renin and acid proteases. This renin was found to consist of one heavy chain with 288 amino acid residues and one light chain with 84 residues connected by a disulphide bridge (Misono et al., 1982a). Its amino acid sequence determined by Edman degradation (Misono et al., 1982a) or that deduced from the nucleotide sequence of its cDNA (Panthier et al., 1982) and human renin sequence similarly deduced from its cDNA nucleotide sequence (Imai et al., 1983) showed greater than 40% sequence identity with porcine pepsin (Sepulveda et al., 1975) or bovine chymosin (Folman & Pedersen, 1977). A greater than 20% homology was observed even with the fundamental enzymes such as penicillopepsin (Hsu et al., 1977). Note-worthy is the conservation of long sequences of amino acids in the vicinity of the two catalytically essential amino acid residues Tyr-75 and Arg-308 are also well conserved among the acid proteases and renin (Fig. 1b), indicating the essential nature of these residues in support of the observations made in the chemical modification studies. A degree of similarity between renin and acid proteases also seems to exist in their tertiary structures which are largely determined by amino acid sequence since the co-ordinate of the three-dimensional structure of a fungal acid protease endothiapepsin accommodates the amino acid residues in the renin structure (Blundell et al., 1983).

While renin and acid proteases share several common features, the restricted substrate specificity and neutral pH optimum distinguish renin from acid proteases. The stringent substrate specificity must be due to selective substrate interactions involving up to seven subsites since renin requires a substrate structure consisting of a minimum of six or seven amino acid residues. The structure of the subsites has not been identified. The structural basis for the neutral pH optimum has not yet been identified either. The amino acid sequence deduced from the nucleotide sequences of the cDNA of mouse renin (Panthier et al., 1982) and human renin (Imai et al., 1983) contains the sequence of the signal peptide and activation peptide. Based on the N-terminal structure of active renin and on postulated signal peptide sequences, the pro-sequence and potential activation peptide sequences were deduced (Corvol et al., 1983; Imai et al., 1983) as shown in Fig. (1a). The conspicuous lack of homology (less than 10%) between the activation peptides of the renin zymogen and pepsinogen suggests different mechanisms of activation for renin and acid proteases (Corvol et al., 1983). The presence of paired basic amino acids -lys...