Angiotensins Lung Converting Enzyme[1] (37257)

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In 1956 Skeggs (1) showed that the conversion of vasoinactive angiotensin I (AT I) to the vasoactive angiotensin II (AT II) took place through the action of converting enzyme which cleaves the decapeptide AT I to form the octapeptide AT II. The importance of serum converting enzyme was suggested in 1964 (2) when following multiple subcutaneous injections of CCl_4 chronic renal hypertensive rats' systolic blood pressures were reduced to normotensive levels and only vasoinactive AT I was found in their serum indicating a failure of converting enzyme. When a mixture of their serum, containing only AT I was incubated with normal serum (normal converting enzyme), this mixture yielded both AT I and vasoactive AT II (3). Confirmatory *in vitro* experiments have shown that the addition of CCl_4 to AT I and normal serum, containing converting enzyme, blocked the conversion to AT II (4).

Ng and and Vane (5) in 1967 reported that considerable converting enzyme activity occurred in the lung. The reaction took place in a matter of seconds whereas conversion by plasma (6) or serum (7) converting enzyme requires more than an hour. A question arises, namely if lung-converting enzyme activity is accomplished in seconds, how can serum from the CCl_4-treated renal hypertensive animals contain vasoinactive AT I? To answer this question AT I was injected into renal CCl_4-treated rats.

Methods. Nineteen Sprague–Dawley rats initially weighing 135 g were divided into 2 groups. The first group of 9 animals was made hypertensive by unilateral nephrectomy followed in 1 week by figure-of-eight ligature to the remaining kidney, A second group of 10 animals served as normotensive controls. All animals were pair fed on Purina chows, and given tap water to drink *ad libitum*. Animals were weighed and blood pressures were recorded biweekly in the unanesthetized rat by the tail microphone method (8). After the renal animals reached hypertensive systolic pressures (greater than 175 mm Hg) for 3 months they were given biweekly subcutaneous injections of CCl_4 (0.15 ml/100 g). Fifteen injections of CCl_4 were given to the hypertensive group. The normotensive control animals were untreated. The renal CCl_4-treated and the untreated controls were anesthetized with amobarbital sodium (10 mg per 100 g intraperitoneally). A tracheal cannula was inserted, vagotomy performed and heparin (500 units intravenously) and pentolinium chloride (3 mg per kg intraperitoneally) were injected. Intraarterial pressures were recorded with a mercury manometer following the injections of 0.007, 0.012, and 0.025 μg of angiotensin I, Val 5 angiotensin II 0.005, 0.010, and 0.015 mch (hypertensin Ciba), norepinephrine 0.3 ml, and 0.3 GU (Goldblatt units) of renin. The animals were then sacrificed and the liver, kidney and adrenals were removed and prepared for histological examination (H & E Stain) with the light microscope.

Results. Blood pressure. Systolic blood pressure in the renal hypertensive group rose from the initial 140 mm Hg to 184 mm Hg, S.E. $\pm$ 1.4, at the 6th month period. After treatment with CCl_4 the mean pressure decreased to 145 mm Hg, S.E. $\pm$ 2.6, which was significantly less than the values in the hypertensive phase ($p < 0.001$). The pressure remained stable in the untreated normotensive controls (137 mm Hg).

Pressor responses. The pressor responses

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TABLE I. Average Systolic Blood Pressures in Renal CCl_4-Treated and Untreated Control Rats. Pressor Rise Following Injections of Angiotensin I and II, Norepinephrine, and Renin.

	Systolic blood pressure, mm Hg			Δ Pressor rise, mm Hg							
				Angiotensin I (μg)			Angiotensin II (μg)			Norepinephrine (ml)	Renin (GU)
	6 mo.	9 mo.	Anesthetic	0.007	0.012	0.025	0.005	0.010	0.015	0.3	0.3
Renal CCl_4	184	145	96	20	28	40	18	30	33	27	56
Normotensive untreated	148	137	67	16	23	31	11	22	31	36	54

to AT I and II were not significantly different from each other. Table I. There was a similar response to injections of norepinephrine and renin in both groups.

Histology. Microscopic examination of the livers showed the changes characteristic of cirrhosis in 4 and only a moderate degree of fatty metamorphosis in the others given CCl_4. The remaining kidney was hypertrophied in the CCl_4-treated group and showed the changes seen in chronic pyelonephritis. Adrenal glands were reported as normal in all animals.

Discussion. The antihypertensive effect of CCl_4 was again (3–4) demonstrated in renal hypertension. As previously reported (3), small subcutaneous injections of CCl_4 over a 3-month period produced cirrhotic changes. In this experiment, 4 of the 9 CCl_4-treated animals showed only a moderate degree of fatty metamorphosis while the others had frank cirrhotic changes. The finding of pyelonephritis in the renal group was secondary to ligature of the kidney. The site of action of lung's converting enzyme has been reported to be on or in the membrane of the capillary cell (9). Previously we reported (7) lung tissues to be normal by light microscopy, following CCl_4 treatment.

By injecting AT I into the renal CCI_4 animals we have studied for the first time the effect of lung-converting enzyme in a pathological state. We observed the rapid conversion of the vasoinactive AT I to the vasopressor AT II immediately after intravenous injection. This means that there was no interference by CCl_4 treatment on lung converting enzyme. Overall, the responses (especially to injected norepinephrine) also indicate that vascular reactivity is normal in CCl_4-treated animals in this experiment which confirms our previous observations (10).

We have shown that serum from hypertensive rats treated with CCl_4, when incubated with renin, yields slightly more angiotensin-like activity (10). Renin substrate concentrations were not decreased (10) and added substrate produced the same pressor response as it did in serum from control animals. The possibility that the serum of the CCl_4-treated animals might bind renin and make it incapable of acting on renin substrate to produce AT I was discarded because AT I was found in this serum (Fig. 1 and Ref. 2, 3, 7).

It was tentatively concluded from these earlier studies that the remission of the experimental hypertension by CCl_4 treatment was due to a failure of the serum converting enzyme to produce AT II from the AT I present. The most likely explanation of the present results, it seems to me, is that *in vivo* prolonged exposure to low concentrations of CCl_4 renders the endogenous AT I incapable of being converted to AT II. Since some time is required (and/or higher concentrations of CCl_4 as in the *in vitro* experiments) injected AT I and II exert their usual effect in the CCl_4-treated animals. Although these postulates are consistent with both the *in vivo* and *in vitro* results there is no proof that the renin–angiotensin system is solely involved in the reduction of the arterial pressure by CCl_4 treatment.

Summary. Elevated systolic blood pressure was reduced to normal after treatment with CCl_4. Histological study of liver tissue primarily showed fatty metamorphosis in all the

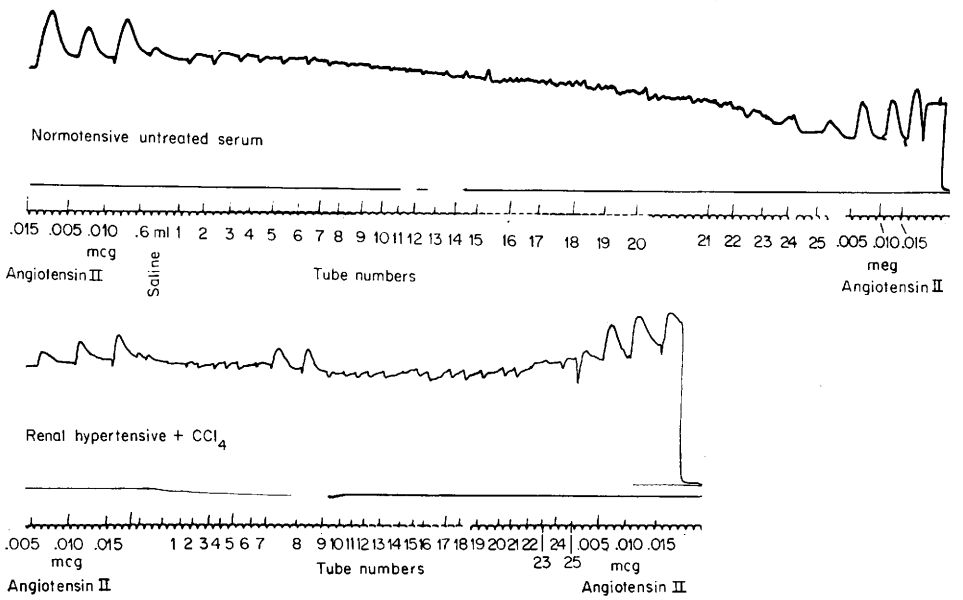

FIG. 1. Separation of angiotensin I from II by paper chromatography (2). Tubes 6–10 identify angiotensin I and angiotensin II in tubes 13–20. No angiotensin I is present in normotensive untreated serum and only angiotensin I is present in the renal hypertensive CCl_4-treated rats serum.

treated animals but cirrhotic changes in only 5 animals. Injection of angiotensin I produced an immediate pressor rise in renal CCl_4 treated rats showing no inhibition of lung converting enzyme. These findings indicate that lung-converting enzyme is probably not involved in the blood pressure reduction in this pathological condition.

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