



HOKKAIDO UNIVERSITY

Title	Intracellular production of adrenal renin in the fetal mouse. An immuno-electron microscopic study
Author(s)	Kon, Yasuhiro; 昆, 泰寛; Hashimoto, Yoshiharu et al.
Citation	JOURNAL OF ANATOMY, 176, 23-33
Issue Date	1991
Doc URL	https://hdl.handle.net/2115/5510
Rights	Copyright © 1991 Cambridge University Press
Type	journal article
File Information	JA176.pdf



Intracellular production of adrenal renin in the fetal mouse. An immuno-electron microscopical study

YASUHIRO KON, YOSHIHARU HASHIMOTO, HIROSHI KITAGAWA*, MAKOTO SUGIMURA AND KAZUO MURAKAMI†

*Department of Veterinary Anatomy, Faculty of Veterinary Medicine, Hokkaido University, Sapporo 060, * Department of Veterinary Anatomy, Faculty of Agriculture, Tottori University, Tottori 680 and † Department of Applied Biochemistry, University of Tsukuba, Ibaraki 305, Japan*

(Accepted 6 November 1990)

INTRODUCTION

Renin as a triggering component of the renin-angiotensin-aldosterone system (RAAS) is produced mainly by juxtaglomerular granulated cells in the kidney, is released into blood vessels or lymphatic vessels and promotes conversion from inactive angiotensinogen into the active angiotensin series (Davis & Freeman, 1976; Vecsei, Hackenthal & Ganten, 1978).

In recent studies, a local renin-angiotensin system has been reported in the kidney (Naruse *et al.* 1982; Taugner & Hackenthal, 1981; Taugner *et al.* 1982), brain (Chabot, Gray, Dubois & Morel, 1989; Ganten *et al.* 1983; McKenzie, Naruse & Inagami, 1985), adrenal gland (Deschepper *et al.* 1986; Mizuno, Hoffman, McKenzie & Inagami, 1988; Urata, Khosla, Bumpus & Husain, 1988), ovary (Lightman *et al.* 1987; Palumbo *et al.* 1989), and testis (Pandey & Inagami, 1986; Pandey, Misono & Inagami, 1984) in addition to the classical system acting throughout the body. In the adrenal gland, particularly, because angiotensin II stimulates the synthesis and release of aldosterone, a paracrine-stimulating function of renin has been proposed in the RAAS (Deschepper & Ganong, 1988).

The present authors have discovered immunohistochemically that the enzyme renin can be detected temporarily in the fetal adrenal gland during Days 14 to 18 of gestation (Kon *et al.* 1990; Kon, Hashimoto, Sugimura & Murakami, 1991). Although it has been suggested that the developmental process of renin-containing cells is accompanied closely by adrenal morphogenesis, the question as to whether renin in the fetal adrenal gland is produced by adrenal cells themselves or is inserted into the cytoplasm from the extracellular fluid or blood has not yet been answered.

In the present study, we have investigated renin-containing cells in the fetal adrenal gland using the protein A-gold immunocytochemical technique (Roth, 1984) and we suggest that renin is produced and released by adrenal cortical cells. Additionally, some new possibilities concerning the production mechanism of intra-adrenal renin are suggested.

MATERIALS AND METHODS

The ages of fetal mice (Balb/c) were dated from the finding of a vaginal plug in the mated female (Day 0 of pregnancy). The mothers were anaesthetised with ether and killed on the 14th, 16th and 18th days of gestation. The adrenal glands, adhering to

the kidneys, were dissected free using a stereo-microscope and fixed in an ice-cold 0.5% glutaraldehyde and 4% paraformaldehyde mixture buffered with 0.1 M cacodylate sodium (pH 7.4) for 2–4 hours. The tissues were then rinsed with 0.1 M cacodylate buffer, dehydrated with an ethanol series and embedded in a commercial acrylic medium, either LR white resin (London Resin Co., UK) or Lowicryl K4M resin (Chemische Werke Lowi, Germany). Briefly, samples for LR white or Lowicryl K4M embedding were dehydrated to 100% ethanol and infiltrated in stages with 1:2, 1:1, 2:1 resin to ethanol mixture, immersed in pure resin and finally polymerised at 55 °C for 24 hours or at –35 °C for 24 hours and later at room temperature for 3–4 days, respectively. Some tissues were fixed in 3% glutaraldehyde, postfixed in 1% osmium tetroxide and embedded in epoxy resin.

The ultrathin sections obtained from the hydrophilic embedding resins were cut with a Porter–Blum MT-1 ultramicrotome, mounted on nickel grids and dried at 37 °C. The sections were then treated with the following immunocytochemical procedures on paraffin sheets in a moisture chamber. Briefly, the sections were rinsed with phosphate-buffered saline (PBS) and treated with 1% bovine serum albumin for 15–30 minutes. They were then incubated with anti-mouse submandibular renin rabbit serum (1:3000) at 4 °C overnight or at room temperature for two hours, rinsed with PBS three times and incubated with colloidal gold (15 nm)-labelled protein A complex (E-Y Laboratories, Inc., USA) at room temperature for thirty minutes–one hour. They were rinsed with PBS and then with distilled water and finally stained with saturated aqueous uranyl acetate and lead citrate; they were studied with a HITACHI HU-12A electron microscope at 75 kV.

Control sections were incubated with normal rabbit serum, PBS or preabsorbed antiserum substituted for primary antiserum. The tissues embedded in epoxy resin were observed using routine procedures.

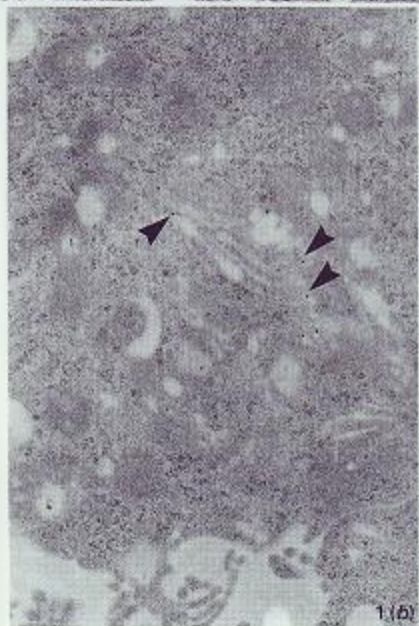
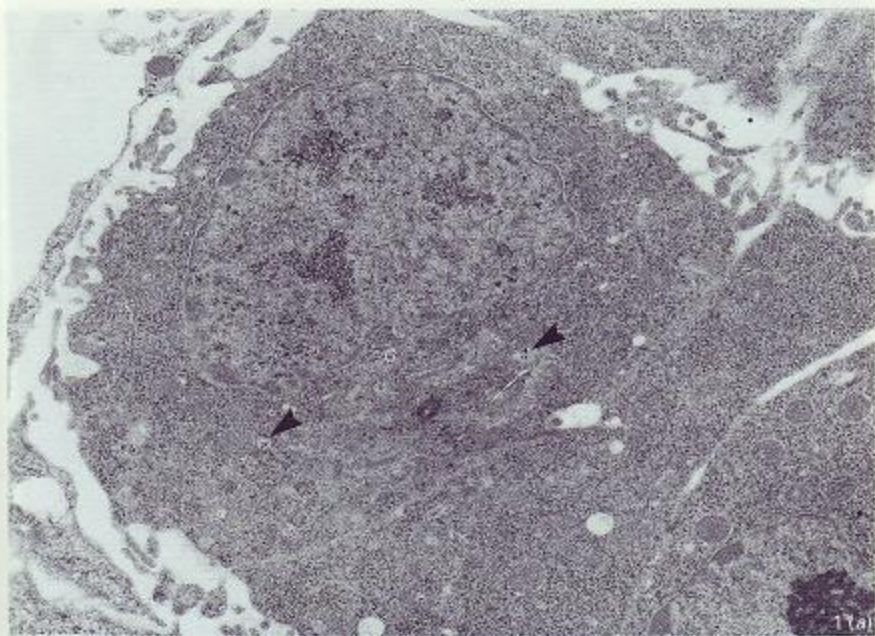
RESULTS

Day 14 of gestation

The Golgi apparatus of the cortical cells adjacent to the medulla showed one or two well-developed regions in the juxta-nuclear position. Large numbers of secretory granules elsewhere contained homogeneous material of moderate electron density. According to the distance from the Golgi apparatus, secretory granules were of various shapes, namely round, oval, comma- or horseshoe-shaped, sometimes they included some minute vacuoles, vesicles or lamellar structures. On Day 14 of gestation, the granules were relatively few in number and measured 200–300 nm in diameter. A small amount of rough endoplasmic reticulum (ER), with no dilated lumina, were observed, whereas smooth ER was not observed anywhere in the cytoplasm.

Immunocytochemically, some gold particles were demonstrated in the Golgi apparatus of the cortical cells adjacent to the medulla (Fig. 1*a*). A small number of specific granules labelled weakly with gold particles were distributed in the Golgi

Fig. 1 (*a-c*). Immuno-electron micrographs after application of anti-renin serum in mouse adrenal cortical cells on Day 14 of gestation. (*a*) The secretory granules (arrowheads) and Golgi apparatus (*G*) reacted slightly. LR white resin. $\times 13800$. (*b*) A few gold particles are located in the terminal portions of the lamellae (arrowheads). LR white resin. $\times 21300$. (*c*) Apparent exocytosis of secretory granules containing immunoreactive gold particles can be seen between the two arrowheads. LR white resin. $\times 57500$.



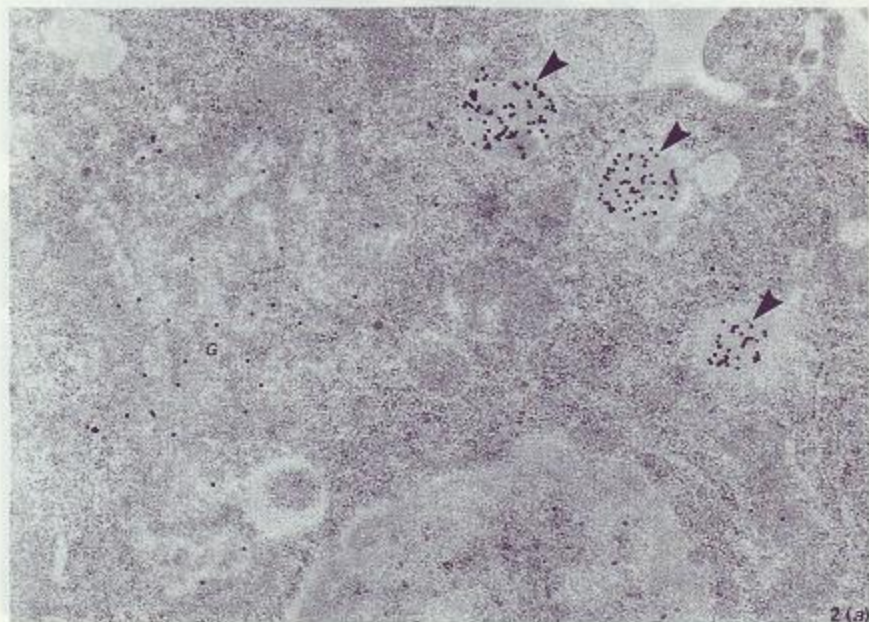


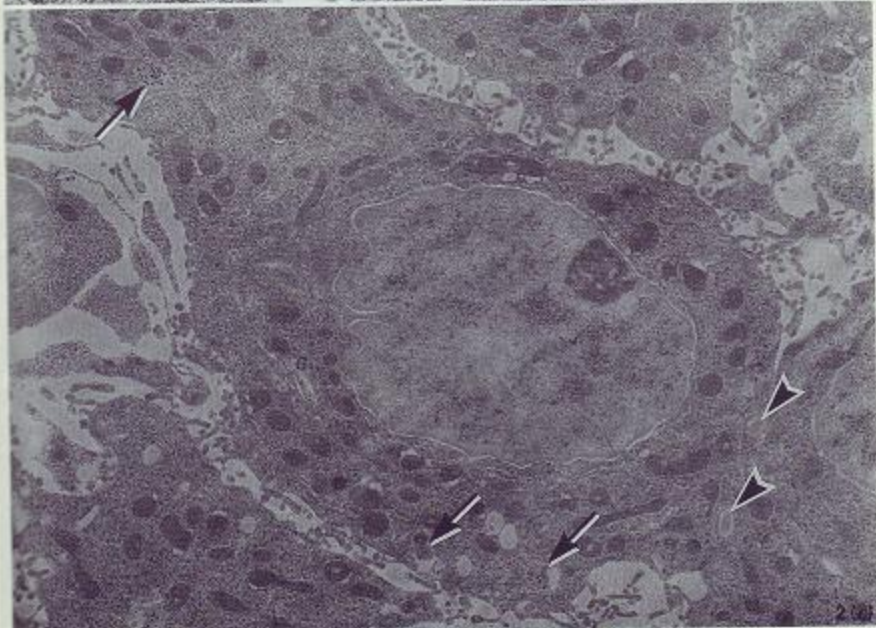
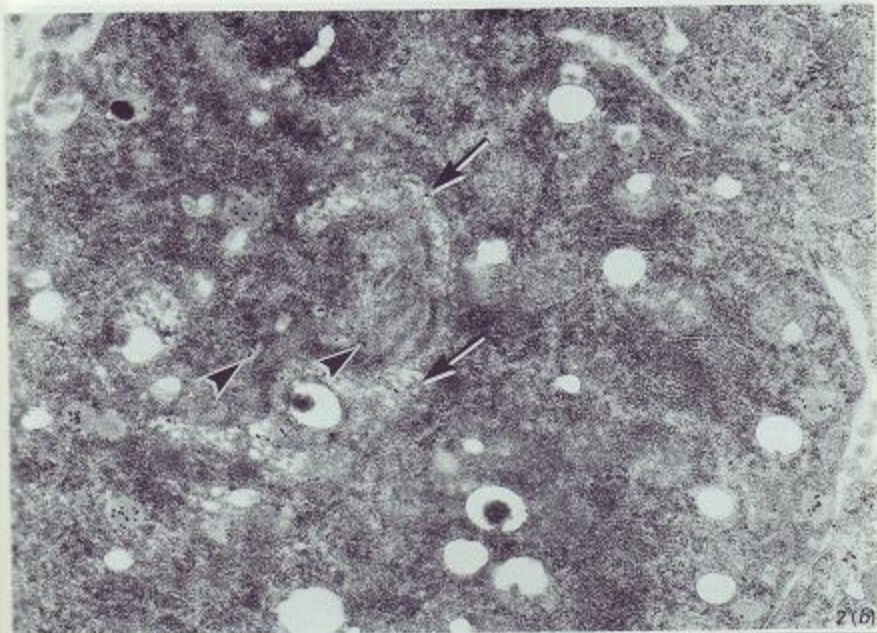
Fig. 2 (a-c). Immuno-electron micrographs of mouse adrenal cortical cells on Day 16 of gestation. (a) Numerous gold particles indicating the presence of renin are found in the Golgi region (G) as well as in specific secretory granules (arrowheads). Lowicryl K4M resin. $\times 27600$. (b) The terminal ampullae (arrows) and protogranules (arrowheads) located in the juxta-Golgi regions reacted with gold particles. LR white resin. $\times 20000$. (c) Gold particles reacted strongly with specific granules located just beneath the cell membrane (arrows), but were not found at all in the Golgi region (G). Some granules did not react with the antibody (arrowheads). Lowicryl K4M resin. $\times 9500$.

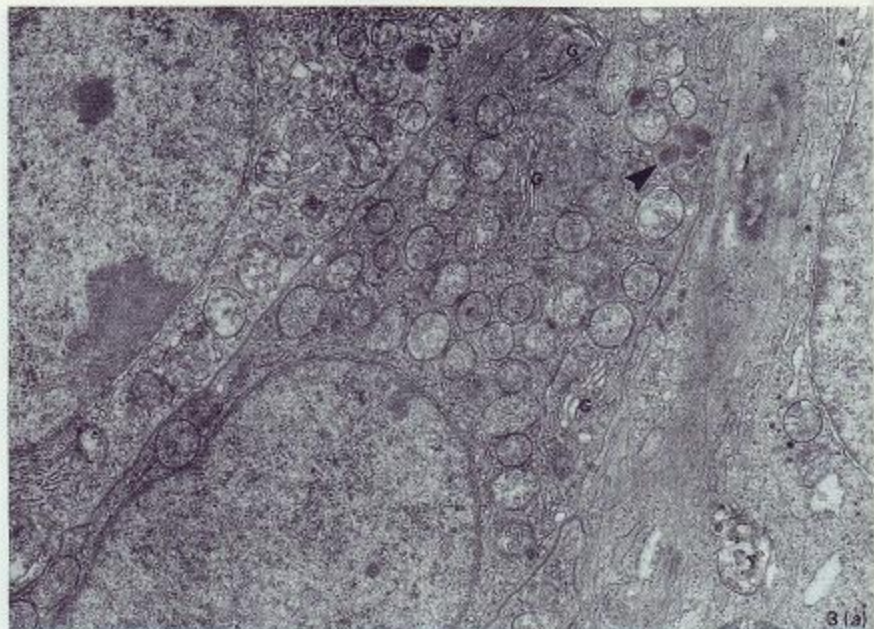
region and beneath the cell membrane. Although only a few particles were observed in the Golgi lamellae, some were located in their terminal portions and in the protogranules (Fig. 1*b*). No particles were shown to localise in the Golgi vesicles, coated vesicles or rough ER. Sometimes, extracellular deposits of particles, suggestive of exocytosis of secretory granules opening into the extracellular space, were demonstrated (Fig. 1*c*).

Day 16 of gestation

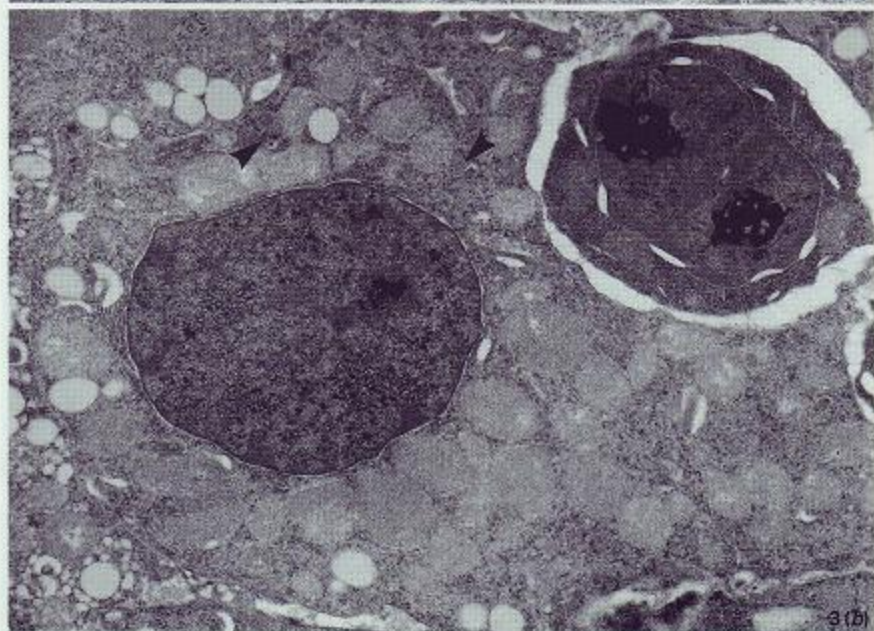
The cortical cells adjacent to the medulla possessed a much more well-developed Golgi apparatus than those on Day 14. They had various shapes, some covering a large area and some divided into 3 or 4 smaller portions. A large amount of rough ER was distributed throughout the cytoplasm, while a great deal of smooth ER was also found. Numerous secretory granules were scattered just beneath the cell membrane; these measured 300–600 nm in diameter.

Immunocytochemically, numerous gold particles were demonstrated in the Golgi region as well as in the specific secretory granules (Fig. 2*a*). Although few or no gold particles were deposited in the Golgi cisternae themselves, the terminal ampullae and





3(a)



3(b)

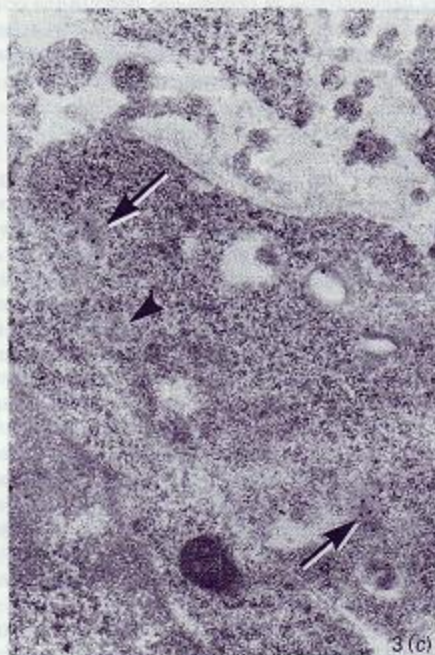


Fig. 3 (a-c). Electron micrographs of mouse adrenal cortical cells on Day 18 of gestation. (a) Golgi apparatus (G) and conglomerations of secretory granules (arrowhead) are scattered throughout the cytoplasm. Epoxy resin. $\times 11600$. (b) Immuno-electron micrograph shows a few weakly reacting specific granules forming clusters (arrowheads). LR white resin. $\times 12000$. (c) Specific granules reacting weakly (arrows) and granules negative (arrowhead) for renin are detected in the cytoplasm. LR white resin. $\times 25000$.

protogranules located near the Golgi apparatus showed a reaction with gold particles (Fig. 2b). On Day 16 of gestation, the reactivity of specific granules was increased as compared with those on Day 14 whereas some granules, even those having the same shape as the reacting granules, did not react. Additionally, it was noted that some cortical cells, in which gold particles reacted strongly with specific granules, were located just beneath the cell membrane, but not contained at all in the Golgi region (Fig. 2c).

Day 18 of gestation

The Golgi apparatus of the cortical cells was scattered throughout the cytoplasm, being divided into a number of portions, while Golgi vesicles were relatively smaller in number than those on Day 16 of gestation (Fig. 3a). A decreased quantity of rough ER was detected, only around the nucleus, while a good deal of smooth ER was distributed throughout the cytoplasm. Small secretory granules were demonstrated in the form of conglomerations just beneath the cell membrane.

Immunocytochemically, cytoplasmic reactivity for renin was greatly decreased. Sometimes only a few specific granules reacted weakly. These measured 200-300 nm

in diameter and were demonstrated in clusters just beneath the cell membrane (Fig. 3*b*). No gold particles were observed in any portions of Golgi apparatus. There was a tendency for granules unreactive to renin, that showed a morphology similar to that of renin-positive granules, to increase in number in comparison with Day 16 of gestation (Fig. 3*c*).

DISCUSSION

Studies on the ontogeny of the adrenal gland have mainly been performed using human fetuses. Much work has been devoted to the 'fetal cortex' in fetal adrenals to determine the developmental and physiological significance of events that occur before parturition (Johannisson, 1968; McNutt & Jones, 1970; Sucheston & Cannon, 1968; Uotila, 1940). The prenatal adrenal cortex is composed of two distinct zones. The outer subcapsular zone, the so-called permanent cortex that increases in size after birth, results in the three layers of the adult cortex, whereas the 'fetal cortex', that occupies the innermost zone of the cortex, regresses completely after birth as a result of haemorrhagic necrosis (Carlson, 1981; Tokuyama, 1980). This may be caused by the collapse of blood capillaries but the mechanism by which it takes place is still poorly understood. In the mouse, the fetal cortex is often called the 'X-zone' and appears on Days 10 to 14 after birth in the boundary regions between the cortex and medulla, synthesising androgen-like materials (Asari, Fukaya, Nishida & Kano, 1977; Holmes & Dickson, 1971; Nishida & Mochizuki, 1963). Since fetal adrenals on Days 14, 16 and 18 of gestation were used in the present study, the tissues studied were those of the permanent cortex adjacent to the medulla and it has been demonstrated, in previous light microscopical studies, that the layer contains a number of renin-immunoreactive cells (Kon *et al.* 1990).

In the present study, apparent ultrastructural changes were noted in the Golgi apparatus, rough ER and smooth ER in the cortical cells. During the development of the adrenal gland, the Golgi apparatus of the cortical cells increased in size and came to be divided into several linked portions; however the Golgi vesicles decreased in number on Day 18 of gestation. Rough ER was developed on Day 16 of gestation, but decreased to surround only the nucleus on Day 18, whereas smooth ER was observed to develop suddenly on Day 18. In view of these findings, it is suggested that protein is synthesised in the cortical cells temporarily during late embryological development. Additionally, it seems that the developed smooth ER acts mainly in steroidogenesis, functioning as it does in the adult cortex after birth.

In the immunocytochemical study, renin was demonstrated in the fetal adrenal gland and this corresponds to the results in the kidney, where a number of renin-containing cells can also be detected on Day 16 of gestation, with positive reactions along the primordial interlobar or arcuate arteries (Kon, Hashimoto, Kitagawa & Kudo, 1989). These results, in both fetal kidney and adrenal gland, suggest that the morphogenesis of these organs, including angiogenesis, steroidogenesis and glomerulogenesis, is the event associated directly or indirectly with the existence of renin, as partly reviewed by Deschepper & Ganong (1988). For instance angiotensin II, produced by the converting action of renin, has been shown to stimulate prostaglandin synthesis in the kidneys while prostaglandins stimulate steroidogenesis in the adrenal gland (Flack, Jessup & Ramwell, 1969; Shebresi & Aiken, 1980).

The present results suggest that adrenal renin can first be detected in the Golgi regions by immunocytochemical techniques and that it may be transformed, via protogranules adjacent to this apparatus, into mature secretory granules. In the general protein-producing cells, however, it has been shown that amino acids in the cytoplasm are built up into individual proteins by the rough ER and transformed into

mature granules via the Golgi apparatus. Prorenin or preprorenin, an inactive prototype of renin, may be produced by rough ER. It was further demonstrated in the present study that no Golgi lamellae showed clear immunoreactivity to renin, but the terminal portions and the protogranules showed exclusive localisation of the gold particles. This suggests the following possibility for the intracellular maturation of renin-containing granules: the cleavage of a prosegment from prorenin may occur on the trans-face of the Golgi apparatus or in the protogranules, as reported in kidney (Taugner, Murakami & Kim, 1986).

During Days 16 to 18 of gestation, because of the tendency for the renin immunoreactivity both in the Golgi apparatus and in the secretory granules to decrease, the following two hypotheses are proposed. (i) Adrenal renin is a temporary enzyme synthesised only in fetal life and it cannot be detected subsequently with our immunohistochemical technique. Although we could not demonstrate renin in adult adrenals, there are a few reports of its detection here (Deschepper *et al.* 1986; Naruse, Naruse & Inagami, 1984). These contradictory results are thought to be caused by the difference in the properties of the anti-serum used. In recent years, there have been many reports that adrenal renin is increased *in vitro* after bilateral nephrectomy (Mizuno *et al.* 1988; Naruse & Inagami, 1982; Naruse *et al.* 1984; Ubeda, Hernandez, Fenoy & Quesada, 1988). Further study is needed to find whether or not adrenal gland renin in adult animals can be detected *in vivo* after bilateral nephrectomy. (ii) The adrenal gland produces prorenin throughout pre- and postnatal life; that is, an unknown enzyme is able to cleave the pro-segment from prorenin only during a short period of fetal life. The enzyme that cleaves this pro-segment in the adult kidney is known to be a cathepsin (Taugner, Bührle, Nobiling & Kirschke, 1985a; Taugner *et al.* 1985b). Recently, we demonstrated that cathepsin B was colocalised in renin granules of the adrenal gland (Kon *et al.* 1991). Moreover, there is another hypothesis in which extrarenal tissues are regarded as sites of renin synthesis, where only inactive prorenin is produced, and no active renin is introduced into the general circulation (Sealey *et al.* 1988). Further investigations are needed on the possibility of prorenin secretion from extrarenal tissues.

SUMMARY

Renin-containing cells in fetal adrenal glands of the mouse were investigated with the protein A-gold immunocytochemical technique. On Day 14 of gestation, a small number of specific granules were weakly immunoreactive and were distributed in the Golgi region. Sometimes, apparent exocytosis of gold-labelled particles could be seen opening into the extracellular space. On Day 16 of gestation, numerous gold particles were demonstrated in the Golgi region as well as in the specific secretory granules. Immunoreactivity of the specific granules was increased as compared with Day 14, though some granules were observed to have no reaction with the antibody. On Day 18 of gestation reactivity for renin decreased, while a few clustered immunoreactive granules were demonstrated just beneath the cell membrane. No gold particles were observed in the Golgi apparatus during this period and more granules negative for renin were noted than on Day 16 of gestation. These results suggest that renin is produced and released temporarily by adrenal cortical cells in the late fetal life of the mouse.

REFERENCES

- ASARI, M., FUKAYA, K., NISHIDA, S. & KANO, Y. (1977). Studies on postnatal development of adrenal X zone in female mice. *Bulletin of Azabu Veterinary College* **2**, 341-353.
- CARLSON, B. M. (1981). Ductless glands, pharyngeal derivatives, and the lymphoid organs. In *Patten's Foundations of Embryology*, 4th ed., pp. 425-439. New York: McGraw-Hill.
- CHABOT, J.-G., GRAY, D. A., DUBOIS, P. M. & MOREL, G. (1989). Presence of angiotensin II in the adult male rat anterior pituitary gland: immunocytochemical study after cryoultramicrotomy. *Experimental Cell Research* **180**, 189-197.
- DAVIS, J. O. & FREEMAN, R. H. (1976). Mechanisms regulating renin release. *Physiological Reviews* **56**, 1-56.
- DESCHAEPPER, C. F. & GANONG, W. F. (1988). Renin and angiotensin in endocrine glands. In *Frontiers in Neuroendocrinology*, vol. 10 (ed. L. Martini & W. F. Ganong), pp. 79-98. New York: Raven Press.
- DESCHAEPPER, C. F., MELLON, S. H., CUMIN, F., BAXTER, J. D. & GANONG, W. F. (1986). Analysis by immunocytochemistry and *in situ* hybridization of renin and its mRNA in kidney, testis, adrenal, and pituitary of the rat. *Proceedings of the National Academy of Sciences of the United States of America* **83**, 7552-7556.
- FLACK, J. D., JESSUP, R. & RAMWELL, P. W. (1969). Prostaglandin stimulation of rat corticosteroidogenesis. *Science* **163**, 691-692.
- GANTEN, D., HERMANN, K., BOYER, C., UNGER, T. & LANG, R. E. (1983). Angiotensin synthesis in the brain and increased turnover in hypertensive rats. *Science* **221**, 869-871.
- HOLMES, P. V. & DICKSON, A. D. (1971). X-zone degeneration in the adrenal glands of adult and immature female mice. *Journal of Anatomy* **108**, 159-168.
- JOHANSSON, E. (1968). The fetal adrenal cortex in the human. *Acta endocrinologica* **130**, Suppl., 7-107.
- KON, Y., HASHIMOTO, Y., KITAGAWA, H. & KUDO, N. (1989). An immunohistochemical study on the embryonic development of renin-containing cells in the mouse and pig. *Anatomia Histologia Embryologia* **18**, 14-26.
- KON, Y., HASHIMOTO, Y., KITAGAWA, H., SUGIMURA, M. & MURAKAMI, K. (1990). Renin immunohistochemistry in the adrenal gland of the mouse fetus and neonate. *Anatomical Record* **227**, 124-131.
- KON, Y., HASHIMOTO, Y., SUGIMURA, M. & MURAKAMI, K. (1991). Adrenal renin is localized in lysosomal granules. *Zeitschrift für mikroskopisch-anatomische Forschung* (in Press).
- LIGHTMAN, A., TARLATZIS, B. C., RZASA, P. J., CULLER, M. D., CARIDE, V. J., MEGRO-VILAR, A. F., LENNARD, D., DECHERNEY, A. H. & NAFTOLIN, F. (1987). The ovarian renin-angiotensin system: renin-like activity and angiotensin II/III immunoreactivity in gonadotropin-stimulated and unstimulated human follicular fluid. *American Journal of Obstetrics and Gynecology* **156**, 808-816.
- McKENZIE, J. C., NARUSE, K. & INAGAMI, T. (1985). The renin-angiotensin system in the rat anterior pituitary: colocalization of renin and angiotensin II in gonadotrophs. *Anatomical Record* **212**, 161-166.
- McNUTT, N. S. & JONES, A. L. (1970). Observations on the ultrastructure of cytodifferentiation in the human fetal adrenal cortex. *Laboratory Investigation* **22**, 513-527.
- MIZUNO, K., HOFFMAN, L. H., McKENZIE, J. C. & INAGAMI, T. (1988). Presence of renin secretory granules in rat adrenal gland and stimulation of renin secretion by angiotensin II but not by adrenocorticotropin. *Journal of Clinical Investigation* **82**, 1007-1016.
- NARUSE, K., INAGAMI, T., CELJO, M. R., WORKMAN, R. J. & TAKI, Y. (1982). Immunohistochemical evidence that angiotensins I and II are formed by intracellular mechanism in juxtaglomerular cells. *Hypertension* **4**, Suppl. II, 1170-1174.
- NARUSE, M. & INAGAMI, T. (1982). Markedly elevated specific renin levels in the adrenal in genetically hypertensive rats. *Proceedings of the National Academy of Sciences of the United States of America* **79**, 3295-3299.
- NARUSE, M., NARUSE, K. & INAGAMI, T. (1984). Immunoreactive renin in mouse adrenal gland. Localization in the inner cortical region. *Hypertension* **6**, 275-280.
- NISHIDA, S. & MOCHIZUKI, K. (1963). Preliminary report on the function of the X-zone of the mouse adrenal cortex. *Archivum histologicum japonicum* **23**, 213-227.
- PALUMBO, A., JONES, C., LIGHTMAN, A., CARCANGIU, M. L., DECHERNEY, A. H. & NAFTOLIN, F. (1989). Immunohistochemical localization of renin and angiotensin II in human ovaries. *American Journal of Obstetrics and Gynecology* **160**, 8-14.
- PANDEY, K. N. & INAGAMI, T. (1986). Regulation of renin angiotensins by gonadotropic hormones in cultured murine Leydig tumor cells. Release of angiotensin but not renin. *Journal of Biological Chemistry* **261**, 3934-3938.
- PANDEY, K. N., MISONO, K. S. & INAGAMI, T. (1984). Evidence for intracellular formation of angiotensins: coexistence of renin and angiotensin-converting enzyme in Leydig cells of rat testis. *Biochemical and Biophysical Research Communications* **122**, 1337-1343.
- ROTH, J. (1984). The protein A-gold technique for antigen localisation in tissue sections by light and electron microscopy. In *Immunolabelling for Electron Microscopy* (ed. J. M. Polak & I. M. Varndell), pp. 113-121. New York: Elsevier.
- SEALEY, J. E., GOLDSTEIN, M., PITARRESI, T., KUDLAK, T. A., GLORIOSO, N., FIAMENGO, S. A. & LARAGH, J. H. (1988). Prolactin secretion from human testis: no evidence for secretion of active renin or angiotensinogen. *Journal of Clinical Endocrinology and Metabolism* **66**, 974-978.

- SIEBRESKI, R. J. & AIKEN, J. W. (1980). Angiotensin II stimulation of renal prostaglandin synthesis elevates circulating prostacyclin in dog. *Journal of Cardiovascular Pharmacology* **2**, 667-678.
- SUCHESTON, M. E. & CANNON, M. S. (1968). Development of zonal patterns in the human adrenal gland. *Journal of Morphology* **126**, 477-492.
- TAUGNER, R., BÜHRLE, C. P., NOBELING, R. & KIRSCHKE, H. (1985a). Co-existence of renin and cathepsin B in epithelioid cell secretory granules. *Histochemistry* **83**, 103-108.
- TAUGNER, R. & HACKENTHAL, E. (1981). Angiotensin II in epithelioid (renin-containing) cells of rat kidney. *Histochemistry* **72**, 499-509.
- TAUGNER, R., HACKENTHAL, E., HELMCHEN, U., GANTEN, D., KUGLER, P. & MARIN-GREZ, M. (1982). The intrarenal renin-angiotensin system. An immunocytochemical study on the localization of renin, angiotensinogen, converting enzyme and angiotensins in the kidney of mouse and rat. *Klinische Wochenschrift* **60**, 1218-1222.
- TAUGNER, R., MURAKAMI, K. & 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.
- TAUGNER, R., WHALLEY, A., ANGERMULLER, S., BÜHRLE, C. P. & HACKENTHAL, E. (1985b). Are the renin-containing granules of juxtaglomerular epithelioid cells modified lysosomes? *Cell and Tissue Research* **239**, 575-587.
- TOKUYAMA, K. (1980). Light- and electronmicroscopy on the adrenal gland of human fetuses. *Journal of the Kurume Medical Association* **43**, 16-31. (In Japanese, abstract in English).
- UHEDA, M., HERNANDEZ, I., FENOY, F. & QUESADA, T. (1988). Vascular and adrenal renin-like activity in chronically diabetic rats. *Hypertension* **11**, 339-343.
- UOTILA, U. U. (1940). The early embryological development of the fetal and permanent adrenal cortex in man. *Anatomical Record* **76**, 183-203.
- URAYA, H., KHOSLA, M. C., BUMPUS, F. M. & HUSAIN, A. (1988). Evidence for extracellular, but not intracellular, generation of angiotensin II in the rat adrenal zona glomerulosa. *Proceedings of the National Academy of Sciences of the United States of America* **85**, 8251-8255.
- VECSÉL, P., HACKENTHAL, E. & GANTEN, D. (1978). The renin-angiotensin-aldosterone system. Past, present and future. *Klinische Wochenschrift* **56**, Suppl. 1, 5-21.