The Effect of Sodium and Calcium on Renin Release *in Vitro*[1] (35677)

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Extracellular cations are known to play an important role in the release of storage products from various secretory cells. The release of oxytocin and vasopressin from the neurohypophysis (1), catecholamines from the adrenal medulla (2), and acetylcholine from nerve endings at the neuromuscular junction (3) are examples of calcium-dependent secretion. Also, extracellular calcium and sodium have been shown to play an important role on the release of insulin from the β cells of the pancreas *in vitro* (4). The present investigation was undertaken to evaluate the effects of calcium and sodium in the *in vitro* release of renin utilizing renal cortical slices from canine kidneys. The results suggest that both cations affect the release of this hormone.

Materials and Methods. Preparation of the renal cortical slices. Dog kidneys were utilized. Without special dietary preparation, young mongrel dogs weighing 15–20 kg were anesthetized with cyclopropane. Through an abdominal incision, both kidneys were excised and immediately placed in ice. A needle was tied in place in each renal artery, and the kidney was perfused with Krebs-Ringer bicarbonate buffer to which 0.2% (w/v) glucose had been added. This perfusion continued until the fluid coming out through the renal vein was clear. The cortex was then removed, thin slices were prepared from it using a Steady-Riggs microtome, and they were immediately placed in buffer of the same composition as above. The renal cortical slices obtained were washed with the same buffer in the cold and within 30 min were used in the experimental incubations as described below. Prior to the incubation, the tissue was kept at 4° at all times.

Experimental incubations with media of various sodium content. Krebs-Ringer bicarbonate buffers of 50, 100, 144, 200, and 300 m*M*/liter sodium were prepared and to each, 0.2% (w/v) glucose was added. The osmolality of the first two buffers was adjusted to that of the 144 m*M* sodium buffer by the addition of sucrose. After the preparation and randomization of the renal cortical slices, a series of tissue samples weighing 1 g each were placed in Erlenmeyer flasks which were kept in the cold. All samples were run in duplicate, and they were kept at 4° prior to the incubation. To each sample 10 ml of the 50, 100, 144, and 300 m*M*/liter sodium buffer were added, respectively, and then they were incubated for 30 min 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 suspending medium was removed and stored at —20° until the time of renin determination.

Experimental incubations in media of various calcium content. Krebs-Ringer bicarbonate buffers of 0, 1.25, 2.5, and 3.75 m*M*/liter calcium were prepared and to each, 0.2% (w/v) glucose was added. As in the case of the sodium study, the slices were randomized and kept in the cold prior to the incubation. Tissue samples of 1 g each were placed in Erlenmeyer flasks which were kept on ice. Then 10 ml of the above buffers were added to each sample, respectively. All samples were run in triplicate. The incubations were carried out for 30 min in a 37° water bath with slow constant shaking under an atmosphere of 95% oxygen and 5% carbon

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dioxide. At the end of the incubation period the suspending medium was removed and stored at —20° until the time of renin determination.

Preparation of renin substrate. Forty-eight hours after bilateral nephrectomy, the blood was drawn from the aorta of the dog into tubes containing EDTA as anticoagulant. Without delay, the blood was centrifuged in the cold, and plasma was separated and stored at —20° until use. This plasma was then used as an autologous substrate for the measurement of renin in each sample.

Renin measurement. An aliquot of suspending medium from each incubated sample was added to 10 ml of the above renin substrate, and the renin activity was measured by known procedures (5). 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 plasma 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 found to be undetectable.

Results. Effect of sodium concentration on renin release in vitro. Five different series of experiments were performed in which the effect of sodium concentration on renin release from renal cortical slices into the incubation medium was studied. A total of ten samples were incubated at each of the different sodium concentrations. Kidneys from different dogs were used for the preparation of each series of samples. The combined data are shown in Fig. 1. In all experiments the renin release was the highest in the samples incubated in the 50 m*M* sodium buffer followed by that released in the samples incubated in the 100 m*M* sodium buffer, as compared to that released in the control samples incubated in the 144 m*M*/liter sodium buffer. This increase in renin release could not be attributed to the effect of changes in osmolality since adjustment was made by the addition of sucrose in the appropriate amount.

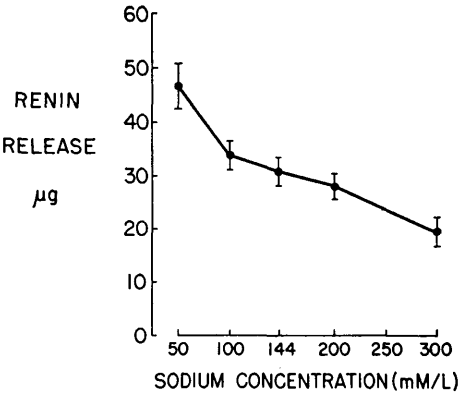

FIG. 1. Renin release from renal cortical slices in response to various sodium concentrations in the incubation medium. Bracketed lines indicate SE.

At concentrations of sodium higher than normal, the renin release from the renal cortical slices into the incubation medium was reduced. In all experiments the lowest renin release was observed in the samples incubated in the 300 m*M* sodium buffer (Fig. 1).

Effect of calcium concentration on renin release in vitro. Six different series of experiments were performed in which the role of calcium concentration on renin release from dog renal cortical slices was studied. The samples of each series were run in triplicate. Thus, a total of 18 samples were incubated at each of the different calcium concentrations. As in the case of the sodium study, kidneys from different dogs were used for the preparation of the samples of each series of experiments. The combined data from these experiments are shown in Fig. 2. The highest amount of renin was released into the incubation medium with a calcium concentration at the level of 2.5 m*M*. Increase of calcium concentration to 3.75 m*M* failed to raise further the release of renin. Omission of calcium from the incubation medium markedly inhibited renin release (Fig. 2).

Since calcium containing buffer was used for the preparation of slices and since in the presence of calcium renin release is enhanced as shown above, it is possible that renin loss from the tissue occurred during the preparation of the slices. Such renin loss may influence the extent to which calcium ions could affect renin release during incubation. Addi-

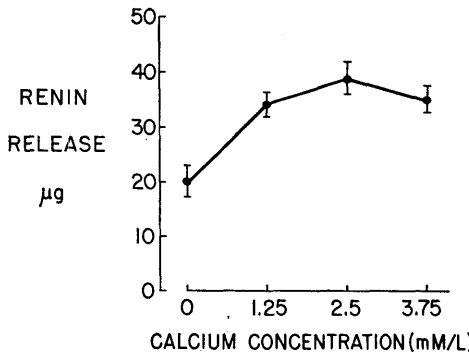

FIG. 2. Renin release from renal cortical slices in response to various calcium concentrations in the incubation medium. Bracketed lines indicate SE.

tional experiments were, therefore, carried out in order to minimize loss of renin from the cells during the preparation of the slices and to find out to what extent calcium ions affect renin release from the storage sites with higher renin content. To accomplish this, the kidney was perfused with buffer to which no calcium was added. The renal cortical slices were then prepared in the usual manner and kept in buffer without calcium until the time of the experimental incubations. The results were similar to those of the experiments described above. In the presence of calcium there was again a greater release of renin reaching maximum amounts in samples incubated in 2.5 m*M* calcium buffer. Again, there was marked reduction of renin release from slices incubated in buffer to which no calcium was added.

Discussion. Although the regulation of renin secretion by the kidney has been the subject of numerous studies, it remains incompletely understood. Previous studies have indicated that the intact kidney responds to hypertonic sodium chloride solutions by secreting less renin and that it responds to catecholamines by secreting more renin than under control conditions. In addition, studies performed in our laboratory have shown that catecholamines and cyclic AMP affect renin production *in vitro* (6), however, little is known of the effect of the ionic environment on renin secretion.

Utilizing a preparation of renal cortical slices, we have made observations on the effect of the cations sodium and calcium on renin release in an attempt to learn whether or not these ions are involved in the release of this hormone by the renal cortex and have examined the effect of the ionic environment that may possibly influence secretory activity of the juxtaglomerular cells. The findings show that changes in concentration of sodium and calcium affect renin release *in vitro*.

It has been suggested that sodium "load" at the level of the macula densa plays a role in regulating renin secretion in the intact animal (7). The present study shows that renin release decreased in an approximate linear fashion with increase of sodium concentration in the incubation medium. These findings are consistent with the view that sodium may inhibit renin secretion (7), and suggest that its action could be a direct one on the renal cells. Although the decrease of renin release in the presence of excess of sodium ions might be considered as a nonspecific effect of extracellular hyperosmolarity, the increase of renin release observed with low sodium concentration could not be explained by osmolarity changes since proper adjustment was made with sucrose.

Calcium has been shown to be necessary for the secretion of several hormones (1–4). The present studies show that renin release is increased with increase of calcium concentration in the incubation medium and that the absence of calcium markedly inhibited renin release. The role, however, that calcium levels might play in the release of renin in the intact animal remains to be determined. In contrast, the demonstration that changes in sodium ion levels affect renin release *in vitro* may be of considerable importance since renin secretion is known to be influenced in the intact organism by sodium intake.

The data obtained in the present study suggest that both calcium and sodium may play a role in renin release. It is possible that these ions act at the juxtaglomerular cell membrane in a mechanism similar to that reported for their action on insulin release by the β cells of the pancreas (4). The relationship, however, of either ion to the mechanism of renin secretion remains to be determined regardless whether it is concerned with the integrity of the cell membrane or whether it

is intimately involved in some other step in the renin secretory process.

Summary. A preparation of dog renal cortical slices suitable for the study of renin release was utilized to study the effect of sodium and calcium ions on the release of this enzyme. An inverse relationship was found between renin release and sodium concentration in the incubation medium. When calcium ion concentration in the incubation medium was the only variable renin release increased with calcium and reached optimal level at around 2.5 m*M* calcium. The omission of calcium markedly inhibited renin release. The results suggest that both sodium and calcium ions may play a role in the release of renin at the cellular level.

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