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The Influence of Ouabain on *in Vitro* Renin Secretion (37970)

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The most tenable hypothesis concerning the role of sodium in the control of renin secretion is that increasing sodium concentration or load in the region of the macula densa inhibits the secretion of renin (11). With one exception, however, (8), *in vitro* studies have demonstrated that renin secretion from tissue slices or cell suspensions is directly proportional to the sodium concentration of the incubation medium (1, 3, 5, 6, 9, 12). A possible explanation for this seeming paradox may be that the sodium receptor controlling renin is intracellular or intratubular (11), and that changing the medium sodium concentration does not necessarily change sodium concentration at these critical sites.

We have monitored renin secretion from rat kidney cortex slices in the presence of ouabain. Under this circumstance, intracellular sodium concentration parallels medium sodium concentration (7).

Methods. Sprague–Dawley rats weighing 250–400 g were bilaterally nephrectomized under sodium pentobarbital anesthesia (Nembutal, 50–60 mg/kg body wt). Kidneys were placed in ice-cold 0.15 *M* NaCl and the capsules removed. Four cortical slices (20–30 mg dry wt) were prepared from each kidney as previously described (2). Each slice was preincubated for 30 min in Krebs–Ringer bicarbonate solution containing 0.2% glucose and equilibrated with 95% O_2 and 5% CO_2 in an oscillating incubator at 37°. The preincubation solutions were then replaced with solutions of varying sodium concentrations (50, 75, 100, 125, and 144 mEq/liter), and the slices were incubated at 37° for 90 min. At 30, 60, and 90 min, two 20-μl samples were taken from each flask for renin determinations. Other slices were incubated identically except 10^{-3} *M* ouabain (Inland Alkaloid Co.) was added to the incubation media.

The renin activity of these samples was determined by radioimmunoassay. Each 20-μl sample of incubation medium was added to 100 μl of rat renin substrate (10); dimercaprol and 8-OH quinoline were added to inhibit the activity of converting enzyme, and this mixture was incubated at 37° for 2 hr. Samples of this mixture were removed at hourly intervals and analyzed for angiotensin I using the New England Nuclear (Boston, MA) renin activity radioimmunoassay kit. The results are presented as ng angiotensin I (hour of incubation of medium with renin substrate)$^{-1}$ (mg dry weight of kidney slice)$^{-1}$ or ng A-I $\times$ hr^{-1} $\times$ mg^{-1}.

Results. The relation between renin secretion and medium sodium concentration is shown in Fig. 1. The data for this figure were derived from 11 renal cortical tissue slices incubated for 90 min in media of sodium concentrations ranging from 50 to 144 mEq/liter. Renin secretion increased as medium sodium concentration increased ($P < 0.001$, least-squares linear regression analysis). The same direct relationship was observed with shorter incubation periods although the slopes of the regression lines were reduced (Table I). These results are in agreement with the majority of previous *in vitro* renin-secretion investigations (1, 3, 5, 6, 9, 12).

The relation between medium sodium concentration and renin was inverse in the presence of 10^{-3} *M* ouabain as is shown in Fig. 2. Data for this figure were obtained from ten renal cortical slices incubated for

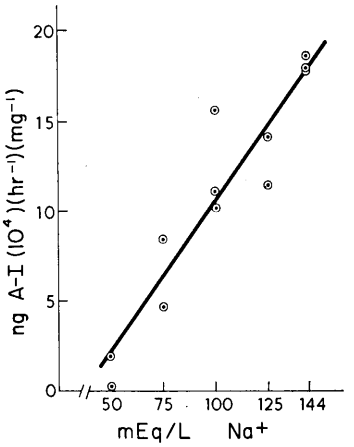

FIG. 1. Renin secretion as a function of sodium concentration of incubation medium. Slices were incubated for 90 min. Renin activity of incubation medium was measured as ng angiotensin I generated per hour of incubation of rat renin substrate with a sample of the slice incubation medium per mg dry weight of the slice.

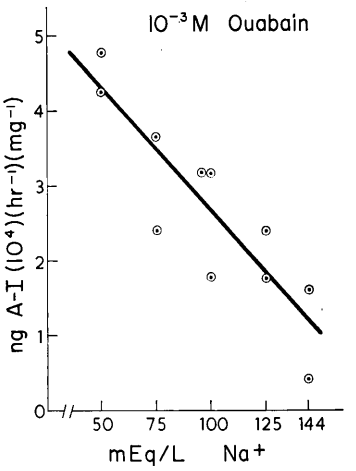

FIG. 2. Renin secretion as a function of sodium concentration of incubation medium in the presence of 10^{-3} *M* ouabain. Slices were incubated for 90 min. Renin activity of incubation medium was measured as ng angiotensin I generated per hour of incubation of rat renin substrate with a sample of the slice incubation medium per mg dry weight of the slice.

90 min in media of the same sodium concentrations as described above, but in the presence of 10^{-3} *M* ouabain. With ouabain, as medium sodium concentration increased, renin secretion decreased ($P < 0.001$, least-squares linear regression analysis). Regression line slopes were less negative with shorter incubation periods, as is shown in Table II.

Discussion. Ouabain is a cardiac glycoside believed to inhibit active potassium transport across epithelial membranes. In its presence, intracellular sodium concentration parallels the sodium concentration of the incubation solution (4, 7). In our studies, when ouabain was added to the incubation solutions, increases in medium sodium concentration were associated with decreases in *in vitro* renin-secretion rate. These results are entirely consistent with Vander's hypothesis (11).

On the other hand, we and others (1, 3, 5, 6, 9, 12) have observed exactly the opposite relation between medium sodium concentration and *in vitro* renin secretion in the absence of ouabain. Renin secretion increases with increasing sodium concentration. For this observation to accord with Vander's hypothesis (11), sodium concentration at the critical site (intracellular, intratubular) must then be inversely related to the concentration of sodium in the incubation medium. Although this could be the case, it seems reasonable to believe that something besides intracellular concentra-

TABLE I. *In Vitro* Secretion of Renin at Varying Sodium Concentrations.

Incubation period (min)	Regression line	Correlation coefficient
30	$y = 0.0353\ x - 1.420$	+0.892
60	$y = 0.1145\ x - 5.0718$	+0.825
90	$y = 0.1662\ x - 6.1259$	+0.930

TABLE II. *In Vitro* Secretion of Renin at Varying Sodium Concentrations with 10^{-3} *M* ouabain.

Incubation period (min)	Regression line	Correlation coefficient
30	$y = -0.0076\ x + 2.02$	+0.875
60	$y = -0.0190\ x + 3.58$	+0.894
90	$y = -0.0333\ x + 5.91$	+0.885

tion might control renin secretion, at least *in vitro*. Studies are currently underway evaluating the possibilities that intracellular potassium concentration, osmolality, or cell volume might control renin secretion *in vitro*.

Summary. Using rat kidney cortex slices, *in vitro* renin secretion was measured as a function of sodium concentration of the incubation medium. We found, as have many others previously, that renin secretion increases with increasing medium sodium concentration. However, in the presence of 10^{-3} *M* ouabain, renin secretion decreased with increasing medium sodium concentration. Since others have shown that intracellular sodium concentration varies directly with medium sodium concentration in the presence of ouabain, it seems unlikely that these results can be explained by a single unifying hypothesis, such as that renin secretion is inversely related to intracellular sodium concentration.

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1. Braverman, B., Freeman, R. H., and Rosterfer, H. H., Proc. Soc. Exp. Biol. Med. **138**, 81 (1971).

2. Churchill, P. C., and Malvin, R. L., Amer. J. Physiol. **218**, 353 (1970).

3. Forgacs, I., and Gaal, K., Acta Physiol. Acad. Sci. Hung. **40**, 139 (1971).

4. Glynn, I. M., Pharmac. Rev. **16**, 381 (1964).

5. Hammersen, G., Karsunky, K. D., Fischinger, J., Rosenthal, J., and Taugner, R., Pflugers Arch. **328**, 344 (1971).

6. Karsunky, K. P., Fischinger, J., and Taugner, R., Pflugers Arch. **312**, R89 (1969).

7. Maude, D. L., Amer. J. Physiol. **216**, 1199 (1969).

8. Michelakis, A. H., Proc. Soc. Exp. Biol. Med. **137**, 833 (1971).

9. Oeklers, W., Molzahn, M., Samwer, K. F., Kreiser, H., and Kutschke, H., Pflugers Arch. **321**, 67 (1970).

10. Schaechtelin, G., Chomety, F., Regoli, D., and Peters, G. Helv. Physiol. Acta **24**, 89 (1966).

11. Vander, A. J., Physiol. Rev. **47**, 359 (1967).

12. Weinberger, M. H., and Rosner, D. R., *in* "Control of Renin Secretion," (T. A. Assaykeen, ed.) pp. 145–150. Plenum Press, New York (1972).

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