Inhibition of Renin Secretion by Angiotensin II.* (30547)

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The renin-angiotensin hormonal system is currently believed to play an important role in the regulation of electrolyte metabolism and arterial blood pressure. Recent studies (1-4) have been concerned with the nature of the stimuli controlling renin secretion. In the present paper we have investigated the possibility that angiotensin II might alter renin secretion in a manner similar to negative feedback mechanisms existing for other hormonal systems. This hypothesis was tested by evaluating the effects of intravenously infused synthetic angiotensin II upon renin secretion in several different experimental situations.

Methods. These studies were performed on dogs anesthetized with pentobarbitol, 30 mg/kg, administered intravenously. *Via* a right flank incision, the right ureter was catheterized with polyethylene tubing, and a clamp was positioned loosely around the aorta proximal to the origin of the right renal artery. By tightening this clamp the mean pressure in the aorta below it could be adjusted within ± 3 mm Hg. Arterial blood pressures above and below the clamp were monitored from a carotid and femoral artery using mercury manometers. To obtain renal venous blood, a polyethelene catheter (2.4 mm, o.d.) was introduced into the left femoral vein, passed up the inferior vena cava and manipulated into the right renal vein. Arterial blood was obtained from a femoral artery catheter. Renal excretory and hemodynamic data were obtained using standard clearance technique. Clearance periods were 10-20 minutes long, with arterial and renal venous blood samples taken in the middle of each period. A prime of creatinine and p-aminohippurate was given intravenously following which these substances were infused at a constant rate, 0.2 ml/min. Creatinine clearance was used as a measure of glomerular filtration rate (GFR), and total renal plasma flow (RPF) was determined by the Fick principle, using PAH. Analytical methods for creatinine, PAH, and Na have been previously described (5). At least 30 minutes after completion of all operative procedures and the administration of the PAH and creatinine prime, control clearances were performed and one of the protocols described in *Results* below was followed.

The method used for estimation of renal venous renin concentration is indirect and has been described in detail(3). In brief, the plasma sample is incubated under standardized conditions for 31 minutes during which time antiogensin II is formed in a quantity directly related to the renin concentration of the plasma. This angiotensin is then extracted into butanol, redissolved in saline, and assayed by comparing its pressor activity to that of standard angiotensin (Val^5 - angiotensin II amide supplied by Ciba) in anesthetized ganglion-blocked rats. Renin concentration of the original plasma is expressed in terms of the nanograms of angiotensin formed (Ang. Equiv.) per ml of original plasma. The existence in these experiments of elevated plasma concentrations of angiotensin (resulting from its intravenous infusion) did not interfere with the renin assay. This was proven by assaying incubated and unincubated samples of the same plasma and finding that the pressor activity of unincubated plasma was usually undetectable and never more than 2.5 ng/ml even during the highest rates of angiotensin infusion. A major explanation for this was found to be the rapid rate of destruction of angiotensin during the initial withdrawal and handling of the blood prior to inactivation of blood angiotensinases by acidification. Moreover, it is evident that the presence of any preformed angiotensin would have spuriously increased the estimated renin concentration whereas, as described below, the infusion of angiotensin actually decreased plasma renin.

Results. Protocol 1: Angiotensin infusion

* Supported by research grant AM 05077-04 from NIH.

during maintenance of control mean renal arterial pressure. After completion of control clearances, Val^5-angiotensin II amide was infused continuously intravenously in a dose range of 25-200 ng/kg min. Simultaneously, as arterial blood pressure increased because of the pressor activity of the infused angiotensin, the aortic clamp above the right renal artery was tightened in order to maintain mean renal arterial blood pressure at the previous control value (110-140 mm Hg). By means of this "static aortic clamping," the effects of angiotensin could be studied with mean renal blood pressure as a constant. Fig. 1 summarizes the data for this group of dogs. In every dog, the angiotensin produced a decrease of renal venous renin concentration. For the series, this decrease was 2.4 $\pm$ 0.63 ng/ml (mean $\pm$ S.E.M.) and was statistically significant ($p = < .02$ by paired samples analysis). In these experiments, changes in renal venous renin concentration are presumed to represent changes in renin secretion. Quantitative evaluation of renin secretion would require the product of the RPF and the renal arterio-venous renin concentration difference. However, Vander and Miller(3) have demonstrated that, in experiments under similar conditions, renal venous renin concentration alone provided an adequate qualitative indication of changes in renin secretion. This was confirmed for the specific conditions of the present experiment by measuring "total renin secretory rates" in several dogs before and during angiotensin infusion. Moreover, it is evident that the small decrease in RPF consistently produced by angiotensin would tend to increase rather than decrease renal venous renin concentration had secretion rate remained unchanged.

The data of Fig. 1 are for samples taken 45 minutes after beginning the angiotensin infusion. Similar reduction of renin secretion occurred within 10 minutes and remained for 105 minutes (no attempt was made to prolong the experiments further). Sodium excretion and RPF were also decreased in every experiment, and GFR decreased in 4 of five. As shown in Fig. 1, these changes were reversed within 30 minutes after stopping the angiotensin and loosening the aortic clamp.

Protocol 2: Angiotensin infusion during maintenance of mean renal arterial pressure at 80-90 mm Hg. Fig. 2 summarizes the data for this group of experiments which were done to test the effects of angiotensin during periods when renin secretion was being stimulated by a reduction in renal arterial blood pressure(1-3). Following completion of control clearances, the aortic clamp was tightened in order to lower mean renal arterial blood pressure to 80-90 mm Hg and was continuously adjusted to maintain this pressure. Thirty to sixty minutes later, further clearances were performed as shown in Fig. 2. As demonstrated previously(3), this reduction of pressure produced an increased renin secretion and a reduction of GFR, RPF, and sodium excretion. These changes have been shown to remain relatively constant during at least 120 minutes of reduced blood pressure(3). After completion of these clearances, angiotensin (6.3-200 ng/kg min) was infused intravenously, and the aortic clamp was further adjusted as required to maintain mean renal arterial pressure at 80-90 mm Hg. By this means, the effects of angiotensin on renal function and renin secretion were measured at a constant reduced renal arterial pressure. In every dog, angiotensin reduced renin secretion despite the continued stimulus of the low renal blood pressure. Indeed, in several dogs, the renal venous renin concentration was reduced below the previous normotensive control value. For the series, the decrease in renal venous concentration was 8.2 $\pm$ 2.45 ng/ml (mean $\pm$ S.E.M.) and was statistically significant ($p = < .02$ by paired samples analysis).

As was true for the experiments of protocol 1, this inhibition of secretion was observable within 10 minutes and continued for at least 105 minutes. RPF was also reduced in every case, but there were no consistent changes in GFR or sodium excretion. The changes produced by aortic clamping and angiotensin were reversible within 30 minutes after cessation of infusion and release of the aortic clamp as shown in the final control data of Fig. 2. Fig. 3 illustrates complete data for a dog in which a dose response study

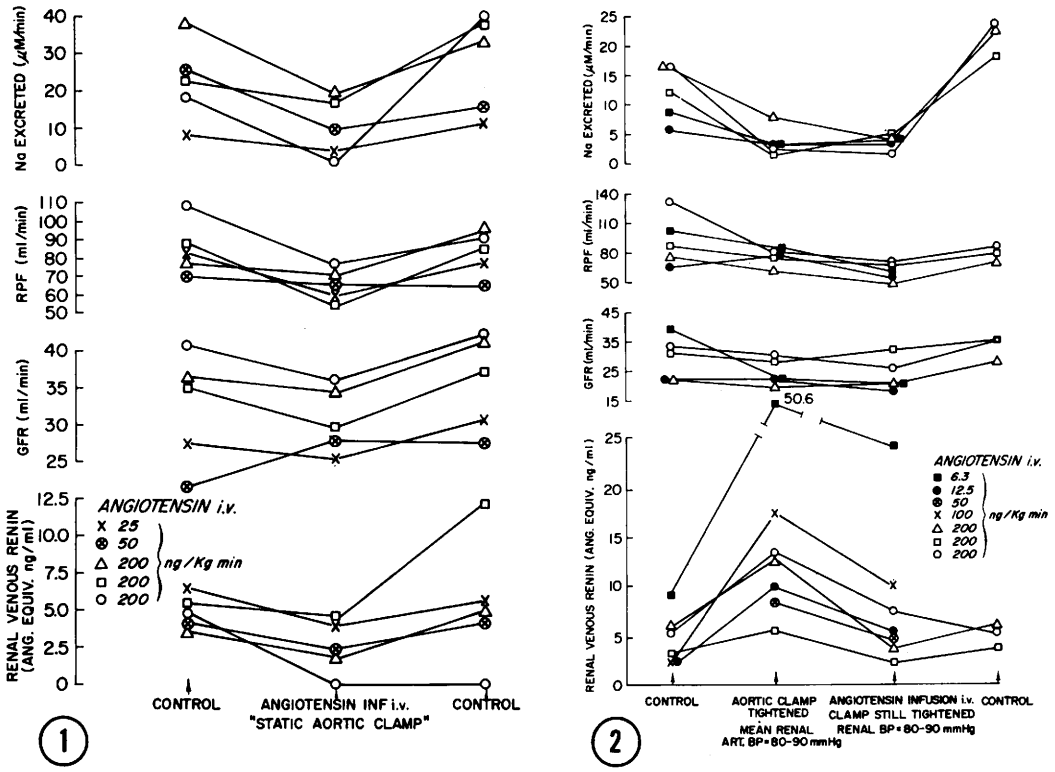

FIG. 1. Effects of angiotensin II infusion on renin secretion and renal function in anesthetized dogs. During angiotensin infusion, an aortic clamp was tightened to maintain mean renal arterial BP at the previous control value ("static aortic clamp"). All data are for the right kidney.

FIG. 2. Effects of angiotensin II infusion on renin secretion and renal function in anesthetized dogs during maintenance of mean renal arterial BP at 80-90 mm Hg. All data are for the right kidney.

was done and in which the angiotensin infusion was stopped while still maintaining mean renal arterial pressure at 80-90 mm Hg.

Protocol 3: Angiotensin infusion during ureteral occlusion. These experiments, summarized in Fig. 4, were performed to determine whether angiotensin could inhibit the renin secretion induced by acute elevation of ureteral pressure(3). After collection of control renal venous samples, ureteral pressure was increased abruptly to at least 60 mm Hg by the retrograde injection of urine into the renal pelvis followed by complete ureteral occlusion. The "ureteral occlusion" samples of Fig. 4 were obtained 30 minutes later, and the angiotensin infusion (100 ng/kg min) was then begun. Despite the continued ureteral occlusion, renal venous renin concentrations were reduced (samples taken 30 minutes after beginning the infusion) in every case. This occurred regardless of whether the arterial pressure was held constant throughout the experiment by means of a "static aortic clamp" (2 exp.) or allowed to rise during the angiotensin infusion (1 exp.)

Discussion. We pointed out above that the infusion of angiotensin did not interfere with the renin assay by contributing significant quantities of preformed angiotensin to the angiotensin generated *in vitro* in the incubation procedure. Two other methodological problems investigated were those concerning angiotensinase and angiotensinogen: a) recovery of standard angiotensin added to the incubation mixture was the same for bloods obtained before and during angiotensin infusion, thus ruling out an increase in angiotensinase as responsible for the changes in the indirectly estimated plasma renin; b) addition of a large quantity of standard renin

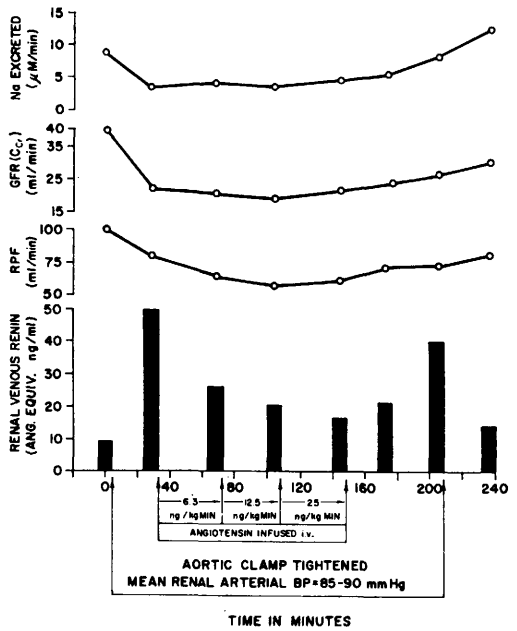

FIG. 3. Effects of reducing renal arterial BP and infusing angiotensin II in an anesthetized dog. All data are for the right kidney.

to plasma samples obtained before and during angiotensin infusion caused the formation of identical amounts of angiotensin, thus ruling out a decrease of plasma angiotensinogen.

A major question raised by these experiments is whether this inhibition of renin secretion by angiotensin occurs physiologically, *i.e.*, is the endogenous plasma (or intrarenal?) concentration of angiotensin great enough to exert an inhibitory effect on renin secretion. There are at least 3 lines of evidence to support this concept: 1) In the first group of dogs in which there was little stimulus to renin secretion, the infusion of 25-50 ng angiotensin/kg min inhibited renin secretion while raising arterial pressure proximal to the clamp only 12-18 mm Hg; such blood pressure changes were frequently observed previously as a result of endogenous angiotensin generation under similar conditions(3). 2) In the second group of dogs in which renin secretion was being stimulated, small doses of angiotensin (6.3-12.5 ng/kg min) caused no further change in blood pressure proximal to the clamp, but still inhibited renin secretion. 3) Several investigators(6,7) have infused angiotensin intravenously in the lower doses that we employed, and have demonstrated resulting plasma angiotensin concentrations similar to those which can occur in non-infused subjects. Although conclusive proof must await more extensive measurements of plasma angiotensin concentrations, all these experiments suggest that physiological rates of renin secretion are the resultant of the strength of the stimulus favoring renin release and the degree of inhibition of release exerted by circulating or intrarenal angiotensin.

A second major question raised by these experiments concerns the mechanism by which angiotensin exerts this inhibitory effect. It is evident that, in the intact animal, an increased angiotensin production would tend to raise blood pressure because of the vasoconstrictor and sodium-retaining (*via* aldosterone) effects of angiotensin. This, in itself, would and probably does constitute a negative feedback control over renin secretion. However, the present experiments demonstrate that a second inhibitory pathway exists which is independent of mean renal arterial pressure which was kept constant in these experiments. Nor is it dependent upon blood pressure changes proximal to the aortic clamp, since sub-pressor doses of angiotensin also inhibited renin secretion. Moreover, it has previously been demonstrated that the infusion of pressor doses of catecholamines coupled with "static aortic clamping" causes stimulation rather than inhibition of renin secretion(4).

The mechanism and pathway by which this pressure-independent inhibition is exerted cannot be determined from these experi-

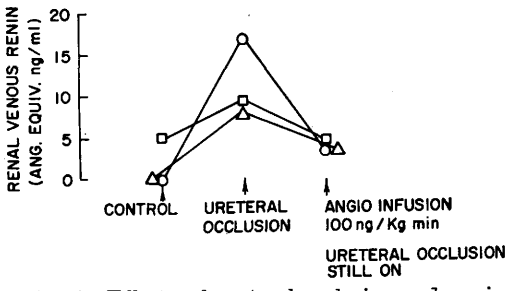

Fig. 4. Effects of ureteral occlusion and angiotensin II infusion on renin secretion in anesthetized dogs. Data are for the right kidney.

ments. RPF was consistently reduced by angiotensin infusion, and GFR and sodium excretion also tended to decrease although less consistently. However, such changes in renal hemodynamics and sodium excretion have previously been shown to be associated with stimulation rather than inhibition of renin secretion(3,4). This is particularly evident as regards the infusion of catecholamines (during "static aortic clamping") or stimulation of the renal nerves, since these procedures produce renal function changes quite similar to those of angiotensin, but stimulate renin secretion(4). It seems likely, therefore, that the inhibitory effect of angiotensin may be exerted directly upon the renin-secreting cells either by angiotensin itself or by some substance liberated extrarenally as a result of the elevated plasma angiotensin. It is interesting that this type of inhibition may be analogous to the enzyme repression which occurs in wholly intracellular metabolic systems.

Summary. Intravenous infusion of angiotensin II (6.3-200 ng/kg min) inhibited renin secretion in dogs under conditions of basal or elevated renin secretion. This inhibition was independent of changes in mean renal arterial blood pressure. We propose a negative feedback effect of circulating angiotensin on renin secretion.

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Received June 9, 1965. P.S.E.B.M., 1965, v120.

Intestinal Transport of D-Xylose.*† (30548)

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The general concept of the intestinal absorption of D-Xylose has recently undergone considerable change. It was recognized early that the rate of xylose absorption is much lower than that of glucose or galactose, and that the transport of xylose was not influenced as much by metabolic inhibitors as that of the two hexoses. It was therefore assumed that, while the absorption of glucose and galactose proceeds "preferentially" involving the "vital" functions of the cell, xylose was taken up by simple passive diffusion(1).

It was shown that the intestinal transport of D-Xylose is carrier-mediated, and that this pentose shares a common carrier with glucose(2). Recently it was discovered that there is an active transport mechanism for intestinal transport of xylose although the capacity of the xylose pump is rather low (3,4).

The structure of the carrier (or better, carrier sites) on the plasma membrane is not known. However, one can obtain some information by studying the structural specificity of the substrates to be transported by the same or similar sites. This is best revealed when examining the mutual competition for a given carrier site between the various substrates of related structure. Since the transport of xylose is carrier-mediated, and since xylose itself does not readily undergo metabolic changes during the transport process, it was tempting to undertake such an investigation using xylose in competition with other simple sugars and sugar-alcohols.

* Aided by U.S.P.H.S. Research Grants.

† The preliminary experiments of this work were carried out at the University of North Carolina by one of us (T.Z.C.) in collaboration with Dr. Lawrence Zollicoffer.