Physiological Evolution of the Renin-Angiotensin System

Hiroko Nishimura, M.D.

SUMMARY

The renin-angiotensin system (RAS) in mammals may participate in the controls of blood pressure and aldosterone secretion, and possibly in the regulation of renal function. It has been shown that renin release is controlled by: 1) two intrarenal receptors, the renal arteriolar receptor and the macula densa; 2) the sympathetic nervous system; and 3) several humoral agents. Recent studies indicate interrelations between the RAS and renal prostaglandins and the kallikrein-kinin system.

Comparative studies have revealed that renal renin and the juxtaglomerular (JG) cells emerged during the early evolution of bony fishes, whereas the macula densa evolved later in the vertebrate phylogeny. Exogenously administered angiotensins and renin produce vasopressor actions in representative species of all vertebrate classes from elasmobranchs to mammals, and increase secretions of mineralocorticoids from the adrenal cortex (interrenal) in amphibians, reptiles, and possibly in teleosts. Angiotensin causes glomerular diuresis in teleosts and lungfishes, which may be ascribed to increased dorsal aortic pressure, while angiotensin may have both glomerular and tubular actions in some amphibians. Intracranial injection of angiotensin stimulates drinking in teleosts, reptiles, and birds, but not in amphibians.

Hemorrhage and acute hypotension are potent stimuli for causing renin release in an agglomerular teleost and a bird. When we consider this fact together with the anatomical evidence that the evolution of the JG cells precedes that of the macula densa, it appears that the RAS has evolved with a close relationship to blood pressure homeostasis. On the other hand, there is no clear evidence that the RAS is activated in
Although the RAS appears to exert several functions in man and other mammals, some of them may be more important in primitive animals, while a similar function remains in mammals as a relic of the primitive system. Comparative approaches provide a perspective of biological history and unique experimental model that will eventually aid in understanding of the underlying mechanisms operating in mammals.

Additional Indexing Words:
Plasma renin activity Renin release Renin in primitive animals Dipsogenic action of angiotensin Blood pressure homeostasis Corpuscles of Stannius Hypocalcin

SESSION 3, "Functions and physiological roles of the renin-angiotensin system (RAS) in the vertebrates", was composed of 2 subjects: 1) effects of exogenously administered angiotensin on various bodily functions; 2) factors influencing endogenous renin level. Subject 2 includes the topics on possible mechanisms causing renin release. Sokabe and Ogawa reviewed the literature extensively in 1974 and summarized the concepts on evolution of the juxtaglomerular apparatus and renal renin activity, chemical structures of angiotensins, and possible physiological roles of the RAS among vertebrates. The present article intends to summarize the current concepts on the above subjects based on the published studies, including more recent works, and a part of the findings presented by participants during the seminar (cited from abstracts). Function of the RAS in fishes, phylogeny of renal effect of angiotensin, and function of the RAS among vertebrates in relation to hydro-mineral regulation were previously reviewed by Nishimura and Ogawa, Sokabe, and Taylor, respectively. Details of the physiology of the RAS in mammals are beyond the scope of this review.

Six questions were proposed during the session on which discussions were focused. Those are: 1) Does the RAS play a role in the control of blood pressure or blood volume? 2) Is the adrenergic nervous system involved in the control of renin release? 3) Does the renin-angiotensin-mineralocorticoid axis exist in nonmammalian vertebrates? 4) Does the RAS regulate renal function by its systemic or intrarenal action? 5) Does the RAS influence calcium metabolism? 6) Does the RAS regulate "drinking" physiologically? Speculations on the functional evolution of the RAS described at the end of this article represent principally the reviewer's concepts on this subject.
RENIN-ANGIOTENSIN SYSTEM IN MAMMALS

1. Possible function of the renin-angiotensin system

It has been shown in several mammalian species that angiotensin stimulates aldosterone secretion from the adrenal cortex. A role of the renin-angiotensin-aldosterone system in the control of blood pressure has been proposed since angiotensin II exerts a potent vasopressor action by constricting peripheral arterioles, and the renin and angiotensin levels are high in some forms of hypertension induced experimentally or by disease in man, and since aldosterone is an important regulator of blood volume and sodium balance. However, the participation of the renin-angiotensin system in maintaining physiological levels of blood pressure is uncertain.

Angiotensin may regulate renal blood flow and glomerular filtration rate by intrarenal feedback mechanisms. Under certain circumstances, angiotensin produces natriuresis, although it is not clear whether angiotensin inhibits Na reabsorption by directly affecting tubular Na transport, or whether the natriuresis is secondary to renal hemodynamic changes produced by angiotensin.

Angiotensin acts on the central nervous system. In rabbits, dogs, and men, angiotensin exerts a greater rise of blood pressure when infused into the vertebral arteries than when given intravenously. The possible site of action is the area postrema in the distal medulla, where the blood-brain barrier is deficient. Intracranial injection of angiotensin stimulates water intake in mammals. Most sensitive dipsogenic sites appear to be located in the medial preoptic areas and the subfornical organ.

Interactions between the renin-angiotensin system and other vasoactive systems have been demonstrated. Administration of angiotensin into the central nervous system appears to release antidiuretic hormone from the neurohypophysis. Infusions of angiotensin into the carotid artery of conscious dog or angiotensin perfusion through the ventriculo-cisternal system of anesthetized dog increased plasma levels of antidiuretic hormone. It has been shown that the brain contains all the components of the renin-angiotensin system. However, the physiological significance of the renin-angiotensin system in the brain remains to be determined.

It has been suggested that the renal kallikrein-kinin system protects the renal vasculature against the constricting action of angiotensin. Although urinary kallikrein excretion is reduced during renal arterial constriction and in some hypertensive diseases, it is not clear whether the renal kallikrein-kinin system may participate in the etiology of hypertension, either independently or in relation to the renin-angiotensin system. Both angiotensin level and urinary kallikrein excretion are high in sodium-depleted subjects. Infusion of angiotensin II into the dog kidney is accompanied by dose-dependent increases of prostaglandins in the venous effluent and blunting of the vasoconstrictor-antidiuretic action of angiotensin II. The renal prostaglandins and renin seem to stimulate each other's formation or release, but oppose each other's action.

2. Mechanisms regulating renin release

This subject was reviewed extensively by Davis and Freeman. It is generally recognized that 3 groups of mechanisms are involved in the control of renin release.
in mammals: 1) 2 intrarenal receptors, the renal arteriolar receptor and the macula densa; 2) the renal sympathetic nerves and an adrenergic receptor; and 3) several humoral agents, including epinephrine, norepinephrine, angiotensin II, vasopressin (antidiuretic hormone), prostaglandins, some steroid hormones, and sodium and potassium ions.

The renal arteriolar receptor, which is presumably located in the juxtaglomerular cells of the afferent arteriole, responds to decreased renal perfusion pressure by increasing renin secretion. The macula densa, a renal tubular receptor, appears to detect changes in either the load or concentration of sodium or chloride at the macula densa site, and transfers the information to the juxtaglomerular cells.

It has been shown that the sympathetic nerve fibers containing adrenergic vesicles innervated the juxtaglomerular cells. Stimulation of renal nerves causes renin release, whereas renal renin content decreased after denervation. It appears that beta adrenergic receptors which are presumably located in the juxtaglomerular cells mediate renin release.

Stimulation of the midbrain or medulla oblongata increased renal renin secretion with a concomitant rise in blood pressure. On the other hand, Zehr and Feig demonstrated that intermittent hypothalamic stimulation suppressed plasma renin activity in unrestrained conscious dogs, presumably by inhibiting a resting "tonic" sympathetic discharge to juxtaglomerular cells. Both responses, enhancement and suppression of renin, were blocked by renal denervation. Zehr et al further showed that repetitive distension of cardiopulmonary receptors which have been shown to modify reflexly peripheral sympathetic outflow caused an acute reduction in renin secretion. This renin secretion was dependent on vagal afferent and renal sympathetic efferent pathways.

Recent studies indicate that inhibition of prostaglandin synthetases with aspirin-like compounds inhibited renin release caused by acute hemorrhage or furosemide and by constriction of renal artery in the rabbit, and renin release from slices of rabbit renal cortex. Intrarenal infusion of a small dose of indomethacin (0.1 mg/Kg/min) significantly reduced prostaglandin synthesis and renin secretion in the dog. Prostaglandins may be a mediator of renin release.

**ACTIONS OF EXOGENOUSLY ADMINISTERED ANGIOTENSIN IN NONMAMMALIAN VERTEBRATES**

1. **Vasopressor action of angiotensin (Fig. 1)**

   Synthetic angiotensins I and II, natural homologous angiotensin (product by the incubation of kidney extract with homologous plasma), and kidney extract exert vasopressor action in the dogfish, teleosts, lungfishes, amphibians, reptiles, and birds. Angiotensin I produces a pressor effect by being converted to angiotensin II, presumably by a converting enzyme in the test animals. Injection of homologous kidney extracts into eels or the bullfrog, *Rana catesbeiana* produces a prolonged pressor effect, whereas injection of angiotensin produces quicker and shorter vasopressor responses. No study was done on the pressor effect of angiotensin or kidney extract in cyclostomes.
2. Increase in steroid secretion by angiotensin (Fig. 1)

Angiotensin or homologous kidney extract (renin preparation) increased aldosterone and/or corticosterone secretions in several nonmammalian animals. Intravenous administration of the homologous kidney extract increased aldosterone, or aldosterone and corticosterone secretion rates into the postcaval vein in the hypophysectomized and anesthetized frog, and increased both aldosterone and corticosterone secretions in the pithed frog. Corticosterone secretion increased in the anesthetized and dexamethasone treated turtle, Pseudemys scripta elegans, after infusion of homologous kidney extract, but no increase in steroid secretion occurred in a crocodile, Caiman sclerops, or cockerel, Gallus domesticus. In these experiments, however, arterial blood pressure, and often postcaval plasma flow also, increased after infusion of the kidney extract.

The adrenal (interrenal) tissue of teleosts does not compose a coherent organ, and it is thus difficult to collect venous effluent from the interrenal tissues to determine angiotensin’s effect on steroid secretion. Taylor and Davis noted that an injection of native carp angiotensin (product by incubation of the kidney extract with homologous plasma) into hypophysectomized and anesthetized frogs increased aldosterone and corticosterone secretions. On the other hand, carp kidney extract
or \([\text{Asn}^1, \text{Val}^5]\) angiotensin II \([\{\text{Asn}^1, \text{Val}^5\}\text{AII}\]) increased blood pressure of the frog, but had no effect on steroid secretion.\(^{42}\) Injection of eel kidney extract or \([\text{Asn}^1, \text{Val}^1]\)AII into intact, hypophysectomized, or Stannicectomized (remove corpuscles of Stannius) eels increased the cortisol level in the arterial blood.\(^{48}\) Since blood pressure increased concomitantly, the possibility cannot be excluded that the increased cortisol secretion may be accounted for by the increased blood flow which perfuses the interrenal cells.

\([\text{Asn}^1, \text{Val}^5]\)AII, added to the incubation medium, failed to stimulate \textit{in vitro} corticoid production in the adrenal cortex from the chicken.\(^{49}\)

3. \textit{Renal action of angiotensin} (Fig. 1)

Administration of angiotensin usually produces dual effects in normotensive man and other mammals: antidiuresis and antinatriuresis at relatively small doses, and natriuresis at higher doses.\(^{10}\) The renal effects of angiotensin have been studied in several nonmammalian species.\(^{38}, 50\) \([\text{Asn}^1, \text{Val}^5]\)AII\(^{38}, 50\) and homologous native angiotensin\(^{48}\) caused diuresis and natriuresis in eel with concomitant increases in glomerular filtration rate (GFR) and dorsal aortic pressure. Diuresis occurred only after clear vasopressor doses of angiotensins, suggesting that angiotensin diuresis might largely be a consequence of increased renal perfusion pressure. Intraarterial infusion of nonpressor doses of synthetic or natural angiotensin did not increase urine flow or urinary Na excretion. However, when low doses of angiotensin were administered via a renal portal vein in the intact Japanese eel, antidiuresis and antinatriuresis occurred (T. Hirano; personal communication). Norepinephrine increased dorsal aortic pressure of eels, as did the angiotensin, but urine flow and filtration rate decreased.\(^{38}\)

Pressor doses of \([\text{Asn}^1, \text{Val}^5]\)AII produced mild to moderate diuresis and natriuresis in the African lungfish, \textit{Protopterus aethiopicus},\(^{39}\) and in the Australian lungfish, \textit{Neoceratodus forsteri}.\(^{40}\)

Pang et al\(^{51}\) infused angiotensin into the Chilean toad, \textit{Calyptocephellia caudioverbera}, through 2 routes, the dorsal aorta and the renal portal vein. In this species, systemic injection of \([\text{Asn}^1, \text{Val}^5]\)AII in as small a dose as 0.1 ng/Kg produced a distinct increase in blood pressure. A high pressor dose of angiotensin administered into unanesthetized toads through the renal arterial system caused diuresis and natriuresis, presumably due to increased renal arterial pressure, but antidiuresis and antinatriuresis occurred after a small (mild pressor) dose of angiotensin. When the renal arterial system and the portal system were individually infused under constant pressure in an \textit{in situ} preparation and angiotensin was given through the portal system, both high and low doses produced antidiuresis and antinatriuresis.\(^{51}\) \([\text{Asn}^1, \text{Val}^5]\)AII injected into the renal portal vein of an isolated toad kidney, \textit{Bufo paracnemis}, also caused antidiuresis and antinatriuresis.\(^{52}\) However, \([\text{Asn}^1, \text{Val}^5]\)AII increased urine flow and Na excretion without change in filtration rate in \textit{Xenopus laevis} which were maintained in distilled water and had high GFR.\(^{53}\) It has been shown that angiotensin II increased water permeability of the urinary bladder in \textit{Bufo paracnemis}.\(^{54}\)

\([\text{Asn}^1, \text{Val}^5]\)AII was infused into the leg vein of the chicken which eventually perfuses the renal tubules independently of glomerular circulation.\(^{55}\) Diuresis and natriuresis in spite of decreased GFR were much more evident at the angio-
4. *Stimulation of drinking by angiotensin* (Fig. 1)

Induction of drinking behavior by angiotensin in various species was reported by Takei, Kobayashi et al., and Hirano et al. Intravenous injection of [Asn\(^1\), Val\(^5\)]AII or partially purified native eel angiotensin into freshwater adapted (500 ng/Kg) or seawater adapted (50 ng/Kg) Japanese eels stimulated water intake. This angiotensin effect was observed in eels with hypothalamic lesions and in decerebrated eels, whereas vagotomy abolished the dipsogenic effect of angiotensin. Freshwater adapted eels started drinking also when the water was replaced by seawater. Injection of [Asn\(^1\), Val\(^5\)]AII at doses of 50 \(\mu\)g to 25 mg/Kg into the frog, Rana brevipoda, submerged in fresh water did not induce drinking, whereas the frogs began to drink water when they were immersed in 50% seawater.

Reptiles (turtles, lizards, and snakes) responded to intraperitoneal injection of [Asn\(^1\), Val\(^5\)]AII by drinking water. However, snakes which lived in arid regions and those under hibernation failed to respond, or responded only to very high doses of angiotensin.

Intracranial injection of [Asn\(^1\), Val\(^5\)]AII induced drinking in the white-crowned sparrow, *Zonotrichia leucophrys gambelii*, white Leghorn cocks, and in the Japanese quail, *Coturnix coturnix japonica*. Sensitive sites to angiotensin are the medial preoptic area and subfornical organ, and the minimum effective dose was 10–50 ng/Kg in the quail, but it was much higher in the sparrow and chicken. The effective dosage and sensitive region in the quail are similar to those of the rat.

Electrical destruction of the subfornical organ of the quail attenuated water intake induced by injecting angiotensin into the preoptic area. When the fluorescent fibers connecting the medial preoptic area and the subfornical organ were cut, injection of angiotensin into the preoptic area did not induce drinking. Thus, it appears that the information generated by angiotensin at the preoptic area is transmitted to the subfornical organ through neural pathways. Takei and Kobayashi found that intracranial injection of noradrenaline and serotonin also induced drinking in the quail. Kobayashi et al. examined drinking response to intraperitoneal administration of angiotensin in various species of birds and mammals. Animals which live in arid areas and do not have free access to water and those which depend on food for most of their ingested water in nature are relatively insensitive to angiotensin.

**FACTORS REGULATING ENDOGENOUS RENIN ACTIVITY IN NONMAMMALIAN VERTEBRATES**

1. *Hemodynamic factors* (Fig. 1)

   a. *Effect of hemorrhage*

   Since kidneys of primitive vertebrates lack a macula densa, they provide a pertinent model for elucidating the function of the vascular receptor, if any, which presumably exists in the granulated cells and responds to hemodynamic factors by
influencing renin release. Toadfish kidneys have neither functioning glomeruli nor a macula densa, but possess granulated epithelioid cells similar to mammalian juxtaglomerular cells in the small arteries and arterioles. Angiotensin from the agglomerular toadfish, *Opsanus tau*, is bound to human angiotensin I antibody, and the radioimmunoassay method is usable for determination of plasma renin activity (rate of angiotensin I generation). The sensitive radioimmunoassay makes it possible to determine changes of plasma renin activity in a given animal by repeatedly drawing a small amount of blood samples.

Plasma renin activity increased markedly in unanesthetized toadfish after a cumulative hemorrhage, a single massive hemorrhage, or after an injection of a vasodilator, papaverine. These results appear to suggest that renin was released in toadfish, in spite of an absence of macula densa, in response to decrease in blood pressure and/or blood volume.

Blaine et al developed a nonfiltering kidney model in the dog, in which the macula densa was made nonfunctional by ligating and sectioning the ureters with a 2-hour period of bilateral clamping of renal arteries. Hemorrhage increased renin secretion from the nonfiltering and denervated kidney by stimulating the afferent arteriolar receptor of the juxtaglomerular apparatus. Sodium delivery to the renal tubule is therefore not essential for renin release after hemorrhage in either the dog or the toadfish.

Although the presence of intact renal nerves is not essential for renin release after hemorrhage, renal nerves participate in increased renin secretion, since renin release occurs after hemorrhage in the innervated nonfiltering kidney of the dog, in which the function of the arteriolar receptor was blocked by intrarenal infusion of papaverine. Thus, it has to be clarified in the toadfish whether a reduction in renal perfusion pressure due to decreased systemic pressure may stimulate a possible vascular receptor in the granulated cells, or whether decreased blood pressure or blood volume may affect the activity of granulated cells reflexly by signals transmitted through the renal nerves.

Chan and Holmes found that acute hemorrhage increased plasma renin activity in intact and hypophysectomized pigeons proportionately to the amount of blood withdrawn. Hypophysectomized pigeons showed low blood pressure and high plasma renin levels, as compared to intact pigeons.

**b. Effect of blood volume**

It has been shown in mammals that cardiopulmonary receptors are involved in renin release. An increase in left atrial pressure, which reflects a change of blood volume, reflexly reduces the rates of renin secretion via vagal afferent and renal sympathetic efferent pathways.

Isosmotic volume expansion produced in the Australian lungfish, *Neoceratodus forsteri*, by infusing isosmotic saline decreased plasma renin activity. The receptor and the pathway through which the stimulus is transmitted, however, remain to be determined.

2. **Autonomic nervous system**

Beta adrenergic receptors, which are presumably located in the juxtaglomerular cells, appear to mediate renin release in mammals. Interaction between the renin-angiotensin system and the adrenergic nervous system has been little studied.
in nonmammalian vertebrates. Systemic injection of a beta adrenergic drug, isoproterenol, into the unanesthetized agglomerular toadfish increased plasma renin activity with a concomitant decrease of blood pressure. It remains to be determined, however, whether the increase in renin activity may be ascribed to direct stimulation of the granulated cells by isoproterenol or whether decreased blood pressure caused renin release. Infusion of propranolol (1 mg/Kg/hr) almost completely inhibited both the vasodepressor response to isoproterenol and the increase in plasma renin activity.

3. Renin activity in sodium-depleted or sodium-loaded animals (Fig. 1)

Since the renin-angiotensin system stimulated aldosterone secretion, and plasma renin activity increased in response to sodium depletion in man and some mammals, evidence for homologous function has been sought in primitive animals. There is no clear indication, however, that the renin-angiotensin system is activated or that it helps to conserve sodium in teleosts or amphibians in hyposmotic media.

Contrarily, plasma renin levels, and plasma renin and cortisol levels in eels showed a transient increase after transfer in the opposite direction, from fresh water to seawater. The histological appearance of juxtaglomerular cells in fishes adapted to either hypo- or hyperosmotic media seems to be compatible with the observed changes in plasma renin activity. Responses of renal renin activity to salinity changes vary among species and are not the same as responses of plasma renin. Renal renin activity and granularity of the juxtaglomerular cells increased in the cockerel, Gallus domesticus, depleted in sodium by maintenance on a low Na diet plus a mercurial diuretic drug.

4. Renal function (Fig. 1)

Functional interrelationships among the macula densa, juxtaglomerular cells, and the glomeruli have been suggested. A possible intrarenal feedback mechanism, in that macula densa senses some changes in composition or flow of renal tubular fluid and influences the rate of glomerular filtration in the same nephron, has been tested using the micropuncture method. In this feedback mechanism, juxtaglomerular cells and the renin-angiotensin system may be involved in regulating single nephron filtration rate. Thurau and coworkers demonstrated that retrograde perfusion of the macula densa with hypertonic sodium chloride solution increased the renin activity in a single juxtaglomerular apparatus. They proposed that the renin-angiotensin system regulated GFR by constricting the afferent arteriole upon receiving the signals transmitted from the macula densa.

Sokabe proposed the hypothesis that the renin-angiotensin system regulated GFR by constricting the efferent arterioles. Plasma renin activity and GFR decreased in the bullfrog by dehydration, whereas both GFR and plasma renin activity increased after infusion of isotonic saline or glucose solution. Constriction of the efferent arteriole was observed microscopically under direct observation of the glomerular circulation.

In teleost fishes, however, increase in plasma renin activity does not correlate with increase in filtration rate. When euryhaline teleosts, which can survive either in seawater or fresh water by maintaining relatively constant internal osmolality,
are introduced from hyper- to hyposmotic media, the kidney plays a role in adapta-
tion in a biphasic manner. Glomerular filtration rate increases immediately
(primary adjustment), and renal tubules become less permeable to water within
a few days (secondary adjustment). Thus, excess water is excreted along the osmotic
gradient as a large amount of dilute urine. However, plasma renin activity de-
creased in eels during freshwater adaptation, and it increased during seawater
adaptation with a concomitant decrease of GFR. Exogenous administrations
of angiotensins, contrarily, increased filtration rate. The functions of endo-
genous and exogenous angiotensins may not be the same. It has to be considered,
also, that measurement of plasma renin activity by the bioassay method may not
be adequate to detect small changes.

5. Calcium metabolism (Fig. 1)
A possible role of calcium in the control of renin release has been suggested in
mammals. Intrarenal infusion of calcium chloride into anesthetized dogs sup-
pressed or increased renin secretion, and chronic calcium loading in Na-
depleted rats suppressed plasma renin activity. Inhibition of renin release by
acute calcium infusion may be related to the finding that hypercalcemia directly
inhibits sodium transport across the thick ascending limb of Henle’s loop, which
results in an increase in sodium delivery to the macula densa.

It has been shown that the corpuscles of Stannius (CS) are involved in calcium
regulation in teleost fishes. Injection of extract from CS into Stanniectomized
killifish or those maintained in Ca-deficient seawater decreases the serum calcium
level. This Ca decreasing substance (hypocalcin) is most likely an acid-
stable peptide hormone, although the mechanism by which this hypocalcin
decreases circulating Ca level is not clear. Recent study by Ma and Copp shows
that the extract from the CS of salmon inhibits active branchial calcium uptake in
the isolated eel gill preparation and inhibits calcium-dependent ATPase which is
isolated from the gill plasma membrane of the rainbow trout. Branchial calcium
permeability decreases in the intact killifish after injection of corpuscle extract.

When goldfish were transferred into 1/3 seawater, plasma levels of Na, K, and
Ca, and water movement in the isolated intestine increased with histological hyper-
function of the CS. These responses were not observed, however, in goldfish
moved into Ca-rich fresh water. The corpuscles of Stannius possess a renin-like
substance which is capable of generating a pressor substance from plasma, although the cells in these glands are not identical to the granulated cells in the kidney, which presumably contain renin. Angiotensin-like substances produced
by incubating CS homogenate from the carp with carp plasma decreased plasma
calcium level of intact eels. It remains to be determined whether CS angiotensin
or renin is chemically the same substance as hypocalcin.

Evolutionary Speculations

Vasopressor actions of angiotensins have been noted extensively among vari-
ous classes of vertebrate. Hemorrhage and hypotension appear to be potent
stimuli for increasing plasma renin activity in an agglomerular teleost, a bird, and
mammals. The observation by Johnston et al that aldosterone increased markedly
after hemorrhage in the American bullfrog may indicate that renin is released, and thus aldosterone increased, in response to hemorrhage in this species. When we consider these physiological observations together with the anatomical evidence that the evolution of the juxtaglomerular cells precedes that of the macula densa, one might speculate that the renin-angiotensin system may have evolved phylogenetically as a humoral regulator of blood pressure homeostasis.

If the renin-angiotensin system regulates blood pressure in primitive animals, why do the cyclostomes and elasmobranchs not possess renin while holocephalians, a cartilaginous fish differing from elasmobranchs, and primitive bony fishes do have renin? Do cyclostomes and elasmobranchs regulate blood pressure by a system differing from that in bony fishes? Since the current determination method of renin is based on the vasopressor biosay in the rat, there remains a possibility that these primitive fishes possess a system similar to the renin-angiotensin system which does not exert pressor action in the rat.

The relation between the autonomic nervous system and the renin-angiotensin system in nonmammalian vertebrates has to be clarified. We need to determine innervation of granulated cells in the renal arteries and arterioles, the effects of electrical stimulation or lesions of the central nervous system and renal nerves on renin release, and the effects of adrenergic drugs and adrenergic blocking drugs on renin release.

The question also arises as to whether the renin-angiotensin-mineralocorticoid system exists in nonmammalian vertebrates. Differing from that in mammals, in which plasma renin activity usually increases in response to sodium depletion, plasma renin activity showed either a decrease or no change in sodium-depleted teleosts. Contrarily, plasma renin activity increased transiently during seawater adaptation. These apparently contradictory findings may be understandable if we consider that angiotensin may stimulate cortisol secretion in teleosts. Cortisol plays an important role in seawater osmoregulation in teleosts by promoting the extrusion of sodium across the gill and increasing water absorption in the gut.

Both plasma renin activity and plasma cortisol increased during seawater adaptation of freshwater eels. However, elevated endogenous mineralocorticoid levels in the toad adapted to distilled water do not accompany the increase in plasma renin activity although exogenous administration of homologous kidney extract or angiotensin increased secretions of aldosterone and corticosterone in the frog.

Does the renin-angiotensin system in primitive animals have only systemic action, such as regulation of blood pressure or stimulation of adrenal steroid secretion, or does it work locally in the kidney? Granulated cells are widely distributed along the renal arteries in primitive animals and show a tendency to be localized near the glomeruli in more advanced animals. If we are allowed to speculate, this distribution pattern of granulated cells appears to suggest some shift of the action of the renin-angiotensin system from a systemic endocrine function to a local regulator of glomerular function along the phylogenetic advancement.

Angiotensin stimulates water intake in various species above teleosts except for amphibians. Does the renin-angiotensin system regulate “drinking” physiologically in nonmammalian vertebrates? In mammals and birds, intracranial injection of a small dose of angiotensin stimulates drinking behavior. In reptiles and
teleosts, large doses of angiotensin injected into the systemic circulation induced drinking behavior. Even decerebrated eels responded to angiotensin by drinking water, suggesting that angiotensin acts in the rhombencephalon or on receptors outside the central nervous system. The fact that angiotensin stimulates drinking in seawater eels, which naturally drink water to compensate for osmotic water loss, by a much smaller dose than in freshwater eels suggests that angiotensin may have a physiological role in drinking behavior in fish. Increase in plasma renin activity during seawater adaptation of eels may stimulate drinking. It will be necessary to determine whether the brains of primitive animals contain renin and whether the intracranial injection of small doses of angiotensin elicits drinking behavior. The hemodynamic effect of angiotensin in drinking behavior has to be considered since the doses injected in reptiles and teleosts are large.

It will be interesting to determine whether an angiotensin-like substance in the corpuscles of Stannius (CS) in teleost fishes acts as hypocalcin. According to criteria for definition of endocrine organs, H. Sokabe (personal communication) proposed to determine: 1) deficiency syndrome, which will be caused by removing CS; 2) whether the deficiency syndromes are eliminated by injecting CS extract; and 3) isolation of active substance. The first two have been shown in several species of teleosts. He further suggests testing the hypocalcemic action of the synthesized native CS angiotensin to determine the identity of hypocalcin and CS-angiotensin. The renin-angiotensin system appears to exert multiple actions and functions in man and other mammals. Some of these functions may be more important in primitive animals, although a similar function remains in man as a relic of the primitive system with little physiological significance, since man is the result of a long history of natural selection of physiological processes (C. Ladd Prosser). The comparative approach will put us into perspective in biological history and phylogenetic relationships.

The function of the renin-angiotensin system may have also changed during the phylogenetic history of the vertebrates, allowing living creatures to adapt to their diverse environments, from the marine to the freshwater environment and from aquatic to terrestrial life. Besides, the mechanisms by which the renin-angiotensin system is operated become more complicated when other hormonal systems, such as the adrenal cortex, neurohypophysial hormones, prostaglandins, and the autonomic nervous system, are involved. Analysis of the functional evolution of the renin-angiotensin system by a comparative approach in relation to the environment and to the evolution of various endocrine organs will aid in better understanding of the underlying mechanisms of the operation in mammals.

The selection of appropriate experimental species for comparative studies is extremely important. The currently available species are not the same as those during vertebrate evolution. One has to consider the evolution of species in the phylogenetic scale in relation to a variety of environments. The majority of physiological studies of angiotensin effects are done by using heterologous angiotensin, and measurement of renin activity is based on the rat vasopressor assay. It is an urgent need that the chemical properties of angiotensins from primitive vertebrates be determined and native angiotensins be synthesized. The effects of native angiotensins should be studied in homologous species. By making an antibody to native angiotensin, renin activity can be measured by the radioimmunoassay method.
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