Expression of the renin gene in extra-renal tissues of the rat

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Expression of the renin gene in several rat organs is demonstrated by the detection of renin mRNA using a ribonuclease-protection technique. In two of these sites, the brain and the liver, renin mRNA levels are unaffected by changes in dietary salt which markedly affect renal renin mRNA levels. The findings provide the basis for an important ubiquitous local regulatory role for the renin–angiotensin system extending beyond the circulation.

INTRODUCTION

Renin is classically considered to be an enzyme that is synthesized by the juxtaglomerular cells of the kidneys and secreted into the circulation, where it cleaves angiotensinogen to release angiotensin I. Angiotensin I is converted into the octapeptide angiotensin II by converting enzyme, a ubiquitous peptidase. Angiotensin II is the active hormone of the renin–angiotensin (RA) system, contributing to cardiovascular homoeostasis by causing arteriolar vasoconstriction and increased adrenal aldosterone biosynthesis [1].

Since the first description by Gould *et al.* [2], extra-renal renin-like activity has been demonstrated in a wide variety of tissues in several species [3]. Initial suggestions that such activity may wholly represent the action of other proteinases on renin substrate [4] have been refuted to some extent by better characterization of the physiochemical properties of the renin-like activity [5] and the demonstration of inhibition of the activity by renin-specific antibodies [6]. However, the source of the tissue renin and the physiological and pathological role(s) of the tissue RA systems remain uncertain. For instance, evidence has been produced for both uptake of circulating renin [7] and for local synthesis [8,9] to account for renin in arterial walls, a potentially important system with regard to both normal control of vascular tone and increased tone in hypertension. Although several functions have been attributed to the tissue RA systems [10], little is known about factors that regulate them and the relationship of these systems to the renal system.

With the cloning of the renin gene [11] and the development of highly specific and sensitive hybridization procedures [12], it has become possible to determine the extent of renin-gene expression outside the kidney. Using these techniques we now have evidence for widespread extra-renal renin-gene expression in the rat, with major differences between organs, providing the basis for an important ubiquitous local regulatory system the role of which may extend beyond the circulation.

EXPERIMENTAL

The RNAase-protection technique used is shown in Scheme 1.

Scheme 1. An illustration of the RNAase-protection technique used to detect renin mRNA

Preparation of RNA

Tissues were taken from 12-week-old female Wistar-Kyoto (WKY) rats immediately after they were killed, frozen in liquid N₂ and stored at −70 °C. Total

cellular RNA from each tissue was prepared by a modification of the method of Auffray & Rougeon [13] as previously described [14]. RNA concentrations were determined from absorbance measurements at 260 nm and checked by comparing ethidium bromide fluorescence of samples run on agarose gels.

Preparation of probe

A 0.75-kb endonuclease-*RsaI* fragment of rat genomic DNA containing the whole of exon 9 of the rat renin gene and flanking 5' and 3' sequences was inserted into the M13 derivative mICE18 which carries the T7 promoter [15]. α - 32 P-labelled anti-message-strand ribo-probe was prepared by transcription *in vitro* [15] in the presence of T7 RNA polymerase (Boehringer) and [α - 32 P]UTP (Amersham International, Amersham, Bucks., U.K.) and gel-purified [16] to remove prematurely terminated transcripts before use.

The *RsaI* fragment was derived from plasmid pRRnE4 containing 4.0 kb of rat renin genomic DNA kindly provided by Dr. Okhubo, University of Kyoto, Kyoto, Japan. Sequence analysis of the 0.75-kb fragment by him and independently by us had shown that it corresponded to the 3' end of the mouse *renI* gene [11] and spanned exon 9. The full rat renin-gene sequence has not been published.

RNAase protection

Hybridization of the probe to the RNA samples and RNAase digestion with single-strand-specific RNAases A and T1 were carried out as described by Melton *et al.* [12], except that the hybridization was carried out at 63 °C and digestion at 37 °C. These conditions had been determined empirically to maximize the signal-to-noise ratio with kidney RNA. The presence of renin mRNA is indicated by the protection of a 350-base fragment (corresponding to exon 9), which was detected by autoradiography after running the products on a 3.5% polyacrylamide/7M-urea gel.

Salt study

Female WKY rats (12 weeks old; $n = 6$ per group) were put on a low-salt diet (0.007 mmol of NaCl/g; deionized water to drink), normal salt diet (0.112 mmol of NaCl/g; deionized water to drink) or a high-salt diet [0.112 mmol of NaCl/g; 1.5% (w/v) NaCl to drink] for 2 weeks. Organs were then removed and pooled for each group before RNA extraction. The low-salt diet was prepared by mixing Casilan (Farley Health Products Ltd., Nottingham, U.K.) with deionized water in a ratio of 25 g of Casilan to 75 ml of water. For the normal-salt and high-salt groups this was supplemented with 1.2 g of NaCl/100 ml of diet. All diets were supplemented with 1.7 g of KCl/100 ml of diet. Between days 12 and 13, 24 h urine samples were collected from three rats from each group, and sodium concentrations were analysed by flame photometry. The mean urine sodium concentrations were 1.3, 69 and 225 mmol/l for the low-, normal- and high-salt groups respectively.

RESULTS

Fig. 1 shows the result of an experiment where the protection technique was applied to RNA from a number of WKY-rat tissues. The presence of renin mRNA is indicated by the detection of a 'protected'

Fig. 1. Extra-renal renin-gene expression in the rat: detection of renin mRNA by RNAase protection: autoradiograph of 3.5%-polyacrylamide/7 M-urea gel

See Scheme 1 and the Experimental section for details of the technique. a, RNA probe; b, protected fragment; M, marker track (32 P-5'-end-labelled phage- ϕ X174 *HaeIII* fragments); P, undigested probe; lane 1, control *E. coli* RNA (250 μ g); lanes 2–9, RNA from WKY-rat tissues (250 μ g per sample unless otherwise stated): 2, kidney (40 μ g); 3, brain; 4, adrenal; 5, aorta; 6, heart; 7, lung; 8, liver; 9, spleen.

fragment (arrow b). As expected, renin mRNA was most abundant in the kidney. A relatively large amount (in terms of fraction of total RNA) of renin mRNA was also detected in the liver and brain. Renin mRNA was detectable in the other tissues examined as well, namely in adrenal, aorta, heart, lung and spleen, but in much smaller amounts. Although we have consistently detected low levels of renin mRNA in the adrenal, aorta and heart by the RNAase-protection method, renin mRNA is not always evident in RNA from lung and spleen. In these tissues the low levels are likely on occasion to be beyond the limits of sensitivity of the method.

Renin production in the kidney responds to changes in salt intake, being stimulated by low salt intake and inhibited by high-salt diets. By affecting aldosterone production in the adrenal, this plays a major role in maintaining extracellular fluid volume in response to changing salt intake [17]. The RNAase-protection assay was utilized to monitor the effect of variations in dietary sodium on renin mRNA levels in the kidney and in two extra-renal sites, namely liver and brain. The data (Fig. 2) clearly show the suppressive effect of increasing sodium intake on kidney renin mRNA levels and the contrasting absence of any such effect in the liver and brain. It should be noted that the level of renin mRNA in the liver in Fig. 1 is atypically high, the levels seen in Fig. 2 being more representative of what we consistently observe.

Fig. 2. Effect of changes in dietary salt on renin mRNA levels in rat kidney, brain and liver

a, RNA probe; b, protected fragment; M, marker track (^{32}P -5'-end-labelled phage- ϕX174 *Hae*III fragments); P, undigested probe; lanes 1–3, kidney RNA (40 μg per sample); lanes 4–6, brain RNA (250 μg per sample); lanes 7–9, liver RNA (250 μg per sample); lanes 1, 4 and 7, low-salt diet; lanes 2, 5 and 8, normal-salt diet; lanes 3, 6 and 9, high-salt diet (the inset shows the kidney tracks after a shorter exposure).

DISCUSSION

Renin mRNA has previously been detected in mouse and rat brain [18] and mouse heart [19] by Northern blotting. By utilizing a more sensitive technique [12] we have extended the number of tissues shown to contain renin mRNA. The findings imply that at least some of the observed renin-like activity in these tissues is indeed due to locally produced renin. Data obtained by using primer extension on mRNA templates (K. Lilleycrop & W. J. Brammar, unpublished work) and RNAase protection (C. Miller & A. J. Carter, unpublished work) have demonstrated the presence of renin mRNA in the liver, testis and brain of mouse strains.

The data clearly show that the RNAase-protection assay detects renin mRNA in the kidney, brain, adrenal and liver. The yields of protected fragments obtained using RNAs from aorta, heart, lung and spleen are lower, but still readily detectable. Although we consistently find protected fragments with aorta and heart RNAs, the levels of renin mRNA in lung and spleen are on occasion beyond the limits of detection by the RNAase-protection technique in our hands.

It is quite normal for the RNAase-protection assay to

produce multiple clustered bands (see Figs. 1 and 2) at the position of the expected protected fragment as a consequence of the base-specific endonucleolytic cleavage of the single-stranded RNA. Although the relative intensities of the various protected species in the 350-base region are variable from one tissue to another, this variation is probably not significant, since it occurs to some extent with RNAs from the same tissue in different experiments [cf. brain RNA, Fig. 1, track 3, versus Fig. 2, tracks 4, 5 and 6]. Control *Escherichia coli* RNA subjected to the same manipulations never shows such bands in the 350-base region.

As plasma renin activity in anephric patients [20] and nephrectomized animals [21] is very low, it is unlikely that any extra-renal contribution to plasma renin concentration is of physiological importance. However, especially with the demonstration of the presence [22] and in some cases local synthesis [23] of other components of the RA system, several local autocrine and paracrine functions have been suggested, including regulation of vascular tone [8], facilitation of noradrenergic transmission [24], hormone secretion [25] and control of gene expression [26]. The human renin gene has three potential promoter elements ('TATA' sequences) where RNA polymerase might bind, and Morris [27] has suggested how use of two of the promoters might produce renin precursors with hydrophilic *N*-terminals incapable of entering the secretory apparatus and therefore locked within the cell to serve intracellular functions.

Vascular wall [28], brain [29] and adrenal [30] renin have all been implicated in the genesis of hypertension in experimental animal models. A particular controversy has existed concerning the source of vascular renin, with some studies [7] suggesting uptake of circulating renin. Although other evidence such as dissociation of vessel-wall levels from plasma levels [28], specific cleavage of synthetic renin substrate in perfused vascular preparations [8] and the demonstration of production of renin by cultured vascular endothelial [31] and smooth-muscle cells [9] have suggested local production of renin, this is the first demonstration of renin mRNA in the extra-renal vascular tree.

A surprising finding was the presence of renin-gene expression in the liver. Although some studies [32] had noticed renin-like activity in the liver, this was attributed to non-specific proteinases [33]. However, Dzau *et al.* [34] found significant renin activity in the mouse liver that was inhibitable by anti-renin antibodies. Taking our observation with the finding of Re *et al.* [35] of high-affinity receptors for angiotensin II in rat liver chromatin and the observation of increased RNA synthesis by isolated hepatic nuclei on exposure to angiotensin II [26], one can postulate a role for an intracellular RA system in the regulation of liver gene expression.

We have started to explore the physiological regulation of the tissue systems by examining the effect of changes in salt intake on renin mRNA levels in the kidney, liver and brain. The brain RA system has been attributed a role in the control of vascular tone, in inducing drinking behaviour and salt appetite, and in causing the release of adrenocorticotropin, vasopressin and lutropin [36]. This system, however, appears to be controlled differently from the renal system, as changes in salt intake which produced marked changes in kidney renin mRNA levels had no effect on brain levels. This is consistent with the observations of Dzau & Sands [37] that various physio-

logical states that markedly affected systemic renin activity had no effect on brain or pituitary activities. Likewise the liver system appears to be independently regulated.

Overall our studies therefore support the concept of a kidney-derived hormonal RA system intimately involved in circulatory homeostasis and independent ubiquitous tissue systems whose roles are likely to extend beyond the circulation. An important step in further defining the functions of the tissue systems will be the localization of precise cellular sites of expression in each tissue by the application of 'in situ' hybridization techniques.

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