Conversion of Angiotensin I to Angiotensin II

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The angiotensin I converting enzyme has two important functions: it inactivates bradykinin and converts angiotensin I to angiotensin II. Inhibition of the enzyme blocks the renin-angiotensin system and decreases systemic blood pressure if the pressure is maintained or increased by renin. The enzyme occurs in a variety of tissues and cell forms. The vascular endothelial cells of the lung and of peripheral blood vessels, and the epithelial cells of the kidney tubules are major sources of the enzyme. In addition to inactivating hypotensive peptides and activating a hypertensive one in the systemic circulation, the enzyme may affect organ functions by hydrolyzing peptides that are formed and released locally.

Although angiotensin I converting enzyme was discovered over two decades ago [1–3], the literature dealing with this protein has increased exponentially only within the last few years. As we now know, angiotensin I converting enzyme does more than its name would indicate, that is, it has functions other than just the cleavage of angiotensin I to angiotensin II and dipeptide. It is present in many tissues and cleaves other peptides such as plasma kinins [4–6]. Because converting enzyme breaks peptidyl-dipeptide bonds in substrates other than angiotensin I, it is referred to in the literature as peptidyldipeptide hydrolase or peptidyl dipeptidase (E.C. 3.4.14.1; 7*). The term dipeptidyl carboxypeptidase [6], although incorrect, has also been used to describe converting enzyme. Converting enzyme is identical with kininase II that cleaves bradykinin [7] (Table I).

The enzyme was discovered by Skeggs and his associates in the mid 1950's [1–3]. They noticed that horse plasma contains an enzyme that converts angiotensin I to angiotensin II. Renin releases the decapeptide angiotensin I from angiotensinogen, the substrate of renin and the decapeptide is in turn converted to the octapeptide angiotensin II by the removal of a histidyl-leucine dipeptide from the C-terminal end. Helmer [8,9] also observed the existence of a factor in plasma that activated his angiotensin preparation in vitro.

Intravenous injections of either angiotensin I or II raise the blood pressure similarly, but angiotensin I is usually inactive or much less active when assayed on isolated tissues in vitro. Thus, an-

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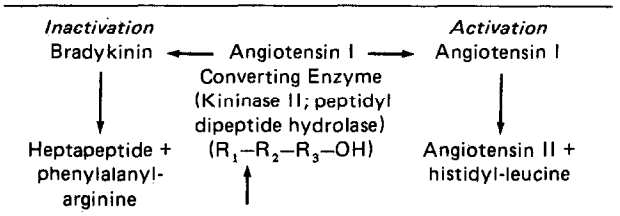
* Enzyme Commission.

TABLE I Mode of Action of the Angiotensin I Converting Enzyme



giotensin I must be converted enzymatically to angiotensin II before becoming fully active in most biological systems in vitro.

Originally plasma was suspected as the site of conversion of angiotensin I. Vane and his associates [10–12] studied the fate of angiotensin I and II by the use of an isolated superfused organ bath system which distinguished the effects of angiotensin I and II in circulating blood. They found that conversion in plasma was too slow to account for formation of angiotensin II in vivo. When they injected angiotensin I intravenously into the dog, they recovered angiotensin II after pulmonary passage. Little or no angiotensin II was found in venous blood when angiotensin I was injected into the renal or femoral artery. Vane and his colleagues [10–12] and Biron and Huggins [13] concluded that the pulmonary circulation is the site of conversion of angiotensin I. Bradykinin is rapidly inactivated during passage through the vascular bed of various organs; the lung is especially active in this respect also [14,15]. Ng and Vane [11] suggested that angiotensin I and bradykinin may be cleaved by the same enzyme in lung, although they proposed that carboxypeptidase N or kininase I could convert angiotensin I to II presumably by the sequential cleavage of the last two amino acids.

Between 1961 and 1967 we independently studied the enzymatic inactivation of bradykinin [4,5,16]. We noted that plasma contains a carboxypeptidase which inactivates bradykinin by the removal of a single amino acid, the carboxyl terminal arginine. This enzyme was named kininase I or carboxypeptidase (arginine carboxypeptidase E.C. 3.4.12.7). Then a second kininase, named kininase II, that cleaved the Pro^7-Phe^8 bond and released phenylalanyl-arginine was found in a bacterial extract, in a microsomal fraction of the homogenized hog kidney cortex and in human plasma [4,5,16].

Thus, the most potent hypertensive peptide, angiotensin I, was activated by the enzymatic removal of the dipeptide His-Leu by the converting enzyme, and the hypotensive bradykinin was inactivated by the cleavage of the dipeptide Phe-Arg. Despite the similarity of these enzymatic processes, proof that kininase II and angiotensin I converting enzyme are the same protein had to wait until the enzymes involved were purified [6,7,17,18] and additional synthetic peptide substrates [19], specific inhibitors [7,20] and antibody to the enzyme became available [21].

BIOCHEMICAL PROPERTIES

The converting enzyme is a protein molecule which has 8 to 26 per cent carbohydrate components [21,22]. It is bound to membranes and, during centrifugation of homogenates, it sediments at high speed with the particulate fraction [23–25]. The enzyme was first extracted and concentrated from a microsomal fraction of kidney [4]; then it was purified from plasma [5]. Cleavage of angiotensin I and bradykinin by the same enzyme was shown first with purified plasma enzyme [6,17], and subsequently with enzyme preparations obtained and purified from lung or kidney [7,17,18,20,22,26–31]. Homogeneous proteins were obtained from human, hog and rabbit lung or kidney. The complete purification of the enzyme from the lung was simultaneously reported by Igic et al. [20] and Cushman and Cheung [27].

Because much of the enzyme sediments with a particulate fraction of homogenized tissue, detergents or organic solvents have been used to extract it from crude homogenates [4,17,21,22,26,31,32]. These harsh methods of extraction as well as the large carbohydrate component may contribute to discrepancies in the reported molecular weight, because the estimates of molecular weight range from 129,000 to 480,000 [21,22,28,30–34]. The enzyme in the rabbit lung was reported to have the lowest molecular weight [22]. The enzyme purified in our laboratory from hog or human lung and kidney had a similar molecular weight [21,30,35]. Converting enzyme requires a divalent cation cofactor for activity [2,17,26,28]. Just as many other peptidases do, the native enzyme contains zinc [22]. In addition to the monomeric form of the hog lung enzyme, a dimer and an aggregate of even larger molecular weight was found [30].

Antibody induced by injecting purified renal enzyme into rabbits [21,35] cross reacts with the lung and plasma enzyme from the hog. The conversion of angiotensin I and the inactivation of bradykinin by hog lung, kidney and plasma enzyme are blocked to a similar extent by the antibody [21]. Antibody to hog kidney enzyme does not cross react with the human enzyme when the antibody is diluted, indicating that there is a species specificity. An apparent cross reactivity of antibody to hog enzyme with rat lung enzyme was reported [36].

The inhibition of the enzyme by antibody depends, however, on the structure of the substrate used. The hydrolysis of bulkier synthetic substrates by renal converting enzyme is inhibited by purified antibody, although the cleavage of smaller ones is not blocked at low concentrations of the antibody. This finding indicates that the antigenic and hydrolytic sites of the enzyme protein differ. Presumably, attachment of the antibody to the antigenic site partially blocks the hydrolytic center by a steric effect [21,35].

Table I summarizes the information on the structure of substrates [7,37]. The general structure is R_1-R_2-R_3-OH. The enzyme cleaves R_1 from R_2. R_1 can be a protected amino acid or a peptide. R_3 should be an amino acid with a free carboxyl terminal but not glutamic acid [18]. R_2 can be any amino acid except proline, because peptides having proline in the R_2 position are not cleaved. This explains why liberated angiotensin II is not broken down further by the converting enzyme [6].

Initially, the naturally occurring substrates, angiotensin I and bradykinin or its fragments, were used to assay enzymatic activity [1–5,7,10–12,17]. This was determined by bioassay or chemical assay or by using labelled substrate [19,20,38]. Then peptide substrates shorter than angiotensin I, but containing the protected C-terminal sequence of angiotensin I, such as Z-Phe-His-Leu [39,40] or Hip-His-Leu [26], were introduced. These peptides were used in chemical assays of the enzyme. When converting enzyme was found to cleave peptide bonds of a wide variety of amino acids [6], other peptide substrates were synthesized. The simplest were protected tripeptides of glycine, such as Hip-Gly-Gly [6,17] or dansyl-Gly-Gly-Gly [20]. Other substrates contain chromophore groups, such as *p*(NO_2)-phenylalanine as in *t*-BOC-*p*-(NO_2)Phe-Phe-Gly [17] or in Z-Phe(NO_2)-Gly-Gly [41].

The assays are based either on measurement of the amount of the dipeptide split off from the substrate or on the protected amino terminal portion of the hydrolyzed substrate molecule. The dipeptide can be assayed by an automated continuous-flow method [42] or in an amino acid analyzer (Erdös, to be published), or in the fluorometer [40,43,44] after coupling it at the histidyl residue to a fluorescent compound. The hippuric acid cleaved from substrates such as Hip-His-Leu or Hip-Gly-Gly can be measured after extraction from the incubation mixture [26] with an organic solvent or by directly recording its release in the ultraviolet spectrophotometer [17]. When radioactive labeled peptide substrates are used [19,20,38,45], the unhydrolyzed substrates and the products have to be separated by chromatography or by electrophoresis. It is not difficult to determine the activity of the enzyme purified or extracted from tissues where it occurs in a high concentration. The assay of the enzyme, however, can be problematic in the presence of contaminating peptidases or when high concentrations of other proteins are present, such as in human plasma.

Skeggs and his associates initially described that chloride ions activate converting enzyme [1]. However, chloride ions affect the hydrolysis of bradykinin and angiotensin I differently. The in vitro conversion of angiotensin I stops almost entirely when the incubation medium lacks chloride ions, although the enzyme inactivates bradykinin in a chloride-free medium [46]. Because of this difference, the inactivation of bradykinin and conversion of angiotensin I were attributed to two separate enzymes [47]. The effect of chloride ions on the rate of hydrolysis depends on the structure of the substrate [46–49], on the concentration of the ions and on the pH [49]. The enzyme has very low activity with Hip-Gly-Gly or angiotensin I as substrate in chloride-free medium, but it still cleaves bradykinin at about 30 to 50 per cent of the optimum rate [46,48]. The cleavage of another pentapeptide substrate, the inhibitor BPF5a or SQ 20475 [17,50], does not require the presence of chloride ions at all. Chloride was called an allosteric modifier of the enzyme [50], and indeed chloride ions lower the Michaelis constant (Km) and increase the V_{max} of bradykinin with the converting enzyme [48]. However, angiotensin I can inhibit hydrolysis of bradykinin by converting enzyme even in a medium low in chloride ions [51]. When the enzyme is immobilized by coupling it to Sepharose-4B, it still cleaves angiotensin I at an appreciable rate in the absence of chloride ions [29,46]. After studying the ultraviolet spectrum of the enzyme in the presence and absence of chloride ions, we hypothesized that the enzyme protein could combine with bradykinin in two different configurations, but that it can cleave angiotensin I in only one configuration [21]. Because the enzyme is bound to membranes in tissues, we do not know how much its conformation can change in situ.

The differences in the kinetics of hydrolysis of bradykinin and angiotensin I may explain another phenomenon, why in some cases in vivo angiotensin I is not converted by the lung enzyme, whereas bradykinin is inactivated by it [52]. Bradykinin has a much higher affinity to the enzyme than angiotensin I, as shown by the lower Km of bradykinin reported by several investigators [7,33,48,53].

OCCURRENCE

Although converting enzyme was discovered in horse plasma [1–3] and kininase II was found in kidney [4] and in human plasma [5], the lung enzyme has been studied most. Centrifugation of homoge-

nized lung tissue yielded a particulate fraction that converted angiotensin I and inactivated bradykinin [17,24,25], but initially the two activities were attributed to two different enzymes [23]. Pulmonary arteries convert angiotensin I faster than vessels taken from other vascular beds [54,55]. The amount of converting enzyme in the homogenized lung varies from species to species. Rat, rabbit or mouse lungs for example have about seven to nine times more activity than the human lung [56].

In the lung, the surface of the vascular endothelial cells is rich in enzyme, as shown by using electron microscopy and tagged antibodies to localize the enzyme [36]. The location of converting enzyme may be on the pinocytic vesicles of the endothelial cells [57,58]. Even pulmonary vascular endothelial cells of the rabbit grown in tissue culture have some enzymatic activity [59]. Human endothelial cells from the umbilical vein grown in tissue culture are an additional source of the enzyme, suggesting that endothelium outside the lung is also important in the metabolism of peptides [60]. In addition to endothelial cells, some kidney cells of epithelial origin contain a very high concentration of converting enzyme [61,62]. By using fluorescent antibody to converting enzyme, we found converting enzyme all along the nephron but especially concentrated in the cells of the proximal tubules [63]. The brush border of the proximal tubules is rich in converting enzyme. This was shown after separating brush border membrane fragments of kidney homogenates of rats by differential centrifugations [62]. A transplanted rat tumor originating from the proximal tubules (MK2) has a high concentration of the enzyme [63].

In addition to lung and kidney, many tissue extracts and various cells contain the enzyme as measured in vitro [7,27,39,64]. For example, high activity has been found in rat testicles [64], in the pituitary gland and in the striatum of rat brain [43], in the caudate nucleus of human brain [65] and in the choroid plexus of dogs and rabbits [66]. Converting enzyme (or kininase II) activity also has been detected in bacteria [16,67–69].

The concentration of the converting enzyme in plasma varies from species to species. For example, guinea pig plasma [17] has the highest level of converting enzyme of the animals tested, but dog plasma has only traces of it. The origin of the plasma enzyme is not definitely established. Recent studies showing higher than normal activity in plasma of patients with sarcoidosis indicate that it may be released from the lung [70,71]. Because renal lymph contains converting enzyme, it may be liberated from the kidney also [72]. Histamine, histamine releasers and anaphylactic shock can release converting enzyme from the perfused blood-free lung [46,73] indicating that damage to the lung may result in loss of enzymatic activity. The concentration of the enzyme in developing edema fluid of the rat lung was much higher than in the perfusion fluid [46].

Recently several investigators reported the existence of different types of angiotensin I converting enzymes that do not cleave bradykinin. They were found in human lung [74,75], guinea pig plasma [76] and in rat submaxillary gland [77]. The enzyme from the latter source, named tonin, forms angiotensin II from either angiotensin I or from the synthetic tetradecapeptide renin substrate. Tonin has a much lower molecular weight than the peptidyl dipeptidase converting enzyme, and its pH optimum is below neutrality. It is not inhibited by inhibitors of the converting enzyme, and it occurs in the submaxillary gland and other organs such as the kidney [77]. An enzyme that has a higher molecular weight than the converting enzyme has been found in hog and guinea pig plasma; its action becomes apparent only in the presence of added cobalt ions (Co^{2+}). This enzyme releases angiotensin II, but it has no kininase activity [76]. Another enzyme with properties resembling tonin, was concentrated from human lung, but its molecular weight was estimated to be an order of magnitude higher [74,75]. It should be mentioned here that converting enzyme can also release angiotensin I from the synthetic tetradecapeptide substrate of renin by the sequential cleavage of C-terminal dipeptides [77a].

INHIBITORS

Angiotensin I converting enzyme is inhibited in vitro by numerous compounds. Among them are dipeptides cleaved from angiotensin I or bradykinin (His-Leu and Phe-Arg; [5,17]) and metal sequestering agents such as EDTA [2,5], o-phenanthroline or 8-OH-quinoline [5,17,37]. Competitive substrates of converting enzyme such as bradykinin or Hip-His-Leu block the conversion of angiotensin I [20,37]. Structural analogues of angiotensin I modified in various positions in the peptide chain inhibit the conversion of angiotensin I [78]. The N-terminal tripeptide portion of bradykinin, Arg-Pro-Pro, is an inhibitor in vitro [49,79]. Among other peptides that occur in the body, insulin, the B chain of insulin, glutathione and a not yet characterized factor in plasma are also converting enzyme inhibitors in vitro [7,20].

Various substances including snake venoms [80,81], proteolytic enzymes [82] and peptides [83,84] can potentiate the action of bradykinin on

the isolated muscles. Although the mode of action of these potentiators is still not completely understood, some of them potentiate the action of bradykinin by inhibiting its breakdown by kininase II of tissues. Potentiating peptides extracted from venoms of Bothrops jararaca and other snakes have yielded compounds that inhibited the conversion of angiotensin I as well [23,85–87]. A number of these peptides have been synthesized. The first one was a pentapeptide, BPF_{5a} [85,86], which is a good inhibitor in vitro, but it is also a substrate of the enzyme [17,50]. This peptide is a less effective inhibitor in vivo and has a shorter duration of action than the longer peptide analogues [87–91]. The nonapeptide SQ 20881 (BPF_{9a}; [88]) and the undecapeptide "Potentiator C" [89] contain a C-terminal Pro-Pro sequence that is not cleaved by the converting enzyme. Thus, they inhibit the hydrolysis of bradykinin or angiotensin I without being substrates of the enzyme. The I_{50} values of the peptide inhibitors are low. In concentrations ranging from 10^{-6} M to 10^{-9} M [20,49,50], they block 50 per cent of the activity of the converting enzyme as assayed with optically active short peptide substrates.

The snake venom peptides are among the few gifts venomous animals have made to mankind. Many investigators have recognized the potential importance of inhibitors such as SQ 20881 since they are tools to help us in the study of converting enzyme. Inhibitors have been employed to establish the identity of kininase II with the converting enzyme and to block the conversion of angiotensin I or the inactivation of a kinin in tissues. They have been used in laboratory tests to determine whether a substrate is cleaved by converting enzyme or by another contaminating enzyme present in an extract. They have been given to man and to experimental animals to explore the importance of the functions of converting enzyme and to specifically block the renin-angiotensin system under a variety of conditions [7].

FUNCTIONS AND INHIBITION OF THE ENZYME

The pulmonary circulation has a high capacity for conversion of angiotensin I and inactivation of bradykinin, but it is not the only vascular bed in which converting enzyme acts on the peptides. Although bradykinin is almost completely inactivated in the pulmonary circulation [14,15], about half of a given dose of labeled angiotensin I was converted during a single passage through rat [20] or dog lung [92]. Ryan et al. [93] estimated a 15 to 20 per cent yield of angiotensin II after perfusing angiotensin I through the rat lung. In the rat [94] and in the guinea pig, intravenous or intra-arterial injections of angiotensin I have nearly equipotent pressor effects which indicate sites of conversion of angiotensin I other than the pulmonary vascular bed (P. E. Ward, personal communication). Part of a single dose of angiotensin I can be converted in the peripheral, splanchnic or renal circulation [94–106]. The hind leg of the dog converted from 30 to 40 per cent of infused angiotensin I [96,104] whereas the isolated kidney of the rat was only 1 to 5 per cent effective [106]. Twenty-six per cent conversion was found in the vasculature supplied by the circumflex coronary artery [104]. The pulmonary circulation, however, is distinguished from other vascular areas by the lack of metabolism of angiotensin II during passage through the lung [15].

The presence of a high concentration of the enzyme in the brush border of the renal-tubular epithelium [62] indicates that in the kidney the enzyme has functions other than cleavage of circulating peptides. It may inactivate bradykinin and convert angiotensin I after they have been filtered at the glomerulus. Indeed, urinary kinins [107] do not originate from blood [108] but are most likely released by renal kallikrein into the nephron at a point distal to the proximal tubules [62].

In man, during cardiac catheterization, a 20 to 40 per cent pulmonary conversion of angiotensin I was described in normal subjects [101,102]. In patients undergoing cardiac surgery, during cardiopulmonary bypass the elevated level of renin brought about an increase in circulating angiotensin I and II. This was taken as an indication of extrapulmonary conversion of angiotensin I in man [109].

In four patients undergoing cardiac catheterization (^{3}H)-Phe^{8}-bradykinin was infused into the pulmonary artery. In plasma collected from the brachial artery 5 to 18 seconds after bradykinin administration, 4 to 16 per cent of the administered radioactivity was present as intact bradykinin and 60 to 70 per cent of the administered radioactivity proved to be phenylalanylarginine released by converting enzyme (J. J. Pisano, personal communication). Thus, converting enzyme is possibly the major kininase in human lung.

Local infusion of either angiotensin I or angiotensin II constricts the arteries and veins of the forearm in man. The vasoconstrictor effect of angiotensin I, but not that of angiotensin II, was abolished by the inhibitor SQ 20881, suggesting that the converting enzyme is active in the human peripheral vasculature [110].

The activity of converting enzyme in plasma of normal subjects and of hypertensive patients was about the same [111]. Furthermore, the amount of

angiotensin I converted in the blood free perfused lung of the spontaneously hypertensive rats of the Okamoto strain was the same as in normal animals [20].

In laboratory experiments the pentapeptide inhibitor, BPF_{5a}, lowered the increased arterial blood pressure during the duration of infusion in rats with renovascular hypertension [87]. The nonapeptide inhibitor, SQ 20881, had a longer-lasting effect [90,91,112]. SQ 20881 blocked the conversion of angiotensin I in anesthetized rats, in unanesthetized rats and in dogs similarly [90,91,112]. Intravenous injection of SQ 20881 in the rat potentiated the vasodepressor effect of bradykinin. The concentrations required for this effect were lower than those used to block the vasopressor action of angiotensin I [90]. Since converting enzyme inhibitors also prolong the vasodepressor effect of bradykinin [90,112,113], it is sometimes difficult to establish from hemodynamic studies the extent of blockade of angiotensin I conversion by the inhibitor.

In experimental animals [114,115], as in man [116], administration of the inhibitor increased the level of renin in blood, probably by eliminating the feedback inhibition of renin release by circulating angiotensin II.

The effect of the inhibitor in man or in animals may depend on the salt intake. In salt-depleted rats, infusion of SQ 20881 resulted in a profound decrease in blood pressure, while producing a neglible effect in the salt-loaded group [117]. In other studies, a low sodium diet and the diuretic mercuhydrin reduced the converting enzyme activity of dog kidney fourfold [118].

In addition to its effects on the systemic blood pressure, SQ 20881 can influence organ function by inhibiting converting enzyme. Long-term alveolar hypoxia produces pulmonary arterial vasoconstriction and pulmonary hypertension in rats. The involvement of the renin-angiotensin system was indicated in experiments in which repeated administration of SQ 20881 reduced right ventricular hypertrophy and pulmonary vascular changes [119]. In mice, alveolar hypoxia lasting over a week increased the activity of lung and serum converting enzymes in parallel manner [120].

Injection of SQ 20881 into anesthetized dogs increased the effect of bradykinin on renal blood flow and prolonged the survival of this kinin during passage through the kidney. The amount of free endogenous kinin increased in the venous blood and in the urine [113]. SQ 20881 increased total renal blood flow and decreased the glomerular filtration rate in dogs. It was assumed that these effects were due to the blocking of the inactivation of bradykinin, although the experiments did not distinguish between the potentiation of the effect of bradykinin and inhibition of the conversion of angiotensin I [121].

The importance of the function of converting enzyme and its inhibition was investigated in circulatory shock. Pretreating dogs, shocked by injection of endotoxin, with SQ 20881 abolished or greatly attenuated the secondary increase in systemic arterial blood pressure that followed the initial sharp decrease [122]. In hemorrhagic shock the secondary increase in systemic blood pressure was significantly lowered by pretreatment with SQ 20881 [122]. Pretreatment or early post-treatment of dogs with SQ 20881 increased the survival rate of the animals in hemorrhagic, but not in endotoxin shock [123]. The beneficial effect of SQ 20881 was attributed to prevention of a severe vasoconstriction in the mesenteric vascular bed that was thought to be responsible for the irreversible hemorrhagic shock. SQ 20881 also blocked intestinal vasoconstriction in cats. In these animals vasoconstriction was induced by volume depletion, and the effect of vasopressin was abolished by hypophysectomy [124].

Hemmorhagic shock also decreased blood flow to the kidney, possibly by activation of the renin-angiotensin system. Renal angiograms indicated that during hemorrhage the diameter of the main renal artery was reduced by 40 per cent and that of the intrarenal branches by 70 per cent. Infusion of converting enzyme inhibitor during decompensation decreased renal vascular resistance and increased the diameter of the renal arteries two to threefold [125].

In addition to blocking the liberation of angiotensin II, SQ 20881 can inhibit the release of angiotensin III (des-Asp^1-angiotensin II). Angiotensinase A removes aspartic acid from the amino terminal end of angiotensin I. The resulting nonapeptide (des-Asp^1-angiotensin I) can be converted by the converting enzyme to a heptapeptide (angiotensin III) that is biologically active since it can stimulate steroidogenesis in the adrenal cortex [126].

SQ 20881 was also used to demonstrate that angiotensin I has intrinsic activity independent of its conversion to angiotensin II. Thus, in some biologic experiments, angiotensin I itself must have been active because inhibition of converting enzyme had no effect on the action of injected peptide. In the isolated perfused dog kidney, angiotensin I diminished inner cortical blood flow and so it affected the intrarenal distribution of blood flow. This effect was not changed by inhibition of converting enzyme with SQ 20881 [127]. A direct action of angiotensin I on the vasculature of the kidney was also suggested by Carriere and Biron [128].

The role of converting enzyme in fluid intake and

retention was also investigated. In some animals the stimulation of this action may be a direct effect of angiotensin I. Angiotensin I has a 1.6 times stronger dipsogenic effect than angiotensin II when given intravenously to rats. The drinking produced by angiotensin I infusion was potentiated, and not blocked by SQ 20881 [129]. Centrally administered SQ 20881, however, blocked the dipsogenic effects of angiotensin I [130–132]. The effect of SQ 20881 on the drinking reflex has not been clearly established yet. Although angiotensin I and angiotensin II have a strong dipsogenic effect in rats [129,133,134], in other species the direct action of angiotensin I on the central nervous system may be different. Choroid plexus of dog and rabbit has a high converting enzyme activity indicating the importance of conversion of angiotensin I in the central nervous system [66]. After intracerebroventricular administration, SQ 20881 reduced the dipsogenic effects of renin or angiotensin I given to cats by the same route [135].

Possibly the most important application of the converting enzyme inhibitors is in the study of experimental and clinical hypertension. That both the pentapeptide (BPF_{5a} or SQ 20881) and the nonapeptide BP_{9a} (SQ 20881) inhibitor can lower the systemic blood pressure of rats if increased by renin, was shown soon after the peptides became available [87,91]. Extensive studies have been made in other animals.

In the dog, acute stenosis of the renal artery increased the blood pressure, after the contralateral kidney was excised, and caused renal vasoconstriction. Subsequent administration of SQ 20881 decreased renal vascular resistance and systemic blood pressure [136,137]. In chronic experiments in dogs, hypertension was induced by constriction of one renal artery. This form of hypertension was prevented by a constant intravenous infusion of the inhibitor. Single injections of the inhibitor were more effective in the early phase of hypertension than in the later phase, possibly because of the importance of salt and water retention in the maintenance of hypertension [115,138]. Similar results were obtained in rabbits [139].

Malignant hypertension was also induced in rabbits by unilateral nephrectomy and by clipping the renal artery of the remaining kidney. Long-term administration of SQ 20881 prevented the development of malignant hypertension during the three week duration of the experiments [140].

Animal studies were soon followed by clinical investigations. SQ 20881 was employed clinically to study the importance of renin in maintenance of blood pressure and in hypertension, and to determine the possibility of the application of the inhibitor in therapy. In clinical studies, it was found that the effect of the converting enzyme inhibitor was much longer lasting than that of angiotensin II antagonists such as Sar^1-Ala^8-angiotensin II [141]. Unfortunately, both angiotensin antagonists are straight-chained peptides and have to be given intravenously. Since peptides of the molecular weight of SQ 20881 usually have a short half-life in the circulation (minutes), the long-lasting effect of SQ 20881 is probably due to its attachment to the surface of the converting enzyme. After giving an intravenous dose of 4 mg/kg to patients, the blockade of converting enzyme lasted for 16 hours [116]. At a dose of 0.5 mg/kg, the effect of inhibitor decreased 50 per cent in 3 hours [142].

In normotensive patients, SQ 20881 had no effect on blood pressure. When persons adhering to a normal sodium diet were kept in the supine position, a sudden upright tilting led to renin release [143]. These subjects, however, could compensate for the inhibition of converting enzyme without significant hemodynamic changes. In sodium-depleted subjects in the same type of experiments, administration of converting enzyme inhibitor decreased the systolic and diastolic blood pressure significantly whereas the heart rate increased. Four of five subjects in the experiments fainted. The results were taken as an indication that angiotensin II is essential for maintaining the blood pressure in persons depleted of sodium [136]. The inhibitor also blocked the rise in the aldosterone level that accompanied increased renin activity in control subjects.

The effect of SQ 20881 was studied extensively in 13 hypertensive patients [116]. In hypertensive patients with an elevated renin level, the blood pressure decreased. In these studies, Gavras et al. [116] observed a dose dependent effect up to 1 mg/kg of inhibitor administered intravenously. Blood pressure was significantly reduced in renovascular, malignant and essential hypertension but not in hypertension associated with chronic renal failure. In man, as in rats [117], the effect of SQ 20881 on the blood pressure was enhanced by sodium depletion. Plasma renin activity increased about threefold and aldosterone decreased to one third of the preinhibition concentration. SQ 20881 decreased the blood pressure in four of five patients with a normal renin level who had not responded to the administration of the angiotensin II antagonist Sar^1-Ala^8-angiotensin II.

As summarized in this article, the angiotensin I converting enzyme has a dual function, it activates a hypertensive peptide and inactivates a hypotensive one. The enzyme is widely distributed in the body. Specific inhibitors are available to study the functions of the enzyme.

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