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The Renin and Iso-Renin–Angiotensin Systems in Rats with Experimental Pituitary Tumors (38585)

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Iso-renin in extrarenal tissue can be determined by means of an enzymatic procedure in which angiotensin formation is measured (1, 2). However, the presence of plasma renin in the tissue can interfere with the iso-renin assay. In previous studies we circumvented this problem by measuring iso-renin in the brain, where the blood-brain barrier excludes such interference, or by using nephrectomized animals in which plasma renin is usually undetectable (2, 3).

Confirming and extending the results of Molteni *et al.* (4) and Nickerson *et al.* (5), we found that rats having an experimental pituitary tumor (MtT/F4) show a negligible plasma renin concentration, probably because of the high plasma concentration of adrenocorticotropic hormone (ACTH), growth hormone, and prolactin and the secondary hypermineralocorticism (3). Despite low plasma renin concentrations in these tumor-bearing rats, we found adrenal gland iso-renin concentrations to be at or above those in controls (3). These rats therefore seemed suitable for a more thorough study of extrarenal iso-renin. Results indicate that all components of the isorenin–angiotensin system are present in extrarenal tissue; they appear to be locally synthesized.

Material and Methods. Measurement of renin and iso-renin. The measurement of plasma renin and tissue iso-renin was based on the method of Boucher *et al.* (1). Rats were killed under light ether anesthesia by rapid bleeding through the abdominal aorta. Within 2 min, tissues from adrenal glands, heart, pituitary tumor and brain were taken, frozen on dry ice and wrapped in parafilm. Tissues were stored at −20° until assay.

Frozen whole adrenal glands were carefully freed from connective tissue, weighed into plastic test-tubes, thawed and frozen again at −20°. They were then homogenized in all-glass tissue homogenizers (Fisher 7727) at 4° in 0.25 molar Tris–phosphate buffer, containing 0.3% EDTA and 0.02% sodium azide and adjusted to pH 5.5 with phosphoric acid, in a dilution of 1:100 (w/v). The homogenate was frozen again in test tubes, thawed and dialyzed for 24 hr at 4° against distilled water. A measured portion of this homogenate was incubated with excess renin substrate prepared from the plasma of nephrectomized dogs.

Samples (200–300 mg) of frozen heart, brain, lung, and tumor tissue were weighed into test-tubes, allowed to thaw, and then frozen again. After homogenization in 3 ml of Tris–phosphate buffer, as described for adrenal glands, the tissue was frozen a third time and then incubated and processed as described elsewhere (2, 3).

Results are expressed as nanograms of angiotensin formed per gram wet weight of tissue (or milliliter of plasma) per hour of incubation at 37°. Specific iso-renin in the adrenal gland was expressed as nanograms angiotensin per milligram of protein per hour. Angiotensin was measured by the rat pressor assay (1).

Renin substrates. Renin substrates from the plasma of nephrectomized dogs, rat and sheep were prepared according to Haas *et al.* (6).

The renin-inhibitor pepstatin (7) was added at a concentration of 2 μg/ml to incubation mixtures which contained either kidney renin with rat plasma substrate or tissue iso-renins with dog plasma substrate.

Angiotensin converting enzyme, angiotensinases. Converting enzyme activity in plasma and tissue was measured by an automated method (8).

The determination of angiotensinase activity in plasma and tissue was based on the method of Strong *et al.* (9). Tris–phosphate buffer (pH 7.4) containing 0.3% bovine serum albumin was used for homogenization

and dilution. Plasma (0.5 ml) and cerebrospinal fluid (0.1 ml from rats and 1 ml when dogs were used) were made up to 3 ml with buffer in each case, and incubated. All other tissues were homogenized in a 1:10 dilution (w/v), centrifuged at 10,000*g* for 10 min and 0.1 ml of the supernatant was incubated with 2.9 ml of the buffer. Valine 5-angiotensin-II-amide (100–500 ng Hypertensin Ciba) was added to the siliconized plastic incubation tubes. Two tubes were kept cold on saline-ice (controls = 100%), two tubes were incubated for 5 min and two tubes for 10 min at 37°. Angiotensin was separated on Dowex 50W-X2 NH_4^+ columns and measured by the rat pressor assay. Overall angiotensinase activity was expressed as the percentage degradation of angiotensin II per minute in the given dilution or calculated as degradation per minute per milligram of protein. Protein was measured by the method of Lowry *et al.* (10).

Animal studies. All experiments were made with male Fisher 344 rats, initial body wt 200 g. An autonomous experimental anterior pituitary tumor (MtT/F4) which was first described by Furth *et al.* (11) was transplanted into 19 rats. For transplantation, tumor tissue was minced into small pieces in a petri dish containing tissue culture medium 199-IX (Grand Island Biological Company, Berkeley, CA) and placed subcutaneously in the left flank. Tumor growth was recognizable 3 wk after transplantation by palpation. Eight rats were killed after 52 days and 11 rats were nephrectomized 16 hr before killing at 52 days. Eight rats which were sham operated and killed after 52 days served as controls.

All values are expressed as mean ± standard error of the mean. For statistical analysis Students' *t* test was used.

Results. Rat kidney renin and iso-renins from brain, tumor, adrenal gland and heart all generated angiotensin with renin substrates from rat, dog and sheep (Table I).

Samples of iso-renin formed more (or much more) angiotensin with dog substrate than with rat or sheep substrate. In contrast, rat kidney renin produced less angiotensin from dog and sheep substrates. Pituitary tumor and brain iso-renin had the highest affinity for dog substrate. Pepstatin (2 μg/ml) inhibited the reaction of rat kidney renin with rat plasma substrate by 70% and the reaction of rat iso-renin from all tissues with dog plasma substrate by 100%.

TABLE I. COMPARISON OF ANGIOTENSIN FORMATION OF RAT RENAL RENIN AND TISSUE ISO-RENINS WITH PLASMA SUBSTRATES FROM DIFFERENT SPECIES.

	Angiotensin formation with renin substrate from:		
	Rat	Dog	Sheep
Kidney renin	100%	42%	35%
Brain iso-renin	100%	8360%	1800%
Tumor iso-renin	100%	1511%	236%
Adrenal iso-renin	100%	337%	101%
Heart iso-renin	100%	202%	101%

Plasma renin was undetectable in tumor-bearing rats (Table II, Fig. 1). Iso-renin concentration was normal in the brain and adrenal gland of tumor-bearing rats, but significantly lower than controls in heart muscle. Sixteen hours after nephrectomy iso-renin was significantly higher in brain, adrenal gland, and heart muscle than in the corresponding tissues of tumor-bearing, non-nephrectomized rats. Nephrectomy did not restore the heart muscle isorenin concentration of tumor-bearing rats to normal values. The iso-renin concentration in MtT/F4 tumor tissue was higher than in all other tissues; nephrectomy seemed to increase it, but not statistically significantly.

Angiotensin-converting enzyme activity in plasma was low in tumor-bearing rats (Table III) and was not affected by nephrectomy. Cerebrospinal fluid and adrenal glands showed no converting enzyme activity. Brain tissue in all three groups of rats showed a high activity of this enzyme, pituitary tumor tissue a low one. In contrast, lung tissue had a very high activity, unaffected by tumor transplantation and nephrectomy. In heart muscle CEA was higher in both groups of tumor-bearing rats than in controls.

Overall angiotensinase activity was unmeasurably low in cerebrospinal fluid (Table IV) and high in brain and tumor tissue. The supernatants from homogenates of adrenal gland and heart tissue degraded angiotensin II to a much greater extent than plasma.

TABLE II. PLASMA RENIN AND ISO-RENIN CONCENTRATIONS IN VARIOUS RAT TISSUES.

	Control (8)	Tumor-bearing (8)	Tumor-bearing nephrectomized (11)
	(ng angiotensin formed per ml or g/hr at 37°)		
Plasma	5.4 ± 0.7	0	0
Cerebrospinal fluid	0	—	—
Brain (frontal cortex)	43.2 ± 1.7	43.8 ± 2.6[c]	51.7 ± 2.1[b]
Pituitary tumor	—	63.8 ± 7.6	93.3 ± 13.6[c]
Adrenal gland	28.3 ± 3.8	35.8 ± 6.3[c]	64.5 ± 7.0[b]
Heart	17.3 ± 1.5	8.8 ± 0.7[b]	12.8 ± 1.0[a]

[a] $P < 0.02$, compared to tumor bearing group.
[b] $P < 0.001$.
[c] Not significantly different.

Discussion. The high affinity of renin substrate from plasma of nephrectomized dogs for tissue iso-renins of the rat, and its low affinity for rat kidney renin, is of practical importance. Use of this substrate for iso-renin assay increases sensitivity and minimizes interference by plasma renin. Tissue iso-renins do generate angiotensin with homologous substrates but they vary in the rate of angiotensin formation. This confirms earlier results with dogs (12). There is evidence that tissue iso-renins utilize local angiotensinogen (13). As with the dog, rat kidney renin and tissue iso-renins are inhibited by pepstatin if naturally occurring substrates are used, but with the synthetic tetradecapeptide renin substrate, we did not obtain complete inhibition of iso-renin.

In rats bearing pituitary tumors, plasma renin is suppressed to undetectable levels (3–5), but adrenal gland iso-renin levels are at or above control levels (3).

Interference by plasma renin in the iso-renin assay in these experiments is excluded for several reasons: (a) Dog substrate reacts very little with a rat plasma renin, (b) the pH of 5.5 used in the iso-renin assay is below the optimal pH (6.5) for rat plasma renin, (c) plasma renin and tissue iso-renin were dissociated, the first being completely eliminated while the latter was at or above control levels, and (d) nephrectomy did not decrease iso-renin. The present results can therefore only be explained by local synthesis of iso-renin in extrarenal tissue. Obviously, tissue iso-renin did not contribute to plasma renin levels in these experiments to any major degree. We have no explanation for the

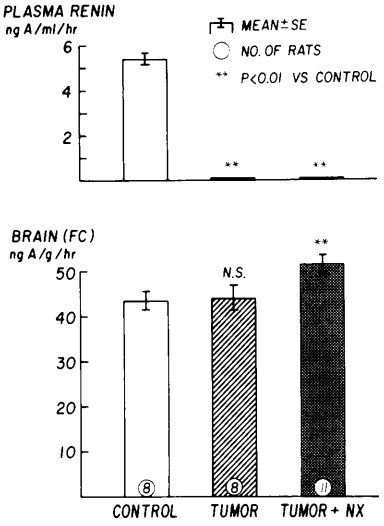

FIG. 1. Dissociation of plasma renin activity and brain iso-renin activity in rats bearing an experimental ectopic pituitary tumor. ** $P < 0.01$ versus controls, NS: no significant difference. Numbers in circles indicate numbers of experimental rats.

increased iso-renin concentration in brain and adrenal gland tissue after nephrectomy. Though this series of experiments does not include nephrectomized rats without tumor, previous experiments in rats and dogs have shown that nephrectomy in itself does not enhance iso-renin concentrations (2, 13, 14). It must be concluded therefore that pituitary hormones released by the tumor contributed to an increased local synthesis of iso-renin. This conclusion is substantiated by the observation that prolactin stimulates production of adrenal gland iso-renin and also by the fact that adrenal iso-

TABLE III. CONVERTING-ENZYME ACTIVITY IN VARIOUS RAT TISSUES.

	Control (8)	Tumor-bearing (8)	Tumor-bearing nephrectomized (11)
	(μM His-Leu formed per ml or g/min at 37°)		
Plasma	0.36 ± 0.01	0.18 ± 0.01[a]	0.19 ± 0.01[a]
Cerebrospinal fluid	0	—	—
Brain	0.91 ± 0.07	0.84 ± 0.04[b]	0.94 ± 0.05[b]
Pituitary tumor	—	0.20 ± 0.03[b]	0.21 ± 0.01[b]
Adrenal gland	0	0	0
Lung	9.51 ± 0.48	9.47 ± 0.74[b]	12.5 ± 1.17[b]
Heart	0.15 ± 0.01	0.20 ± 0.08[b]	0.19 ± 0.01[a]

[a] $P < 0.01$ compared to controls.
[b] Not significantly different.

TABLE IV. ANGIOTENSINASE ACTIVITY IN VARIOUS RAT TISSUES.

	mg protein per incubation mixture	% degradation of A[a]/min	% degradation of A per mg protein/min
Plasma	37	3.6±0.45	0.10
Cerebrospinal fluid	0.31	0	0
Brain	0.75	4.5±0.6	5.9
Pituitary tumor	0.62	4.5±0.7	7.?
Adrenal gland	1.39	4.1±0.5	2.9
Heart	1.55	5.8±0.8	3.7

[a] A = Valine-5-angiotensin II amide (Hypertensin Ciba). Values represent means of five or more individual measurements.

renin concentration is correlated with tumor weight (3). It is noteworthy that iso-renin concentration is highest in fast-growing pituitary tumor tissue, since it has been suggested that angiotensin stimulates nucleotide and protein synthesis (14). High renin concentrations have also been described in other renal (15) and extrarenal tumors (16). Iso-renin in heart muscle was significantly lower in rats with high plasma levels of pituitary hormones, although converting enzyme activity was higher.

Tissue iso-renin, converting enzyme, and angiotensinases reacted in different ways to the one stimulus in this experiment; this difference is also evident in other physiopathological conditions such as experimental hypertension and electrolyte imbalance in dogs, where plasma renin and tissue isorenins are frequently dissociated (12, 13 and unpublished data). These findings support the concept that the components of the tissue iso-renin–angiotensin system are synthesized locally and regulated to some extent independently of the classical kidney and plasma renin–angiotensin system.

Components of the iso-renin–angiotensin system do not always move in parallel. In the adrenal gland, for example, iso-renin concentration was high while converting enzyme was undetectable. Angiotensinases in the adrenals were also less active than in other tissues but higher than in plasma. It has been shown that angiotensin I and other angiotensin metabolites are metabolically active in the adrenal gland (17). Whether decreased converting enzyme activity indicates increased local angiotensin I production or an increased angiotensin I/angiotensin II ratio remains to be investigated.

From these and previous observations it is evident that the intrinsic iso-renin–angiotensin system is a complex local enzymatic system which may be physiologically important for regulation in the adrenal glands, brain, heart, and possibly other organs.

Summary. Renin, iso-renin, angiotensin I. angiotensin-converting enzyme, and angiotensinases were measured in plasma and in various extrarenal tissues of rats. Despite complete suppression of plasma renin in rats bearing pituitary tumors iso-renin and all other components of the renin–angiotensin system were found to be at or above control concentrations. The results strongly suggest

that there is local synthesis of iso-renin in extrarenal tissues.

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