REVIEW

Comparative Study of Renin-Containing Cells. Histological Approaches

Yasuhiro KON

Laboratory of Experimental Animal Science, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo 060–0818, Japan

(Received 24 March 1999/Accepted 11 June 1999)

ABSTRACT. The juxtaglomerular apparatus is known to be the functional unit of renin control. In the present review, the author will describe the comparative characteristics of renin-containing (RC) cells as well as extrarenal distribution, paying special attention to developmental and topographical approaches. The characteristic locality of RC cells suggests that the secretion of renin is performed at a site beside the adventitia or via the glomerular capillaries. Ontogenetical and phylogenetical investigations of RC cells have provided interesting findings on their morphogenesis. Analysis of the endocrine kidney after unilateral obstruction of the ureter provides some findings about the origin of RC cells and the processing of renin granules. Observation of developing adrenal renin suggests that there is important involvement of angiotensin II produced by renin synthesis in the morphogenesis of the adrenal gland in the fetal stage. Coagulating gland (CG) renin is characterized by testosterone-regulated and exocrine mechanisms. Recently, all or some of the components of the renin-angiotensin system (RAS) have been reported to be synthesized and secreted outside of classical organs or tissues. In the future, the real function of local RAS will be clarified by using gene targeting in mice.—KEY WORDS: coagulating gland, development, immunohistochemistry, kidney, renin-angiotensin system.

J. Vet. Med. Sci. 61(10): 1075–1086, 1999

The relationship between the kidney and hypertension was confirmed by Tigerstedt and Bergmann, who reported the creation of hypertension in rabbits with injections of homogenized kidney extracts and termed this active factor "renin" [109]. Thus, one century has passed since the discovery of the renin-angiotensin system (RAS). The first histological study of juxtaglomerular (JG), namely renin-containing (RC) cells, was made by Ruyter, who observed specialized granule-containing smooth muscle cells in the walls of the afferent arterioles [92].

The juxtaglomerular apparatus (JGA) known as the functional unit of renin control, is classically located at the hilus of the glomerulus and consists of tubular and vascular elements (Fig. 1). The tubular element, the macula densa (MD), closely contacts the glomerular vascular pole and contains densely packed nuclei. The vascular element of the JGA is composed of afferent and efferent arterioles containing the JG cells, and the extraglomerular mesangium (EGM) contiguous with the intraglomerular mesangium. The above definition and compartmentalization correspond generally to the mammalian JGA. In the present review, the author will describe the comparative characteristics of RC cells as well as extrarenal distribution, with special reference to developmental and topographical approaches. As a result of recent advances in biological science there is a tendency to ignore more traditional methods of investigation, resulting in the omission of interesting findings. The author is of the opinion that "seeing is believing".

OVERVIEW OF RAS

It is now established that a trigger enzyme, renin, secreted from JG cells changes liver-derived angiotensinogen in blood circulation into angiotensin I. Angiotensin I is metabolized by angiotensin-converting enzyme (ACE) localized in the lung into angiotensin II, which is the most effective hormone regulating the contraction of vascular smooth muscle [18]. In JG cells, inactive prorenin is released by a constitutive pathway, while active renin is released by a regulated pathway, observed morphologically as exocytosis (Fig. 1). In molecular biology, renin genes from several animal species have been cloned [2, 10, 34]. A single renin locus is identified in the human and rat. Some mouse strains, for example C57BL/6, have a single renin locus (Ren-1), whereas other strains, for example DBA/2, have duplication of the renin structural gene (Ren-1, Ren-2) [89, 99].

In situ hybridization, northern blot analysis and immunohistochemical techniques employing bilateral nephrectomy or colchicin injections are useful for detection of hepatic angiotensinogen [11, 27, 91]. Angiotensinogen in the human liver is synthesized as a precursor composed of 485 amino acid residues, cleaved into a signal peptide composed of 33 amino acid residues and glycosylated angiotensinogen, and then immediately released by a possibly constitutive pathway [15]. Because of the large size of the angiotensinogen molecule compared with the decapeptide, angiotensin I, it has been hypothesized that angiotensinogen may have additional functions in the case of inflammation [35]. Renin localized in postcapillary venules of lymph nodes and Peyer's patches may play a

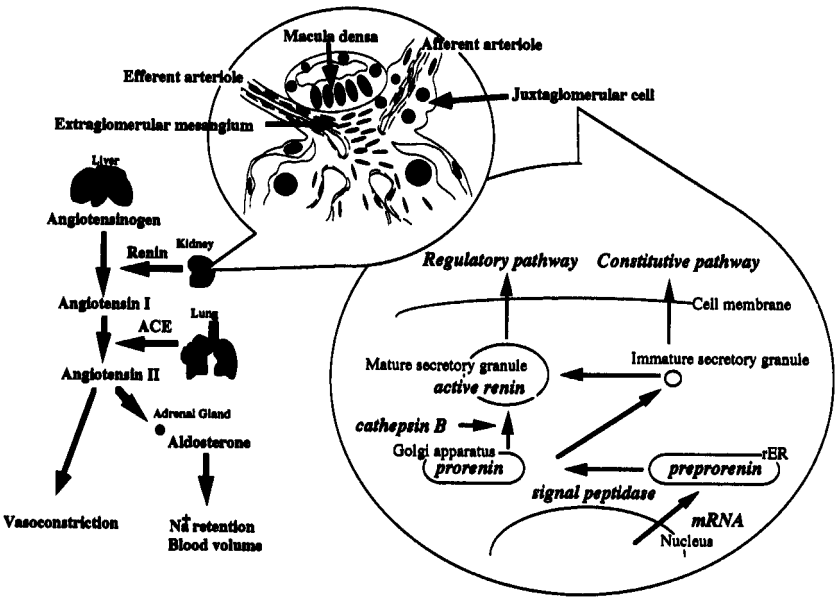

Fig. 1. Schematic view of renin-angiotensin system.

role in immunological defense, in concern with this circulating angiotensinogen (Kon, personal communication). ACE, which exists as both a membrane-bound and soluble type, has been extensively characterized and purified from several sources, including serum, lung and seminal fluid [84], and subtyped into two isozymes, somatic ACE and testicular ACE [37, 97]. Several ACE inhibitors have been investigated for clinical therapeutics against hypertension, arteriosclerosis and brain dysfunction [13, 102].

The angiotensin II type-1 (AT1) receptor, cloned from several animals, is a member of the seven-transmembrane, G protein-coupled receptor family. Two AT1 receptor subtypes, AT1A and AT1B, have been isolated in the rat and mouse [39, 94]. In the rat, AT1 receptor is expressed in the liver, heart, brain, lung, anterior pituitary, adrenal gland, and kidney [29]. Angiotensin II type-2 (AT2) receptor has been reported to be expressed at high levels in the fetal brain, but is only sparsely expressed in adult animals [75]. Studies using various nonpeptide antagonists against AT1 and AT2 receptors suggest that most, if not all, of the classically recognized functions of the RAS are mediated by AT1 receptor [110].

ONTOGENETICAL DEVELOPMENT OF RC CELLS IN THE KIDNEY

Comparative analyses of RC cells in domestic animals have been carried out immunohistochemically [60]. Generally, an overwhelming number of RC cells found as swollen epithelioid figures are localized at the tunica media of afferent arterioles just near the vascular poles according to many histological text books. However, unignorable quantities of RC cells are also found at the tunica media of efferent arterioles and the tunica adventitia of interlobular arteries, and in the mesangial regions of glomeruli (Fig. 2). This characteristic locality of RC cells suggests that the secretion of renin is performed at a site beside the adventitia or via the glomerular capillaries, rather than through the arteriolar endothelium.

To demonstrate the origin of RC cells, the developmental relationships between morphogenesis and RC cells have been investigated in mice [59, 73], rats [8, 20], pigs [21, 59], humans [12, 88] and sheep [49]. In mouse embryos, RC cells associated with arterial trees are first demonstrated on day 13 of gestation (Fig. 3). On day 16, the most intensive immunoreactivities are observed in the walls of the afferent, efferent and interlobar arteries and even in the mesangial regions. From day 18 to 7 days after birth, the immunoreactivities of the intrarenal arteries disappear distally. In pig embryos with a crown rump length (CRL) of 0.8–2.0 cm, RC cells are found at the ventral wall of the dorsal aorta, the omphalo-mesenteric (cranial mesenteric) artery, the mesonephric arteries, the mesonephric afferent glomerular vessels and inside the mesonephric glomeruli (Fig. 3). When the CRL is 5.1–14.0 cm, RC cells are not demonstrated at the aortic wall, while they are still observed on the afferent arterioles and in the vascular pole region of the degenerated mesonephros. In embryos with a CRL of 14.1–30.0 cm, intensive immunoreactivity is found at only the afferent arterioles in the metanephros. The difference of RC cell development between mouse and pig meso- and metanephroi is mainly due to the respective rates of glomerulogenesis. In mouse, the transient mesonephros is formed by day 12 of gestation, but begins to regress by day 13.5. In pig, however, the mesonephros is the largest transient organ of all domestic animals, suggesting that the cytological development and the functional maturation of mouse JGA require a more curtailed condensed period and,

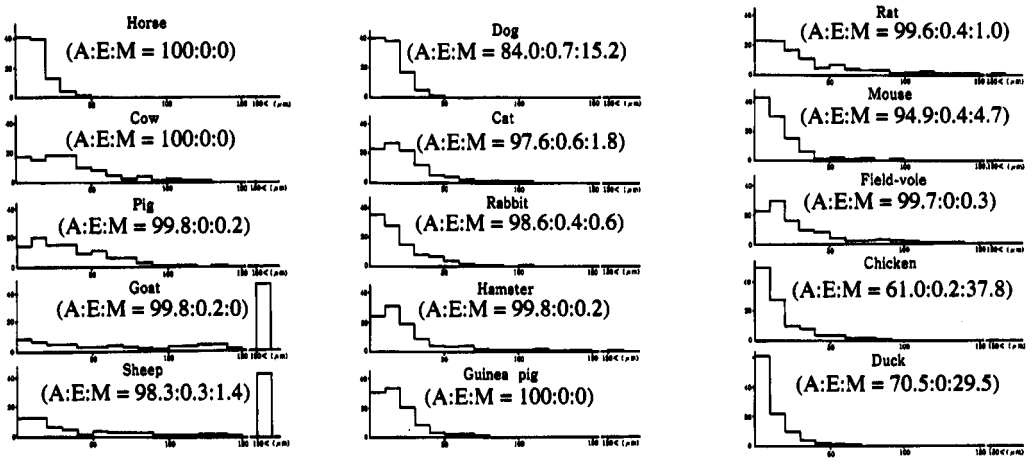

Fig. 2. Frequency and distribution of renal RC cells in several domestic animals [60]. The horizontal and vertical lines show the distance from afferent vessel and the frequency of RC cells, respectively. A:E:M is the ratio of the frequency of cells located in afferent, efferent and mesangial regions.

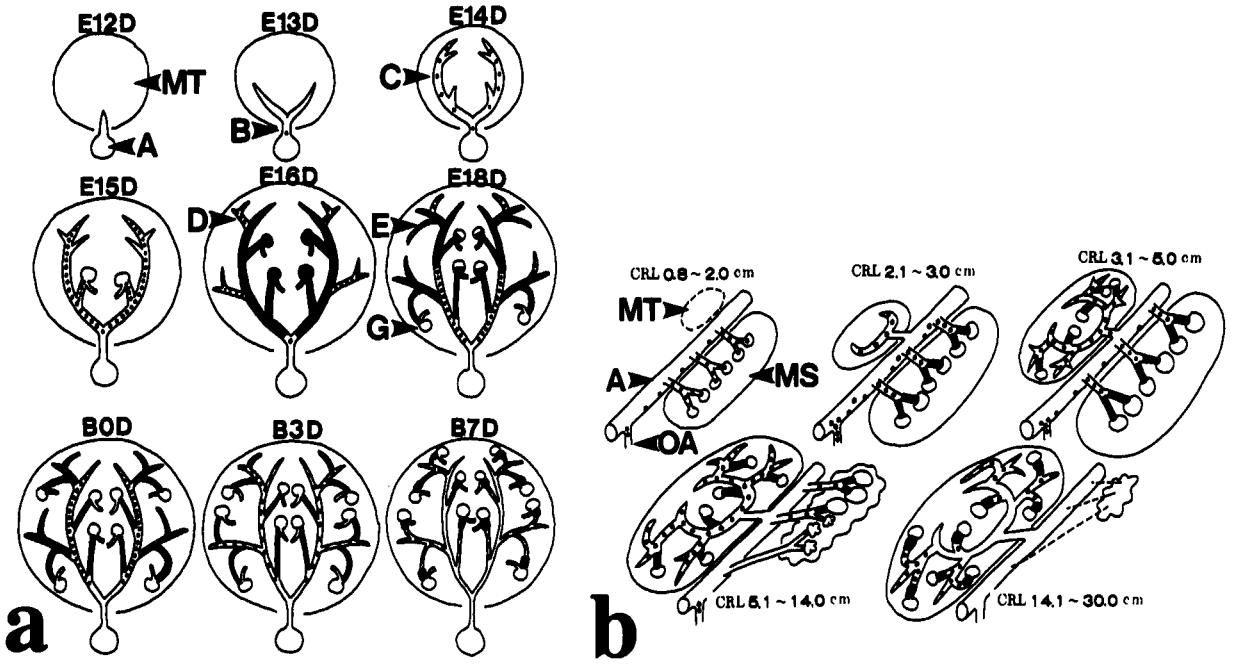

Fig. 3. Development of RC cells in the metanephros of fetal mice (a) and in meta- and mesonephroi of fetal pigs (b) [59].

consequently, show an apparent delay in functional maturation as compared with that of pig.

In sheep, at day 44 of gestation, RC cells are already demonstrated in the walls of renal, interlobar, and afferent vessels. Then they are detected throughout the intrarenal arterial trees. However, MD is not yet detected, suggesting a non-functioning JGA [49]. The RAS cannot be stimulated by furosemide before day 106 of gestation and the response, including renin release after day 110, increases with gestational age [98, 111]. In late gestational periods, RC cells distributed throughout the intrarenal arterial trees, tend to decrease or disappear gradually, while they are located predominantly in the afferent glomerular vessels, corresponding to the morphological and physiological maturation of the JGA in sheep in late fetal life [100]. The intrarenal smooth muscle cells constituting vessel walls potentially possess the ability to synthesize renin [17], as demonstrated by experimental hydronephrosis using the mouse kidney [63, 64, 66] and by restriction of renal blood flow [71] or inhibition of ACE [38]. Because of elevated

angiotensin II levels in newborn lambs [7], the RAS in the perinatal period plays an important role in the maintenance of circulatory homeostasis and in adaption to the changes of environment [83]. RC cells are widely distributed along the blood vessels in adult small ruminants [60], for instance, in interlobular arteries, where all RC cells in other animals totally disappear in adults [59]. Sympathetic nerve fibers possess many varicosities in contact with vascular walls on the way to the glomerular vascular pole [31], and axon-Schwann cell complexes are frequently observed around the JG cells [4]. Additionally, functional heterogeneity of RC cells has been demonstrated by reports that isoprenaline-sensitive RC cells are located in the afferent arterioles only at some distance from the glomerulus [6]. Previous studies have also shown that endogeneous renal sympathetic activity modulates renin gene expression along the renal microvasculature [85]. These findings may suggest that there are two populations of RC cells receiving several signals for renin release. RC cells close to the glomerulus would be sensitive to MD (sodium chloride) signals, whilst RC cells further away from the glomerulus would be sensitive to vascular innervation (beta-adrenergic) signals.

Peripolar cells, located at the boundary region between the external and internal glomerular epithelial cells, have been suggested to have some role as part of the JGA [28, 93]. Some small ruminants, including sheep, goats and deer, have many RC cells located at a distance from the glomerulus, and have also been recognized as possessing increased numbers of peripolar cells. Additionally, in the newborn sheep kidney, granulated peripolar cells are present in greater numbers than in kidneys from fetal lambs or adult sheep [1]. The relationship between RC cells and peripolar cells in these small ruminants supplies an interesting homeostasis in intrarenal regulation.

PHYLOGENETICAL STUDY OF RC CELLS

It is known in mammals that the MD and EGM are elements of a feedback control system. The humoral concentration in distal nephric tubules is sensed by MD cells and this information is transmitted to the JG cells via the EGM. According to previous phylogenetical results, the number of constitutional elements of the JGA decreases as the class of animals becoming more primal [55, 57, 58, 101] (Fig. 4). In birds, for instance, both the MD and EGM are juvenile [14]. In fish, these two elements are missing in

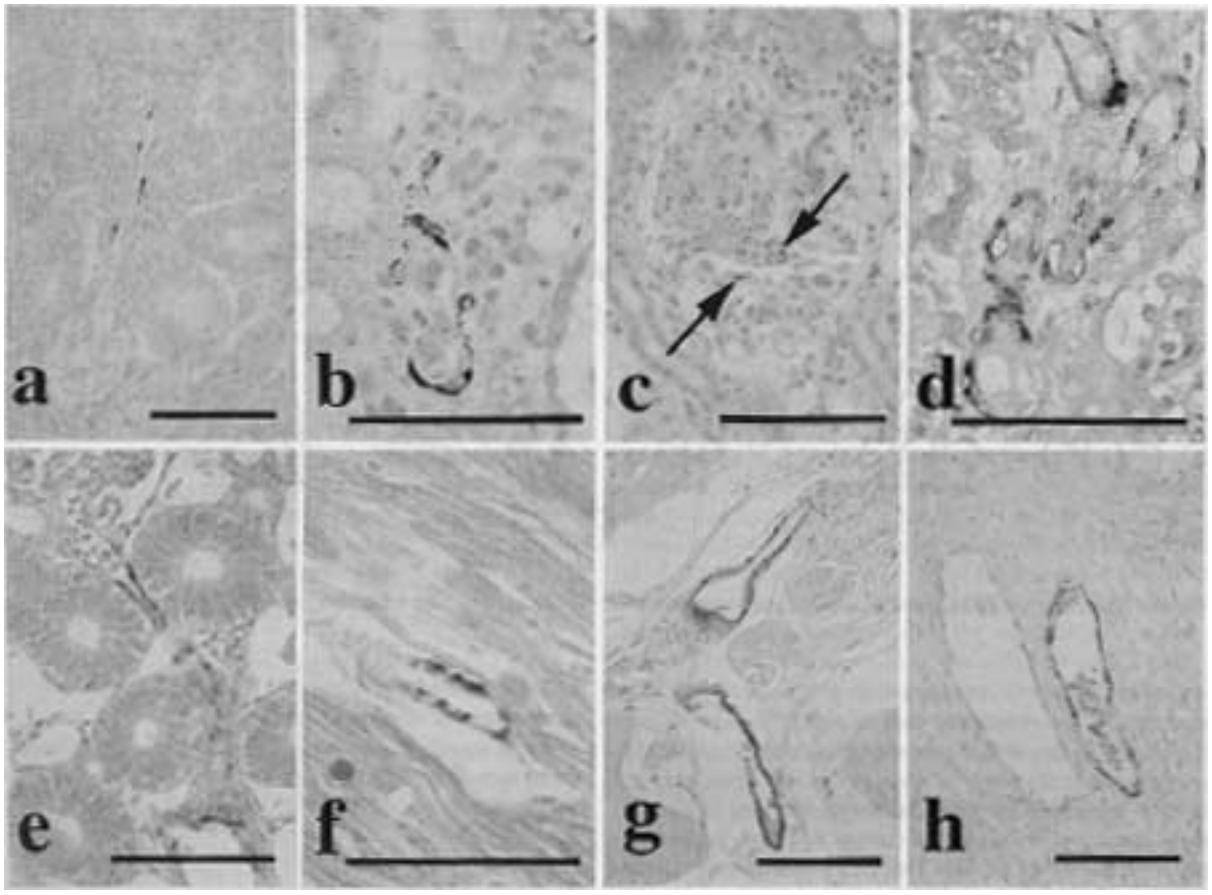

Fig. 4. Phylogenetical detection of RC cells [57, 58]. a: Carp kidney. b: Bullfrog kidney. c: Craw frog kidney. d: Python kidney. e: Elasmobranch kidney. f: Elasmobranch conus arteriosus. g: Elasmobranch gill. h: Elasmobranch rectum. bar=100 μm.

all their JGA [57]. Both secretory granules of JG cells and renin activity are first demonstrated in holocephalian [81] or elasmobranch fish [68].

The cyclostomes, which are one of the lowest vertebrates, possess a combined glomerulus getting a blood supply directly from the aorta [72]. In one of the cyclostomes, RC cells, MD and EGM are not observed, indicating that there is neither a structure nor possible function of JGA. There is functional RAS in elasmobranchs [104, 112]; however, RC cells are distributed widely to extrarenal tissues, including the aorta, intestine and gill (Fig. 4).

In the Cyprinidae and Salmonidae fishes, numerous RC cells are demonstrated mainly in the areas of the tunica media of the arterioles or the small arteries [57]. In each cell, the immunoreactivities are polarized toward the adventitial side rather than the medial side. In carp, three types of granules are observed and their possible transformation are suggested. The diameter of the granules is around 230 nm in carp, and no evidence for fusion of granules can be ascertained, while those of the rat and mouse JG cells are about 1 μm or greater, coalescing to form larger ones (fusion of granules). The duration from formation to secretion of granules in fish JG cells may be much shorter than that in mammalian JG cells. It has been reported that the administration of angiotensin increases blood pressure [80], and that the experimental reduction of aortic blood pressure or renal perfusion pressure of fishes also increases the activity of plasma renin [3, 79]. These findings suggest that the hormonal system for control of blood pressure by the RAS also exists in the teleost fishes.

In amphibians, the JGA consists of afferent and efferent arterioles and JG cells, and there is no EGM [58]. The MD-like structures are observed only in Rana species. RC cells are generally localized over a wide range in the walls of afferent vessels. In *Xenopus* species, RC cells are demonstrated numerously in the intraglomerular region. Amphibian species demonstrate multivesicular body-like granules commonly in the JG cells, like those of fish, chickens and mammals [55, 57, 58]. Although it has been postulated that renin granules are modified lysosomal granules, based on the cytological similarities between multivesicular bodies and juvenile renin granules [105], no JG cells appear to take up the colloidal carbon in chicken kidneys [56]. It was also observed that the secretory granules fuse occasionally in the JG cells of *Rana* and *Triturus* species, suggesting that the fusion of granules is one of the morphological presentations for granular synthesis, which occurs exclusively in higher vertebrates.

EXPERIMENTAL PRESENTATION OF RC CELLS

The kidney mainly has two distinct functions, those performed by the exocrine and endocrine systems. Analysis of the endocrine kidney after unilateral obstruction of the ureter provides some interesting findings about the origin of RC cells and the processing of renin granules [63, 64, 66] (Fig. 5). At 1 month after ligation of the ureter, a large number of RC cells are detected in this hydronephrotic kidney; on the other hand, there are very few in the non-ligated side. At 6 months after ligation, no difference is demonstrated between ligated and non-ligated kidneys. This finding corresponds to the results by morphometrical analysis, whole mount immunohistochemistry and the Northern blot analysis. In the ligated side, a large number of RC cells are clearly identified, but they decrease with time, suggesting that RC cells near the glomerulus are true secreting cells, whereas those lying a long distance from the glomerulus appear spontaneously, corresponding with the reports of ontogenetical studies [59]. The pathways of intrarenal vessels and their dynamics during experimental hydronephrotic periods have been investigated for the purpose of demonstrating the relationship between the vascular system and pathological conditions such as chronic pyelonephritis and stone pyelonephrosis [36, 82]. In JG cells of hydronephrotic kidneys, there is no agreement in results, although their morphology and intrarenal renin activity have been reported [5, 9, 42]. However, the developmental changes during experimental periods were not sufficiently observed. The physiological functions resulting in an increase in renin activity at 1 month after ligation are explained by the following two hypotheses. First, external pressure due to urine affects the activation of the mechanical baroreceptors [41, 115]. Second, the obstruction of urinary tubules is extended to the macula densa, playing a role in the feedback mechanism of renin secretion [18, 108].

Although there has been much *in vitro* evidence that renin is released from JG cells [26, 33], no morphological consensus has been reached since there are only a few reports regarding exocytosis or other release mechanisms for renin [70, 87, 106]. Because of activation of JG cells in experimental hydronephrosis at 1 month after ligation, this is a useful model for investigation of renin release. Here, two types of granules, electron dense and lucent, are observed, suggesting two hypothetical mechanisms for renin release [63]. The first type occurs with the formation of exocytotic channels somewhat different in morphology from those found in other endocrine cells. The second type occurs via diacrine secretion, the materials being discharged into the cytoplasmic cytosol from an opening in the granular membrane and then transported into the intercellular spaces in a way that is not morphologically detectable.

LOCAL RAS

Recently, some or all of the components of the RAS have been reported to be synthesized and secreted outside of classical organs or tissues [19, 44]. This extrarenal or local renin has been demonstrated in the brain, pituitary and pineal glands, submandibular glands, adrenal glands, testes, ovary, uterus, oviduct, placenta, macrophage, lymph nodes, liver and eye. The details of these local RAS have been reviewed in other journals [43–45]. Although the functions and roles of these local renins are not well known, the concept of the

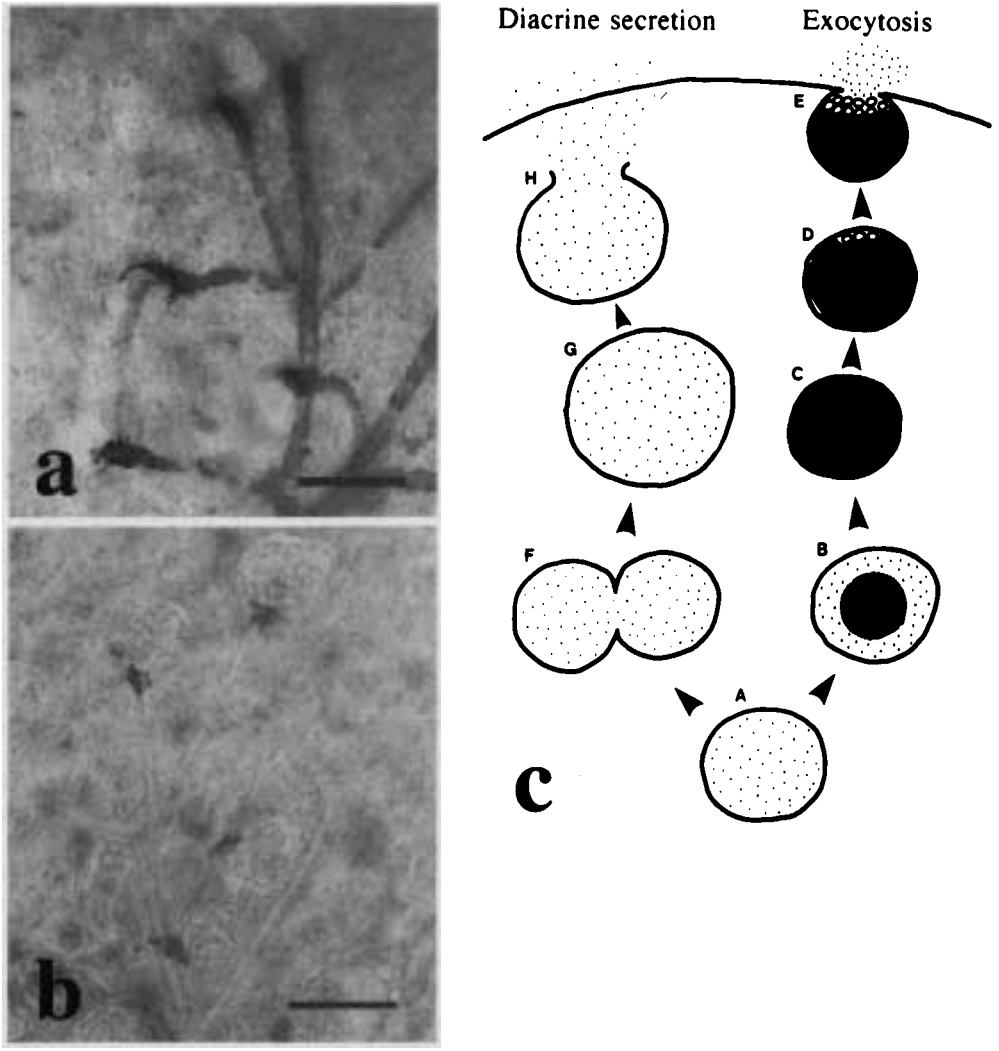

Fig. 5. Renin detection with whole-mount immunohistochemistry in experimental hydronephrosis of mice at one month (a) and 6 months (b) after operation [63, 64, 66]. bar=100 μm. c: Schematic drawing of renin release.

RAS as a circulating endocrine system alone is in question (Fig. 6). The local expression of RAS components may be involved in the regulation of an individual tissue function via endocrine, paracrine, autocrine or possibly also via exocrine mechanisms.

Recently, it has been suggested by using angiotensinogen-deficient mice that astrocytes producing angiotensins are required for functional maintenance of the blood-brain barrier [40]. In ACE-deficient mice, the fertility of homozygous male, but not female mutants is greatly reduced, despite being potent and having sperm of normal appearance [67]. Additionally, renin-deficient mice have shown lowered blood pressure and altered renal morphology. In the future, the real function of local RAS will be clarified by using such mutant mice [16].

DEVELOPMENT OF RC CELLS IN ADRENAL GLANDS

As one component of local RAS, renin is released from adrenal glands, but its volume is very low (Fig. 7). On day 13 of gestation in mice, the capsule, the cortex, and the medulla are still immature; however, immunoreactivity for renin is first demonstrated in the parenchymal cells [61]. On day 16 of gestation, the intensively reactive RC cells are numerously localized only in the cortical zone, sometimes showing mitotic figures. By immunoelectron microscopy, numerous gold particles are demonstrated in the terminal portions of the Golgi apparatus and protogranules as well as in specific secretory granules, suggesting that the cleavage of a prosegment from prorenin may occur on the maturating face of the Golgi apparatus or in the protogranules, as reported in kidney [62, 107]. Both renin and cathepsin B are co-localized in identical granules, indicating that many

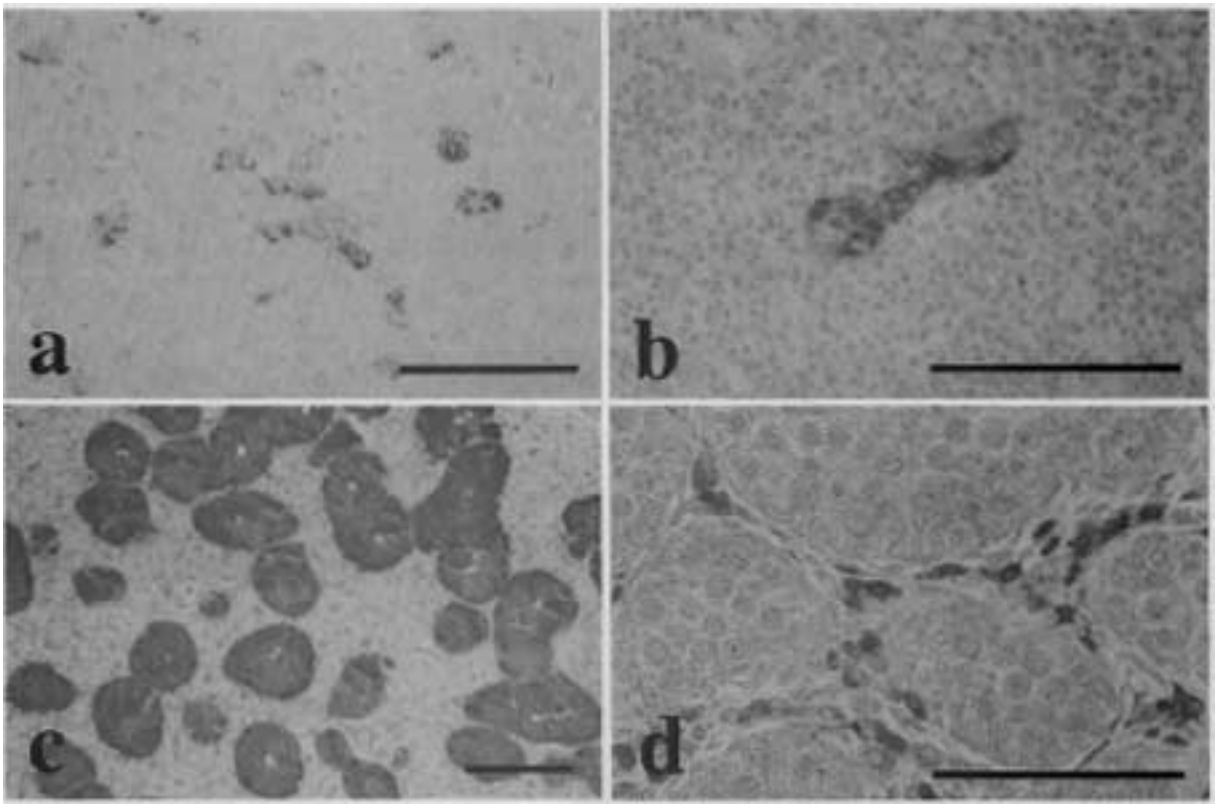

Fig. 6. Extrarenal renin expression in various tissues [44, 48]. a: Hepatocytes in rat. b: Endothelial cells of postcapillary venule in rat submandibular lymph node. c: Epithelial cells of granulated ducts in mouse submandibular gland. d: Leydig cells in fetal mouse testis. bar=100 μm.

renin gold particles have homogeneous intragranular distribution, whereas those for cathepsin B are distributed heterogeneously [65]. The co-existence of renin and cathepsin B suggests the possibility that cleavage of the prosegment from an inactive renin precursor also plays an important role in the production of adrenal active renin. On day 18 of gestation, renin is decreased or undetectable, whereas cathepsin B is still demonstrated at the same level as on day 16. Although adrenal renin is a temporary enzyme detected with immunohistochemical techniques, there have been many reports that adrenal renin increases *in vitro* after bilateral nephrectomy [74, 77]. There is a hypothesis in which extrarenal tissues are regarded as sites of renin synthesis to produce only inactive prorenin, and no active renin is introduced into the general circulation [96].

These views are of some significance in elucidating the ontogenetical development of the extrarenal RAS. Because the initial appearance of renin immunoreactivity is observed in adrenal cortical cells accompanied by cell divisions at 13–14 days of gestation, there is an assumption that the angiotensin series also appears in the gland at or after the fetal period [12]. Angiotensin II is known to have various functions in tissues: for instance, in the bovine adrenal gland it is a potent promoting factor for the mitosis of cortical cells [30]. Furthermore, angiotensin II has also been shown to be a significant stimulating factor for angiogenesis and prostaglandin synthesis in several organs, and the prostaglandin in the adrenal gland has also been reported to stimulate steroidogenesis [24, 25]. These reports suggest that there is the important involvement of angiotensin II produced by renin synthesis in the morphogenesis of the adrenal gland in the fetal stage of the mouse.

COAGULATING GLAND (CG) RENIN

Renin from the CG in mice has a yet undetermined functional role [22, 46]. However, it has been emphasized that the CG renin is significant because large amounts of both mRNA and protein can be detected in this organ, as well as in JG cells in the kidney [23, 50–52] (Fig. 8). Immunoreactive deposits of renin are localized in many granules of epithelial cells of the CG, as well as surface membrane and luminal extracts.

It has been reported that steroid hormones, especially testosterone, influence renin synthesis in the submandibular gland, the anterior pituitary gland and other extrarenal tissues, although intrarenal renin is not influenced by testosterone [76, 113]. Developmental observations provide evidences that RC cells in the CG of mice are physiologically expressed after birth, especially during the

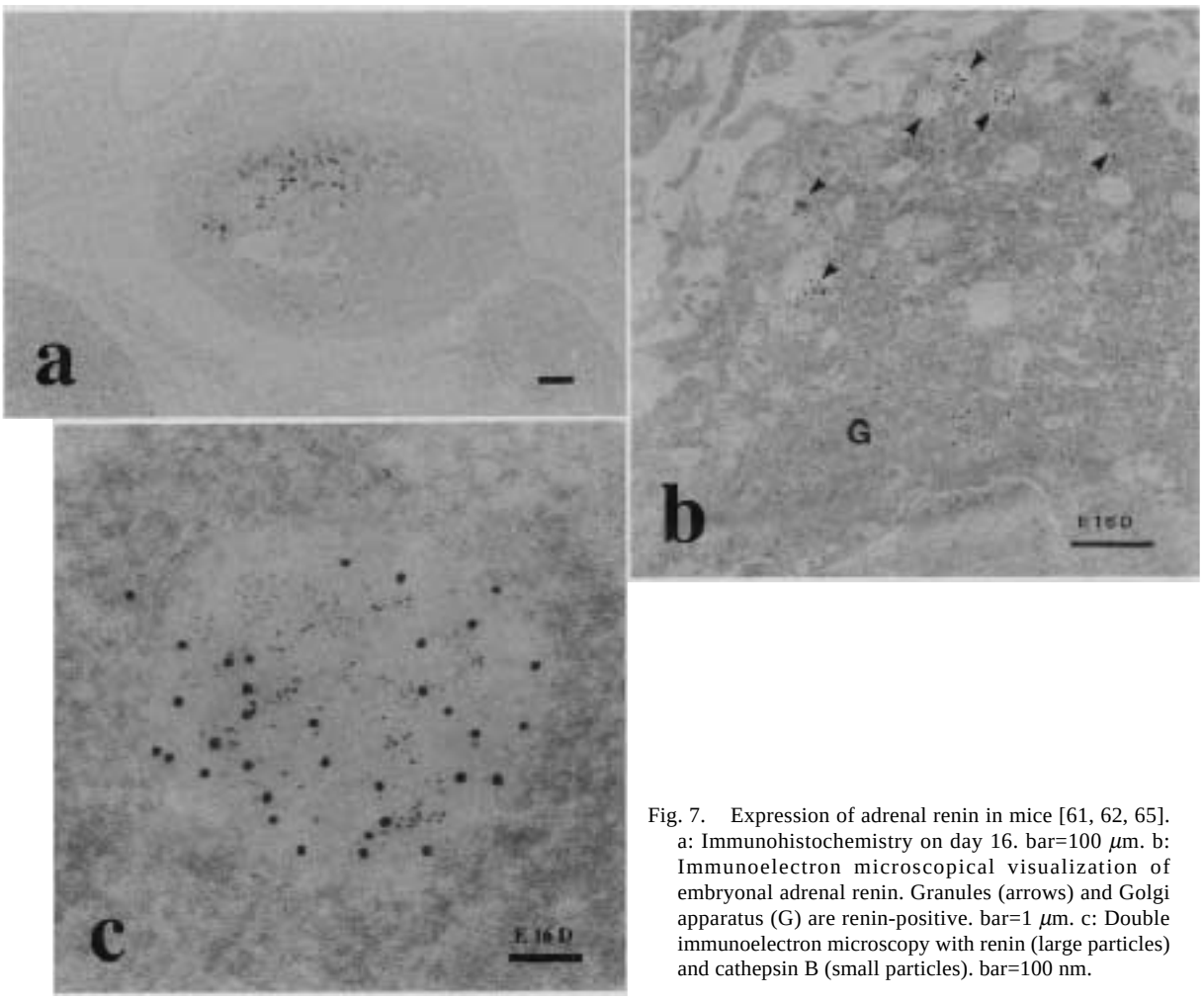

Fig. 7. Expression of adrenal renin in mice [61, 62, 65]. a: Immunohistochemistry on day 16. bar=100 μm. b: Immunoelectron microscopical visualization of embryonal adrenal renin. Granules (arrows) and Golgi apparatus (G) are renin-positive. bar=1 μm. c: Double immunoelectron microscopy with renin (large particles) and cathepsin B (small particles). bar=100 nm.

adolescent period. After castration, RC cells are not demonstrated in terminal ducts of the CG, whereas weak renin immunoreactivity is still observed in the main duct [52, 53]. After testosterone administration to castrated mice, large numbers of RC cells can be detected. It appears that the expression of CG renin is mainly regulated by the testis, especially by testosterone.

As a result of immunoelectron microscopical observations, two exocrine secretory pathways for renin have been proposed [54]. The first is a general exocrine pathway, and the other is a lysosome-dependent pathway. Because renin is secreted first toward the seminiferous lumen, the secretory style of CG renin is classified as an exocrine function, while that in the kidney is an endocrine or paracrine function secreted toward the intercellular spaces, blood or lymphatic circulation. The granular convoluted tubule cells of the mouse submandibular glands also produce renin, as well as epidermal growth factor, nerve growth factor, kallikrein, proteinase A and tonin [32, 48, 69, 86, 90, 114]. In particular, because it has been reported that renal/pancreatic kallikrein of the submandibular gland is secreted from both the basolateral and apical surfaces [86], the possibility of bipolar secretory pathways for CG renin cannot be ignored.

CG renin released by an exocrine mechanism performs certain functions for the male urogenital or the female genital organ. Renin protein, but not detectable mRNA, is observed in the epithelial cell lines of the uterus at the 1st day after mating, suggesting that CG renin is transferred into the female uterus by this process [47]. Additionally, the signal for angiotensinogen mRNA is detected in the epithelial cells of the uterus by hybridohistochemistry after mating. The function of angiotensin II cleaved from angiotensinogen is the production of prostaglandin in several organs as well as the control of blood pressure and the water-mineral balance [18, 78]. Moreover, prostaglandin has been shown to stimulate steroidogenesis in the adrenal gland [95], and renin release in association with β-adrenergic receptors [103]. It is possible that CG renin also plays a role in production of the angiotensin series, and functions in association with prostaglandin. These findings suggest that the renin-angiotensin-prostaglandin system is dependent on

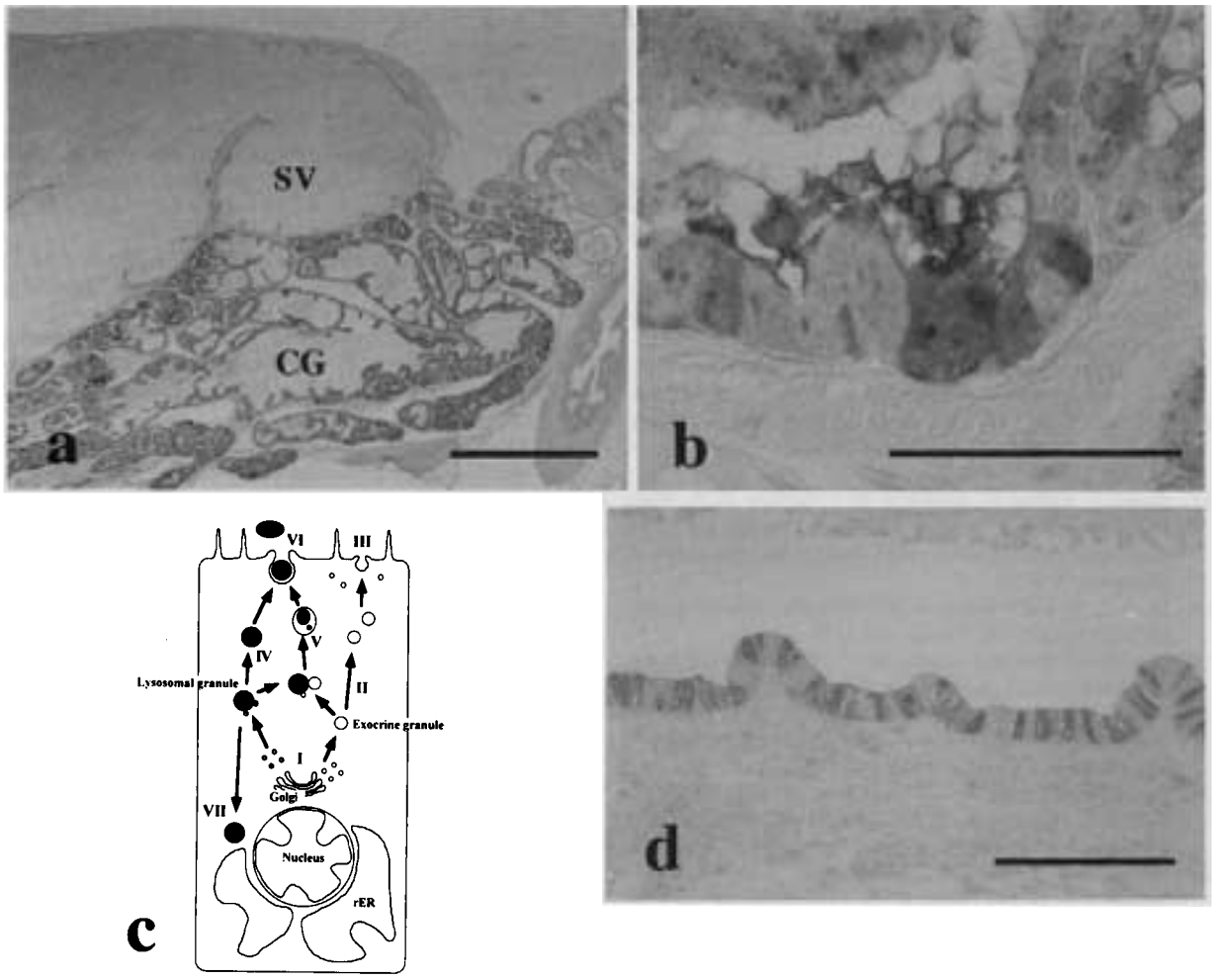

Fig. 8. Renin detection with immunohistochemistry in the epithelial cells of mouse coagulating gland [50, 52–54]. bar: a=1mm, b=100 mm. c: A hypothetical diagram of morphological renin processing in the coagulating gland. d: Renin in uterine epithelial cells at one day after mating of a C57BL/6 male and C57BL/6 female. bar=100 μm.

male sexual maturation and affects certain aspects of female reproduction as well, working in local-feedback and local-enhancing mechanisms.

REFERENCES

1. Alcorn, D., Cheshire, G. R., Coghlan, J. P. and Ryan, G. B. 1984. Peripolar cell hypertrophy in the renal juxtaglomerular region of newborn sheep. *Cell Tissue Res.* 236: 197–202.
2. Aldred, G. P., Fu, P., Crawford, R. J. and Fernley, R. T. 1992. The sequence and tissue expression of ovine renin. *J. Mol. Endocrinol.* 8: 3–11.
3. Bailey, J. R. and Randall, D. J. 1982. Renal perfusion pressure and renin secretion in the rainbow trout, Salmo gairdneri. *Can. J. Zool.* 59: 1220–1226.
4. Barajas, L. and Müller, J. 1973. The innervation of the juxtaglomerular apparatus and surrounding tubules: a quantitative analysis by serial section electron microscopy. *J. Ultrastruct. Res.* 43: 107–132.
5. Barrett, G. L., Morgan, T. O. and Alcorn, D. 1990. Stimulation of renin synthesis in the hydronephrotic kidney during sodium depletion. *Pflügers Arch.* 415: 774–776.
6. Baumbach, L. and Skøtt, O. 1986. Renin release from different parts of rat afferent arterioles *in vitro*. *Am. J. Physiol.* 251: F12–F16.
7. Broughton-Pipkin, F., Kirkpatrick, S. M., Lumbers, E. R. and Mott, J. C. 1974. Renin and angiotensin-like levels in foetal, new-born and adult sheep. *J. Physiol.* 241: 575–588.
8. Brühl, U., Taugner, R. and Forssmann, W. G. 1974. Studies on the juxtaglomerular apparatus. I. Perinatal development in the rat. *Cell Tissue Res.* 151: 433–456.
9. Bührle, C. P., Nobiling, R. and Taugner, R. 1985. Intracellular recordings from renin-positive cells of the afferent glomerular arteriole. *Am. J. Physiol.* 249: F272–F281.
10. Burnham, C. E., Hawelu-Johnson, C. L., Frank, B. M. and Lynch, K. R. 1987. Molecular cloning of rat renin cDNA and its gene. *Proc. Natl. Acad. Sci. U.S.A.* 84: 5605–5609.
11. Campbell, D. J. and Habener, J. F.1987. Cellular localization of angiotensinogen gene expression in brown adipose tissue

and mesentery: quantification of messenger ribonucleic acid abundance using hybridization in situ. *Endocrinology* 121: 1616–1626.
12. Celio, M. R., Groscurth, P. and Inagami, T. 1985. Ontogeny of renin immunoreactive cells in the human kidney. *Anat. Embryol.* 173: 149–155.
13. Chobanian, A. V., Haudenschild, C. C., Nickerson, C. and Drago, R. 1990. Antiatherogenic effect of captopril in the Watanabe heritable hyperlipidemic rabbit. *Hypertension* 15: 327–331.
14. Christensen, J. A., Morild, I., Mikeler, E. and Bohle, A. 1982. Juxtaglomerular apparatus in the domestic fowl (*Gallus domesticus*). *Kidney Int.* 22: (*Suppl.* 12): S24–S29.
15. Clauser, E., Gaillard, I., Wei, L. and Corvol, P. 1989. Regulation of angiotensinogen gene. *Am. J. Hypertens.* 2: 403–410.
16. Coffman, T. M. 1998. Gene targeting in physiological investigations: studies of the renin-angiotensin system. *Am. J. Physiol.* 274: F999–F1005.
17. Darby, I., Aldred, P., Crawford, R. J., Fernley, R. T., Niall, H. D., Penschow, J. D., Ryan, G. B. and Coghlan, J. P. 1985. Renin gene expression in vessels of the ovine renal cortex. *J. Hypertens.* 3: 9–11.
18. Davis, J. O. and Freeman, R. H. 1976. Mechanisms regulating renin release. *Physiol. Rev.* 56: 1–56.
19. Deschepper, C. F. and Ganong, W. F. 1988. Renin and angiotensin in endocrine glands. *Front. Neuroendocrinol.* 10: 79–98.
20. Dhiab al Naimi, A. A. H. and Bearn, J. G. 1981. The juxtaglomerular apparatus and glomerular granular cells in fetuses and neonates of the rat. *J. Anat.* 133: 607–623.
21. Egerer, G., Taugner, R. and Tiedemann, K. 1984. Renin immunohistochemistry in the mesonephros and metanephros of the pig embryo. *Histochemistry* 81: 385–390.
22. Fabian, J. R., Field, L. J., McGowan, R. A., Mullins, J. J., Sigmund, C. D. and Gross, K. W. 1989. Allele-specific expression of the murine Ren-1 genes. *J. Biol. Chem.* 264: 17589–17594.
23. Fabian, J. R., Kane, C. M., Abel, J. and Gross, K. W. 1993. Expression of the mouse Ren-1 gene in the coagulating gland: localization and regulation. *Biol. Reprod.* 48: 1383–1394.
24. Fernandez, L. A., Twickler, J. and Mead, A. 1985. Neovascularization produced by angiotensin II. *J. Lab. Clin. Med.* 105: 141–145.
25. Flack, J. D., Jessup, R. and Ramwell, P. W. 1969. Prostaglandin stimulation of rat corticosteroidogenesis. *Science* 163: 691–692.
26. Fray, J. C., Lush, D. J. and Valentine, A. N. 1983. Cellular mechanisms of renin secretion. *Fed. Proc.* 42: 3150–3154.
27. Gaillard-Sanchez, I., Bruneval, P., Clauser, E., Belair, M. F., da Silva, J. L., Bariety, J., Camilleri, J. P. and Corvol, P. 1990. Successful detection by in situ cDNA hybridization of three members of the serpin family: angiotensinogen, α1 protease inhibitor, and antithrombin III in human hepatocytes. *Mod. Pathol.* 3: 216–222.
28. Gardiner, D. S., Jackson, R. and Lindop, G. B. 1992. The renin-secreting cell and the glomerular peripolar cell in renal artery stenosis and Addison's disease. *Virchows Arch. A Pathol. Anat. Histopathol.* 420: 533–537.
29. Gasc, J. M., Shanmugam, S., Sibony, M. and Corvol, P. 1994. Tissue-specific expression of type 1 angiotensin II receptor subtypes. An in situ hybridization study. *Hypertension* 24: 531–537.
30. Gill, G. N., Ill, C. R. and Simonian, M. H. 1977. Angiotensin stimulation of bovine adrenocortical cell growth. *Proc. Natl. Acad. Sci. U.S.A.* 74: 5569–5573.
31. Gorgas, K. 1978. Structure and innervation of the juxtaglomerular apparatus of the rat. *Adv. Anat. Embryol. Cell Biol.* 54: 3–83.
32. Gresik, E. W., Chung, K. W., Barka, T. and Schenkein, I. 1980. Immunocytochemical localization of nerve growth factor, epidermal growth factor, renin and proteinase A in the submandibular glands of Tfm/Y mice. *Am. J. Anat.* 158: 247–250.
33. Hackenthal, E., Schwertschlag, U. and Taugner, R. 1983. Cellular mechanisms of renin release. *Clin. Exp. Hypertens. A Theory Pract.* A5: 975–993.
34. Hobert, P. M., Fogliano, M., O'Connor, B. A., Schaefer, I. M. and Chirgwin, J. M. 1984. Human renin gene: structure and sequence analysis. *Proc. Natl. Acad. Sci. U.S.A.* 81: 5026–5030.
35. Hoj-Nielsen, A. and Knudsen, F. 1987. Angiotensinogen is an acute-phase protein in man. *Scand. J. Clin. Lab. Invest.* 47: 175–178.
36. Holle, G. and Schneider, H. J. 1961. Über das Verhalten der Nierengefässe bei einseitiger experimenteller Hydronephrose. *Virchows Arch. A Pathol. Anat. Histopathol.* 334:475–488.
37. Hubert, C., Houot, A. M., Corvol, P. and Soubrier, F. 1991. Structure of the angiotensin I-converting enzyme gene. Two alternate promoters correspond to evolutionary steps of a duplicated gene. *J. Biol. Chem.* 266: 15377–15383.
38. Ice, K. S., Geary, K. M., Gomez, R. A., Johns, D. W., Peach, M. J. and Carey, R. M. 1988. Cell and molecular studies of renin secretion. *Clin. Exp. Hypertens. A Theory Practice* 10: 1169–1187.
39. Iwai, N. and Inagami, T. 1992. Identification of two subtypes in the rat type I angiotensin II receptor. *FEBS Lett.* 298: 257–260.
40. Kakinuma, Y., Hama, H., Sugiyama, F., Yagami, K., Goto, K., Murakami, K. and Fukamizu, A. 1998. Impaired blood-brain barrier function in angiotensinogen-deficient mice. *Nature Med.* 4: 1078–1080.
41. Kaloyanides, G. J., Bastron, R. D. and DiBona, G. F. 1973. Effect of ureteral clamping and increased renal arterial pressure on renin release. *Am. J. Physiol.* 225: 95–99.
42. Kelemen, J. T. and Endes, P. 1965. Die juxtaglomerulären granulierten Zellen bei einseitiger Hydronephrose der Ratte. *Virchows Arch. A Pathol. Anat. Histopathol.* 339: 301–303.
43. Kon, Y. 1995. Extrarenal expression of renin-angiotensin system concerning the regulation of blood pressure. *J. Hokkaido Vet. Med. Assoc.* 39: 1–4 (in Japanese).
44. Kon, Y. 1996. Local renin-angiotensin system: especially in coagulating glands of mice. *Arch. Histol. Cytol.* 59: 399–420.
45. Kon, Y. 1996. The history of renin-producing cells. *Gut Intest. Hormones* 14: 1–7 (in Japanese).
46. Kon, Y. and Endoh, D. 1996. Expression of renin in coagulating glands. *Histol. Histopathol.* 11: 257–266.
47. Kon, Y. and Endoh, D. 1999. Expression of angiotensinogen in the uterus induced by coagulating gland renin in mice. *Anat. Histol. Embryol.* 28: 119–124.
48. Kon, Y. and Endoh, D. 1999. Renin in exocrine glands of variable mouse strains. *Anat. Histol Embryol.* 29: (in press).
49. Kon, Y., Alcorn, D., Murakami, K., Sugimura, M. and Ryan, G. B. 1994. Immunohistochemical studies of renin-containing cells in the developing sheep kidney. *Anat. Rec.* 239: 191–197.
50. Kon, Y., Endoh, D., Fukamizu, A., Murakami, K., Hashimoto, Y. and Sugimura, M. 1992. Comparative study of renin ex-

pression in the coagulating glands of C57BL/6 and Balb/c mice. *J. Anat.* 181: 335–343.
51. Kon, Y., Endoh, D., Fukamizu, A., Murakami, K., Hashimoto, Y. and Sugimura, M. 1993. A simple method of hybridohistochemistry for detection of renin mRNA in the mouse kidney. *J. Vet. Med. Sci.* 55: 461–463.
52. Kon, Y., Endoh, D., Fukamizu, A., Murakami, K., Yamashita, T. and Watanabe, T. 1996. Detection of coagulating gland renin by hybridohistochemistry. *Anat. Histol. Embryol.* 25: 289–294.
53. Kon, Y., Endoh, D., Murakami, K., Hashimoto, Y. and Sugimura, M. 1995. Expression of renin in coagulating glands is regulated by testosterone. *Anat. Rec.* 241: 451–460.
54. Kon, Y., Endoh, D., Murakami, K., Yamashita T., Watanabe, T. and Hashimoto, Y. 1995. Morphological evidence of exocrine function in coagulating gland renin of mouse strain C57BL/6. *Anat. Rec.* 243: 200–207.
55. Kon, Y., Hashimoto, Y., Kitagawa, H. and Kudo, N. 1984. Morphology and quantification of juxtaglomerular cells of the chicken kidney. *Jpn. J. Vet. Sci.* 46: 189–196.
56. Kon, Y., Hashimoto, Y., Kitagawa, H. and Kudo, N. 1986. Localization of acid phosphatase in chicken juxtaglomerular cells and its functional meaning. *Jpn. J. Vet. Sci.* 48: 925–931.
57. Kon, Y., Hashimoto, Y., Kitagawa, H. and Kudo, N. 1987. Morphological and immunohistochemical studies of juxtaglomerular cells in carp, Cyprinus carpio. *Jpn. J. Vet. Sci.* 49: 323–331.
58. Kon, Y., Hashimoto, Y., Kitagawa, H. and Kudo, N. 1988. Distribution and morphology of the renin-containing cells in the amphibian kidneys. *Jpn. J. Vet. Sci.* 50: 325–332.
59. Kon, Y., Hashimoto, Y., Kitagawa, H. and Kudo, N. 1989. An immunohistochemical study on the embryonic development of renin-containing cells in the mouse and pig. *Anat. Histol. Embryol.* 18: 14–26.
60. Kon, Y., Hashimoto, Y., Kitagawa, H., Kudo, N. and Murakami, K. 1986. Immunohistochemical demonstration of juxtaglomerular cells in the kidneys of domestic mammals and fowls. *Jpn. J. Vet. Res.* 34: 111–123.
61. Kon, Y., Hashimoto, Y., Kitagawa, H., Sugimura, M. and Murakami, K. 1990. Renin immunohistochemistry in the adrenal gland of the mouse fetus and neonate. *Anat. Rec.* 227: 124–131.
62. Kon, Y., Hashimoto, Y., Kitagawa, H., Sugimura, M. and Murakami, K. 1991. Intracellular production of adrenal renin in the fetal mouse. An immuno-electron microscopical study. *J. Anat.* 176: 23–33.
63. Kon, Y., Hashimoto, Y., Murakami, K. and Sugimura, M. 1992. An immunoelectron-microscopical observation of mouse juxtaglomerular cells in the case of experimental hydronephrosis. *Acta Anat.* 144: 354–362.
64. Kon, Y., Hashimoto, Y., Murakami, K. and Sugimura, M. 1992. Whole-mount preparation of renin-containing cells in hydronephrotic mice. *J. Vet. Med. Sci.* 54: 589–590.
65. Kon, Y., Hashimoto, Y., Sugimura, M. and Murakami, K. 1993. Adrenal renin is localized in lysosomal granules. *Anat. Histol. Embryol.* 22: 324–327.
66. Kon, Y., Takahashi, S., Fukamizu, A., Murakami, K., Hashimoto, Y. and Sugimura, M. 1992. Morphological and Northern blot analysis of juxtaglomerular cells in experimental hydronephrotic mice. *Anat. Rec.* 232: 393–400.
67. Krege, J., John, S. W., Lagenbach, L. L., Hodgin, J. B., Hagaman, J. R., Bachman, E. S., Jennette, J. C., O'Brien, D. A. and Smithies, O. 1995. Male-female differences in fertility and blood pressure in ACE-deficient mice. *Nature* 375: 146–148.
68. Lacy, E. R. and Reale, E. 1990. The presence of a juxtaglomerular apparatus in elasmobranch fish. *Anat. Embryol.* 182: 249–262.
69. Ledoux, S., Gutkowska, J., Garcia, R., Thibault, G., Cantin, M. and Genest, J. 1982. Immunohistochemical localization of tonin in rat salivary glands and kidney. *Histochemistry* 76: 329–339.
70. Lindop, G. B. 1987. Morphological aspects of renin synthesis, processing, storage and secretion. *Kidney Int.* 31 (*Suppl.* 20): S18–S24.
71. Lindop, G. B. and Lever, A. F. 1986. Anatomy of the renin-angiotensin system in the normal and pathological kidney. *Histopathology* 10: 335–362.
72. Logan, A. G., Moriarty, R. J., Morris, R. and Rankin, J. C. 1980. The anatomy and blood system of the kidney in the river lamprey, *Lampetra fluviatilis. Anat. Embryol.* 158: 245–252.
73. Minuth, M., Hackenthal, E., Poulsen, K., Rix, E. and Taugner, R. 1981. Renin immunocytochemistry of the differentiating juxtaglomerular apparatus. *Anat. Embryol.* 162: 173–181.
74. Mizuno, K., Hoffman, L. H., McKenzie, J. C. and Inagami, T. 1988. Presence of renin secretory granules in rat adrenal gland and stimulation of renin secretion by angiotensin II but not by adrenocorticotrophin. *J. Clin. Invest.* 82: 1007–1016.
75. Nakajima, M., Mukoyama, M., Pratt, R. E., Horiuchi, M. and Dzau, V. J. 1993. Cloning of cDNA and analysis of the gene for mouse angiotensin II type-2 receptor. *Biochem. Biophys. Res. Commun.* 197: 393–399.
76. Naruse, K., Naruse, M., Obana, K., Demura, R., Demura, H., Inagami, T. and Shizume, K. 1986. Renin in the rat pituitary coexists with angiotensin II and depends on testosterone. *Endocrinology* 118: 2470–2476.
77. Naruse, M., Naruse, K., Inagaki, T. and Inagami, T. 1984. Immunoreactive renin in mouse adrenal gland. Localization in the inner cortical region. *Hypertension* 6: 275–280.
78. Needleman, P., Marchall, G. R. and Sobel, B. E. 1975. Hormone interactions in the isolated rabbit heart. Synthesis and coronary vasomotor effects of prostaglandin, and bradykinin. *Circ. Res.* 37: 802–808.
79. Nishimura, H., Lunde, L. G. and Zucker, A. 1979. Renin response to hemorrhage and hypotension in the aglomerular toadfish, *Opsanus tau. Am. J. Physiol.* 237: H105–H111.
80. Nishimura, H., Norton, V. M. and Bumpus, F. M. 1978. Lack of specific inhibition of angiotensin II in eels by angiotensin antagonists. *Am. J. Physiol.* 235: H95–H103.
81. Nishimura, H., Ogawa, M. and Sawyer, W. H. 1973. Renin-angiotensin system in primitive bony fishes and a holocephalian. *Am. J. Physiol.* 224: 950–956.
82. Nobiling, R., Bührle, C. P., Hackenthal, E., Helmchen, U., Steinhausen, M., Whalley, A. and Taugner, R. 1986. Ultrastructure, renin status, contractile and electrophysiological properties of the afferent glomerular arterioles in the rat hydronephrotic kidney. *Virchows Arch. A Pathol. Anat. Histopathol.* 410: 31–42.
83. Olson, A. L., Perlman, S. and Robillard, J. E. 1990. Developmental regulation of angiotensinogen gene expression in sheep. *Pediat. Res.* 28: 183–185.
84. Oparil, S. 1983. Angiotensin I converting enzyme inhibitors and analogues of angiotensin II. pp. 250–270. *In*: Hypertension: Physiopathology and Treatment (Genest, J., Kuchel, O.,

Hamel, P. and Cantin, M. eds), McGraw-Hill, New York.
85. Page, W. V., Perlman, S., Smith, F. G., Segar, J. L. and Robillard, J. E. 1992. Renal nerves modulate kidney renin gene expression during the transition from fetal to newborn life. *Am. J. Physiol.* 262: R459–R463.
86. Penschow, J. D. and Coghlan, J. P. 1993. Secretion of glandular kallikrein and renin from the basolateral pole of mouse submandibular duct cells: an immunocytochemical study. *J. Histochem. Cytochem.* 41: 95–103.
87. Peter, S. 1976. Ultrastructural studies on the secretory process in the epithelioid cells of the juxtaglomerular apparatus. *Cell Tissue Res.* 168: 45–53.
88. Phat, V. N., Camilleri, J. P., Bariety, J., Galtier, M., Baviera, E., Corvol, P. and Menard, J. 1981. Immunohistochemical characterization of renin-containing cells in the human juxtaglomerular apparatus during embryonal and fetal development. *Lab. Invest.* 45: 387–390.
89. Piccini, N., Knopf, J. L. and Gross, K. W. 1982. A DNA polymorphism, consistent with gene duplication, correlates with high renin levels in the mouse submandibular gland. *Cell* 30: 205–213.
90. Rall, L. B., Scott, J., Bell, G. I., Crawford, R. J., Penschow, J. D., Niall, H. D. and Coghlan, J. P. 1985. Mouse prepro-epidermal growth factor synthesis by the kidney and other tissues. *Nature (Lond.)* 313: 228–231.
91. Richoux, J. P., Cordonnier, J. L., Bouhnik, J., Clauser, E., Corvol, P., Menard, J. and Grignon, G. 1983. Immunocytochemical localization of angiotensinogen in rat liver and kidney. *Cell Tissue Res.* 233: 439–451.
92. Ruyter, J. C. 1925. Über einen merkwürdigen Abschnitt der Vasa afferentia in der Mäuseniere. *Z. Zellforsch.* 2: 242–248.
93. Ryan, G. B., Coghlan, J. P. and Scoggins, B. A. 1979. The granulated peripolar epithelial cell: a potential secretory component of the renal juxtaglomerular complex. *Nature (Lond.)* 277: 655–656.
94. Sandberg, K., Ji, H., Clark, A. J., Shapira, H. and Catt, K. J. 1992. Cloning and expression of a novel angiotensin II receptor subtype. *J. Biol. Chem.* 267: 9455–9458.
95. Saruta, T. and Kaplan, N. M. 1972. Adrenocortical steroidogenesis: the effects of prostaglandins. *J. Clin. Invest.* 51: 2246–2251.
96. Sealey, J. E., Goldstein, M., Pitarresi, T., Kudlak, T. T., Glorioso, N., Fiamengo, S. A. and Laragh, J. H. 1988. Prorenin secretion from human testis: no evidence for secretion of active renin or angiotensinogen. *J. Clin. Endocrinol. Metab.* 66: 974–978.
97. Sibony, M., Segretain, D. and Gasc, J. M. 1994. Angiotensin-converting enzyme in murine testis: step-specific expression of the germinal isoform during spermiogenesis. *Biol. Reprod.* 50: 1015–1026.
98. Siegel, S. R. and Fisher, D. A. 1980. Ontogeny of the renin-angiotensin-aldosterone system in the fetal and newborn lamb. *Pediat. Res.* 14: 99–102.
99. Sigmund,, C. D. and Gross, K. W. 1991. Structure, expression, and regulation of the murine renin genes. *Hypertension* 18: 446–457.
100. Smith, F. G., Lupu, A. N., Barajas, L., Bauer, R. and Bashore, R. A. 1974. The renin-angiotensin system in the fetal lamb. *Pediat. Res.* 8: 611–620.
101. Sokabe, H. and Ogawa, M. 1974. Comparative studies of the juxtaglomerular apparatus. *Int. Rev. Cytol.* 37: 271–327.
102. Stier, C. T., Benter, I. F., Ahmad, S., Zuo, H. L., Selig, N., Roethel, S., Levine, S. and Itskovitz, H. D. 1989. Enalapril prevents stroke and kidney dysfunction in salt-loaded stroke-prone spontaneously hypertensive rats. *Hypertension* 13: 115–121.
103. Suzuki, S. and Hashiba, K. 1986. The mechanism of the control of renin release by beta-adrenergic receptors. *Jpn. Heart J.* 27: 871–880.
104. Takei, Y., Hasegawa ,Y., Watanabe, T. X., Nakajima, K. and Hazon, N. 1993. A novel angiotensin I isolated from an elasmobranch fish. *J. Endocrinol.* 139: 281–285.
105. Taugner, R. and Metz, R. 1986. Development and fate of the secretory granules of juxtaglomerular epithelioid cells. *Cell Tissue Res.* 246: 595–606.
106. Taugner, R., Bührle, C. P. and Nobiling, R. 1984. Ultrastructural changes associated with renin secretion from the juxtaglomerular apparatus of mice. *Cell Tissue Res.* 237: 459–472.
107. Taugner, R., Murakami, K. and Kim, S. J. 1986. Renin activation in juvenile secretory granules? Immunocytochemical experiments with an antiserum directed against the prosegment of human renin. *Histochemistry* 85: 107–109.
108. Thurau, K., Schnermann, J., Nagel, W., Horster, M. and Wohl, M. 1967. Composition of tubular fluid in the macula densa segment as a factor regulating the function of the juxtaglomerular apparatus. *Circ. Res.* 20–21 (*Suppl.* II): 79–90.
109. Tigerstedt, R. and Bergmann, P. G. 1898. Niere und Kreislauf. *Skand. Arch. Physiol.* 8: 223–271.
110. Timmermans, P., Wong, P., Chiu, A., Herblin, W., Benfield, P., Carini, D., Lee, R., Waxler, R., Saye, J. and Smith, R. 1993. Angiotensin II receptors and angiotensin II receptor antagonists. *Pharmacol. Rev.* 45: 205–251.
111. Trimper, C. E. and Lumbers, E. R. 1972. The renin-angiotensin system in fetal lambs. *Pflügers Arch.* 336: 1–10.
112. Uva, B., Masini, M. A., Hazon, N., O'Toole, L. B., Henderson, I. W. and Ghiani, P. 1992. Renin and angiotensin converting enzyme in elasmobranchs. *Gen. Comp. Endocrinol.* 86: 407–412.
113. Wagner, D., Metzger, R., Paul, M., Ludwig, G., Suzuki, F., Takahashi, S., Murakami, K. and Ganten, D. 1990. Androgen dependence and tissue specificity of renin messenger RNA expression in mice. *J. Hypertens.* 8: 45–52.
114. Wilson, P. A., Scott, J., Penschow, J. D., Coghlan, J. P. and Rush, R. A. 1986. Identification and quantification of mRNA for nerve growth factor in histological preparations. *Neurosci. Lett.* 64: 323–329.
115. Wyker, A. T., Ritter, R. C., Marion, D. N. and Gillenwater, J. Y. 1981. Mechanical factors and tissue stresses in chronic hydronephrosis. *Invest. Urol.* 18: 430–436.