Stimulation of Angiotensinogen Formation by Renin and Angiotensin[1] (37011)

ALBERTO NASJLETTI AND GEORGES M. C. MASSON

Research Division, Cleveland Clinic Foundation, and Cleveland Clinic Educational Foundation, Cleveland, Ohio 44106

It has recently been shown that angiotensinogen, the substrate of renin, is synthesized in the liver and that angiotensinogen levels in plasma are dependent on rates of production as well as on rates of destruction (1–3). While destruction of the substrate is accomplished mostly by the enzyme renin, little is known about the factors regulating angiotensinogen formation. In the present paper we have investigated the possibility that angiotensin II, the effector of the renin-angiotensin system might alter angiotensinogen formation through a feed-back mechanism similar to that existing for renin secretion (4).

Methods. Female Sprague–Dawley rats (weighing 200–250 g) were used. They were fed a commercial chow and given tap water to drink.

First series. Experimental animals received one single sc injection of 40 Goldblatt units of rat renin in 0.4 ml of saline and sacrificed 2, 4 and 8 hr later after anesthesia with ether. Blood (0.8 ml) was withdrawn from the aorta for determination of renin and angiotensinogen concentration. The liver was cannulated and perfused for determination of rates of angiotensinogen formation. Control animals subjected to the same procedure received injections of saline.

Second series. Angiotensin II (Hypertensin, Ciba) was administered by intravenous infusion at rates of 16 to 100 ng/min during periods of 3 or 8 hr into conscious rats. Animals infused during 3 hr were used for liver perfusion. Since this time interval would likely be too short for significant changes in plasma levels of renin and angiotensinogen to occur, the latter determinations were carried out in animals infused during 8 hr. Normal rats infused with saline were used as controls. An indwelling catheter was inserted into the jugular vein the day prior to the infusions. The exposed end of the catheter was carried under the skin to emerge between the ears and was protected by a saddle. At the time of infusion the saddle was connected to a steel coil attached to a swivel thus permitting free but restrained movement within the cage. The volume of fluid infused amounted to 0.8 ml/hr. Blood samples were taken from the jugular vein through the inserted catheter.

Third series. Angiotensin I and II were infused into the perfusion system during the first hour of perfusion of livers from normal untreated rats. Rates of infusion varied between 16 and 80 ng/min. The volume of fluid infused was kept constant and amounted to 0.8 ml/hr.

The liver perfusion was performed *in situ* according to the procedure of Mortimore *et al.* with some modifications (2). In short, after etherization and laparotomy, the inferior vena cava and the hepatic artery were ligated, and both portal vein and supradiaphragmatic inferior vena cava were cannulated. After flushing, the liver was inserted into a closed system consisting of a membrane oxygenator and a pump. The perfusing fluid amounting to 50 ml consisted of a tissue culture medium (Medium 199, Grand Island Biological Co.) containing 4% of bovine albumin and 20% of washed erythrocytes from normal rats. Samples of 5 ml were removed from the system at times 0, 2, 4 and 6 hr and centrifuged, and the "plasma" was stored to be used for determination of angiotensinogen by an exhaustion technique (2). Results are expressed as

[1] This work was supported in part by Grants HL 6835 and 5126 from the National Heart and Lung Institute.

TABLE I. Effect of Renin and Angiotensin II on Angiotensinogen and Renin Concentration in Plasma.

Groups	No. of animals	Angiotensinogen (ng angiotensin/ml)	*p* value	Renin (ng angiotensin/ml/hr)	*p* value
Control	6	355 ± 24		52.7 ± 23.7	
Renin[a] 2 hr	5	106 ± 25	0.001	740 ± 318	0.001
Renin[a] 4 hr	8	203 ± 65	0.001	421 ± 98	0.001
Renin[a] 8 hr	8	539 ± 124	0.005	115 ± 65	0.05
Saline infusion during 8 hr	6	354 ± 23		44 ± 8.5	
Angiotensin, 100 ng/min during 8 hr	6	965 ± 170	0.001	15.8 ± 3.3	0.01

[a] Renin was injected sc as a single dose of 40 Goldblatt units. Determinations in plasma were carried out 2 hr (Group 2), 4 hr (Group 3) and 8 hr later (Group 4).

nanograms of angiotensin II released per gram of liver or as nanograms of angiotensin II released per gram of liver per hour of perfusion.

In some experiments, blood was withdrawn from the animals in the presence of EDTA, centrifuged and used for determination of angiotensinogen (5) and renin concentration (6). Results are expressed as nanograms of angiotensin II per milliliter of plasma and as nanograms of angiotensin II per milliliter of plasma per hour of incubation, respectively. Values presented are means ± SD.

Results. Effect of renin treatment. The effects of renin varied according to the time of blood sampling. At first there was a sharp decrease in plasma angiotensinogen, followed by a rebound with values significantly above normal after 8 hours (Table I). On the other hand, plasma renin concentration increased to a peak about 10 times above normal on the second hour, then slowly declined with values still significantly elevated at the end of the observation.

Determination of angiotensinogen formation by the liver of the renin-treated rats showed a significant stimulation 2 hr after the renin injection (Fig. 1). Calculated rates of angiotensinogen formation averaged 50 ± 6.07 ng/g of tissue/hr of perfusion compared with 18.9 ± 4.5 ng/g of tissue/hr in control animals. Rates of formation were further increased 4 and 8 hr after the renin injection, to reach, respectively, the values of 68 ± 12.4 and 83 ± 24.7 ng/g of tissue/hr of perfusion. All differences between experimental and control values are highly significant, $p < .001$.

Effects of angiotensin treatment. Intravenous infusion of angiotensin II at the rate of 100 ng/min into conscious animals during a period of 8 hr more than doubled plasma angiotensinogen concentration and significantly decreased plasma renin concentration

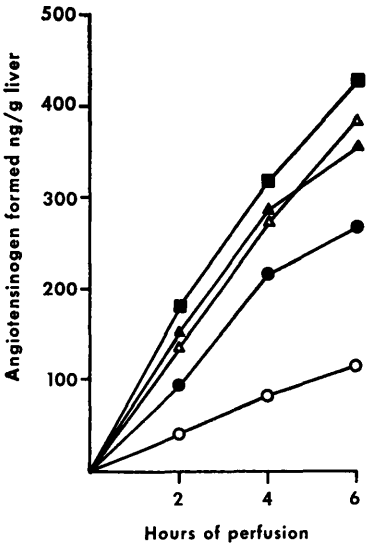

FIG. 1. Amounts of angiotensin formed during perfusion of livers of rats 2 hr (●), 4 hr (△) and 8 hr (■) after injection of rat renin and of rats which had been infused with 100 ng/min of angiotensin II during 3 hr (▲); (○) control values.

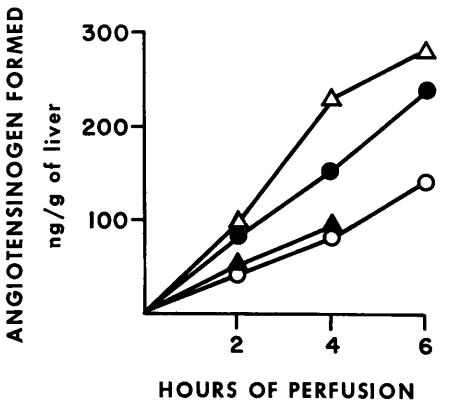

FIG. 2. Amounts of angiotensin formed by the liver of normal rats following infusion of angiotensin into the perfusing system during the first hour of perfusion. Rates of infusion were: angiotensin I, 16 ng/min (▲) and 80 ng/min (△); angiotensin II, 16 ng/min (●) and saline (○).

(Table I). Infusion of saline in control animals had no significant effect on either parameter.

Intravenous infusion of angiotensin II at the same rate of 100 ng/min during 3 hr caused a marked stimulation of substrate formation by the perfused liver (Fig. 1) with rates of formation averaging 71 ± 27 ng/g of tissue/hr of perfusion compared with the control value of 18.9 ± 4.5 ng/g of tissue/hr. Lowering the infusion rate to 16 ng/min still stimulated formation with rates averaging 68 ± 35 ng/g of tissue/hr.

Effects of angiotensin added to the perfusing medium. Continuous addition of angiotensin II at the rate of 16 ng/min to the perfusate during the first hour of the perfusion significantly increased the rates of angiotensinogen formation (Fig. 2). In six observations, rates averaged 37.4 ± 5.87 ng/g of tissue/hr compared with control value of 20 ± 2.5 ng/g of tissue/hr ($p < .001$).

By contrast, angiotensin I given at the same dose level of 16 ng/min resulted in a rate of angiotensinogen formation of 23.7 ± 4.85 which is not significantly different from normal. However, increasing the dose of angiotensin I to 80 ng/min caused a significant stimulation with values averaging 51.6 ± 15.33 ng/g of tissue/hr ($p < .005$).

Discussion. The present experiments confirm previous observations which showed that increases in plasma renin due to the administration of large doses of the enzyme cause an initial decrease in plasma levels of angiotensinogen followed by recovery and an eventual increase to values above normal despite the persistence of an elevation in plasma renin concentration (7, 8). The initial fall in plasma substrate can be attributed to an increased consumption resulting from the high levels of circulating renin. On the other hand, the secondary rise very likely reflects an increase in angiotensinogen synthesis as demonstrated here by the fourfold increase in rates of substrate production following injection of renin.

Infusion of angiotensin II into conscious animals caused a marked fall in plasma renin concentration which likely results from the negative feedback effect of the peptide on renin release from the kidneys (9). This was associated with a consistent increase in both hepatic production and plasma levels of angiotensinogen, thus suggesting that the elevated formation of substrate observed in renin treated animals is due to the effects of the high levels of circulating angiotensin rather than to a direct effect of renin itself (10). The possibility that low plasma levels of angiotensinogen may directly stimulate substrate synthesis cannot be entirely discarded. Such a mechanism has been demonstrated for albumin (12).

When angiotensin II was added to the medium perfusing the livers from untreated normal rats, it also increased substrate production significantly, thus indicating that the polypeptide can act directly on the liver and stimulate the synthesis and release of angiotensinogen. Angiotensin I was also effective although the doses required were higher than those of the octapeptide. Whether this effect of angiotensin I is direct or mediated through its conversion into angiotensin II is not clear. However, the presence of the converting enzyme in the liver tends to support the latter possibility (11).

The present observations strongly suggest that the levels of plasma angiotensinogen are determined by two main variables: first the rate of consumption by the enzyme renin and second, the rate of formation and release of angiotensinogen by the liver (2). Further-

more, they indicate the existence of a dynamic equilibrium between both variables which would tend to maintain plasma levels of angiotensinogen within a normal range in spite of small variations in plasma renin. This would explain why plasma substrate concentration was not significantly altered by renin administration in spite of a twofold increase in circulating renin (7).

From the present results, it is suggested that angiotensin, the product of the reaction of renin on angiotensinogen, exerts a feedback effect on the production and release of both the enzyme and its substrate as a way of maintaining their plasma levels or plasma renin activity within the normal range. The first feedback effect, already well documented (4), consists of an inhibition of renin release. The second effect, as suggested here, would consist of the stimulation of angiotensinogen formation. Although these two effects would tend to neutralize each other as far as plasma renin activity is concerned, it may be that differences in latent periods are a significant factor as shown in the sequence of events which follow administration of renin. We have demonstrated that there is at first a fall in plasma substrate levels which is likely accompanied by an increase in circulating angiotensin. Angiotensin in turn would cause an immediate cessation of renin release to be followed later on by an increased production of angiotensinogen. This increased production, together with a decreased destruction resulting from the shutoff of endogenous renin and the normal decay of exogenous renin may then explain the subsequent rebound in plasma substrate levels. We, therefore, suggest that the dual effects of angiotensin, first on renin release, then on synthesis of angiotensinogen permits a finer and smoother readjustment to changes in plasma renin activity. This stimulatory effect of angiotensin on substrate production might explain the increased rates of formation observed in both adrenalectomized and partially hepatectomized rats, situations in which plasma renin concentration and activity are markedly elevated (2, 13).

Finally, it is suggested that angiotensin II together with other hormonal factors (2) plays a major role in the regulation of angiotensinogen formation hence in the total homeostasis of the renin angiotensin system.

Summary. In rats administration of renin decreased plasma angiotensinogen and increased plasma renin, while administration of angiotensin had the opposite effects. However, both agents stimulated angiotensinogen formation by the liver. The stimulating effect of angiotensin II, and to a lesser extent of angiotensin I, was also demonstrated by adding these peptides to the perfusate of an isolated liver preparation. We propose a positive feedback effect of circulating angiotensin on angiotensinogen formation and release.

We thank Dr. F. M. Bumpus for the supply of angiotensin I and Mrs. Kathleen Rice for technical assistance.

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Received July 17, 1972. P.S.E.B.M., 1973, Vol. 142.