Mechanism of action of angiotensin II and antidiuretic hormone on renin secretion

ROBERT E. SHADE, JAMES O. DAVIS, J. ALAN JOHNSON, R. W. GOTSHALL, AND W. S. SPIELMAN

Department of Physiology, University of Missouri School of Medicine, Columbia, Missouri 65201

produce inhibition of renin secretion by acting through a vasoconstrictor action, either hormone could conceivably produce changes in renal sodium excretion in addition to their renal nerves (1, 7). Since both antidiuretic hormone (ADH) and angiotensin II (AII) inhibit renin secretion in the absence of a functional macula densa. High control rates of renin secretion were induced by sodium depletion prior to the acute experiment. After control observations, AII was infused into the renal artery of the single remaining nonfiltering kidney at a rate of 7 ng/100 ml of renal blood flow for 30 min and renin secretion was measured at 15 and 30 min. Thirty minutes after stopping the AII infusion recovery observations were made. This was followed by a 30-min infusion of ADH at a rate of 5 µU/ml of renal blood flow. In a second series of experiments the same protocol was followed except that the order of infusion of AII and ADH was reversed. Renin secretion in nonfiltering kidneys decreased from 496 to 253 and 246 ng angiotensin/min (P < .02) with AII as the first infusion, and from 523 to 194 and 38 ng angiotensin/min (P < .01) when AII was infused second. ADH infusion decreased renin secretion in nonfiltering kidneys from 698 to 136 and 134 ng angiotensin/min (P < .02) with ADH given first, and from 305 to 174 (P < .03) and 134 (P < .02) when the second infusion. ADH had no effect on renal blood flow or arterial blood pressure. In some instances AII decreased renal blood flow. These observations show that both AII and ADH inhibited renin secretion in the absence of a functional macula densa.

PRESVIOUS INVESTIGATORS have demonstrated that intravenous infusion of either angiotensin II (3, 5, 7, 16) or vasopressin (5, 13, 15) decreases plasma renin activity. Since these decreases in plasma renin activity were accompanied by little or no change in renal blood flow, the data were interpreted to indicate a decrease in renin secretion. Renin secretion is primarily controlled by two intrinsic mechanisms and further modulation of renin secretion is provided by the extrarenal influence of the renal nerves (1, 7). Since both antidiuretic hormone (ADH) (4, 6) and angiotensin II (1, 3) are known to produce changes in renal sodium excretion in addition to their vasoconstrictor action, either hormone could conceivably produce inhibition of renin secretion by acting through a macula densa mechanism, a vascular receptor mechanism, or by direct inhibition of the juxtaglomerular cells.

Previous experiments performed in our laboratory have revealed that factors affecting the intrarenal vascular receptor mechanism can be studied under conditions in which the macula densa mechanism is nonfunctional (2, 7, 10) by use of a nonfiltering kidney model (2). The present experiments were designed to determine whether ADH or angiotensin II inhibits renin secretion in the nonfiltering kidney in the absence of a functional macula densa.

METHODS

A nonfiltering kidney was produced on the left side in 12 female mongrel dogs weighing 16-21 kg according to the method of Blaine et al. (2). The procedure consisted of ligating the left ureter and placing a serrefine clamp on the left renal artery for 2 hr under sterile conditions; 3 days later the right kidney was removed and the experiment was performed on the nonfiltering kidney the following day. To achieve a high control rate of renin secretion to facilitate study of an inhibitory response, each dog was sodium depleted by administering a diuretic (2 ml Mercuhydrin im) on the day of ureteral ligation and clamping of the renal artery; this was followed by the diuretic and a low-Na diet (<3 mEq/day) for the next 3 days. On the day of the experiment the dog was anesthetized with pentobarbital (15-30 mg/kg), polyvinyl catheters were placed in the femoral artery and vein, and an electromagnetic flow probe (Carolina Medical Electronics) was placed around the left renal artery through a flank incision. An 18-gauge needle was placed in the left renal vein to obtain renal venous blood samples and a 22-gauge needle was inserted into the left renal artery for intrarenal infusions. Arterial pressure was measured with a Statham model P 23 Db pressure transducer. Arterial pressure and renal blood flow were recorded on a Sanborn model 7700 recorder.

After surgical preparation normal saline was infused into the renal artery at 0.59 ml/min. Sixty minutes later, two pairs of arterial and renal venous blood samples were collected for determining the control rate of renin secretion. In the first series of dogs, angiotensin II (Hypertensin-Ciba) in normal saline was infused into the renal artery at 0.59 ml/min and at a rate which was calculated to increase blood angiotensin II concentration by 7 ng/100 ml. This
level of angiotensin II infusion has previously been shown to produce an increase in angiotensin II comparable to that observed in sodium depletion in sheep (3). Samples for determination of renin secretion were collected at 15 and 30 min after beginning the angiotensin II infusion. Two recovery values for renin secretion were obtained 30 min after replacing the angiotensin II infusion with an infusion of normal saline at 0.59 ml/min. Following these recovery observations, a second intrarenal infusion of ADH (vasopressin, Parke, Davis & Company) in normal saline was started at a concentration calculated to increase the renal arterial blood ADH concentration by 5 μU/ml; the infusion rate was continued at 0.59 ml/min. Renin secretion was determined after 15 and 30 min of ADH infusion and two recovery values were obtained after 30 min of normal saline infusion. In a second group of experiments, the same protocol was followed except that the order of infusion of angiotensin II and ADH was reversed; the first experimental infusion contained ADH and the second was an angiotensin II infusion. All blood removed for sampling was immediately replaced with an equal volume of fresh donor blood from a normal dog.

**Analytical procedures.** Arterial and renal venous blood samples for plasma renin activity determinations were collected in chilled tubes containing 0.1 ml of 10% EDTA for each 10 ml of blood. The samples were immediately cooled in an ice bath and centrifuged in the cold for separation of plasma. Plasma samples were then stored frozen until they were processed for angiotensin generation by the method of Schneider et al. (12). Two-milliliter samples of plasma were dialyzed against a phosphate buffer (pH 5.4) in the cold (4 C). Following dialysis, NaCl and diisopropylfluorophosphate (DFP) were added and the samples were incubated 37 C for 3 hr. Following incubation, the samples were placed in a boiling water bath for 10 min and then chilled in an ice bath. Each sample was diluted to 4 ml with phosphate buffer (pH 8.3), stirred and centrifuged, and the supernatant frozen until assayed. Samples were assayed by a pressor response in the pentobarbital-anesthetized, pentolinium-blocked rat with use of angiotensin II as a standard. Renin secretion (ng angiotensin/min) was calculated by subtracting arterial plasma renin activity from plasma renin activity and multiplying the difference by the renal plasma flow. Hematocrits were determined in duplicate by a microhematoctrit method. At the end of each experiment a solution of lissamine green dye was injected into the renal artery to verify the lack of glomerular filtration (2). Examination of the superficial renal tubules with a dissecting microscope revealed that dye failed to appear in the renal tubules in all of the dogs used in this study. In addition, recent studies from the laboratory have produced further evidence that this type of kidney preparation is nonfiltering (9).

The data were analyzed for statistical significance by Student t test for paired differences.

**RESULTS**

The results for the first group of experiments are presented in Fig. 1. Intrarenal infusion of angiotensin II produced decreases in renin secretion in each of the five dogs. Renin secretion decreased from mean values of 516 and 474 during control periods to 253 (P < .03) and 246 (P < .03) after 15 and 30 min of angiotensin II infusion. The apparent decrease in renal blood flow was the result of a large decrease in one of the five dogs in this experiment. A small decrease in renal blood flow occurred in another dog but no change was detected in the three remaining animals; for the group, renal blood flow was unchanged (P > .05). Also, there were no consistent or significant changes in mean arterial pressure during angiotensin II infusion. After 30 min for a recovery period, renin secretion was 272 and 335 ng angiotensin/min but this change was not statistically significant. The infusion of ADH decreased renin secretion to 174 (P < 0.3) and 134 ng angiotensin/min (P < .02) after 15 and 30 min of infusion (Fig. 1). There were no significant changes in renal blood flow or mean arterial pressure during ADH infusion. Thirty minutes after stopping the ADH infusion, renin secretion increased to an average of 503 ng angiotensin/min (P < .01).

In a second group of experiments the same protocol was used and the order of infusion of the two peptides was reversed. These results are presented in Fig. 2. Infusion of ADH decreased renin secretion in each of the seven dogs from an average control level of 698 ng angiotensin/min to 136 and 134 after 15 and 30 min of infusion (P < .03 for both). Again, ADH infusion produced no significant changes in renal blood flow or mean arterial pressure. Renin secretion decreased from mean values of 516 and 474 during control periods to 253 (P < .03) and 246 (P < .03) after 15 and 30 min of angiotensin II infusion. The apparent decrease in renal blood flow was the result of a large decrease in one of the five dogs in this experiment. A small decrease in renal blood flow occurred in another dog but no change was detected in the three remaining animals; for the group, renal blood flow was unchanged (P > .05). Also, there were no consistent or significant changes in mean arterial pressure during angiotensin II infusion. After 30 min for a recovery period, renin secretion was 272 and 335 ng angiotensin/min but this change was not statistically significant. The infusion of ADH decreased renin secretion to 174 (P < 0.3) and 134 ng angiotensin/min (P < .02) after 15 and 30 min of infusion (Fig. 1). There were no significant changes in renal blood flow or mean arterial pressure during ADH infusion. Thirty minutes after stopping the ADH infusion, renin secretion increased to an average of 503 ng angiotensin/min (P < .01).

**FIG. 1.** Effects of intrarenal arterial infusion of angiotensin II followed by ADH infusion in sodium-depleted dogs. BP and RBF are abbreviations for arterial pressure in millimeters Hg and renal blood flow in milliliters per min. Deviations are SEM.

**FIG. 2.** Effects of intrarenal arterial ADH infusion followed by angiotensin II infusion in sodium-depleted dogs. BP and RBF are abbreviations for arterial pressure in millimeters Hg and renal blood flow in milliliters per min. Deviations are SEM.
secretion increased toward the control level (average = 523, \(P < .05\)) after 30 min of recovery. The infusion of angiotensin II decreased renin secretion to 205 (\(P < .01\)) at 15 min and 38 (\(P < .01\)) at 30 min. In this case, intrarenal infusion of angiotensin II produced significant reductions in renal blood flow. Following a 30-min recovery period, renin secretion increased significantly to 341 ng renin/min (\(P < .02\)) and renal blood flow returned to the control level.

**DISCUSSION**

Intravenous infusions of angiotensin II have been reported to produce decreases in renal venous renin activity in the anesthetized dog with suprarenal aortic constriction (5, 15). In one of these reports changes in renal perfusion pressure were prevented by adjusting the aortic constriction, but there were decreases in renal plasma flow, glomerular filtration rate, and sodium excretion (15). Blair-West and associates (3) have recently demonstrated that chronic intravenous and intrarenal infusions of angiotensin II in conscious sheep prevented the normal increase in plasma renin activity observed after 24 hr of sodium depletion. These authors postulated that angiotensin II inhibited renin secretion by a direct negative feedback on the juxtaglomerular cells since there were no observed changes in arterial blood pressure or renal sodium excretion.

Similar results have been reported for infusion of ADH. Bunag, Page, and McCubbin (5) found that intravenous and intrarenal infusions of ADH produced decreases in renal venous renin activity with and without changes in renal blood flow and mean arterial pressure in dogs with aortic constriction. Vander (15) demonstrated that intravenous infusion of ADH at 10 \(\mu\)U/min but not at 1 \(\mu\)U/min decreased renal venous renin activity in anesthetized dogs. Recently, Tagawa et al. (14) demonstrated that acute intravenous infusions of ADH that produced increases in plasma ADH concentration as small as 1 \(\mu\)U/ml significantly decreased plasma renin activity in sodium-deprived, conscious dogs.

The present results provide direct confirmation that ADH and angiotensin II inhibit renin secretion. In addition, since these peptides were infused in the nonfiltering kidney preparation, the results also show that inhibition of renin secretion occurred in the absence of a functional macula densa. Thus, inhibition of renin secretion in this situation could be mediated by a vascular receptor in the renal afferent arteriole or by a direct influence on the juxtaglomerular cells (1). The lack of change in renal blood flow and arterial blood pressure with ADH and, in some instances, with angiotensin II suggests that inhibition was the result of a direct effect on the juxtaglomerular cells. These results, however, do not rule out the possibility of changes in afferent arteriolar hemodynamics. It is conceivable that vasoconstriction in the efferent arteriole was associated with vasodilatation in the afferent arteriole leading to an increase in afferent arteriolar pressure with no change in total renal resistance and renal blood flow. In this event, inhibition of renin secretion might result from an action on a vascular receptor mechanism.

It should be pointed out that it is unlikely that a decrease in renal cortical blood flow resulted with either ADH or angiotensin II infusion, and thereby contributed to the reduction in renin secretion. During ADH infusion renal blood flow was consistently unchanged. With angiotensin II, renal blood flow was not significantly changed in the first series of dogs and the dose used here is far below that reported by others (11) to produce changes in regional renal blood flow which might include a decrease in cortical flow through the kidney.

Will physiological increases in plasma levels of ADH or angiotensin II inhibit renin secretion? The rate of angiotensin II infusion used for the present experiments increased intrarenal blood levels of angiotensin II by 7 ng/100 ml. This is within the range of the increase in angiotensin II observed after 24 hr of sodium depletion in sheep (3). Similarly, the rate of intrarenal ADH infusion increased intrarenal blood ADH levels by 5 \(\mu\)U/ml; this resulted in an increase in blood ADH concentration comparable to that observed with a nonhypotensive hemorrhage (18). It is suggested, therefore, that one of the important physiological mechanisms of action for both ADH and angiotensin II includes an influence of these peptides on either the vascular receptor or on the juxtaglomerular cells directly.

We are grateful to Robert Monroe and Elizabeth Culley for technical assistance. The angiotensin II was graciously supplied by Ciba Pharmaceutical Company Summit, N.J.

This work was supported by Public Health Service grants H1 10612 and HL 08510.

Received for publication 14 September 1972.


