Plasma Renin Activity and Renin-Substrate Concentration in Patients with Liver Disease

By Carlos R. Ayers, M.D.

ABSTRACT

Peripheral venous renin activity was determined by the method of Boucher in 15 patients with cirrhosis of the liver and ascites who were on an unrestricted sodium intake. Positive sodium balance was present in all subjects. Marked elevation of mean plasma renin activity (43 ng angiotensin II/min per liter, SD ± 38) was present. The mean plasma renin activity in 16 normal ambulatory subjects on an unrestricted sodium intake was 10.7 ng (SD ± 4.9) of angiotensin II/min per liter. The difference was highly significant, P < 0.01.

A zero-order enzyme reaction occurred in all but 1 patient; the first-order enzyme reaction in this 1 patient suggests a low renin-substrate concentration in liver disease. The renin-substrate concentration was determined in 18 patients with liver disease (13 with cirrhosis and 5 with hepatitis) and in 10 normal subjects. The renin substrate was expressed in equivalents of angiotensin II formed/100 ml plasma. The mean renin substrate in 13 patients with cirrhosis and ascites was 17,193 ng (SD ± 6,556); the normal mean renin substrate was 34,385 ng (SD ± 5,679), P < 0.01. The lowest renin substrate concentration was seen in 2 patients with severe hepatitis.

ADDITIONAL KEY WORDS hepatitis ascites angiotensinogen angiotensin II cirrhosis of the liver

Many factors have been shown to be important in the pathogenesis of ascites. In cirrhosis of the liver, the scarring process leads to an intrahepatic and portal venous hydrostatic pressure resulting in transudation of fluid into the peritoneal cavity (1). This removal of fluid from the intravascular volume results in stimulation of the juxtaglomerular apparatus of the kidney which increases its secretion of renin. This enzyme interacts with angiotensinogen to form angiotensin I, which in turn is acted upon by a converting enzyme to produce angiotensin II. Angiotensin II has been shown to be the primary controller of the rate of aldosterone secretion (2-5).

Aldosterone secretion rate and excretion are increased in patients with cirrhosis of the liver with sodium retention and ascites formation (6, 7). Renin activity was elevated in a few patients studied (8). The present study was undertaken to evaluate more fully the renin-angiotensin system in patients retaining sodium due to cirrhosis of the liver and ascites formation. The renin activity and angiotensinogen concentration were studied in a group of untreated patients with sodium retention due to cirrhosis of the liver and ascites formation. Angiotensinogen concentration was also studied in patients with hepatitis and without ascites formation.

Methods

Of the 20 patients studied, 15 had cirrhosis of the liver and ascites formation and 5 had hepatitis. Sodium excretion was less than 10 mEq/24 hr in the group with cirrhosis and ascites. The studies were performed on the first hospital day.
before the use of diuretics and low sodium diet. Peripheral venous blood samples were collected with the patient supine or sitting at bedside. All samples were obtained at noon. The normal subjects were ambulatory laboratory personnel and medical students with no salt restriction.

The plasma renin activity was determined using a modification of the Boucher method (9). Aliquots of plasma were incubated either 1, 2, or 3 hr. A stream of nitrogen at 80°C was used for evaporation instead of flash evaporation. Saline was used as the final vehicle instead of 20% ethanol because of the vasopressor effect of ethanol in the rat (10, 11). The sample was assayed in a 115- to 125-g rat given pentobarbital anesthesia, then vagotomized, and given the ganglionic blocking agent, pentolinium. A four point assay method was used when the quantity of the sample permitted. An enzyme activity curve was plotted from the results. The rate of angiotensin II formation was determined from the enzyme activity curve.

Angiotensinogen concentration was determined in a group of patients with cirrhosis of the liver and ascites and in a separate group with hepatitis. Human renin extract prepared from post-mortem kidneys by the method of Haas and Goldblatt (12) was added to peripheral venous plasma. An enzyme activity curve was performed on each patient. Complete utilization of angiotensinogen was considered to be present when the enzyme activity curve became flat, and no further angiotensin II was formed. Angiotensinogen concentration is reported in terms of the maximum amount of angiotensin II that could be formed with prolonged incubation.

**Results**

The amount of angiotensin II formed after 1, 2, and 3 hr of incubation of plasma was plotted on linear graph paper. A zero-order enzyme reaction occurred in all the normal
subjects and in all but 1 patient with cirrhosis and ascites. The enzyme activity curve for the group of 16 normal subjects is shown in Figure 1 with a representative enzyme activity curve for a patient with cirrhosis and ascites. The enzyme activity curve in this patient was markedly elevated and the enzyme reaction was also zero-order.

The total amount of angiotensin II formed after 3 hr of incubation, and the rate of formation of angiotensin II in the normal group and in the patients with cirrhosis and ascites are illustrated in Figure 2. The normal subjects formed 219 ng (SD ± 91) of angiotensin II/100 ml of plasma after 3 hr of incubation at a rate of 10.7 ng (SD ± 4.9)/liter per min. The patients with cirrhosis and ascites formed a mean of 939 ng (SD ± 526) of angiotensin II/100 ml of plasma after 3 hr of incubation at a rate of 43 ng (SD ± 38) of angiotensin II/liter per min. The difference between the two groups in the total amount of angiotensin II formed and the rate of formation of angiotensin II was highly significant (P < 0.01).

The enzyme activity curve of the 1 patient with cirrhosis and ascites who did not have a zero-order enzyme reaction is demonstrated in Figure 3. In this patient with "hepatorenal syndrome," the initial reaction was very rapid.

A renin enzyme activity curve demonstrating depletion of angiotensinogen in a patient with cirrhosis and ascites with the hepatorenal syndrome.

FIGURE 3

FIGURE 4

Renin enzyme activity curves in a patient with cirrhosis and ascites before and after renin was added to the plasma. The flattening of the curve after the addition of renin suggests complete utilization of the angiotensinogen.

The curve then became flat suggesting angiotensinogen deficiency.

Angiotensinogen concentration was further determined in patients with liver disease by

FIGURE 5

The maximum amount of angiotensin II formed after the addition of excess renin in normal subjects, patients with cirrhosis and ascites and patients with hepatitis.

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measuring the maximum amount of angiotensin II that could be formed in plasma after the addition of excess renin; a typical enzyme activity curve is depicted in Figure 4. It was zero-order before the addition of renin but after the addition of renin to an aliquot of the same plasma, it became flat indicating complete utilization of angiotensinogen. The angiotensinogen concentration in Figure 5 is expressed as the maximum amount of angiotensin II that can be formed after prolonged incubation. The mean value for the group of 10 normals was $34,385 \text{ ng (SD± 5,679)}$ of angiotensin II/100 ml of plasma, and that of the 13 patients with cirrhosis and ascites was $17,193 \text{ ng (SD± 6,556)}$. The difference was significant ($P<0.01$). The angiotensinogen concentration in 5 patients with hepatitis is also shown in Figure 5. The amount of angiotensinogen present was markedly reduced in 2, slightly reduced in 1, and normal in 1.

**Discussion**

Transudation of fluid from the intravascular space into the peritoneal cavity in some way stimulates the juxtaglomerular apparatus to secrete renin at an increased rate. Whether the stimulus for increased renin secretion is mediated through a change in mean arterial pressure as proposed by Tobian et al. and Skinner et al. (13, 14), or by decreasing the sodium load to the macula densa area of the convoluted tubule (15), is still being debated. In any event these data show clearly that patients with cirrhosis and ascites have hyper-reninemia. These data also support those obtained by Genest and associates in three similar patients (8). Angiotensinogen concentration was reduced in the present study. Thus, the addition of a gross excess of renin to plasma samples from these patients resulted in the production of less angiotensin than plasma similarly treated from normal individuals. The question arises as to potential practical significance of this finding.

It has been suggested (16) that the rate of angiotensin II formation, and thus aldosterone secretion, is dependent upon angiotensinogen concentration. Angiotensinogen is an $\alpha 2$-globulin produced by the liver. If this globulin is ever rate-limiting in the renin enzyme reaction, it should be so in cirrhosis of the liver with ascites formation. In this situation there is probably a decreased production rate of angiotensinogen, as well as an increased rate of its utilization due to the excess renin. In the present study, the enzyme activity curves were all zero-order in normals and in the patients with cirrhosis and ascites with one exception. Thus, in nearly all cases substrate was sufficiently plentiful to allow sustained formation of angiotensin II for 3 hr or more. The patient who was an exception had the "hepatorenal syndrome" with a markedly reduced arterial blood pressure and extremely high renin activity. His initial enzyme reaction rate (Fig. 3) was very rapid, so after approximately 60 min, angiotensinogen was apparently exhausted and no more angiotensin was formed. These observations, and the previously observed high rates of secretion of aldosterone in patients with cirrhosis and ascites, indicate that angiotensinogen is probably never rate-limiting for the renin enzyme reaction in the circulation and seldom in the laboratory.

Other factors participating in the renin reaction could influence the rate of the reaction in patients with liver disease. It has been shown that carbon tetrachloride-treated rats (17) have a deficiency of converting enzyme; this enzyme could be decreased in liver disease. The methods used here do not assess the concentration of converting enzyme directly. It would seem reasonable to expect that the initial rate of formation of angiotensin II would be influenced by converting enzyme concentration; however, converting enzyme, which presumably is not consumed in the reaction, would not be expected to influence the total amount of angiotensin II formed after prolonged incubation.

The low angiotensinogen concentration in the patients with hepatitis and without ascites demonstrates that this globulin can be decreased due to decreased production. However, the increased utilization of substrate by
the excess renin is also undoubtedly an important determinant of the ultimate plasma concentration of angiotensinogen.

It is unlikely that the decreased peripheral resistance (18) and low arterial blood pressure seen in patients with liver disease are due to a deficiency of hepatic factors participating in the renin enzyme reaction. Finding high renin concentration and a quantity of angiotensinogen large enough to support a zero-order reaction in patients with cirrhosis and ascites supports the concept that the primary difficulty is related to the resistance to the vasoactive effect of angiotensin II (19) that these patients show.

References
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CARLOS R. AYERS

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