Converting Enzyme in Rat Serum[1] (34769)

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Experimental renal hypertension has been lowered to normotensive pressure levels after CCl_4 treatment (1, 2). Considered in the framework of the renin–angiotensin system, our work suggested that this reversal of hypertension is due to an abnormality of angiotensin's coverting enzyme (3). Bumpus *et al.* (4) have shown that plasma converting enzyme is in the liver and presumably the liver synthesizes this enzyme. Plasma converting enzyme has been found to convert the vasoinactive decapeptid to the vasoactive octapeptid antiotensin II within minutes by Carline (5); although Skeggs *et al.* (6) found only 50% conversion in plasma in 1½ hr. Ng and Vane (11) have shown the conversion of angiotensin I to II to take place within 4 to 8 sec in the lung. Since the time required for conversion of angiotensin I to II in serum has not been determined, nor has it been studied in a disease state, this paper attempts (i) to determine the speed of conversion of angiotensin I to II in the serum of normal and liver damaged CCl_4-treated rats, and (ii) to examine the lung tissue histologically.

Methods. Renal hypertension was produced in 13 Sprague-Dawley rats by bipolar ligation of one kidney, followed in 1 week by contralateral nephrectomy. Thirty other rats served as normotensive controls without surgical intervention. Both groups were fed Purina chows, and given tap water *ad libitum*. Arterial pressure was recorded twice weekly by the tail microphonic method (7) in the preheated, unanesthetized animal. After systolic pressure in the renal group had remained at hypertensive levels (+175 mm Hg) for 2 months, 10 hypertensive and 5 normotensive control animals were begun on biweekly subcutaneous injections of CCl_4 (0.15 ml/100 g). Three hypertensive and 25 normotensive rats served as untreated controls. All animals were bled after the 20th injection of CCl_4 during the 28th week of the experiment. Blood samples were centrifuged and the serum frozen. The animals were sacrificed; and kidneys, adrenals, lung, and liver were removed for histological study.

In group A, 2-ml samples of serum were taken from each rat in the untreated controls and pooled. The same was done for the treated and untreated hypertensive groups. Each of the 3 groups was then incubated with 0.25 μg of angiotensin I at 37°. The reaction was stopped at 1-, 10-, 20-, 30-, 60-, and 120-min intervals by lowering the pH to 5.5 and boiling. All mixtures were assayed for pressor responses in the vagotomized, pentolinium-blocked rat using 0.5-ml volumes except for the 1- and 2-hr samples, where 0.1-ml injections were used. Selected serum samples from each incubation interval were chromatographed for separation of angiotensin I from angiotensin II using the qualitative method of Wisenbaugh *et al.* (9).

In group B, 2 ml of pooled serum from the same animals was used but angiotensinases were altered in the serum by lowering the pH to 4 for 30 min at 25°, then returned to pH 7.0. Sera from each of the 3 groups were incubated with angiotensin I for varying time intervals. The mixtures were injected as above to record pressor responses in the assay rat.

Results. Systolic blood pressure of more than 175 mm Hg (mean 184) was found in the hypertensive animals for at least 2 months. The mean blood pressure of the control group was 155 mm Hg. After 20 CCl_4

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injections during the 28th week, the pressure of the hypertensive treated group was reduced to a mean of 145 mm Hg which was significantly less than the value in the hypertensive phase ($p < .01$). The untreated hypertensive group pressures during the 28th week remained elevated and averaged 187 mm Hg.

Histologically, the kidney, liver, and adrenals of the untreated control animals were normal. The lung tissue showed slight focal atelectasis and/or slight pulmonary edema in most animals. Most of the animals treated with CCl_4 showed a moderate degree of fatty metamorphosis in the liver and some had frank cirrhotic changes. The kidneys showed chronic pyelonephritic changes in both renal treated and untreated groups whereas adrenals were normal in all of the rats. The lung tissue resembled the control.

Pressor responses were similar for the hypertensive treated group and untreated control group (Fig. 1). The hypertensive sera was not tested for pressor responses. The extended incubation times of 10, 20, and 30 min of angiotensin I with norml serum and renal CCl_4 serum showed decreased pressor responses upon lengthening the incubation time. After 1 hr of incubation, both the untreated control and hypertensive treated sera produced increasing pressor responses.

Serum from the hypertensive group showed only angiotensin II activity by chromatographic separation of angiotensin. Neither angiotensin I nor angiotensin II activity could be demonstrated in the untreated controls or in the hypertensive treated animals.

The addition of angiotensin I to group A

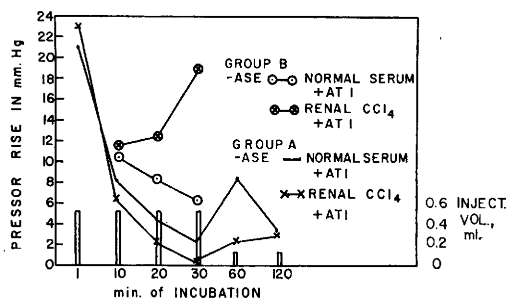

FIG. 1. Pressor responses following the incubation of angiotensin I with 2 types of sera. Vertical bars refer to the volume injected (ordinate at right).

TABLE I. Group A Chromatographic Separation of Angiotensin I and II after Variable Time Incubations of 3 Types of Sera with a Constant Amount of Angiotensin I.

Symbols 0, + = presence or absence of angiotensin I and II.

Serum + angiotensin I	Incubation time (min)	Angiotensin type	
		I	II
Normal control		+	0
Renal hypertensive	1	+	0
Renal CCl_4		+	0
Normal control		0	+
Renal hypertensive	60	+	+
Renal CCl_4		+	0
Normal control		0	+
Renal hypertensive	120	0	0
Renal CCl_4		+	+

(unaltered serum samples) for 1-min incubation with sera from normal, treated, and untreated renal animals showed by chromatography only angiotensin I activity (Table I). Likewise chromatographic separation of angiotensin after 1 hr of incubation showed angiotensin II to be present in the untreated and hypertensive groups; however, angiotensin I remained in the renal CCl_4-treated sera. After 2 hr of incubation, angiotensin I and II were found in the serum of the renal treated group.

In group B samples, those sera which had angiotensinase destroyed, then incubated with angiotensin I, generally produced pressor rises which were higher than that produced from sera in which angiotensinases were not altered. The untreated controls pressor responses were decreased with the lengthened incubation time however; and the renal CCl_4-treated sera had an increased pressor responsiveness with the longer incubation time (Fig. 1) which may be due to the availability of converting enzyme and renin substrate in the serum from the CCl_4-treated animals.

Discussion. The return of normotension was again demonstrated in the renal hypertensive rat under chronic subcutaneous treatment with CCl_4. Only the serum from the

renal hypertensive group showed the presence of angiotensin and that was all angiotensin II, as has been found in man (8) and dogs (9, 10). Incubation of serum from the renal CCl_4-treat group with angiotensin I added showed only angiotensin I activity at 60 min, showing no converting enzyme activity; however, after 120 min, angiotensin I and II was found. The hypertensive treated and the untreated control sera likewise showed conversion of angiotensin I to II with increasing the time to the 1- and 2-hr periods. These data present a slower rate of conversion of angiotensin I to II in serum than that which occurs in the lung (11) which was found to take place in one circulation time of 3 sec. This slower conversion in the serum may be related to the presence of angiotensinase in the serum which destroys angiotensin I (12). After 30 to 60 min of incubation, converting enzyme activity seems to be able to overcome the angiotensinase effect or the angiotensinases are decreased to that degree which enables converting enzyme to act. Bumpus *et al.* (4) found that prolonged incubation of liver homogenates with angiotensin increased angiotensinase activity. Although angiotensinase was altered by pH changes in group B, this method does not destroy all angiotensinases present (13).

A possible explanation for the rapid conversion of angiotensin in the lung may be that it does not have the opportunity to react with serum angiotensinases. This position is negated by Biron *et al.* (14) who found that a very short *in vivo* half-life of angiotensinase which suggests a comparatively rapid generation rate by the enzymatic system acting upon the angiotensin II precursors. Another possible explanation for the rapid conversion of angiotensin in the lung may be its sensitivity to oxygen and carbon dioxide tensions; however, Ng and Vane (11) found that after various incubation times concentrations of the enzyme in venous blood were within the same range as those for arterial blood. Carretero *et al.* (15) have studied converting enzyme by the acute injection of angiotensin I and found no difference between a CCl_4-treated normotensive and an untreated control group of rats. An important difference in studying converting enzyme must be noted since Ng and Vane studies (11) and those of Carretero *et al.* (15), reflect the immediate effects after infusions, while this experiment reflects the constant maintained serum levels of angiotensin in the animal. Biron *et al.* (14) have found that CCl_4 administration can reduce the clearance of angiotensin by the rat liver.

This experiment does not show any significant histological change in lung tissue after CCl_4 treatment. CCl_4 liver damage, produced by subcutaneous injections in the rat, was accompanied by an altered converting enzyme activity accompanied with a fall in blood pressure from hypertensive levels. Both liver and lung seem to be an important site for converting enzyme activity; however, other organs as the kidneys and other systemic tissues have been found by Biron (16) to account for about one third of the overall activity. The areas of converting enzyme activity being varied, it must be determined which site is paramount; or, if interrelated, which come into action in a normal state, in chronic pathological conditions and in changing physiological states.

Summary. It seems therefore, that the liver plays an important role in the conversion of angiotensin I to II. The speed of this reaction is much slower than that which occurs in the lung. The slower conversion that occurs in the serum may be due to the interplay of liver converting enzyme with angiotensinases. The angiotensinase effect is overcome with the passage of time enabling converting enzyme to reduce the vasoinactive decapeptid to the vasoactive octapeptid angiotensin II.

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