VASOACTIVE PEPTIDES[1]

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Reports on the biochemical and pharmacological aspects of vasoactive peptides have been appearing with increasing frequency in the recent literature. The field, which opened slightly over thirty years ago with the discovery of "angiotonin" or "hypertensin," and was broadened nine years later by the addition of bradykinin, has since expanded to include a large number of peptides that often have as their only common property the ability to alter the physiology of the mammalian cardiovascular system. Most of these "vasoactive peptides" exert a wide variety of physiological effects in addition to their cardiovascular activity; in certain cases the cardiovascular aspect is the minor component in the particular peptide's spectrum of activity. For purposes of simplicity we have in many cases limited the discussion of each of these peptides to include only their relationship to the cardiovascular system. We have chosen to divide the vasoactive peptides into three major sections, namely, angiotensin peptides, kinins, and amphibian skin peptides. Various aspects of each of these groups has been the subject of a number of recent reviews and symposia (1–18); the reader is referred to these discussions for further consideration of details we have been forced to omit. Due to the extensive work that has been done with the biochemical, pharmacological, and physiological aspects of vasopressin and oxytocin, we have found it impossible to include a consideration of these peptides in this review.

ANGIOTENSIN PEPTIDES

Angiotensin II, the active octapeptide formed from the precursor decapeptide angiotensin I, is a potent vasoconstrictor and hypertensive peptide. The wide spectrum of physiological and pharmacological properties of the octapeptide are only gradually being realized. Angiotensin will be discussed in terms of its formation, metabolism, and principal physiological actions. The biochemistry and the methods of assay of the enzyme responsible for converting angiotensin I to angiotensin II, i.e., converting enzyme, will be treated in conjunction with angiotensin metabolism. Also considered in this

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section are substance A and pepsitensin, two peptides similar to angiotensin in their hypertensive properties.

Renin-angiotensin system.—It has long been suspected that the kidney exerts a controlling effect on the circulatory system; Tigerstedt & Bergman (19) produced the first direct evidence of this in 1898 when they observed an increase in blood pressure following injection of crude saline extracts of kidney into anesthetized rabbits. They referred to this pressor material as "renin." Renewed interest in this area followed Goldblatt's discovery in 1934 that constriction of one renal artery in the dog produced experimental hypertension (20). Pickering & Prinzmetal (21) reconfirmed the presence of renin in kidney tissue and subsequently characterized it as a protein on the basis of its nondialysability and rapid destruction at temperatures exceeding 60°. Evidence has recently been presented that the juxtaglomerular cells of the kidney are the source of renin (22, 23). The release of renin may well be signaled by a decrease in sodium load at the macula densa (24–27).

Kohlstaedt, Helmer & Page (28) reported that with increasing purification renin had increasingly stronger pressor effects in in vivo systems, and the opposite effects in in vitro systems; the addition of plasma to the in vitro system restored the pressor activity. This led them to suggest that renin was probably an enzyme and not a direct pressor substance. The mechanism by which renin acts was first elucidated by Munoz and his associates (29), who discovered that renin liberated a pressor substance from a blood pseudoglobulin fraction; the precursor molecule was later named renin substrate or angiotensinogen. Hog angiotensinogen has been identified as a glycoprotein with the mobility of α_2-globulins (30–32). On the basis of electrophoretic distribution, angiotensinogen is primarily located either in that fraction with albumin mobility (rat and man) or in the α_2-globulin fraction (dog and hog) (33). Hog angiotensinogen has a molecular weight of approximately 57,000 (32–34); that of human angiotensinogen is 42,300 (35). Angiotensinogen production has been considered to occur in the liver (36, 37); however, Dauda, Szokol & Devenyi (38) have found identical blood angiotensinogen levels in the hepatic vein and inferior vena cava in both normal and nephrectomized rats. The unexpectedly low content of the hepatic venous effluent does not support the role of the liver in angiotensinogen production. Skeggs et al (39) have shown that tryptic digestion releases a tetradecapeptide—asp-arg-val-try-ile-his-pro-phe-his-leu-leu-val-tyr-ser—from angiotensinogen, and have suggested that the aspartyl residue is N-terminal and that the tetradecapeptide is linked to the remainder of the angiotensinogen molecule by an ester linkage. Renin then acts on the tetradecapeptide, cleaving the leucyl-leucine bond specifically (Figure 1).

The peptide released by renin was investigated and named simultaneously by two groups in 1940. Observations that this substance was inactivated by proteolytic enzymes and by boiling in acid, and that it was insolu-

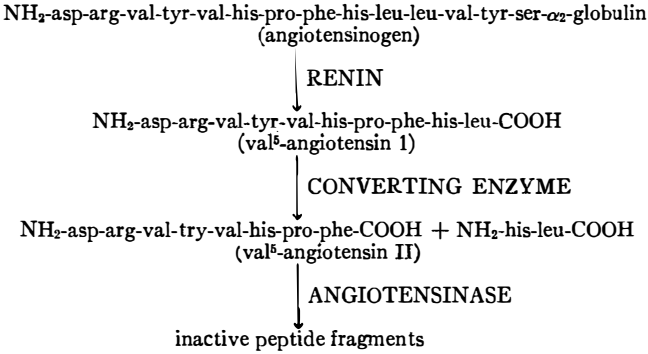

FIGURE 1. The metabolism of bovine angiotensin.

ble in organic solvents, led Braun-Menendez and his associates (40) to conclude that it was a polypeptide, which they named "hypertensin." Page & Helmer (41) found that this substance was heat stable; they named it "angiotonin." Confusion resulting from the dual nomenclature was resolved by adoption of the name "angiotensin" for the pressor substance (42).

Bovine angiotensin contains ten amino acids, eight of which are different (43); horse (44) and hog (45, 46) angiotensins are also decapeptides but contain nine different amino acids. Elliott & Peart (47) elucidated the sequence of bovine angiotensin as asp-arg-val-tyr-val-his-pro-phe-his-leu. Lentz et al (48) have reported that horse angiotensin differs only by the replacement of the val^5 residue of bovine angiotensin with an ile^5 residue; human angiotensin is identical to horse angiotensin (49). Attempts to obtain a purified angiotensin fraction led to the discovery that there were two angiotensin peptides, one formed by the action of renin on angiotensinogen (angiotensin I), and the other formed from angiotensin I (angiotensin II) (50). Two years later Skeggs et al (51) established that angiotensin II was an octapeptide differing from angiotensin I only by the absence of the two C-terminal amino acids, histidine, and leucine. The chemical sequence of the angiotensin peptides, their precursors, and the activation sequence are illustrated in Figure 1.

Synthesis of angiotensin peptides.—The final proof of structure of the angiotensin peptides required that the postulated sequence be synthesized and compared to the natural product. Bumpus and his associates (45, 46) first synthesized biologically active ile^5-angiotensin II by the stepwise condensation of dipeptide units. At the same time the asn^1-ile^5-angiotensin II analog was prepared by Rittel et al (52). Schwyzer et al (53) have described the preparation of val^5-angiotensin II and its asn^1-derivative by condensation of smaller units. Merrifield (54) has introduced a solid phase technique for the synthesis of polypeptides; amino acid residues are sequentially added to the N-terminal position of a polypeptide chain which is

chemically joined to an insoluble support resin. Ile^5-angiotensin II (55) and ile^5-angiotensin I (56) have been successfully made by this method.

Metabolism of angiotensin.—The discovery of two forms of angiotensin (50) coupled with Helmer's work with rabbit aortic strips showing that angiotensin II was the vasoactive principle (57) resulted in a search for the substance responsible for the conversion of angiotensin I to angiotensin II. Skeggs and his colleagues (50) first observed the presence of a plasma enzyme capable of making this conversion in the presence of chloride ion and other halide ions. This converting enzyme liberates angiotensin II by specifically cleaving the phe^8-his^9 bond of angiotensin I, releasing the dipeptide histidyl-leucine (48). Observations that angiotensin I injected intravenously into animals caused a rapid rise in blood pressure very similar in magnitude and onset to that produced by angiotensin II led to the assumption that in vivo the conversion of angiotensin I to angiotensin II occurs rapidly in the blood (37). Ng & Vane (58), investigating in vivo conversion in the dog by the blood bathed organ technique, confirmed the rapid conversion of angiotensin I, but found that in 15–20 sec (corresponding to one circulation time) there was only 14% conversion in blood. Arterial infusions of angiotensin I resulted in 30% conversion, whereas intravenous infusion resulted in 80% conversion. These results suggested that angiotensin I was lost in peripheral vascular beds and that the lung may play a role in angiotensin conversion. Ng & Vane found that in the 4–8 sec that blood remains in the pulmonary circulation there was more conversion of angiotensin I to angiotensin II than after 120 sec in the blood alone. This work was verified the following year in dogs (59) and in rats, cats, dogs, and rabbits (60). Bakhle, Reynard & Vane (61) have demonstrated that the enhanced biological activity of the lung effluent is due to the conversion of angiotensin I to angiotensin II, and not to the release of endogenous catecholamines. Angiotensin I conversion occurred in isolated lungs perfused with Krebs solution as well as in in vivo preparations, indicating that the enzymatic activity was in the lung tissue itself and not the result of activation of the plasma enzyme during passage through the pulmonary circulation. The ability of cell-free extracts of dog lung to convert angiotensin I to angiotensin II confirmed this hypothesis (62). Ryan and his colleagues (63) perfused rat lungs in situ with ^{14}C-leu^{10}-angiotensin I in Krebs-Henseleit solution; angiotensin I was converted to angiotensin II during a single circulation through lungs. All radioactivity was recovered either as angiotensin I or histidyl-leucine, illustrating that the lung-converting enzyme, like the plasma enzyme, is a dipeptidase. However, several groups have reported the recovery of both free leucine and histidyl-leucine in lung effluents following perfusion with high concentrations of angiotensin I (64–66). Following injection of angiotensin I into the pulmonary artery at 10,000 times the physiological level, approximately 50% of the injected material was recovered in the aorta; 53% of the material recovered had been converted into angiotensin II. Leucine was four

times as abundant as histidyl-leucine (65). It seems most probable that at concentrations that saturate the lung-converting enzyme an endopeptidase cleaves leucine from angiotensin I to produce the biologically inert nonapeptide des-leu^{10}-angiotensin I. The possible significance of such a peptide was tested by Ng & Vane (67), who perfused lungs with an undecapeptide related to angiotensin I but containing an extra histidine residue at position 10 (ile^{5}-phe^{8}-his^{9}-his^{10}-leu^{11}-angiotensin I). Between 40–50% of this undecapeptide disappeared in transit through the lungs of either dog or cat, probably by cleavage of the terminal histidyl-leucine, leaving his^{9}-angiotensin II (des-leu^{10}-angiotensin I). The fact that this peptide was not converted to angiotensin II by cleavage of his^{9} suggests that there is no stepwise formation of angiotensin II from angiotensin I by carboxypeptidase.

Ng & Vane (59) and Bakhle, Reynard & Vane (61) were unable to demonstrate angiotensin I conversion in femoral, carotid, renal, or total peripheral vascular beds of the intact animal or the isolated perfused kidney by the blood-bathed organ technique. Although Oparil, Sanders & Haber (65) found no evidence of conversion in the liver or hindlimb, 7–10% of the immunoreactive material in the venous effluent after a single circulation was angiotensin II following injection of 1,000–10,000 times physiological levels of angiotensin I into the renal artery. Franklin, Peach & Gilmore (68) perfused angiotensin I into the renal arteries of dogs and observed an immediate increase in renal vascular resistance. Since angiotensin I is biologically inert, they interpreted the results as indicating generation of angiotensin II, and estimated the extent of renal conversion at 19%. Gilmore, Merrill & Peach (69) have observed that injection of angiotensin I or II into the renal arteries of in situ blood-perfused kidneys resulted in an immediate decrease in renal blood flow. The renal vascular responses to injections of angiotensin I and II were blocked by the concomitant administration of angiotensin II antiserum, but not by angiotensin I antiserum; such findings indicate the presence of a kidney converting enzyme. Carriere & Biron (70) have reported that intrarenal infusion of angiotensin I caused decreased cortical blood flow rate, decreased urine volume, and decreased sodium excretion, and have also suggested that the observed effects are due to the intrarenal conversion of angiotensin I to angiotensin II.

A number of tissue homogenates have been shown to contain converting enzyme activity. Huggins & Thampi (71), using acetone powders prepared from crude homogenates, have reported approximately equal activity levels in rat liver, diaphragm, lung, and ileum. Heart, brain, plasma, and kidney contained significant, but lower, levels; there was essentially no activity in aorta and uterus. Roth and his associates (72), using rat tissue homogenates which had been frozen and thawed six times, showed a similar distribution pattern, with the exception that the lungs had a much higher activity relative to the next most active tissue, small intestine. Aiken & Vane (73) have studied the converting enzyme content of several tissues by observing the changes in response to angiotensin I produced by bradykinin potentiat-

ing factor, a pentapeptide that also serves as a converting enzyme inhibitor. They reported 30–45% conversion of angiotensin I to angiotensin II in the pulmonary arteries of rat, rabbit, and cat. Dog carotid and rat aorta showed approximately 10% conversion; rat colon, ileum, and uterus also showed low levels of conversion. Horky et al (74) have demonstrated the presence of converting enzyme activity in the abdominal lymph of anesthetized rats. Thampi & Huggins (75) have reported that subtilisin BPN' (Nagarse), a bacterial protease, has the ability to liberate histidyl-leucine from angiotensin I, thus demonstrating the ability of a bacterial protease to act as a converting enzyme.

Destruction of angiotensin II (and angiotensin I) is carried out by a number of distinct enzymes referred to as angiotensinases. Incubation of angiotensin II with hemolyzed human red blood cells or diluted human plasma rapidly destroyed pressor activity (76). Three enzymes capable of hydrolyzing angiotensin I and II have been described in normal human plasma (77, 78). Angiotensinase A_1 is an aminopeptidase, requiring Ca^{2+}, with a pH optimum of 7.4; it specifically hydrolyzes the asn^1-analog of angiotensin I. Angiotensinase A_2 is also a Ca^{2+} requiring aminopeptidase, with a pH optimum of 6.8; it specifically hydrolyzes asp^1-angiotensin I, the naturally occurring peptide. Angiotensinase B is an endopeptidase hydrolyzing all angiotensin analogs studied. The presence of at least two distinct aminopeptidases has been confirmed by several other groups (79–82). Analogs with the naturally occurring α-L-asp^1-configuration were inactivated much more rapidly in serum or plasma than those with α-D-, β-L-, β-D, or des-asp^1-configurations (83). The angiotensinase activity in red cells of rabbit has been reported to be much greater than that in plasma (84). An endopeptidase was isolated capable of cleaving the arg^2-val^3, try^4-ile^5, and ile^5-his^6 bonds of ile^5-angiotensin II; the hydrolysis of the tyrosyl-isoleucyl bond is apparently the first step of this inactivation process (85). An angiotensinase G has been found to appear in the γ-globulin zone of rabbit plasma following hepatic injury (86).

When asn^1-val^5-angiotensin II was incubated with rat plasma, 120 sec were required for 50% destruction of activity to occur (83); when incubated with human plasma, the half-life was 10–20 min (87). Hodge, Ng & Vane (88) found a half-life of 146 sec in the dog; this would mean that if angiotensinase activity is restricted to the blood, angiotensin II would have to circulate from 10–20 times before a 50% reduction in activity occurred. They found, however, that in the vascular beds studied 47–76% of infused angiotensin disappeared in one circulation. Biron, Meyer & Panisset (89) reported that extraction percentages averaged 70% for α-angiotensin II and 63% for the β-analog for a single circulation through hepatoportal, renal, and femoral vascular beds. Leary & Ledingham (90) and Osborne et al (91) have reported similar results. Hodge, Ng & Vane (88), working with dogs, have demonstrated 47% removal of angiotensin II following perfusion through the head, 62% through the liver, 71% through the hindquar-

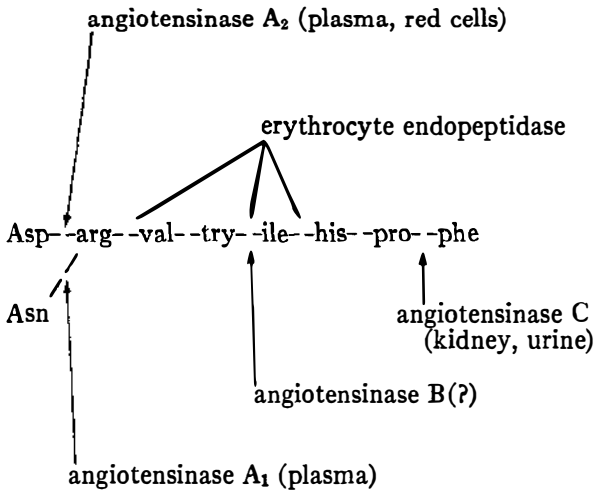

FIGURE 2. The site(s) of action of the principle angiotensinases. After Kokubu et al (85).

ters, 75% through the kidneys, and 76% through the body below the diaphragm. The lungs do not remove angiotensin II (88–90). The major role in angiotensin inactivation thus seems due to tissue enzymes.

Methot et al (92) first reported the presence of liver angiotensinase; the pressor response of angiotensin II following perfusion via the portal system was reduced 70% in comparison to that injected into the jugular vein. Johnson & Ryan (93) have found that aqueous extracts of rabbit liver are capable of hydrolyzing all the peptide bonds of asn^1-val^5- and asn^1-ile^5-angiotensin II. After dialysis against EDTA, these extracts degrade both forms primarily by release of the C-terminal phenylalanine residue. The perfused rat liver inactivates a number of angiotensin II analogs at similar rates, suggesting a variety of active enzymes (94).

The kidney destroys α-aspartic acid analogs of angiotensin II at less than half the rate of asn^1-val^5-angiotensin II and does not inactivate the β-analog (94). Several angiotensinases differing with respect to their pH optima, Ca^{2+} activation, and DFP inactivation have been isolated from renal tissue (95). Hess (96) has suggested that some renal angiotensinase activity may be related to arylamidase activity. Angiotensinase (97, 98) has been partially purified from swine kidney cortex; it specifically cleaves the pro^7-phe^8 bond of angiotensin II. Matsunaga & Masson (99) have analyzed the responses of neutral angiotensinases in liver, kidney, and erythrocytes to various inhibitors in an attempt to distinguish among them. Angiotensinase activities in the liver cell sap and the erythrocytes were found to be similar; the kidney enzyme was distinct. Figure 2 demonstrates several of the angiotensinases and their sites of action.

Angiotensin protective cofactor.—Haas et al (100, 101) have reported the presence in blood of a cofactor that prevents the retention, or inhibition of the pressor effect, of intraarterially injected angiotensin II. A large portion of the angiotensin I injected into a femoral artery of the conscious dog failed to pass through the arterial bed of the hindlimb. The addition of a cofactor obtained from human, bovine, or canine blood insured the full systemic pressor effect of the angiotensin II injected into a femoral artery. This cofactor does not protect angiotensin II from destruction by angiotensinase under in vitro incubation conditions.

Berman and his associates (102) have reported similar findings. The injection of angiotensin II into the femoral artery of human subjects produced no blood pressure rise, whereas intravenous injection of the same doses caused significant rises; intraarterial injection of human cofactor together with angiotensin II produced an elevation of systemic pressure to a level 80% of that caused by intravenous administration. Studies in dogs indicated that the cofactor was a potent vasodilator; the ability of the cofactor to restore the pressor effect of intraarterially injected angiotensin II may be reproduced by the vasodilatory drugs papaverine and aminophylline. These workers feel that angiotensin II fails to affect the systemic pressure when injected intraarterially due to reduced flow in the injected artery, dispersion of the bolus, and the decrease in peak concentration. Cofactor thus functions by causing vasodilation.

Ng, Teh & Whelan (103) have observed that blood or plasma added to perfusing Krebs solution potentiated the vasoconstrictor effects of angiotensin II on the isolated artery of the ear of the rabbit. The relatively greater increase in the vasoconstrictive effect of angiotensin II in the presence of blood or plasma may well be due to the presence of cofactor in the blood.

Plasma converting enzyme.—Skeggs et al (51) obtained a partially purified converting enzyme preparation from horse plasma by ammonium sulfate fractionation, dialysis, and isoelectric precipitation at pH 5.2. They found that this preparation had a pH optimum of 6.5 in phosphate buffer and 8.0 in Tris buffer, required the presence of halide ions for activity, and was inhibited by the chelating agent agent EDTA, indicating a metal ion requirement. The converting enzyme was stable at pH 4 to 7.8, but was entirely destroyed in 20 min at pH 3 at 25°. Dorer et al (104) have used a combination of ammonium sulfate precipitation, CM-cellulose filtration, and alumina gel elution to increase the specific activity of the enzyme in hog plasma from 0.007 units/mg protein (one unit is that amount of enzyme hydrolyzing one μmole of angiotensin I to histidyl-leucine and angiotensin II in I hr at 37°) to 0.51 units/mg. Working with the CM-cellulose fraction, they recorded an activity of 0.22 μmole angiotensin II formed/hr/mg protein. The activity was proportional to the enzyme concentration, with maximal activity in sodium phosphate buffer (pH 7.8). Maximum activation occurred at a chloride concentration of 0.1 *M*. No activation was observed

with Ca^{2+}, Mg^{2+}, Mn^{2+}, Co^{2+}, or Zn^{2+} (concentrations from 1 to 100 μM); Zn^{2+} proved strongly inhibitory. Dialysis of the enzyme against EDTA removed activity; Zn^{2+}, Co^{2+}, and Mn^{2+} at concentrations of 30 μM restored activity. Huggins et al (105) have reported several kinetic properties of the converting enzymes from horse and dog plasma. Ca^{2+}, Ba^{2+}, Mg^{2+}, Mn^{2+}, Fe^{2+}, Ni^{2+}, Co^{2+}, Cu^{2+}, Zn^{2+}, and Sn^{3+} have no effect on the enzyme activity; Hg^{2+} and Pb^{2+} were found to be inhibitory. Chloride ion was an effective activator, although other halides were also active. Bicarbonate anion was found to be stimulatory and cyanide ion inhibitory. The enzyme seemed to require free sulfhydryl groups, since *p*-chloromercuribenzoate, N-ethylmaleimide, and Hg^{2+} inhibited, while cysteine and dithiothreitol did not activate or significantly inhibit; phenylmethylsulfonylfluoride was a potent inhibitor. Dimercaprol has been shown to inhibit the plasma enzyme (106). Bumpus, Smeby & Page (107) reported that DFP inhibited enzyme preparations from heart and aorta, but had no effect on the plasma and liver enzymes.

Huggins et al (105) have also described several of the properties of the converting enzyme prepared by ammonium sulfate fractionation of dog lung homogenates, and compared these with the properties of the plasma enzyme. The pH optima for both enzymes appear to be 7.25 in phosphate buffer. The lung concerting enzyme exhibited maximum formation of angiotensin II after 8 min incubation at 37°; the plasma enzyme required 45 min. Production of angiotensin II was linear with enzyme concentration of either enzyme. The apparent Km for the plasma enzyme was 4.8×10^{-5} *M;* that for the lung enzyme was 5.2×10^{-6} *M*. They concluded from this comparative study that the lung and plasma enzymes are distinct. Fitz, Boyd & Peart (108) have investigated the human plasma-converting enzyme. EDTA, EGTA, 8-quinolinol, and oxalic acid interfered with enzyme activity; sodium citrate, 2:2′-dipyridyl, chromotropic acid, and desferrioxamine had no effect. Ca^{2+}, Zn^{2+}, and Co^{2+} reversed EDTA inhibition; Fe^{2+}, Cu^{2+}, and Mn^{2+} did not restore activity. Lee, Larue & Wilson (109) have combined the techniques of ammonium sulfate fractionation, Sephadex G-200 filtration, and DEAE-cellulose chromatography to obtain an enzyme preparation from porcine plasma capable of hydrolyzing 50 μmoles of substrate/mg/min; it was inhibited by angiotensin II, but not by histidyl-leucine. This same group (110) has also reported the partial purification of the human plasma-converting enzyme by ammonium sulfate precipitation and Sephadex G-200 gel filtration; a 67-fold increase in activity was achieved. Comparison of the enzymes from human and hog plasma showed that both enzymes had molecular weights estimated at 150,000. The Km for the human enzyme was 4.5×10^{-5} *M;* that for the hog enzyme was 4.2×10^{-5} *M*. Both enzymes exhibited the chloride ion requirement, EDTA inhibition, and pH optima between 7.2 and 7.8.

Lung converting enzyme.—Hollemans, Van der Meer & Kloosterziel

(111) homogenized human lung slices for 20 min until the temperature reached 60°; they then centrifuged this preparation and discarded the precipitate. The supernatant contained converting enzyme activity. Presumably the high temperature selectively destroyed angiotensinase activity while apparently not altering converting enzyme levels. Converting enzyme activity was blocked by EDTA, illustrating that the lung enzyme also has a cationic requirement. Trasylol, an inhibitor of peptidases involved in kinin formation (112), also inhibited activity; DFP had no influence. Cushman & Cheung (113) partially purified converting enzyme from rabbit lung; they reported that the enzyme was particulate and succeeded in using detergent to solubilize it. Bakhle, Reynard & Vane (61) homogenized dog lung perfused free of blood for 30 sec at full speed in an MSE blade homogenizer. This homogenate was centrifuged at 1000 × g for 20 min to give the low speed pellet (P_1). P_2 was obtained by centrifuging the supernatant at 20,000 × g for 20 min; P_3 resulted from centrifugation at 105,000 × g for 60 min, and S_3 was the final supernatant. Converting enzyme activity was greatest in the P_3 pellet. Although angiotensinase activity was most concentrated in S_3, P_3 also contained significant angiotensinase, thus rendering the determination of converting enzyme levels somewhat difficult. Enzyme activity was accelerated by chloride and inhibited by EDTA; trasylol, 2-mercaptoethanol (10^{-3} *M*), and 8-hydroxyquinoline (10^{-4} *M*) did not inhibit. Bakhle & Reynard (114) subsequently obtained an angiotensinase-free enzyme preparation from dog lung by using a 78,400 × g pellet rather than the 105,000 × g pellet as the enzyme source. Converting enzyme was stable at −20° for at least five months; it displayed a pH optimum in phosphate and Tris buffers of 7.0. Zn^{2+} and Ni^{2+} inhibited enzyme activity; no divalent cationic stimulation could be demonstrated. EDTA and 2,3-dimercaptopropanol inhibited activity; only Co^{2+} and Mn^{2+} partially restored activity in the presence of these chelating agents. N-ethylmaleimide did not inhibit. The enzyme showed a chloride ion requirement, with maximal activation occurring between 0.17 *M* and 0.3 *M* sodium chloride.

Sander & Huggins (115) have combined the techniques of marker enzyme analysis and electron microscopy to establish the subcellular localization of the lung-converting enzyme as the plasma membrane of the capillary endothelial cells. Such a site had previously been suggested on the basis of the rapid activation of angiotensin I, which occurs as this peptide makes a single passage through the pulmonary circulation (62). Smith & Ryan (116) have suggested on the basis of electron microscopy studies that both inactivation of bradykinin and activation of angiotensin I occur in pinocytotic vesicles formed on the surface of the lung capillary endothelial cells. It seems difficult to conceive that pinocytosis could occur with sufficient speed to explain the rapid conversion of angiotensin I to angiotensin II. However, since pinocytotic vesicles are formed by evaginations of the plasma membrane, this model is consistent with the findings of Sander & Huggins.

Methods of assay of converting enzyme.—Converting enzyme activity is determined by rate of catalysis of angiotensin I conversion. One of the most common assay techniques has utilized the rise in blood pressure induced by injecting angiotensin II intravenously into the pentolinium-treated rat (117, 118) or cat (41, 119). However, this method must be used with caution, since tissue and blood angiotensinases and angiotensin II-stimulated catecholamine release may mask angiotensin II levels actually present. Isolated smooth muscle preparations have also been used for assay; among these are guinea pig ileum (120, 121), rabbit intestine (122), and rat and rabbit uterus (123). Since both angiotensin I and angiotensin II are active in several of these systems, the two peptides must often be separated before angiotensin II levels can be accurately assessed. The first method for measuring the activity of converting enzyme was based on an eight tube countercurrent distribution procedure for the separation of the two peptides (51). Several chromatographic procedures for separation have since appeared in the literature (53, 124). Boucher et al (125) have separated angiotensin II from angiotensin I on Dowex Resin 50W-X8.

The much greater biological activity of angiotensin II over that of angiotensin I on certain isolated tissues has been utilized to monitor angiotensin II concentrations and converting enzyme levels selectively. Helmer (57) found that spirally cut rabbit thoracic aortic strips respond to angiotensin II but not to angiotensin I. Bumpus et al (126) and Andersen (127) have used the isolated rabbit uterus in a similar manner. Regoli & Vane (128) have described the use of the rat colon; Ng & Vane (59) have reported this latter organ to be 10–50 times more sensitive to angiotensin II than to angiotensin I. Using these methodologies angiotensin I and angiotensin II need not be separated before assay.

Huggins & Thampi (71) have developed a radiometric assay for converting enzyme. Following incubation of ^{14}C-leu^{10}-angiotensin I with the enzyme preparation, the radioactive histidyl-leucine formed is isolated by glass-fiber paper chromatography and counted. Results obtained with this methodology are comparable to those obtained by bioassay on the isolated rabbit aorta. Gregerman & Kowatch (129) have described a double isotope assay for nanogram quantities of angiotensin II. The peptide is reacted with ^{3}H-I-fluoro-2,4-dinitrobenzene and ^{14}C-dinitrophenylangiotensin II is added as an indicator. The ^{14}C-indicator allows for accurate correction, eliminating the problem of variable recoveries.

Shore, Burkhalter & Cohn (130) determined histamine concentrations by condensation with *o*-phthaldehyde. The product gave a stable fluorescence at 450 nm following activation at 360 nm. Gregerman (131) has adapted this method for quantitating histidyl-leucine at levels of less than one nanomole. This methodology has also been successfully applied to the assay of converting enzyme by following the release of histidyl-leucine from angiotensin I (132).

Radioimmunoassay has the significant advantage of very high selectivity for either angiotensin I or II. However, the small molecular weights of these peptides has made antibody production difficult; some success has been achieved by chemically linking the peptides to larger molecules. Deodhar (133, 134) produced antibodies to angiotensin II by immunization with an angiotensin-protein complex. Radioimmunoassays for angiotensin II in blood have been developed (135–139). Results obtained using these assays are high compared to bioassay data (140, 141). Boyd, Landon & Peart (142) have described a radioimmunoassay sensitive to angiotensin II concentrations of less than 30 picograms, based on a high titer antibody raised against angiotensin II absorbed onto charcoal. As a tracer, ^{125}I-angiotensin II-amide was employed. Results were similar to those afforded by bioassay. A radioimmunoassay for angiotensin I has also been reported (143). Cain et al (144) have utilized charcoal-bound angiotensin II to induce the formation of antibodies. Such antibodies showed only slight cross reaction with angiotensin I (2–5%) and negligible reaction with angiotensin II devoid of the C-terminal phenylalanine. This lack of cross-reactivity is apparently due to the location of the immunoreactive sites of the angiotensin molecules near the C-terminal end (145). Worobec (146) has induced antibodies of the 7S γ-globulin class against angiotensin I and angiotensin II by using for immunization the peptides absorbed to polyvinylpyrrolidone. Rabbits immunized with angiotensin II did not form cross-reacting antibodies for angiotensin I, while animals immunized with angiotensin I did show low levels of activity against angiotensin II. Antisera evoked against both species of angiotensin were active in complement fixation tests. Hexapeptide and heptapeptide fragments of angiotensin II formed by the aminopeptidase activity have been found to cross-react with angiotensin II antibodies. The presence of relatively high concentrations of these degradation products in venous blood necessitates the use of arterial blood samples for radioimmunoassay (144, 147). These radioimmunoassay techniques have been successfully applied to the determination of converting enzyme activity, both in plasma and lung (65, 108, 111).

The lack of a rapid assay for angiotensin II coupled with the limited availability of pure angiotensin I has stimulated the development of several synthetic substrates for the converting enzyme. Cushman & Cheung (113) reported that converting enzyme cleaved hip-his-leu to hippuric acid and histidyl-leucine at approximately one-ninth of the rate at which angiotensin I was hydrolyzed. The extent of histidyl-leucine formation can be determined colorimetrically with ninhydrin (113) or spectrophotometrically at 254 nm (148). Yang and her associates (149–151) have used hip-gly-gly, Z-pro-leu-gly (Z = carbobenzoxy) and *t*-Boc-phe-(NO_2)-phe-gly at 310 nm. Piquilloud, Reinharz & Roth (132, 152) have reported that Z-phe-his-leu and Z-pro-phe-his-leu were hydrolyzed by the converting enzyme; Z-phe-his-leu was reported to be cleaved about ten times faster than Z-pro-

phe-his-leu or angiotensin I. Hydrolyses were followed by the formation of histidyl-leucine, which was determined fluorometrically.

Physiological actions of angiotensin.—Angiotensin II is one of the most powerful pressor agents known, acting through a complex mechanism involving a direct constrictor effect on the smooth muscle of vascular walls, a pressor response mediated through the central nervous system, and direct and indirect effects on the heart itself. Angiotensin II is believed to exert its effect through binding with specific receptor sites in or on the cell (153, 154). Tritiated angiotensin II injected into the left ventricle of adult rats induced significant ultrastructural endothelial changes, such as an increase in pinocytotic activity and a widening of intracellular spaces. These changes were followed by preferential localization of tritiated material in the nuclei of vascular and cardiac muscle cells, thus suggesting that ^{3}H-angiotensin II or certain metabolic fragments may have specific effects on nuclear function (155). Local administration of angiotensin II results in constriction of small arteries and arterioles (156, 157). Increased resistance to blood flow is produced in most vascular beds when angiotensin II is administered directly and reflex modulation eliminated (158, 159). Blair-West, McKenzie & McKinley (160) have observed that angiotensin II contracted rat portal vein by direct action on smooth muscle; this action was independent of the presence of norepinephrine. Keatinge (161) has also presented evidence that angiotensin II caused depolarization and contraction of arterial smooth muscle cells by direct action on the muscle cells and not through the mediation of adrenergic neurons. The effect on venous smooth muscle is apparently variable. Pulmonary, mesenteric, and portal veins showed constriction; no effect was detected on femoral, superior venal caval, inferior venal caval, or saphenous veins (162).

The ability of angiotensin II to activate the sympathetic nervous system has implicated the central nervous system in the cardiovascular response to angiotensin II. Bickerton & Buckley (163) demonstrated that infusion of angiotensin II into the cerebral circulation of the dog, with the head connected to the body by the nervous system alone, led to a rise in systemic pressure that could be blocked by prior treatment with an *alpha*-adrenergic receptor-blocking drug. Infusion of angiotensin II into the common carotid artery of the rabbit showed greater pressor effects than did intravenous infusion (164, 165). Lowe & Scroop (166) demonstrated that injection of angiotensin II doses that had no effect intravenously into the vertebral artery resulted in increased blood pressure, heart rate, and cardiac output, with no effect on total peripheral resistance. Subsequent work showed that these effects were due to a withdrawal of vagal tone to the heart (167), probably mediated through the area postrema in the floor of the fourth ventricle (168). Microinjections of angiotensin II-amide into the hypothalamus of cats caused prolonged pressor response, which could be

blocked by ganglionic blocking agents; thus, the hypothalamus also appears to be one of the sites for elicitation of the central pressor effect of angiotensin II (169). Intravenous administration of angiotensin II produced vasoconstriction in the hand of human subjects by a mechanism attributed to the central sympathetic action (170, 171). In intact animals this centrally mediated peripheral hypertensive response may be reflexly inhibited by carotid sinus baroreceptor activation secondary to increased cerebral perfusion pressure (172). The reverse mechanism may also be operable; Sweet & Brody (173) have presented evidence that angiotensin II and reduced renal pressure inhibit reflex vasodilatation, produced by elevation of arterial blood pressure, by what appears to be a central mechanism.

The existence of direct myocardial actions of angiotensin II is still subject to some dispute. Early work showing that angiotensin II produced a marked reduction in coronary flow and increased amplitude of beat had suggested that angiotensin II possesses a direct myocardial effect (174). Koch-Weser (175) illustrated in papillary muscle a positive inotropic effect characterized by a shortened time to peak tension. However, subsequent work indicated that myocardial effects arose secondarily to peripheral vascular effects (158), ganglionic stimulation (176), or the release of catecholamines from intramyocardial adrenergic nerve terminals, (177, 178). Thompson (179) has found that the accelerator response to sympathetic nerve stimulation was significantly greater during than before angiotensin II infusions. The mechanism involved seems to be facilitation of norepinephrine release from nerve terminals (180–182). Several investigators have even suggested a negative inotropic effect for angiotensin II (183, 184). Recently, Dempsey et al (185) have elicited a positive inotropic effect in normal and denervated ventricular myocardium at angiotensin II concentrations from 10^{-10} to 10^{-6} g/ml which they suggest is independent of intact adrenergic nerves or endogenous catecholamine stores. A positive inotropic effect of angiotensin II on isolated kitten ventricular papillary muscle is greater than that produced by equimolar amounts of norepinephrine, and is not altered by prior administration of *beta*-adrenergic blocking compounds (175, 186). These observations support a direct action of angiotensin II on cardiac cells. Angiotensin II has been shown to constrict the coronary arteries (187–189); such constriction could well be a significant component of the overall myocardial response, further complicating the issue.

Angiotensin II has been shown to interact with peripheral and central autonomic neurons, sympathetic ganglia, and the adrenal medulla; an indirect cardiovascular effect is produced through an increase in the levels of circulating catecholamines. Angiotensin II stimulated the release of adrenal catecholamines both in vivo (40, 190) and in vitro (191). This effect persisted after denervation and seemed to be due to a direct polarizing action on the chromaffin cells (192). Angiotensin I also seemed active in this system; sustained catecholamine secretion was induced from the isolated adrenal medulla by administering angiotensin I via perfusion (193). Angiotensin II

inhibited the uptake of ^{3}H-norepinephrine in the brain (194), mesenteric blood vessels (195), and perfused heart (196). This effect may be due to inhibition of norepinephrine uptake at the level of the neuronal membrane (194) or facilitation of the release of neurally bound norepinephrine (181, 182, 197–199). Peach and his colleagues (200) have shown that endogeneous myocardial norepinephrine levels were increased in vivo following intravenous angiotensin II administration. The absence of this increase in bilaterally adrenalectomized rabbits suggested that this effect was secondary to angiotensin II stimulation of the adrenal glands, and hence increased circulating catecholamine levels. Palaic & Panisset (201) have demonstrated that angiotensin II blocked norepinephrine uptake and retention and produced a depletion of endogenous norepinephrine in the stimulated vas deferens. The accumulation of norepinephrine was impaired by continuous pre- and post-ganglionic stimulation and was dependent on the frequency of stimulation, thus suggesting a possible modulating role for angiotensin II in adrenergic neurotransmission. Angiotensin II has also been reported to increase the synthesis of norepinephrine from ^{3}H-dopamine (202) and to cause the release of acetylcholine from cholinergic nerve endings (203). A 240% increase in acetylcholine release from the cat parietal cortex was caused by 10^{-8} *M* angiotensin II, apparently by action on the release mechanism (204). Sympathetic ganglionic responses to angiotensin II differed markedly with the dose (205). Depending on the dose used, ganglionic transmission may be modified by direct action of angiotensin II on the ganglionic cells or by indirect cholinergic and adrenergic processes (206).

The renin-angiotensin system seems to be a major regulator of aldosterone secretion in man. Angiotensin II stimulated the zona glomerulosa of the adrenal cortex to produce aldosterone (207–209); this stimulation was apparently a specific process, which could not be duplicated by other pressor agents. Stimulation occurred without an accompanying increase in cortisol secretion and persisted as long as the angiotensin II infusion was applied; it occurred at mildly pressor doses but could not be consistently demonstrated with subpressor doses (210). Renin and angiotensin II administration increased aldosterone secretion and the width of the zona glomerulosa; nephrectomy lowered aldosterone secretion (211–213).

Angiotensin II has recently been implicated in a wide variety of apparently unrelated physiological effects. Fluid transfer by isolated everted sacs of rat jejunum, ileum, and intact colon prepared from adrenalectomized, nephrectomized rats was significantly stimulated by angiotensin II concentrations of 10^{-10} g/ml (214, 215). Cycloheximide and puromycin specifically inhibited this angiotensin II stimulated colonic fluid transfer, suggesting that the stimulation of fluid transfer is secondary to an effect on protein synthesis at the ribosomal level (216). Khairallah & Davila (217) have shown that angiotensin II stimulated RNA turnover and synthesis in rat myocardial cells, lending support to the concept that certain of the effects of angiotensin II may be mediated by synthesis of specific proteins. Pressor

doses of angiotensin II have been shown to increase plasma levels of adrenocorticotropic hormone in man significantly, apparently by altering cortisol production (218). McGiff et al (219) have demonstrated that infusion of angiotensin II into the renal artery causes release from the kidney into the venous blood of a substance that has the chromatographic and bioassay properties of a PGE prostaglandin. Angiotensin II has been shown to decrease plasma free fatty acid levels (220, 221); Iizuka, Eckstein & Abboud (222) suggested that this lowering may occur through a cholinergic mechanism that inhibits the release of free fatty acids from adipose tissue in the presence of an intact nerve supply.

Pepsitensin.—Croxatto & Croxatto (223) found that incubation of plasma globulins with pepsin at pH 2 to 6 resulted in the formation of a hypertensive and vasoconstrictor peptide, which they called pepsitensin. Pepsitensin was very similar in pharmacological activity to angiotensin (224). Paiva, Bandiera & Prado (225) were unable to separate a mixture of angiotensin and pepsitensin by paper chromatography, but were able to obtain a separation with paper electrophoresis at pH 2.5. Blair (226) reported that minor differences were observed in the activities of pepsitensin and angiotensin on the guinea pig ileum and rat uterus. Franze de Fernandez, Paladini & Delius (227) prepared pepsitensin by incubating ox plasma with pepsin at pH 6.0; a highly purified pepsitensin was isolated from the incubation mixture by fractional precipitation, solvent extraction, column chromatography, countercurrent distribution, and paper chromatography. This pepsintensin was reported to be identical to val^5-angiotensin I. However, Hochstrasser, Bachuber & Werle (228) obtained a pepsitensin from incubation of pepsin with denatured bovine plasma protein at pH 3.0 which was not identical to angiotensin I. This peptide contained N-terminal aspartic acid and the same amino acids in the same proportion as did angiotensin I, except for leucine which occurred twice. The additional leucine was probably C-terminal, thus making pepsitensin differ from val^5-angiotensin I only by possessing an extra leucine residue at position eleven.

Substance A.—Huggins & Walaszek (229) have reported that a polypeptide, named substance A, could be produced when fraction IV-4 of human plasma protein was incubated with crude α-amylase preparations; this material was hypertensive in dog and rabbit. Substance A was also liberated by the crystalline protease BPN′ (Nagarse) obtained from *Bacillus subtilis* (230). It is most probable that it was *Bacillus subtilis* contamination of the crude α-amylase preparations that was responsible for the liberation of Substance A by these preparations. Walaszek, Bunag & Huggins (231) have observed that Substance A resembled an angiotensin octapeptide. Substance A is a polypeptide of 32 amino acids with alanine at the C-terminal end and aspartic acid at the N-terminal end (232).

KININS

Bradykinin is the prototype of an ever-increasing number of peptides that are structurally and physiologically related to bradykinin; these peptides are collectively termed kinins. The kallikrein-kininogen-kinin system, the kininase system, bradykinin potentiating peptides, the relationship between bradykininase activity and converting enzyme activity, and the cardiovascular actions of the kinins will be discussed sequentially. Several peptides only indirectly related to bradykinin will also be considered: the avian peptide ornithokinin, the bee venom peptides melittin and MCD-peptide, and certain long acting vasodilator peptides from hog lung and intestine.

Kinin peptides.—In 1949, Rocha e Silva, Beraldo & Rosenfeld (233) described the trypsin and snake venom catalyzed release from a plasma globulin fraction of a vasoactive peptide that they called "bradykinin." Bradykinin (arg-pro-pro-gly-phe-ser-pro-phe-arg) is the most important of the mammalian plasma kinins, a group that includes lys-bradykinin (kallidin I) and met-lys-bradykinin (kallidin II). Kinin peptides have been reported from the venoms of the wasp *Vespa vulgaris* (234, 235), the hornet *Vespa crabro* (236), and the scorpion (237). Pisano (238, 239) has demonstrated the presence of a major (90%) and at least two minor kinins from the venom of the wasp *Polistes*. The major kinin has been determined to be an octadecapeptide named polisteskinin; treatment of this kinin with trypsin yielded one of the minor peptides, gly-bradykinin. Dunn & Perks (240) have isolated from turtle plasma what appeared to be the naturally occurring bradykinin analog thr^6-bradykinin. Phyllokinin has been isolated from amphibian skin; it will be considered in a subsequent section. Urokinin (241) and GPCGK-kinin (242) have subsequently been identified as identical to bradykinin or its naturally occurring analogs. Greenbaum et al (243) have described the isolation of PMN-kinin, a kinin peptide formed from a plasma kininogen of man or rabbit by enzymes of polymorphonuclear leucocytes. This kinin, although displaying many of the properties of bradykinin, has been differentiated from bradykinin and related analogs on the basis of column chromatography, discriminative effects (on isolated tissue, blood pressure, and relaxation of rat duodenum), molecular weight, and end-group analysis. Clostridin (1), a peptide very similar to bradykinin, is produced from plasma by a *Clostridia* protease; colostrokinin (244) is produced from colostrum by a protease in saliva and urine. Colostrokinin is composed of 17 amino acid residues of which the N-terminal is phenylalanine and the C-terminal serine (245). Chapman et al (246) have reported the existence of the peptide neurokinin in association with the pathogenesis of migraine headaches. The name "neurokinin" has also been applied to a peptide formed by incubating substance P with trypsin (247, 248). These two "neurokinins" are apparently unrelated.

Kallikrein-kininogen-kinin system.—Kinins are produced by the action of a group of highly specific peptidases (kallikreins or kininogenases) on a plasma α_2-globulin substrate, kininogen (249). They may also be released by incubation of kininogen with trypsin, pepsin, or certain snake venom proteases. Kallikreins have been isolated from mammalian urine, plasma, gut, pancreas, and salivary, lacrimal, and sweat glands; there is an apparent multiplicity of similar kallikreins in many of these organs (11). Fritz et al (250) have separated five active kallikreins from hog pancreas; these are indistinguishable in biological and chemical properties, but may be distinguished electrophoretically. Using various chromatographic and electrophoretic techniques, Kagen, Leddy & Becker (251) and Webster (252) found in human serum a single kallikrein, Eisen & Glanville (253) two kallikreins, and Colman, Mattler & Sherry (254) three kallikreins; two of these latter may have been monomeric and polymeric forms of the same enzyme.

Kallikrein is present in blood and in pancreas in an inactive form known as kallikreinogen or prekallikrein (255, 256). It is presently believed that the inactive prekallikrein is a precursor molecule distinct from the active kallikrein, and not merely an inactive complex of kallikrein and a circulating inhibitor (257, 258). Acid pH or denaturation by acetone precipitation or water dilution (259), adding antigen-antibody aggregates (260), exposing plasma to highly charged molecules or surfaces such as heparin, glass, or injured vascular endothelium (261), addition of Hageman factor (blood clotting factor XII) (262, 263), cooling at 37–0° (264), and treatment with plasmin (265, 266) are among those factors that have been demonstrated to activate prekallikrein.

Many workers currently feel that endogenous plasma kinin formation involves three major sequential pre-enzyme to enzyme transformations (267). Activation of Hageman factor (by contact), the initiating step, and activation of plasma prekallikrein, which in turn liberates kinin, are well established. Intermediate between these stages may be PF/dil (plasma permeability factor activated by dilution) (268, 269), or both PF/dil and a kininogenase (kininogenase II, 270). Webster (271) has reported the existence in human plasma of two additional as yet unidentified enzymes (enzymes III and V), which are also sequentially activated in the intermediate phase of kinin formation. The kinin activation sequence is outlined in Figure 3.

Kallikrein inhibitors have been discovered to occur naturally in mammalian systems. In 1930, Kraut, Frey & Werle (256) extracted a kallikrein inhibitor from a number of bovine organs; lung, parotid gland, and pancreas were the best sources. Elucidation of the amino acid sequence of the bovine parotid gland and lung kallikrein inhibitor (272) showed it to be identical to the bovine trypsin inhibitor (273). Mammalian plasma and serum have also been demonstrated to inhibit kallikrein (274, 275). These plasma kallikrein inhibitors seem to be distinct from serum trypsin inhibitors; purified serum trypsin inhibitors, even in large excess, do not inhibit the kallikreins (276). The naturally occurring serum inhibitor of C'1-ester-

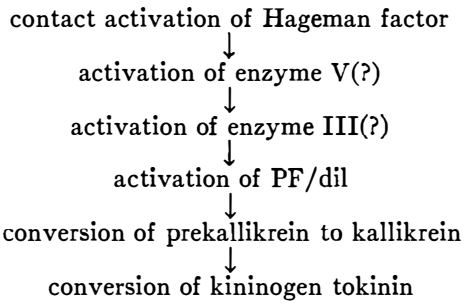

FIGURE 3. The sequence of enzymatic activations required for kinin release. Question marks indicate that the position of that particular step in the activation sequence in not firmly established.

ase, the activated form of the first component of complement, has been shown to inhibit kallikrein (*277*), thus indicting a possible association of the kinin system with the complement system as well as with the blood clotting system (through the Hagemen factor).

Two distinct kininogens, kininogen I and kininogen II, have been isolated from human (*278, 279*) and bovine (*280, 281*) plasma. Both human and bovine plasma kininogens are glycoproteins with molecular weights of approximately 50,000 (*282*). Bovine kininogen II is an acidic glycoprotein (*282*) (kininogen) in which the kinin moiety is located in the inner part of the peptide chain (*283*). Trypsin, snake venoms, and pancreatic kallikrein release kinin from bovine kinogen II, whereas bovine plasma kallikrein releases kinin only from kininogen I (*281, 284*). Following reduction and carboxymethylation of all disulfide groups of kininogen II, pancreatic kallikrein was unable to release kinin from this molecule. However, trypsin and snake venom enzymes could liberate kinin both from the chemically modified kininogen and from native kininogen. These results suggest that a tertiary structure of kininogen II is required for the hydrolysis (*285, 286*). Vogt (*270, 287*) postulated a dual enzymatic system in plasma to release kinins; kininogenase I acting on kininogen I and kininogenase II (activated by Hageman factor) acting on kininogen II. Buluk, Czokalo & Malofiejew (*288*) have confirmed Vogt's hypothesis and have successfully distinguished a kallikrein activated Hageman factor (contact kallikrein) from a kallikrein activated by plasmin (plasmin kallikrein).

Kininase system.—The first observation of a kinin-inactivating enzyme in plasma was made by Werle, Gotze & Keppler in 1937 (*289*). The rates of kinin destruction by sera varied from species to species (*237, 290*); guinea pig serum was especially active in this regard. Bradykinin has a half-life of about 17 sec in the blood stream of the cat or dog (*291*). Erdos & Sloane (*292*) have described an enzyme from human plasma, carboxypeptidase N

or plasma kininase I, that inactivates bradykinin and the kallidins by cleaving off the C-terminal arginine residue. Yang & Erdos (293) have described a second kininase in human plasma, kininase II or dipeptide hydrolase, that inactivates bradykinin by cleaving the C-terminal dipeptide phenylalanyl-arginine; this enzyme will be discussed subsequently in more detail. These kininases may be distinguished from pancreatic carboxypeptidases A and B (293, 294). Lys-bradykinin and met-lys-bradykinin may be converted to bradykinin by plasma aminopeptidases (295–297).

When bradykinin was infused into isolated organs, 30% disappeared in the hind legs, 70% in the kidneys, 50% in the liver, 65% in the head, and 80% in the lungs (298). The almost complete pulmonary removal of bradykinin has been confirmed in rats and dogs (299, 300). This pulmonary inactivating system is apparently specific for bradykinin; eledoisin (298) and polisteskinin (300) were unaffected by passage through the lungs. Ryan, Roblero & Stewart (301) have reported 95–99% inactivation of bradykinin, 92–96% inactivation of lys-bradykinin, and 80–83% inactivation of met-lys-bradykinin. Experiments in which blood-free lung preparations were perfused with labeled bradykinin have shown no measurable radioactivity retained by the lung and all peptide fragments recoverable in the effluent. In one circulation through blood-free lungs, five peptide bonds were found to be hydrolyzed: arg^1-pro^2, pro^3-gly^4, gly^4-phe^5, ser^6-pro^7, and pro^7-phe^8. Effects of 2-mercaptoethanol (ME) and a pentapeptide bradykinin potentiating factor (BPF) suggested that only two of these hydrolyses are of primary importance: arg^1-pro^2 and ser^6-pro^7. Under control conditions 2-(^{14}C-pro)-bradykinin was metabolized to pro^2-pro^3 and arg^1-pro^2-pro^3-gly^4, and to arg^1-pro^2-pro^3-gly^4-phe^5-ser^6 when ME, an inhibitor of arg^1-pro^2 hydrolysis (302), was added to the perfusion solution. In the presence of ME, ^{3}H-phe^8-bradykinin was hydrolyzed to one radioactive peptide, phe^8-arg^9. Under these circumstances only two hydrolyses could have occurred: ser^6-pro^7 and pro^7-phe^8. When the BPF and ME were used together, perfusion of ^{3}H-phe^8-bradykinin led to formation of pro^7-phe^8-arg^9, indicating that hydrolysis of ser^6-pro^7 may be primary while hydrolysis of pro^7-phe^8 is secondary and may depend upon prior hydrolysis of ser^6-pro^7 (301).

Bradykinin potentiating peptides.—The venom of the South American poisonous snake *Bothrops jararaca* has been shown to contain a BPF capable of increasing some of the pharmacological activities of bradykinin both in vivo and in vitro on the guinea pig ileum, rat uterus, and rabbit duodenum, as well as its hypotensive effects in cats and dogs (303, 304). BPF has been separated into several different active fractions and the amino acid composition of nine purified peptides established (305). Seven of these have been synthesized (306). The smallest of these is the pentapeptide PCA-lys-try-ala-pro (307). It has been suggested that bradykinin potentiation by BPF could be due to the blockade of peptide inactivation by blood or

tissue kininases possibly present in the smooth muscle, at sites close to the pharmacological receptors (303, 304). However, observations that the concentration of an agent that enhanced the effect of bradykinin on isolated preparations of smooth muscle was smaller than that required to inhibit kininase activity of tissue homogenates (308) and that slow inactivation of kinin incubated with guinea pig ileum segments, contrasted with the rapid and intense potentiation of the smooth muscle contraction of this preparation (309), caused this interpretation to be questioned. Camargo & Ferreira (310) have demonstrated that both BPF and dimercaprol inhibited the contractions induced by bradykinin on isolated rat duodenum and terminal ileum. Neither BPF nor dimercaprol affected the relaxation induced by bradykinin on rat duodenum. These results were interpreted as indicating that different types of receptors are involved in the action of brandykinin on rat intestine, and that other factors besides the inhibition of agonist destruction participate in the mechanism of potentiation of kinin action by BPF. BPF has also been shown to inhibit the angiotensin I converting enzyme from dog lung (61–62).

The venom of the Japanese poisonous snake *Agkistrodon halys blomhoffii* has been reported to contain two or more bradykinin potentiating peptides (311); five peptides (potentiators A, B, C, D, and E) were subsequently purified. Of the five potentiators, B and C had the strongest bradykinin potentiating activity on guinea pig ileum. The sequence of B was determined as PCA-gly-leu-pro-pro-arg-pro-lys-ile-pro-pro; that of C was PCA-gly-leu-pro-pro-gly-pro-pro-ile-pro-pro. These structures were confirmed by chemical synthesis (312). BPPE was determined to be PCA-lys-try-asp-pro-pro-pro-ser-pro-pro (313). Potentiators B and C have also been shown to inhibit the converting enzyme strongly (312).

Relationship between converting enzyme and bradykininase activity.— Ng & Vane (59), noting that both bradykinin and angiotensin I almost completely disappeared in a single circulation through the lung, and that both peptides contained the sequence prolyl-phenylalanine near their C-terminal ends, first suggested that the pulmonary inactivation of bradykinin and the conversion of angiotensin I to angiotensin II might be caused by the same enzyme. Bakhle (62) found that the 105,000 × g (P_3) fraction obtained from homogenized dog lung inactivated bradykinin faster than it converted angiotensin I, but the final supernatant (S_3), which contained predominantly angiotensinase activity, also inactivated bradykinin. ME, BPF, EDTA, and 8-hydroxyquinoline all prevented bradykinin inactivation by P_3; neither ME nor 8-hydroxyquinoline inhibited the converting enzyme. Ferreira et al (305) tested the effects of various fractions of *Bothrops jararaca* extract on converting enzyme and bradykininase activities in an attempt to show differential effects on the two activities. Those fractions that were the most active in potentiating the action of bradykinin on the guinea pig ileum were generally the most active converting enzyme inhibitors, and

yet comparison between specific samples suggested some separation of activities. Scholz & Biron (314) noted that intravenous administration of ME to normal anesthesized rats and dogs reduced by 25% the pulmonary inactivation of bradykinin without modifying the pulmonary activation of angiotensin I; they concluded that the peptidases responsible for the pulmonary metabolism of these two peptides are not identical. Freer & Stewart (315) have reported further evidence for this nonidentity. High levels of infusion of ME into rat lungs completely blocked pulmonary kininase with no effect on angiotensin I conversion. BPF inhibition of kininase reached a maximum at 250 μg/kg/min, with 30% residual destruction of bradykinin, while significant inhibition of angiotensin I conversion begins at higher levels. One rat was found that converted angiotensin I normally but had no pulmonary kininase. Sander, West & Huggins (316) found that in a particulate converting enzyme preparation from rabbit lung, the bradykininase activity was independent of the presence of chloride ion, whereas the converting enzyme activity displayed an absolute chloride ion requirement. However, bradykinin, met-lys-bradykinin, and bradykinin potentiating peptide C competitively inhibited the conversion of angiotensin I to angiotensin II; thus these bradykininase substrates are capable of binding at the active site of the converting enzyme.

Yang & Erdos (149–150) have demonstrated that kininase II, an enzyme previously isolated first in a microsomal fraction of kidney cortex and later in human blood plasma and urine (293, 317), which inactivated bradykinin by cleaving the pro^7-phe^8 bond to liberate phenylalanyl-arginine, also cleaved histidyl-leucine from angiotensin I to give angiotensin II. They feel that this enzyme, renamed dipeptide hydrolase, functions as both a converting enzyme and a bradykininase. Dipeptide hydrolase was purified 200-fold from hog plasma and concentrated from homogenates of lung and kidney. Dialysis against EDTA inhibited enzyme activity; Zn^{2+}, Co^{2+}, and Mn^{2+} restored activity. Chloride ion accelerated activity. *o*-Phenanthroline, porcine insulin, the B chain of insulin, BPF, phenylalanyl-arginine, histidyl-leucine, and hip-his-leu all inhibited the action of this enzyme on both bradykinin and angiotensin I (151). Although the relationship between converting enzyme activity and bradykininase activity is still somewhat uncertain, as yet unpublished results from our laboratory support those workers who have suggested the nonidentity between converting enzyme activity and bradykininase activity. A series of cyclohexane derivatives have been found to inhibit both converting enzyme and bradykinase; however, their order of potency as converting enzyme inhibitors differs markedly from the order of potency as bradykininase inhibitors. Bradykinase activity seems less stable than does converting enzyme activity; this is consistent with the observation of Freer & Stewart (315) that one rat that activated angiotensin I normally failed to destroy bradykinin.

Physiological actions.—All kinins thus far tested have been demon-

strated to display similar physiological actions with only quantitative differences; thus bradykinin will be used as a prototype of the kinin family in discussing physiological activity. Bradykinin is a strong vasodilatory peptide, which when injected into the systemic circulation causes a decreased vascular permeability in all mammalian species that have been tested. The peptide produced vasodilation in most systemic arterioles (10); Hilton (318) has demonstrated an increased blood flow within cat gastrocnemius muscle following intraarterial injection of bradykinin. The venous vascular system responds somewhat differently. Bradykinin produced dilation in the resistance vessels of the human forearm (319). Several groups have reported that bradykinin caused constriction of lung vessels (320–323), rabbit ear (324), and rabbit skin (325). Guth, Cano & Jaramillo (326) demonstrated a reduction of the perfusion flow in cat and dog hind limb and rat hind quarters; Shimamoto et al (327) described a bradykinin-induced constriction of the rabbit saphenous vein. Sekiya et al (328) reported that bradykinin injected in situ into the central artery of the rabbit ear caused a dilation of artery and vein and an increased venous flow. Administered topically around the vessels in the rabbit in situ, bradykinin caused simultaneous arteriodilation and venoconstriction; in the isolated rabbit ear and guinea pig and rat hind quarters, bradykinin decreased the outflow. Bradykinin apparently constricted the veins of the rabbit and guinea pig directly without catecholamine release.

Subsequent to decreased peripheral resistance and blood pressure, an increased heart rate, an increased cardiac output, an increased force of myocardial contraction, and a redistribution of regional blood flow appear. Rowe et al (329) found that infusion of bradykinin directly into the right atrium of dogs produced an increased cardiac rate and a decreased systemic arterial pressure. Cardiac output significantly increased; systemic, pulmonary, and coronary resistance fell. Following increases in cardiac output, coronary blood flow, and myocardial oxygen consumption, arterial hemoglobin and hemocrit rose. Maxwell, Elliott & Kneebone (330), Bergamaschi & Glasser (331), and Nakano (332) have all published similar findings. On the isolated hearts of guinea pig, rabbit, cat, and dog, bradykinin produced an increase in coronary flow (333).

Cardiac actions of bradykinin may be secondary to reflex activation of the sympatho-adrenal system (334) and peptide induced direct release of catecholamines from the adrenals (335). Lang & Pearson (334) demonstrated a biphasic response of the blood pressure of intact animals following intravenous bradykinin injection. Following an initial brief fall, the arterial pressure returned toward control levels, often overshooting control values and producing a secondary pressor phase. These workers have further demonstrated that this biphasic response is mediated via activation of central and peripheral sympathetic reflexes. Reichgott & Melmon (13) have blocked the secondary rise in peripheral resistance in primates by simultaneously infusing the ganglionic blocking agent trimethaphan with bradykinin.

Under these conditions no rise in heart rate occurred and the cardiac output decreased.

Melmon et al (336) have suggested that bradykinin may play a major role in the adaptation of the neonatal circulation to extra-uterine life. This hypothesis is based on observations that the neonate is capable of producing bradykinin, and that this bradykinin produces vascular responses, such as constriction of the umbilical artery and veins and ductus arteriosus and dilation of the pulmonary artery, which allows conversion of fetal to adult circulatory patterns.

Ungar & Parrot (337) originally suggested that salivary kallikrein is released during salivary secretion and that this kallikrein is the mediator of the atropine-resistant vasodilation produced by parasympathetic nerve stimulation in the submaxillary gland. In supporting this hypothesis, Hilton & Lewis (338), Hilton (339), and Lewis (340) have maintained that kinins are the mediators of functional hyperemia or vasodilation in salivary, pancreatic, tongue, and sweat glands. Gautvik (341) has recently presented still further support for this hypothesis. Working with perfused cat submandibular gland, he observed that reduction of kininogen II in the plasma perfusate resulted in reduced vasodilatory response; omission of kininogen from the perfusates resulted in little or no vasodilation in the submandibular gland. Addition of partially purified kininogen II restored the vasodilatory response to parasympathetic stimulation.

Holdstock, Mathias & Schachter (342) and Bhoola & Schachter (343) have demonstrated that bradykinin in low concentrations increased capillary permeability in the skin of guinea pigs and rabbits. At higher concentrations Lewis (344) has reported the accumulation and migration of leucocytes. Gabbiani, Badonnel & Majno (345) have shown that intraarterial injection of bradykinin produced vascular leakage predominantly from the venules; those vascular segments affected were the same whether the peptide was administered intraarterially or locally.

Ornithokinin.—Werle, Hochstrasser & Trautschold (346) have found that birds possess a system analogous to the mammalian kallikrein-kininogen-kinin system. Ornithokinin has been prepared by treating acidified plasma with ornithokallikrein from hen pancreas. This peptide apparently contains twelve amino acid residues, five of which are not constituents of any of the known kinins. Ornithokinin also differs in physiological activity from bradykinin; ornithokinin is hypotensive in hens but not in dogs, and contracts the hen ileum, a tissue upon which mammalian kinins have no effect.

Bee venom peptides.—Breithaupt & Habermann (347) isolated from bee venom by gel filtration and chromatography two peptides that caused mast cell degranulation. These two peptides were termed melittin and mast cell degranulating (MCD) peptide. Melittin has been previously reported (348)

and its structure determined (349). It is a basic peptide with twenty-six amino acid residues: gly-ile-gly-ala-val-leu-lys-val-leu-thr-thr-gly-leu-pro-ala-leu-ile-ser-trp-ile-lys-arg-lys-arg-gln-gln. Breithaupt & Habermann (347) found that MCD-peptide was also strongly basic, and consisted of 22 amino acid residues. MCD-peptide depressed the blood pressure of rats and increased capillary permeability upon intracutaneous injection. Melittin was less active on rat skin vessels, but more active on the skin capillaries of rabbit.

Long acting vasodilatory peptides.—Extracts of hog lung and small intestine contain as yet unidentified peptides which when administered intravenously increase peripheral blood flow and cause systemic hypotension; both effects are long lasting (350, 351). The intestinal peptide, consisting of 28 amino acid residues, was found in methanol extracts of intestine; it produced systemic vasodilation, hypotension, and increased cardiac output (352). This intestinal peptide is a vasodilator in isolated dog lung; it is effectively removed from blood during passage through the liver (353).

Amphibian Skin Peptides

Besides bradykinin and angiotensin, a number of other peptides capable of exerting vascular effects have been isolated. Many of these peptides have been found in amphibian skin. Erspamer (18) has recently published a review of them. This discussion will be restricted to the cardiovascular actions of these peptides; the reader is referred to this excellent review for a consideration of their systemic properties. Erspamer has divided the active peptides derived from amphibian skin into four categories on the basis of their general structural and biological characteristics: eledoisin-like peptides (the tachykinins, so called because their effects are rapid in onset as compared to the relatively slow-acting bradykinins), bradykinin-like peptides, caerulin-like peptides, and alytesin-like peptides: these same classifications will be maintained in this discussion. These peptides exert widespread effects on mammalian systems, and may be differentiated from one another on the basis of their varying activities on different organ systems. Their structures are indicated in Figure 4.

Eledoisin-like peptides.—Included in the eledoisin family are eledoisin itself, physalaemin, phyllomedusin, and uperolein. Substance P, although of mammalian origin, is biologically and chemically similar to the eledoisin peptides and is often considered with this family. Eledoisin is an undecapeptide first isolated from the posterior salivary glands of *Eledone moschata* (354, 355); it is a potent vasodilator and hypotensive agent in most animal species (356). In man, eledoisin produces a marked increase in both systolic blood flow and total blood flow in the muscle, but only a very slight increase in these parameters in the skin (357). Physalaemin, first isolated in pure form from methanol extracts of *Physalaemus bigilonigerus,* has also been found in skin extracts of other Physalaemus species (18, 358). This peptide

produced hypotension in the dog (359) and increased capillary permeability in the guinea pig, rat, and man (360). The hypotensive activity of physalaemin is three to four times stronger than that due to eledoisin (361). Both eledoisin and physalaemin, together with bradykinin, possess strong inotropic properties in rabbit atria; they are devoid of significant chronotropic activity and are powerful agents for lengthening the refractory period. No adrenergic component was detected in their stimulant effect (362). Nakano (363) has reported that the intravenous administration of both peptides results in decreased mean systemic arterial pressure and total peripheral resistance in dogs; heart rate, pulmonary arterial pressure, myocardial contractile force, and cardiac output were increased. Intraarterial administration caused increased blood flows in the coronary, brachial, and femoral arteries without significant changes in heart rate, mean systolic arterial pressure, and myocardial contractile force. Nakano feels that the cardiovascular changes induced by the intravenous administration of these peptides are most likely due to their direct vasodilator action on the peripheral vasculatures. Increases in heart rate and myocardial contractile force are probably due to reflex sympathetic stimulation. There is no significant loss of eledoisin in one minute in the vascular beds, including the pulmonary circulation of the cat; it is removed mainly by the kidneys (298).

Phyllomedusin has recently been found in the skin of the South American frog *Phyllomedusa* (364); it is quite similar in biological properties to physalaemin (365). Uperolein was reported in the skin of the Australian amphibian *Uperoleia rugosa* (366).

Substance P was discovered by von Euler & Gaddum in 1931 in crude acid alcohol extracts of equine brain and intestine (367). Chang & Leeman (368) have recently isolated it from bovine hypothalamus, and determined it to be an undecapeptide similar to eledoisin and physalaemin (369); the proposed structure has been confirmed by synthesis (370). Substance P has

Eledoisin	PCA-Pro-Ser-Lys-Asp-Ala-Phe-Ile-Gly-Leu-Met-NH_2
Physalaemin	PCA-Ala-Asp-Pro-Asn-Lys-Phe-Tyr-Gly-Leu-Met-NH_2
Substance P	Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2
Phyllomedusin	PCA-Asn-Pro-Asn-Arg-Phe-Ile-Gly-Leu-Met-NH_2
Bradykinin	Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg
Phyllokinin	Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg-Ile-Tyr (SO_3H)
Caerulein	PCA-Gln-Asp-Tyr(SO_3H)-Thr-Gly-Trp-Met-Asp-Phe-NH_2
Phyllocaerulein	PCA-Glu-Tyr(SO_3H)-Thr-Gly-Trp-Met-Asp-Phe-NH_2
Alytesin	PCA-Gly-Arg-Leu-Gly-Thr-Gln-Trp-Ala-Val-Gly-His-Leu-Met-NH_2
Bombesin	PCA-Gln-Arg-Leu-Gly-Asn-Gln-Trp-Ala-Val-Gly-His-Leu-Met-NH_2
Ranatensin	PCA-Val-Pro-Gln-Trp-Ala-Val-Gly-His-Phe-Met-NH_2

FIGURE 4. The structure of vasoactive polypeptides isolated from amphibian skin.

been found to elicit tachycardia and a fall in systemic blood pressure due mainly to peripheral vasodilation in all mammals. In man, intravenous administration is followed by an increase in blood flow, particularly in the skin and striated muscles. Cardiac output is increased as a consequence of the rise in heart rate, whereas the stroke volume, as well as the intracardiac and pulmonary blood pressure, remain unchanged (371). Dilation of the pulmonary and coronary circulations has been reported; catecholamine release does not seem to be involved (372). Lembeck & Hettich (373) have observed that vasodilatation and a fall in blood pressure were the predominant effects of Substance P. A marked effect on the generation of spontaneous activity in cardiac Purkinje tissue has also been observed following substance P treatment. Increases of 20–70% in spontaneous frequency occurred. In cells with marked pacemaker activity the diastolic potential was reduced and the rate of spontaneous depolarization increased (374). Like eledoisin, physalaemin and substance P are not inactivated in the pulmonary circulation (375).

Bradykinin-like peptides.—Bradykinin has been observed in the skin extracts of the European brown frog *Rana temporaria* (376). Phyllokinin [bradykinyl-ile-try (SO_3H)], the bradykinin analog val^1-thr^6-bradykinin, and the apparent bradykinin precursor bradykininyl-val-ala-pro-ala-ser have also been reported in amphibian skin (377, 378). These peptides display the same general activities as does bradykinin.

Caerulein-like peptides.—Caerulein was initially isolated from methanol extracts of the skin of the Australian amphibian *Hyla caerulea* (379), and the structure established and the synthesis accomplished shortly thereafter (380, 381). The tyrosine-sulfate residue is essential for activity; desulfated caerulein is virtually inactive (382). Caerulein is rapidly inactivated by chymotrypsin and subtilisin, but is resistant to trypsin degradation (383). Caerulein has marked structural similarities to human gastrin II and cholecystokinin-pancreozymin; it is also similar to these peptides in pharmacological activity, particularly in the gastro-intestinal tract. The activity ratio between intestinal and cardiovascular actions of caerulein highly favored intestinal motility (384). However, a strong vasodilating effect on the pancreatic vascular region was observed as caerulein doses which stimulated pancreatic external secretion but produced no significant cardiovascular changes (385). Thus vascular changes may be involved in the production of the intestinal effects.

Following intravenous injection into the dog, caerulein caused a hypotension proportional to the dose. It was more potent than bradykinin, but considerably less potent than physalaemin. Caerulein-induced hypotension has a lower onset and much greater duration than that produced by either bradykinin or physalaemin; this hypotension is enhanced, particularly in duration, by *alpha*-adrenergic blocking agents. Tachyphylaxis was absent.

Caerulein also lowered the blood pressure in humans and in the rabbit. In the cat its actions were erratic, less intense, and there was often tachyphylaxis; the chicken was even less sensitive than the cat. Rat gave the most erratic responses of all; hypertension predominated at low doses and hypotension at large doses. Capillary permeability was markedly increased in man following intradermal injection of caerulein (386, 387).

The skin of the South American amphibian *Phyllomedusa sauvagei* contains a peptide, phyllocaerulein, closely resembling caerulein in its pharmacological actions. It proved to be a nonapeptide, and was physiologically indistinguishable from caerulein even in parallel bioassay (388).

Alytesin-like peptides.—Methanol extracts of the skin of two European amphibians of the discoglossid family, *Bombina bombina* and *Bombina variegata,* contained the tetradecapeptide bombesin. Alytesin, a peptide possessing a structure very similar to that of bombesin and displaying the same biological actions, has been detected in the skin of another European amphibian of the same family, *Alytes obstetricans* (389). Alytesin produced hypertension and marked tachyphylaxis in the dog; this effect was not altered by pretreatment of the animal with *alpha*-adrenergic blocking agents. It evoked a less intense hypertensive response than did val[5]-angiotensin II but the effect produced by alytensin had a much greater duration (390).

Nakajima, Tanimura & Pisano (391) have isolated the undecapeptide ranatensin from the skin of the frog *Rana pipiens.* Ranatensin elevated blood pressure with a potency about one-tenth that of angiotensin in the dog and rabbit, most probably by a direct peripheral vasoconstrictor action; tachyphylaxis did not occur. The peptide lowered blood pressure in the monkey with a potency similar to that of eledoisin; the mechanism seemed to involve a direct peripheral action on vascular smooth muscle; here tachyphylaxis did not occur. Ranatensin did not alter the blood pressure of cats; the action in guinea pig was variable with a rapid onset of tachyphylaxis. The effect on the rat was also variable and apparently related to the basal blood pressure. With high basal pressures, ranatensin produced a hypotensive response; low basal pressures resulted in a hypertensive response. In the rat the response to ranatensin seemed to involve the release of noradrenaline from peripheral nerve endings (392).

Discussion

Current research in the field of vasoactive peptides has become increasingly concentrated on the mechanisms of action of these peptides at the molecular level. The fact that angiotensin II is a potent vasoconstrictor has long been established; interest now centers on such areas as the specific interactions of the angiotensin peptide with receptor sites in the smooth muscle of the vascular wall and the various factors influencing these interactions. Introduction of the Merrifield solid-phase methodology for peptide synthesis has made the preparation of any number of analogs of the natu-

rally occurring peptides relatively simple, thus making possible detailed structure-activity studies. It is now possible to prepare synthetic peptides of greater biological activity than the natural peptides which would then function as potent pharmacological tools for inducing a desired elevation or reduction of systemic blood pressure. Similarly, inhibitors of the activation or of the physiological response of the natural peptides can be prepared and used pharmacologically to block the effect of endogenous peptides.

Also of increasing interest is the role of the lung in the metabolism of the vasoactive peptides. The lung had long been considered to function only for gaseous exchange and maintenance of acid-base balance; it is now known to play an important role in the activation and degradation of such active metabolites as angiotensin I, bradykinin, serotonin, ATP, and prostaglandins, to name only a few. But the overall significance of the lung in these processes has not yet been established. What is the biological advantage in having these reactions occur in the pulmonary circulation? Is it to make available to the coronary circulation and the heart itself those materials such as angiotensin II, which are activated or released in the pulmonary circulation? Is it to protect the heart from those molecules such as bradykinin, which are destroyed during passage through the lung? These questions remain to be answered. The interrelationship between the metabolic activities of the lung toward several vasoactive peptides has not yet been pursued to its fullest extent. As an example, the relationship between converting enzyme activity and bradykininase activity is still subject to some dispute. Is the lung responsible for establishing a balance between the hypertensive angiotensin II and the hypotensive bradykinin? Or are converting enzyme activity and bradykininase activity independent?

The possible participation of the lung in certain pathological processes has yet to be investigated. The potential role of the renin-angiotensin system in the kidney has been the subject of extensive work, and yet the possibility of contributions of derangements in converting enzyme activity to altered systemic pressure has not been examined. Thus extensive possibilities exist for investigations in the area of vasoactive peptides; such work is necessary to provide the answers for these as yet unsolved problems.

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