Is Angiotensin Excreted in Urine?

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If a part of circulating angiotensin is excreted in urine, the study on this important pressor substance could be performed with great convenience by treating the urine instead of blood, which has been generally used in this sort of investigation.^{1,2,3)} But papers concerning urinary angiotensin have been completely lacking in the literature up to date. Bumpus⁴⁾ stated that angiotensin would not appear in urine because many destructive enzymes are present in both blood and urine. However, since angiotensin is a peptide of low molecular weight (about 1,000) and dialyzable through cellophane membrane, it may be expected that the circulating angiotensin is filtered through glomerulus into tubular fluid, and at least a part of filtered angiotensin is excreted in urine escaping reabsorption or destruction, if any.

This study was undertaken in an effort to clarify the problem of urinary excretion of angiotensin.

METHODS

Angiotensin

Valyl-5-angiotensin II aspartyl- β -amide ("Hypertensin", CIBA), synthesized and kindly supplied by CIBA, was used throughout this study.

Purification Procedure

Freshly voided urine was immediately acidified with HCl to pH 1.0. An aliquot (25-100 ml.) of the urine was saturated with sodium chloride, and extracted twice with one volume of *n*-butanol. To the combined butanol extract (two vol.) one vol. of petroleum ether was added, and the mixture (three vol.) was re-extracted with 0.7 and 0.3 vol. of 0.1 N HCl. The aqueous extract was percolated through a column (0.6 $\times$ 4 cm) of Dowex -50 X-2, H⁺ form, prepared according to Boucher *et al.*,²⁾ the column was then washed with 0.2 N sodium acetate-acetic

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acid buffer, pH 6.0, until the pH of the effluent rose to 6.0 (usually 6–7 ml. required). Finally angiotensin was eluted with 4.0 ml. of 0.1 N NaOH. The alkaline (pH 12.0) eluate was neutralized with 2 N HCl. This solution was clear, faintly yellowish and ready for assay.

When higher purification was desired, the eluate was acidified to pH 1.0, saturated with sodium chloride, extracted twice with 4 ml. of *n*-butanol, and the extract (8 ml.) was mixed with 4 ml. of petroleum ether, re-extracted once with 1 ml. of distilled water. The final aqueous extract was also ready for bio-assay after neutralization.

Added angiotensin (0.05–0.2 μ g) in urine (25–100 ml.) was quantitatively recovered with this procedure. Adrenaline or noradrenaline was scarcely recovered (1–2 per cent), indicating no contamination with catecholamines. Vasoactive principles of peptide nature, such as vasopressin, bradykinin, etc., were not investigated, but most urine extracts had no vasoactivity, suggesting the absence of these substances in concentrations to interfere with the assay.

Bioassay

Albino rats from Wister strain, both sexes, weighing 130–200 g., were used as test animals. They were anesthetized with intraperitoneal injection of pentobarbital (4 mg./100 g. of body weight), spontaneous undulation of their blood pressure was minimized by subcutaneous injection of 10 mg. of hexamethonium. Right carotid artery was canulated, the canula was connected to a mercury manometer, the movement of which was recorded on a smoked drum. Injections were given through another canulation into right femoral vein in doses ranging 0.05–0.5 ml. using tuberculin syringe. The rat preparation responded well to 0.003–0.005 μ g. of angiotensin, with 15–20 mm Hg elevation of blood pressure, so that it permitted the accurate estimation of angiotensin in low concentrations down to 0.03–0.05 μ g./4 ml. of eluate.

Infusion of Angiotensin

An ampule of "Hypertensin" was quantitatively dissolved in 500 ml. of 5 per cent glucose, giving a concentration of 1 μ g./ml. The solution was infused into an antecubital vein of test subjects in a dose sufficient to cause marked elevation of blood pressure (0.03–0.1 μ g./Kg./min.). 60–120 μ g. of total angiotensin was given in 30–40 minutes. Immediately before and after the infusion, the subjects were asked to empty their bladder, the post-infusion urine was acidified and extracted as described above. No accident was encountered during or after the infusion.

RESULTS

Stability of Angiotensin in Urine

To test the stability of angiotensin in urine, synthetic angiotensin was added

to human urine to make a concentration of $0.05 \mu\text{g./ml.}$, and the urine was divided into several parts which were stored separately at different pHs and temperatures for various time intervals. Then they were directly assayed on the rat preparation after pH adjustment to neutral.

Table I shows the results. In acidified urine (pH 1-2), angiotensin was quite stable at any temperatures for at least 2 days. When the acidification was omitted (pH 5.8-7.0), added angiotensin lost its activity by 20-30 per cent in 30 minutes, 30-50 per cent in 60 min., and 70-90 per cent in 90-120 min., at 37°C ; the stability increased with the decrease of temperature.

TABLE I. Stability of Angiotensin in Urine
Figures Are Expressed in Per Cent Decrease of Initial Activity

pH 1 — 2						
Days	1	2	10			
Room temperature	0	0	—			
4°C	0	0	0			

pH 5.8 — 7.0						
Minutes	15	30	60	90	120	24hr
37°C	0	20	20	50	70	—
	0	20	30	50	70	
	0	30	50	70	90	
Room temperature					100	100
		0	0		30	
	—	0	20	—	50	
4°C		20	20			0
			0		0	
	—	—		—	10	
						50
						100

Estimation of Angiotensin in Human Urine

Six healthy persons, two patients with moderate essential hypertension and two with renal hypertension were studied. Freshly voided one or two hour urine was extracted and assayed. But none of these extracts showed any distinct vasoactivity, pressor or depressor.

One of the renal patients, aged 13, female, was suffering from severe hypertension of 230/130 mmHg, and was proved to have left renal arterial stenosis. Her ischemic kidney was later removed surgically, remarkable drop of her blood pressure to 130/80 mmHg resulted from the operation. Thus her hypertension was undoubtedly of renal origin, but no angiotensin was detectable in her urine in spite of repeated examinations.

Excretion of Angiotensin during Its Infusion

Six persons were infused with angiotensin (total doses, 60–120 $\mu\text{g.}$) in 30–40 minutes as described above. The urines collected immediately after infusion were extracted and assayed. All of six extracts gave either no response or equivocal pressor effects corresponding to 0.001–0.002 $\mu\text{g.}$ of angiotensin even with the maximum doses of 0.5 ml. Assuming these minimum responses as caused by angiotensin, the total amounts calculated in the extracts never attained to 0.04 per cent of the infused doses.

It was concluded that the infused angiotensin is not excreted in urine in measurable amounts.

DISCUSSION

Why circulating angiotensin is not excreted in urine? It is not due to destruction of angiotensin filtered through glomerulus in the urinary tract or bladder, because the substance is not inactivated so rapidly in 30–40 minutes, the time interval allowed to elapse in the present study for urine collection after the initiation of the infusion. Is circulating angiotensin in a protein-bound form, and not filtered through glomerulus? Are there any other mechanisms responsible for the absence of it in urine? These are not answered by the present study.

Recently, Helmer wrote to us in personal communication, "Page told me that he tried to find angiotensin in urine but he could not find it."

Thus the possibility to study urinary angiotensin hoped in the beginning of this investigation was proved unrealizable.

SUMMARY

The problem whether circulating angiotensin is, as catecholamines, excreted in urine was investigated in the hope that the study of renin-angiotensin system would be greatly facilitated, if it is the case in human or in experimental animals.

Though the assay method used here could accurately estimate angiotensin in urine in low concentrations down to 0.05 $\mu\text{g./100 ml.}$, no angiotensin was found in the urine of healthy or hypertensive subjects including a patient with unilateral renal arterial stenosis with severe hypertension.

Infusion of angiotensin in doses enough to cause violent elevation of blood pressure resulted in no definite excretion of it in urine.

It is concluded that the circulating angiotensin is not excreted in urine in any significant amount.

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