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Renin Secretion by Rat Kidney Slices *in Vitro*[1] (37819)

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Several investigators have reported the use of kidney cortical slices to study the release of renin *in vitro* (1–3). It has been difficult to demonstrate stimulation of renin secretion in this *in vitro* system, and the suggestion has been made that renin secretion from kidney slices may be occurring at a maximal rate (1).

A variety of drugs including epinephrine, norepinephrine, furosemide, and prostaglandin E stimulate renin secretion in the intact animal (4–6). In addition, the state of sodium balance of the animal is important in the control of renin secretion (7). Therefore, it would be significant to be able to study the effects of these drugs *in vitro*. The present study was designed to further look at the *in vitro* system. The results suggest that renin secretion from kidney slices is taking place at a maximal rate and that the system is not responsive to usual stimuli.

Methods. Male Sprague–Dawley rats weighing between 100 and 200 g were killed by a blow on the head and the left kidney rapidly removed. The kidney was cut longitudinally and three slices weighing between 50 and 100 mg were prepared using a Stadie–Riggs microtome. Two of the slices were placed in separate Erlenmeyer flasks containing Robinson's medium, pH 7.4 (8). The third slice was immediately frozen in dry ice and acetone and stored at −20°. The flasks were placed in a Dubnoff shaking metabolic incubator at 37°, gassed with 100% O_2, and preincubated for 30 min.

At the end of this period, the slices were transferred to a second set of flasks containing fresh Robinson's medium, gassed with 100% O_2, and incubated for 60 min. Samples of medium (0.25 ml) were removed from each flask at 30–60 min and frozen at −20° until assayed. At the end of the 60 min the tissue was removed, frozen in dry ice and acetone, and stored at −20° until assayed.

Assay of tissue content of renin was carried out on the supernatant obtained by homogenizing the tissue in Tris buffer (100 m*M*, pH 7.4). The renin concentration in the Robinson's medium and in the supernatant from tissue homogenates was determined by incubating an aliquot with excess renin substrate and measuring the amount of angiotensin I formed by radioimmunoassay (Schwarz/Mann). Renin substrate was prepared from dog plasma by the method of Skeggs (9). The incubation of 50 μl of Robinson's medium or supernatant with substrate was carried out in 100 m*M* phosphate buffer, pH 7.4, for 60 min at 37°. 8-Hydroxyquinaline and BAL were added to the incubation mixture to inhibit angiotensinase and converting enzyme activity.

The zero-order kinetics of this reaction mixture was confirmed by addition of varying amounts of renin to a constant amount of substrate (10). The concentrations of renin in the Robinson's medium and in the supernatant of tissue homogenates were within the range required to maintain zero-order kinetics.

Three experimental protocols were used. (a) The flask containing one of the paired slices was gassed with 100% O_2 and the other flask gassed with 100% N_2. (b) The flask containing one of the paired slices was

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used as control, and the following drugs added to the other (experimental) flask after removal of the 30-min sample: epinephrine (5 μg/ml), norepinephrine (5 μg/ml), furosemide (20, 40, 200 μg/ml), prostaglandin E_1 (1 μg/ml), sodium chloride (200 m*M* final concentration). Comparison of rates of renin secretion were made during the final 30 min of incubation. (c) Slices from animals pretreated with a high sodium diet (2% NaCl in drinking water for 2 weeks) or pretreated with furosemide (10 mg/kg intraperitoneally before killing) were compared to slices obtained from animals drinking tap water or receiving an equal volume of saline intraperitoneally. Renin concentration in Robinson's medium and tissue slices was expressed as nanograms of angiotensin I formed per 100 milligrams of slice wet weight (ng/100 mg). Comparisons between slices were analyzed using a paired Student's *t* test. All intergroup comparisons were analyzed using an unpaired student's *t* test. Both tests were carried out at a significance level of 0.05.

Results. Renin concentration in slices and Robinson's medium (Fig. 1). The average renin concentration of kidney slices in 30 experiments before incubation was not statistically different from the average renin

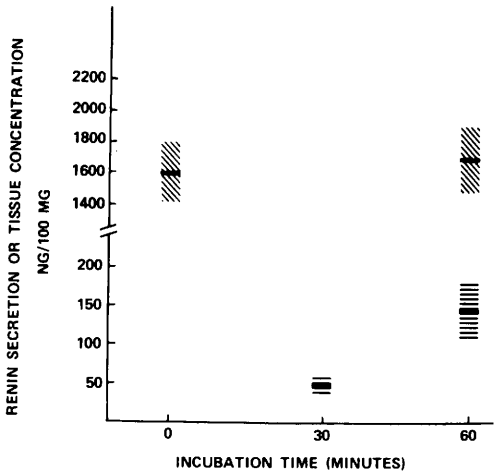

FIG. 1. Renin secretion into Robinson's medium during 30- and 60-min incubation periods (horizontally hatched bars) and tissue concentration of renin (diagonally hatched bars) in control experiments. Bars represent mean ± SEM.

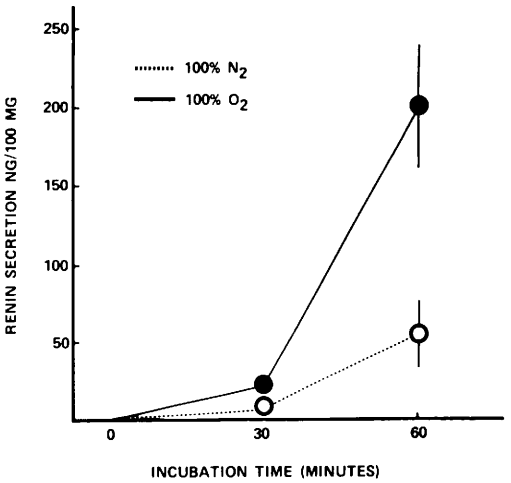

FIG. 2. Effect of a 100% nitrogen atmosphere on renin secretion during two 30-min incubation periods in 5 experiments. Vertical lines indicate SEM.

concentration of the slices after 1 hr of incubation ($P > 0.9$). The amount of renin released into the Robinson's medium was determined during two 30-min incubation periods (0–30, 30–60 min) in 55 experiments. The renin released into the medium increased threefold from the first 30 min of incubation to the second 30 min of incubation ($P < 0.001$). The renin released to the medium during the first and second 30-min incubation periods represents 3.0 and 4.7% of the concentration of renin in the slice prior to incubation, respectively.

The effect of 100% nitrogen on renin release and slice renin concentration (Fig. 2). During the first 30 min of incubation, the amount of renin released into the medium from slices incubated in 100% N_2 was not statistically different from the renin released in 100% O_2. Secretion of renin from slices in 100% N_2 was significantly less than those in 100% O_2 during the second 30-min period ($P < 0.02$). The secretion of renin by slices incubated in an atmosphere of either O_2 or N_2 increased from the first 30 min to the second 30 min ($P < 0.01$ and $P < 0.05$, respectively). The concentration of renin in the slice after incubation in 100% N_2 was not statistically different from the renin concentration of slices after incubation in 100% O_2 ($P > 0.5$).

TABLE I. The Effects of Various Drugs Added to the Incubation Medium on Renin Secretion by Rat Kidney Slices.

Drug	Conc. μg/ml	N	Renin secretion (ng/100 mg) Control[a]	Expt.[a]	P
Epinephrine	5	5	28.5 ± 4.2	29.4 ± 6.9	$p > 0.9$
Norepinephrine	5	5	28.5 ± 4.2	45.6 ± 7.8	$p > 0.1$
Furosemide	20	5	47.4 ± 3.6	58.8 ± 15.3	$p > 0.4$
	40	5	48.6 ± 9.3	69.3 ± 12.9	$p > 0.2$
	200	5	34.2 ± 16.2	24.6 ± 7.2	$p > 0.5$
Protaglandin E_1	1	5	35.7 ± 12.9	38.4 ± 14.4	$p > 0.9$
NaCl	200[b]	10	84.6 ± 16.5	83.7 ± 12.9	$p > 0.9$

[a] mean ± sem.

[b] millimolar concentration.

Effect of drugs known to alter renin secretion in vivo (*Table I*). There was no significant change in the secretion of renin following the addition of epinephrine, furosemide, prostaglandin E_1, or NaCl to the Robinson's medium. Norepinephrine did appear to show a slight increase, but the difference was not significant.

Effects of prior treatment with high-salt diet and furosemide. When furosemide was injected intraperitoneally 45 min prior to killing of the rats, the release of renin from the kidney slices was not statistically different from that released from the slices of control rats injected with isotonic saline (276 ± 190 vs 209 ± 32 ng/100 mg) ($P > 0.2$). A prominent diuresis was noted from the rat following the furosemide injection. The renin content of slices from furosemide-treated rats (1660 ± 190 ng/100 mg) did not differ from the renin content of the slices from control rats (1353 ± 168 ng/100 mg) ($P > 0.2$).

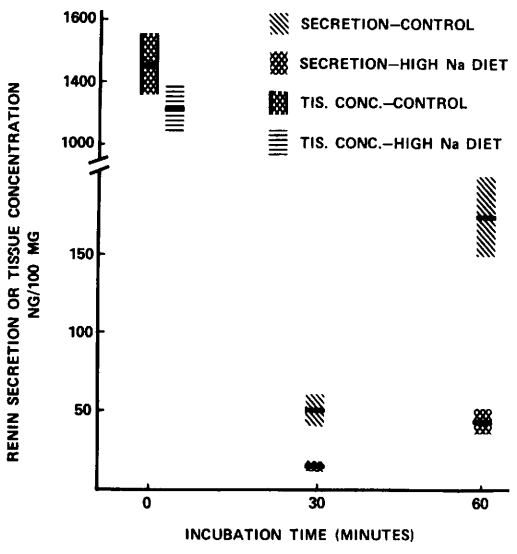

FIG. 3. Effect of a high sodium chloride diet on the release of renin and the slice concentration. Data is presented as mean ± SEM.

Slices from rats on a high-NaCl diet (Fig. 3) secreted renin into the Robinson's medium at a slower rate than slices from rats on a control diet during both 30-min incubation periods. The concentrations of renin in the slices from the two groups of rats immediately after removal from the rats were not different.

Discussion. Braverman *et al.* have estimated that *in vitro* renin release is tenfold greater than in the intact rat (1). Our results show renin release during the second 30 min of incubation to be threefold greater than during the first, suggesting the incubation of kidney slices *in vitro* accelerated the secretion of renin into the Robinson's medium. When the renin secreted during our second period was expressed as a percentage of the total concentration in the slice, our values were lower than the 10–15%/hr reported by Braverman and DeVito (1, 2). We feel that because of the longer incubation time (2–7 hr) used by these investigators, the rate of renin release may have been accelerated to a greater extent in their preparations. The concentration of renin in the slice did not change during the combined 60 min of incubation. Since the rate of release tripled during this period, the data suggest there is not a direct correlation between renin release and the concentration of renin

in the slice. DeVito *et al.* were also unable to show a correlation (2).

Release of renin into the medium was depressed by a nitrogen atmosphere, but during the same period the tissue concentration of renin remained constant, suggesting renin release in both oxygen dependent and an active process. Slices incubated under nitrogen did secrete renin, and the rate of secretion increased during the second 30-min period. These data suggest that anaerobic metabolism is supporting the release when the incubation is carried out under nitrogen, since it is doubtful a passive washout would increase with time.

Intrarenal arterial infusions of epinephrine and norepinephrine have been reported to increase the secretion of renin in the intact kidney (4). Furthermore, Johnson *et al.* have shown that these agents cause an increased renin secretion in nonfiltering kidneys (macula densa inoperative), and norepinephrine still caused a stimulation in the model when the intrarenal baroceptors were blocked with papaverine (11). These findings suggest norepinephrine and epinephrine may have a direct effect on the juxtoglomerular cells. We were unable to show stimulation with epinephrine, and norepinephrine caused a marginal increase although not significant. DeVito *et al.* (2) also did not find stimulation when using these drugs in the slice preparation, but Michelakis *et al.* did find a stimulatory effect on renin with both agents using an *in vitro* dog kidney homogenate system (12). The possible differences between the two *in vitro* systems which would account for the varied effects from the catecholamines are not understood but may be due to the different species used.

Furosemide has been shown to stimulate renin secretion in intact dog kidneys despite the replacement of fluids lost through diuresis (5). These data suggest that the action of furosemide is not due to volume depletion but through a direct action on the macula densa of the distal tubule. When furosemide was added to the incubating kidney slices, stimulation of renin release could not be shown. Furosemide pretreatment of rats did not show stimulation of renin release *in vitro*. The rate of release from the slices could not be elevated with any of the drugs tested. This may be due to an absence of a direct stimulation on the macula densa cells by these drugs.

However, this data is also compatible with the previous suggestion that secretion by the *in vitro* system may be maximal. The negative effect on renin secretion from the administration of furosemide to the animal or directly to the flask supports this concept.

Summary. This study was performed in order to evaluate the status of renin release from rat kidney slices. The results suggest that renin release *in vitro* is an active process occurring under both aerobic and anaerobic conditions. The rate of renin release is accelerated during the *in vitro* incubation, and any further stimulation with the addition of renin-stimulating drugs to the medium was not demonstrated. The rate of release could be depressed by placing the rat on a high-salt diet.

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