The Effect of Angiotensin on Renin Production and Release *in Vitro*[1] (36060)

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The physiologic regulation of renin production and secretion depends upon various hormonal and neural factors. Renin production is stimulated by decreased blood volume and decreased renal perfusion pressure. Renin production is also stimulated by sympathetic nerve stimulation (1) and by the infusion of catecholamines in animals (1, 2) and man (3), and we have shown that catecholamines exert a direct stimulatory effect on renin production by the renal cells (4). In addition, the sodium in the fluid reaching the macula densa has been suggested as being one mechanism controlling renin secretion.

Other factors have been found to inhibit renin production *in vivo*. A negative feedback effect of circulating angiotensin on renin secretion has been proposed (5), but the mechanism by which it exerts its inhibitory effect is not understood. In addition, we have shown that intravenous infusion of angiotensin to normal man in pressor and subpressor amounts markedly inhibited plasma renin activity (3). From published studies, it is not clear whether the action of angiotensin on renin production is due to its effect on sodium balance and renal hemodynamics or to a direct inhibitory effect on the renin-producing cells. In the present study, the possibility that angiotensin might influence the production and release of renin by a direct inhibitory action on the renal cells was investigated utilizing a renal cell suspension system free of hemodynamic or neurogenic influence. Since the method we employed does not provide information concerning the rate of synthesis and the rate of destruction of renin as independent processes, only their algebraic sum is known, *i.e.*, "net production" and "net release" of renin.

Materials and Methods. Materials. The materials used were angiotensin II (1-L-asparaginyl-5-L-valyl angiotensin octapeptide; Hypertensin, Ciba); collagenase, product of Nutritional Biochemicals Corp., Cleveland, OH, and MEM Eagle, Spinner modified medium without glutamine (Baltimore Biological Laboratory, Baltimore, MD to which 1.3 ml of 200 m*M* L-glutamine (Robbin Laboratories, Inc., Chapel Hill, NC) were added/100 ml (4).

Methods. The preparation of the renal cell suspension was performed as described in detail in a previous report (4). In brief, both kidneys were excised from young mongrel dogs under pentobarbital anesthesia and placed in ice. The renal cortex was removed after perfusing the kidney with psysiological saline followed with medium containing 0.3% collagenase. The cortex was then minced and the fragments obtained were digested in the collagenase solution for 1 hr. The cells obtained were washed to remove adherent collagenase and then resuspended in medium and used in the experimental incubations as described below. Microscopic examination of the cell suspensions revealed intact cells, some of which had the appearance of smooth muscle cells. Small segments of tubules and arterioles were also present, but intact glomeruli were not identified. All samples were kept in ice prior to the incubation. In each experiment, kidneys from a different dog were used for the preparation of the renal cell suspension.

Experimental incubations. In each experiment, 10 ml of the cell suspension were added to each flask. All samples were run in duplicate. Each time, two samples were not

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incubated but were processed for measurement of renin levels to be used as nonincubated controls and another two were incubated in medium alone to serve as incubated controls. As experimental variable, angiotensin was added to other incubated samples. All incubations were carried out for 1 hr in a 37° water bath with slow constant shaking under an atmosphere of 95% oxygen and 5% carbon dioxide. At the end of the incubation period, the suspensions (including the supernate) were homogenized, and immediately stored at —20° until the time of renin determination. Under identical conditions, experiments were performed in which duplicate samples were incubated with, or without, angiotensin and the renin release into the incubation medium was measured.

Renin measurement. To measure the renin in each sample, autologous plasma was used as substrate. Forty-eight hours after bilateral nephrectomy, the blood was drawn from the aorta into tubes containing EDTA as anticoagulant. Without delay, the plasma was separated by centrifugation in the cold and stored at —20° until use.

An aliquot of each sample was added to 10 ml of the above renin substrate, and the renin activity was measured by known procedures (6, 7). The adequacy of substrate in the plasma was checked in each experiment by using graded quantities of sample and showing that the quantity of angiotensin generated was proportional to the quantity of aliquot of the sample. The renin activity, therefore, measured in this study may be considered to represent renin concentration. In all experiments, the renin activity in the plasma substrate prior to addition of the sample was undetectable.

Results. Effect of angiotensin on net renin production. Eight experiments were performed in which angiotensin was added to cell suspension samples. In all experiments, the suspensions that were incubated with angiotensin had lower concentrations of renin activity than did the control suspensions incubated without angiotensin. When all experiments were considered as a group (Fig. 1), the decrease in production of renin in suspensions incubated with angiotensin was significant ($p<.01$). At the conclusion of the

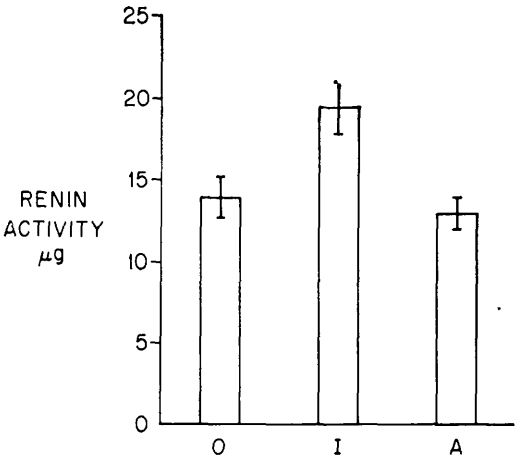

FIG. 1. Inhibition of renin production by angiotensin in renal cell suspensions: 0 = nonincubated controls; I = incubated control suspensions; A = suspensions incubated with angiotensin (5 μg/ml added at the beginning and at the middle of the incubation period). The brackets indicate SE. The inhibition of renin production by angiotensin was significant ($p<.01$).

incubation of the cell suspensions, aliquots of the suspensions injected into nephrectomized rats failed to elicit pressor responses, indicating that the angiotensin added during the incubation had been largely inactivated.

Effect of angiotensin on renin release in vitro. In four series of experiments, after the incubation with angiotensin had been completed, the supernatant was removed, and the cells were then resuspended in medium and homogenized. Renin activity was measured separately in supernatant and cells. All samples were run in duplicate. The results of a representative experiment are shown in Table I. The renin-inhibiting effects of angiotensin were reflected both in the supernatant and the cellular fractions.

Renin release in response to various doses of angiotensin. In five series of experiments, the effect of different concentrations of angiotensin on renin release by the renal cells was studied. Kidneys from a different dog were used in each series of experiments. All samples were run in duplicate and the amount of renin released into the incubation medium was determined. In all experiments, angiotensin exerted a dose-dependent inhibition of renin release. The results of a representative experiment are shown in Fig. 2. There ap-

TABLE I. Renin Activity in a Cell Suspension.[a]

Experimental variables	Renin activity (μg)		
	Supernatant	Sediment	Total/flask
1. Controls, nonincubated			14.4 ± 0.6
2. Controls, incubated	8.4 ± 0.4	9.8 ± 0.4	18.2 ± 0.7
3. Angiotensin (5 μg added at the beginning and at the middle of the incubation period per ml of suspension)	5.6 ± 0.35	8.0 ± 0.4	13.6 ± 0.6

[a] Duplicate samples were incubated with angiotensin for 1 hr; each flask contained 10 ml of renal cell suspension; at the end of the incubation, the cells (sediment) and supernatant were separately assayed for renin.

peared to be a dose–response relationship between the renin released and the amount of angiotensin added per sample. Such relationship seems to apply essentially to a certain dose range.

Discussion. Although much has been written on the renin–angiotensin system, the specific signals perceived by the renal juxtaglomerular cells to produce and secrete renin are still unknown. Previous studies have indicated that the intact kidney responds to angiotensin by secreting less renin than under control conditions and a negative feedback effect of circulating angiotensin on renin secretion has been proposed (5). From previous studies, however, it was not clear whether angiotensin acted directly on the renin-producing cells or through changes in renal hemodynamics and sodium balance. In order to test the hypothesis that angiotensin might, as chemical agent, act directly on renal cells to modify renin production or release, we utilized a renal cell suspension capable of producing renin in the absence of neural or hemodynamic factors. We have found that angiotensin had a direct inhibitory effect on net renin production and release. It seems, therefore, that the negative feedback effect of angiotensin on the *in vivo* secretion of renin by the kidney may be exerted, at least in part, directly on the renin-producing cells by angiotensin itself or by some substance liberated intrarenally in the presence of angiotensin.

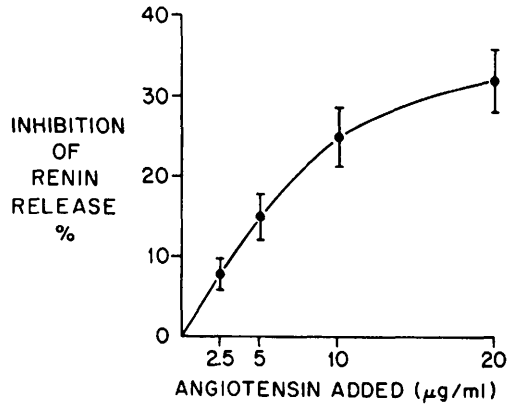

FIG. 2. Inhibition of renin release from renal cells in response to various doses of angiotensin: Bracketed lines indicate SD.

Summary. A preparation of a renal cell suspension system suitable for renin studies was utilized to investigate the *in vitro* effects of angiotensin which has previously been shown to influence renin production *in vivo*. The addition of angiotensin to the incubated samples caused a significant inhibition of renin production and release. It is suggested that the renin-inhibitory effect of angiotensin in the intact animal might be due, at least in part, to a direct chemical action on the renal cells.

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