Transcription of (pro)renin mRNA in the rat adrenal cortex, and the effects of ACTH treatment and a low sodium diet

M M Ho and G P Vinson

Department of Biochemistry, St Bartholomew's and The London School of Medicine & Dentistry, Queen Mary & Westfield College, Mile End Road, London E1 4NS, UK

(Requests for offprints should be addressed to G P Vinson)

(M M Ho is now at the Rayne Institute, Department of Medicine, University College London Medical School, 5 University Street, London WC1 6JJ, UK)

Abstract

Transcription of the (pro)renin gene in the adult rat adrenal gland was studied by non-isotopic *in situ* hybridization.

In glands from control (untreated) animals, transcription was relatively sparse, and occurred mostly in the outer zona fasciculata. Treatment with ACTH increased the apparent signal in both the glomerulosa and in fasciculata zones.

A low sodium diet initially enhanced the transcription signal specifically in the glomerulosa, but as the regime

was extended from 5 days to more than 2 weeks, the signal was also increased dramatically in the zona reticularis.

The results emphasize the potential importance of the intraglandular renin–angiotensin system, particularly under conditions of chronic stimulation. They also suggest that angiotensin II, as well as being the major regulator of the glomerulosa, may also have some role in inner adrenocortical zone functions.

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Introduction

Although the renin–angiotensin system (RAS) has been primarily considered to be a circulating hormonal system, its presence in a variety of tissues locally has been well documented (Ganten *et al.* 1976, Campbell 1987, Deschepper & Ganong 1988, Mulrow 1992). Renin-like activity has been detected in the adrenal glands of rat (Ryan 1967, Ganten *et al.* 1974, Naruse & Inagami 1982, Doi *et al.* 1983, 1984a, Inagami *et al.* 1984, Nakamaru *et al.* 1985) and other species (Hayduk *et al.* 1970, Ganten *et al.* 1976, Naruse *et al.* 1983, 1984, Racz *et al.* 1992). Nephrectomy elicits a rapid and marked increase in the adrenal renin activity indicating that this enzyme is endogenous to the adrenal rather than due to contamination by plasma renin of renal origin (Naruse & Inagami 1982, Doi *et al.* 1984a,b, Inagami *et al.* 1984). Further evidence for the adrenal production of renin comes from *in vitro* studies using adrenal capsular tissue explants (Shier *et al.* 1989) or rat adrenal glomerulosa cell cultures (Yamaguchi *et al.* 1990). Mizuno *et al.* (1988) suggested that renin is stored in the rat adrenal gland and that storage granules are released into the extracellular space in the same fashion as from the granules of the juxtaglomerular cells of the kidney.

Other components of the RAS, including angiotensin-converting enzyme (ACE) and angiotensinogen, have also been detected in the rat (Cushman & Cheung 1971,

Mendelsohn 1984). The presence of ACE in the adrenal gland, and specifically within the capillary endothelial cells in the adrenal cortex, has been shown by immunocytochemistry and [³H]captopril autoradiography (Caldwell *et al.* 1976, Del Vecchio *et al.* 1980, Strittmatter *et al.* 1986). Angiotensinogen mRNA has also been detected in rat (Campbell & Habener 1986, Ohkubo *et al.* 1986), mouse (Dzau *et al.* 1987) and human (Racz *et al.* 1992) adrenals, and using *in situ* hybridization, Campbell & Habener (1989) demonstrated that angiotensinogen mRNA was present in the fibroblast-like cells immediately adjacent to the adrenal capsule of control rats, as well as in the adrenal itself (Campbell & Habener 1986, 1987).

Although earlier studies showed (pro)renin gene expression in rat adrenals (Deschepper *et al.* 1986, Dzau *et al.* 1987), the precise localization of adrenal (pro)renin mRNA in normal adult rats is not yet entirely clear. Deschepper *et al.* (1986) used a mouse renin cDNA probe on rat adrenal sections, but obtained a high background of non-specific positive signal in their *in situ* hybridization data, though Brecher *et al.* (1989) were able to compare the amounts of (pro)renin mRNA detected in adrenal capsular tissue (zona glomerulosa) and decapsular tissue (inner cortex and medulla) from nephrectomized rats using dot blot hybridization, but inevitably without cellular precision. The present study, using rat renin antisense oligonucleotide for non-radioactive *in situ* hybridization, was designed to identify the precise localization of

(pro)renin mRNA in normal female adult rat adrenals, and to study its regulation by adrenocorticotrophin (ACTH) administration and dietary sodium.

Materials and Methods

General laboratory chemicals and reagents were obtained from Sigma Chemical Company Ltd (Poole, Dorset, UK) and were molecular biology grade. B.D.H. (Poole, Dorset, UK) and Merck, Sharp and Dohme Ltd (Hoddesdon, Herts, UK) chemicals and solvents were Analar grade.

Adult female Wistar rats between 12 and 14 weeks of age and weighing 200–250 g, were obtained from established breeders and maintained briefly at Queen Mary and Westfield College. ACTH-treated rats, fed on a normal diet, were injected daily with Depot Synacthen (Ciba-Geigy, Horsham, Sussex, UK; 100 µg/100 µl per animal, i.m.) for 1, 3 or 5 days. The dosage of ACTH used here has been shown to have a good response in rat adrenals (Vazir *et al.* 1981). Control rats were similarly injected daily with 0.9% (w/w) NaCl, 100 µl/animal. Sodium-restricted rats were fed on wholemeal flour diet (Fattah *et al.* 1977) containing less than 0.1% (w/w) NaCl, with added 1% (w/w) CaCO₃ for 5 and 17 days. The same diet with 1% (w/w) NaCl added was used for dietary control rats. Five rats were used in each control or experimental group.

The antisense rat renin oligonucleotide probe was obtained commercially (Oncogene Science Inc., distributed by Cambridge BioScience, Cambridge, Cambs, UK), and enzymatically labelled with a digoxigenin (DIG)-labelled nucleotide tail with incorporation of several DIG-dUTP molecules at their 3'-end, using terminal transferase (Boehringer Mannheim, Lewes, Sussex, UK) to increase sensitivity, thus allowing the detection of small amounts of target mRNAs. The labelled probes were reconstituted in sterile water and stored at -20 °C until used. Tissue preparations and hybridization methods were as previously described (Ho & Vinson 1995). Both frozen and paraffin wax-embedded sections were used. In order to determine the optimal condition for *in situ* hybridization, a range of proteinase K concentrations were used. Sections (8 µm) were digested by proteinase K (0.625–5 µg/ml; Boehringer Mannheim) for 30 min at 37 °C. RNase-treated control sections were incubated in pre-warmed

RNase cocktail (HT Biotechnology Ltd, Cambridge, Cambs, UK) for 90 min at 37 °C. Proteinase K and RNase reactions were stopped by post-fixation in 0.4% (w/v) paraformaldehyde for 15 min. Sections were acetylated by using 0.25% (v/v) acetic anhydride in 0.1 mol/l tri-ethanolamine (TEA) and 0.9% (w/v) NaCl solution and incubated for 10 min. After acetylation, sections were dehydrated and dried at room temperature. Sections were covered with hybridization buffer (50% (v/v) formamide, 10 mmol Tris/l, pH 7.5, in 4 × SSC (1 × SSC contains 150 mmol NaCl/l and 15 mmol trisodium citrate/l), 1 × Denhardt's solution, 500 µg sheared salmon sperm DNA/ml, 10% (v/v) dextran sulphate) containing DIG-labelled probes (0.5 pmol/15 µl per section) and incubated overnight at 37 °C in a humidified environment. The unhybridized probes were removed by washing the sections with decreasing concentrations of SSC for 40 min and for a further 15 min in Tris-buffered saline (TBS, pH 7.6, 50 mmol/l) with added 0.1% (v/v) Triton X-100 and 0.1% (w/v) BSA at room temperature. The specific binding of oligonucleotide probes was detected by incubating the sections with alkaline phosphatase-conjugated sheep anti-DIG Fab fragments (Boehringer Mannheim) for 5 h. Sections were washed in TBS followed by incubation with nitroblue tetrazolium (NBT, 330 µg/ml) and 5-bromo-4-chloro-3-indolyl phosphate (BCIP, 165 µg/ml) in a dark, humidified environment. The optimal substrate incubation time for detecting adrenal (pro)renin mRNA was about 22 h.

Results

It should be noted that the dark staining observed in the connective tissue capsule was considered to be non-specific in nearly all cases. To ensure reproducibility of results, experiments were repeated a minimum of three times, with adrenals from five animals in each group on each occasion. A negative control is shown in Fig. 1. Since renin is produced by proteolytic cleavage from its precursor preprorenin, the renin mRNA detected in this investigation codes for the precursor rather than for active renin specifically.

A remarkable increase in both size and weight (more than 100%, from a mean of approximately 70 mg (control)

Figure 1 *In situ* hybridization using rat renin antisense oligonucleotide probe on RNase-pretreated rat adrenal section showing negative staining. G=zona glomerulosa, F=zona fasciculata, R=zona reticularis. (× 70)

Figure 2 Localization of (pro)renin mRNA using *in situ* hybridization in control rat (a) cortex and (b) zona reticularis and medulla. Positive signals are shown by dark staining. M=medulla. (× 140)

Figure 3 Localization of (pro)renin mRNA using *in situ* hybridization in adrenal (a) cortex and (b) zona reticularis and medulla from rat treated with ACTH for 1 day. (× 140)

Figure 4 Localization of (pro)renin mRNA using *in situ* hybridization in adrenal (a) cortex and (b) zona reticularis and medulla from rat treated with ACTH for 3 days. (× 140)

Fig. 1

Fig. 2a

Fig. 2b

Fig. 3a

Fig. 3b

Fig. 4a

Fig. 4b

to 140–200 mg (ACTH-treated) per gland pair) was observed in adrenals from rats treated for 3 or 5 days; these glands were intensely hyperaemic. In control, untreated animals small amounts of (pro)renin mRNA were detected in the adrenal cortex, predominantly in the zona fasciculata (Fig. 2a), whereas a much stronger signal was seen in the medulla (Fig. 2b). There was no observable change in the amount of (pro)renin mRNA detected in the inner cortex after 1 day of ACTH treatment, although the positive signal was at this stage also observed in the zona glomerulosa (Fig. 3). Further ACTH treatment for 3 or 5 days increased the (pro)renin mRNA in the entire adrenal cortex, and positive signals were detected in the subcapsular region as well as the inner cortex (Figs 4 and 5). Moreover, continuous administration of ACTH also gradually increased the amount of (pro)renin mRNA in the zona reticularis (Figs 4b and 5b). The (pro)renin mRNA in the medulla was not altered by ACTH treatment (Figs 4b and 5b).

Low sodium diet has no effect on ACTH or inner adrenocortical zone function (Vinson *et al.* 1992). The localization of (pro)renin mRNA was also changed remarkably in the sodium-restricted rat adrenal in which, after 5 days, a positive signal was observed only in the zona glomerulosa and some fasciculata cells next to the outer cortex (Fig. 6a). The amount of (pro)renin mRNA detected in the medulla was slightly reduced in these rats (Fig. 6b). After 17 days of dietary sodium restriction, a positive (pro)renin mRNA signal was seen in the reticularis as well as in the glomerulosa (Fig. 7).

Discussion

These observations demonstrate transcription of the (pro)renin gene and the localization of its mRNA in adult rat adrenal glands. In contrast with previous reports showing that (pro)renin mRNA and/or renin activity were detected predominantly in the zona glomerulosa of rat adrenals (Doi *et al.* 1984a, Deschepper *et al.* 1986, Brecher *et al.* 1989), the present data showed that in the normal adult female rat adrenal cortex, small amounts of (pro)renin mRNA were found predominantly in the outer zona fasciculata, but not in the zona glomerulosa (Fig. 2a). Although the significance of the local RAS in the adrenal inner cortex receives very little attention, it has been shown that long-term angiotensin II administration slightly stimulates the growth of the rat zona fasciculata, and increases plasma corticosterone concentration *in vivo* (Nussdorfer *et al.* 1981, Mazzocchi *et al.* 1985). Other reports also demonstrated that angiotensin II enhances the secretory activity of fasciculata cell preparations in other species *in vitro* (Hepp *et al.* 1977, McKenna *et al.* 1978, Pham-Huu-Trung *et al.* 1981), while McEwan *et al.* (1996) showed that angiotensin II, like a low sodium diet, increased cell proliferation in the reticularis as well as in

the glomerulosa. It is, of course, well documented that angiotensin II is a specific zona glomerulosa stimulant and an important regulator of aldosterone secretion in most mammalian species (Glöz & Sugar 1962, Müller 1988). However, angiotensin II receptors are also found in rat adrenocortical inner zones though at a lower concentration than in the glomerulosa (Douglas *et al.* 1978). Thus it is possible that the local RAS may play a role in the regulation of the growth and functions of the adrenal inner cortex, and these possibilities are given further support by our studies on the actions of ACTH and a low sodium diet. The functions of the inner adrenal cortex are thought to be controlled almost exclusively by ACTH. Nevertheless, adrenal renin is also influenced by ACTH (Ganten *et al.* 1974) and administration of ACTH to normal rats significantly increases adrenal renin content (Doi *et al.* 1984a), while, *in vitro*, ACTH increases renin activity in cultured rat glomerulosa cells (Yamaguchi *et al.* 1992). The present results also show that adrenal (pro)renin gene expression was enhanced by ACTH administration *in vivo* (Figs 3–5), and that this induction occurs throughout the cortex. Although the physiological significance of ACTH-induced renin activity in the adrenal gland is not entirely clear, the results certainly suggest that it has a role both in inner zone as well as glomerulosa function.

This concept is supported by the effects of sodium depletion. Early work by Deane *et al.* (1948) showed that sodium deficiency causes widening of the adrenal glomerulosa with cellular hypertrophy and hyperplasia. It stimulates both systemic and local adrenal RASs and induces a gradual hypertrophy of the endoplasmic reticulum, Golgi network and the mitochondrial compartment of rat glomerulosa cells *in vivo* (Mazzocchi *et al.* 1985, Robba *et al.* 1988). Aguilera & Catt (1978) also reported that a low sodium diet enhances the glomerulosa aldosterone response to a chronic infusion of angiotensin II. Sodium-depleted diets also increase adrenal renin as well as aldosterone secretion, while a high sodium diet decreases both (Ganten *et al.* 1974, Doi *et al.* 1983, 1984a, Nakamaru *et al.* 1985), and these effects have been attributed to the increase in activity of the local RAS (Aguilera & Catt 1978). The adrenal RAS, which is stimulated by low sodium intake (Kifor *et al.* 1991, Chiou *et al.* 1995), is thought to enhance the responsiveness of the glomerulosa cells to circulating angiotensin II and results in a maximal increase in aldosterone secretion (Menachery *et al.* 1991). This hypothesis supports earlier observations that the prior use of an ACE inhibitor prevents the zona glomerulosa responsiveness to angiotensin II (Müller *et al.* 1984) and the hypertrophy of glomerulosa cells (Mazzocchi & Nussdorfer 1984) in sodium-restricted rats. The present observations certainly support the view that the zona glomerulosa was the main site for (pro)renin synthesis in sodium-restricted rats (Fig. 6a). These results are, as may be expected, somewhat different from those obtained with ACTH treatment. Nevertheless, it is

Fig. 5a

Fig. 5b

Fig. 6a

Fig. 6b

Fig. 7a

Fig. 7b

Figure 5 Localization of (pro)renin mRNA using *in situ* hybridization in adrenal (a) cortex and (b) zona reticularis and medulla from rat treated with ACTH for 5 days. ($\times 140$)

Figure 6 Localization of (pro)renin mRNA using *in situ* hybridization in adrenal (a) cortex and (b) zona reticularis and medulla from rat subjected to dietary sodium depletion for 5 days. ($\times 140$)

Figure 7 Localization of (pro)renin mRNA using *in situ* hybridization in frozen adrenal (a) cortex and (b) zona reticularis and medulla from rat subjected to dietary sodium depletion for 17 days. (a = $\times 280$, b = $\times 70$)

important to emphasize that both of these treatments, ACTH and low sodium, affect (pro)renin transcription throughout the gland, and it is particularly striking that a

more prolonged low sodium diet enhanced the (pro)renin mRNA signal in reticularis cells as well as in the glomerulosa. This accords with the observation by McEwan *et al.*

(1996) that a low sodium diet, like angiotensin II treatment, increases proliferation in the reticularis as well as in the glomerulosa. The results emphasize the potential importance of the tissue RAS, perhaps particularly in extreme conditions of stimulation, such as in the regulation of adrenocortical function.

In this study significant amounts of (pro)renin mRNA were detected in the adrenal medulla in both control and stimulated conditions (Figs 2b–6b). As early as 1967, Ryan suggested that renin might act as a local hormone in modulating adrenal medullary catecholamine secretion. Renin and its mRNA have been detected in human pheochromocytoma cells (Racz *et al.* 1992), and ACE is localized in the rat adrenal medulla (Strittmatter *et al.* 1986). In addition, angiotensin II binding sites have been found in membrane homogenates of rat adrenal medulla (Healy *et al.* 1985). Stimulation of angiotensin receptors on adrenal medullary cells increases the influx of calcium (Zimlichman *et al.* 1987), and causes translocation of protein kinase C from soluble to particulate fractions (Stachowiak *et al.* 1990, 1991). Angiotensin II induces *c-fos* expression in adrenal medullary cells *in vivo* and *in vitro* (Stachowiak *et al.* 1990). It also has the ability to influence the activity of the sympathetic nervous system, and since it stimulates catecholamine biosynthesis (Lewis & Reit 1965) and release (Robinson 1967, Peach 1977) in the adrenal medulla. The local RAS may play a role in the regulation of catecholamine excretion. It is clear, however, that its regulation is quite different from that of the cortex, since (pro)renin gene transcription in the medulla was relatively unaffected by the treatments that so clearly changed the distribution of the transcription of this gene in the cortex.

References

- Aguilera G & Catt KJ 1978 Regulation of aldosterone secretion by the renin–angiotensin system during sodium restriction in rats. *Proceedings of the National Academy of Sciences of the USA* **75** 4057–4061.
- Brecher AS, Shier DN, Dene H, Wang SM, Rapp JP, Franco Saenz R & Mulrow PJ 1989 Regulation of adrenal renin messenger ribonucleic acid by dietary sodium chloride. *Endocrinology* **124** 2907–2913.
- Caldwell PRB, Seegal BC, Das M & Soffer RL 1976 Angiotensin-converting enzyme: vascular endothelial localisation. *Science* **191** 1050–1051.
- Campbell DJ 1987 Circulating and tissue angiotensin system. *Journal of Clinical Investigation* **79** 1–6.
- Campbell DJ & Habener JF 1986 Angiotensinogen gene is expressed and differentially regulated in multiple tissues of the rat. *Journal of Clinical Investigation* **78** 31–39.
- Campbell DJ & Habener JF 1987 Regional distribution of angiotensinogen messenger RNA in rat adrenal and kidney. *Journal of Hypertension* **4** S385.
- Campbell DJ & Habener JF 1989 Hybridization *in situ* studies of angiotensinogen gene expression in rat adrenal and lung. *Endocrinology* **124** 218–222.
- Chiou CY, Williams GH & Kifor I 1995 Study of the rat adrenal renin–angiotensin system at a cellular level. *Journal of Clinical Investigation* **96** 1375–1381.
- Cushman DW & Cheung HS 1971 Concentration of angiotensin-converting enzyme in tissues of the rat. *Biochimica et Biophysica Acta* **250** 261–265.
- Deane HW, Shaw JH & Greep RO 1948 The effect of altered sodium or potassium intake on the width and cytochemistry of the zona glomerulosa of the rat's adrenal cortex. *Endocrinology* **43** 133–153.
- Del Vecchio PJ, Ryan JW, Chung A & Ryan US 1980 Capillaries of the adrenal cortex possess aminopeptidase A and angiotensin-converting enzyme activities. *Biochemical Journal* **186** 605–612.
- Deschepper CF & Ganong WF 1988 Renin and angiotensin in endocrine glands. *Frontiers of Neuroendocrinology* **10** 79–98.
- Deschepper CF, Mellon SH, Cumin F, Baxter JD & Ganong WF 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 USA* **83** 7552–7556.
- Doi Y, Atarashi K, Franco Saenz R & Mulrow PJ 1983 Adrenal renin: a possible regulator of aldosterone production. *Clinical and Experimental Hypertension* **A5** 1119–1126.
- Doi Y, Franco Saenz R & Mulrow PJ 1984a Evidence for an extrarenal source of inactive renin in rats. *Hypertension* **6** 627–632.
- Doi Y, Atarashi K, Franco Saenz R & Mulrow PJ 1984b Effect of changes in sodium or potassium balance and nephrectomy on adrenal renin and aldosterone concentrations. *Hypertension* **6** 1124–1129.
- Douglas J, Aguilera G, Kondo T & Catt K 1978 Angiotensin II receptors and aldosterone production in rat adrenal glomerulosa cells. *Endocrinology* **102** 685–696.
- Dzau VJ, Ellison KE, Brody T, Ingelfinger J & Pratt RE 1987 A comparative study of the distributions of renin and angiotensinogen messenger ribonucleic acids in rat and mouse tissues. *Endocrinology* **120** 2334–2338.
- Fattah DI, Whitehouse BJ & Vinson GP 1977 Biosynthesis of aldosterone from 18-hydroxylated precursors by rat adrenal tissue *in vitro*. *Journal of Endocrinology* **75** 187–195.
- Ganten D, Ganten U, Kubo S, Granger P, Nowaczynski W, Boucher R & Genest J 1974 Influence of sodium, potassium and pituitary hormones on iso-renin in rat adrenal glands. *American Journal of Physiology* **227** 224–229.
- Ganten D, Hutchinson JS, Schelling P, Ganten U & Fischer H 1976 The iso-renin angiotensin systems in extra-renal tissue. *Clinical and Experimental Pharmacology and Physiology* **3** 102–126.
- Glöz E & Sugar K 1962 The effect of synthetic angiotensin II on the synthesis of aldosterone in the adrenals. *Journal of Endocrinology* **24** 299–302.
- Hayduk K, Boucher R & Genest J 1970 Renin activity content in various tissues of dog under different physiopathological states. *Proceedings of the Society for Experimental Biology and Medicine* **134** 252–255.
- Healy DP, Maciejewski AR & Printz MP 1985 Autoradiographic localization of angiotensin II binding sites in the rat adrenal gland. *Endocrinology* **116** 1221–1223.
- Hepp R, Grillet C, Peytremann A & Vallotton MB 1977 Stimulation of corticosteroid biosynthesis by angiotensin I [Des–Asp1]–angiotensin I, angiotensin II and [Des–Asp]–angiotensin II in bovine adrenal fasciculata cells. *Endocrinology* **101** 717–725.
- Ho MM & Vinson GP 1995 Endocrine control of the distributions of bFGF, IGF-I and TGF- β 1 mRNAs in adult rat adrenals using non-radioactive *in situ* hybridization. *Journal of Endocrinology* **144** 379–387.
- Inagami T, Pandey K, Nakamaru M, McKenzie C, Misono KS, Naruse K & Naruse M 1984 Advances in the biochemistry of the renin–angiotensin system: intracellular renin–angiotensin system in

- rat adrenal zona glomerulosa cells and rat testicular Leydig cells. In *Endocrinology*, pp 399. Eds F Labrie & L Proulx. Amsterdam: Elsevier Science Publishers.
- Kifor I, Moor TJ, Fallo F, Sperling E, Menachery A, Chiou CY & Williams GH 1991 The effect of sodium intake on angiotensin content of the rat adrenal gland. *Endocrinology* **128** 1277–1284.
- Lewis GP & Reit E 1965 The action of angiotensin and bradykinin on the superior cervical ganglion of the cat. *Journal of Physiology* **179** 538–553.
- McEwan PC, Lindop GB & Kenyon CJ 1996 Control of cell-proliferation in the rat adrenal gland *in vivo* by the renin-angiotensin system. *American Journal of Physiology, Endocrinology and Metabolism* **34** E192–E198.
- McKenna TJ, Island DP, Nicholson WE & Liddle GW 1978 Angiotensin stimulates cortisol biosynthesis in human adrenal cells. *Steroids* **32** 127–136.
- Mazzocchi G & Nussdorfer GG 1984 Long-term effects of captopril on the morphology of normal rat adrenal zona glomerulosa. *Experimental and Clinical Endocrinology* **84** 148–152.
- Mazzocchi G, Rebuffat P, Robba C, Malendowicz LK & Nussdorfer GG 1985 Trophic effects of potassium loading on the rat zona glomerulosa: permissive role of ACTH and angiotensin II. *Acta Endocrinologica* **108** 98–103.
- Menachery A, Braley LM, Kifor I, Gleason R & Williams GH 1991 Dissociation in plasma renin and adrenal ANG II and aldosterone responses to sodium restriction in rats. *American Journal of Physiology* **261** E487–E494.
- Mendelsohn FA 1984 Localisation of angiotensin-converting enzyme in rat forebrain and other tissues by *in vitro* autoradiography using ¹²⁵I-labelled MK351A. *Clinical and Experimental Pharmacology and Physiology* **11** 431–435.
- Mizuno K, Hoffman LH, McKenzie JC & 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.
- Mulrow PJ 1992 Adrenal renin: regulation and function. *Frontiers of Neuroendocrinology* **13** 47–60.
- Müller J 1988 Alterations in aldosterone biosynthesis and secretion in long-term experiments and diseases. In *Regulation of Aldosterone Biosynthesis*, pp 108–110. Ed J Müller. Berlin: Springer-Verlag.
- Müller J, Hofstetter L, Schwenderner-Canlas P, Brunner DB & Lund EG 1984 Role of the renin-angiotensin system in the regulation of late steps in aldosterone biosynthesis by sodium intake of potassium-deficient rats. *Endocrinology* **115** 350–356.
- Nakamaru M, Misono KS, Naruse M, Workman J & Inagami T 1985 A role of the adrenal renin-angiotensin system in the regulation of potassium-stimulated aldosterone production. *Endocrinology* **117** 1772–1778.
- 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 USA* **79** 3295–3299.
- Naruse M, Sussman CR, Nause K, Jackson RV & Inagami T 1983 Renin exists in human adrenal tissue. *Journal of Clinical Endocrinology and Metabolism* **57** 482–487.
- Naruse M, Naruse K, Inagaki T & Inagami T 1984 Immunoreactive renin in mouse adrenal gland: localisation in the inner cortical region. *Hypertension* **6** 275–280.
- Nussdorfer GG, Robba C, Mazzocchi G & Rebuffat P 1981 Effects of angiotensin II on the zona fasciculata of the rat adrenal cortex: an ultrastructural stereologic study. *Journal of Anatomy* **132** 235–242.
- Ohkubo H, Nakayama K, Tanaka T & Nakanishi S 1986 Tissue distribution of rat angiotensinogen mRNA and structural analysis of its heterogeneity. *Journal of Biological Chemistry* **261** 319–323.
- Peach MJ 1977 The renin-angiotensin system. *Physiological Reviews* **57** 313–370.
- Pham-Huu-Trung MT, Lombes M, Bogoy A & Girard F 1981 Combined effects of angiotensin II and ACTH on cortisol production: *in vitro* studies on isolated guinea-pig adrenal cells. *Journal of Steroid Biochemistry* **14** 929–934.
- Racz K, Pinet F, Gasc JM, Guyene TT & Corvol P 1992 Coexpression of renin, angiotensinogen, and their messenger ribonucleic acids in adrenal tissues. *Journal of Clinical Endocrinology and Metabolism* **75** 730–737.
- Robba C, Rebuffat P, Mazzocchi G & Nussdorfer GG 1988 Long-term trophic effect of sodium restriction on the rat adrenal zona glomerulosa. I. Its partial independence of the renin-angiotensin system. *Experimental and Clinical Endocrinology* **91** 43–50.
- Robinson RL 1967 Stimulation of the catecholamine output of the isolated, perfused adrenal gland of the dog by angiotensin and bradykinin. *Journal of Pharmacology and Experimental Therapeutics* **156** 252–257.
- Ryan JW 1967 Renin-like enzyme in the adrenal gland. *Science* **158** 1589–1590.
- Shier DN, Kusano E, Stoner GD, Franco Saenz R & Mulrow PJ 1989 Production of renin, angiotensin II and aldosterone by adrenal explant cultures: response to potassium and converting enzyme inhibition. *Endocrinology* **125** 486–491.
- Stachowiak MK, Sar M, Touminen RK, Jiang HK, An S, Iadarola MJ, Poisner AM & Hong JS 1990 Stimulation of adrenal medullary cells *in vivo* and *in vitro* induces expression of c-fos proto-oncogene. *Oncogene* **5** 69–73.
- Stachowiak MK, Poisner AM, Jiang HK, Hudson PH & Hong JS 1991 Regulation of proenkephalin gene expression by angiotensin in bovine adrenal medullary cells: molecular mechanisms and nature of the second messenger systems. *Molecular and Cellular Neuroscience* **2** 213–220.
- Strittmatter SM, Desouza EB, Lynch DR & Snyder SH 1986 Angiotensin-converting enzyme localized in the rat pituitary and adrenal glands by [3H]captopril autoradiography. *Endocrinology* **118** 1690–1699.
- Vazir H, Whitehouse BJ, Vinson GP & McCredie E 1981 Effects of prolonged ACTH treatment on adrenal steroidogenesis and blood pressure in rats. *Acta Endocrinologica* **97** 533–542.
- Vinson GP, Whitehouse BJ & Hinson J 1992 *The Adrenal Cortex*. Prentice Hall, Englewood Cliffs: New Jersey.
- Yamaguchi T, Naito Z, Stoner GD, Franco Saenz R & Mulrow PJ 1990 Role of the adrenal renin-angiotensin system on adrenocorticotrophic hormone- and potassium-stimulated aldosterone production by rat adrenal glomerulosa cells in monolayer culture. *Hypertension* **16** 635–641.
- Yamaguchi T, Tokita Y, Ranco Saenz R, Mulrow PJ, Peters J & Ganten D 1992 Zonal distribution and regulation of adrenal renin in a transgenic model of hypertension in the rat. *Endocrinology* **131** 1955–1962.
- Zimlichman R, Goldstein DS, Zimlichman S, Stull R & Keiser HR 1987 Angiotensin II increases cytosolic calcium and stimulates catecholamine release in cultured bovine adrenomedullary cells. *Cell Calcium* **8** 315–325.

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