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Tissue Kallikrein Levels and Synthesis Rates Are Not Changed by an Acute Physiological Dose of Aldosterone (42152)

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Abstract. Tissue kallikrein content and rate of synthesis have been measured in the kidney and submaxillary gland of the rat after a single physiological dose of aldosterone, and during the time in which the physiological effects of aldosterone are evident. No change in the renal cortical tissue content of immunoreactive kallikrein, in either adrenalectomized or sham-operated animals, was seen within 3 hr of the administration of aldosterone. The kininogenase activity of these samples also did not change. The rates of kallikrein synthesis in the submaxillary gland and the whole kidney were measured by specific immunoprecipitation of kallikrein after 20 min of [^{35}S]methionine incorporation, and again no aldosterone effect was seen. We conclude that, under the conditions of these experiments, aldosterone does not induce the synthesis of tissue kallikrein in either the submaxillary gland or the kidney.

Renal kallikrein (EC 3.4.21.8) levels have been shown to change in response to a variety of stimuli involving mineralocorticoid effects. A sodium-restricted diet, for example, resulted in a significant increase in urinary kallikrein excretion, kidney kallikrein concentration, and rate of kallikrein synthesis (1–3). Administration of deoxycorticosterone to rats or fludrocortisone to man increased urinary kallikrein excretion, whereas spironolactone, a mineralocorticoid receptor antagonist, decreased urinary kallikrein excretion in man (4, 5). Furthermore, addition of aldosterone to a renal cortical cell suspension increased the production of kallikrein, as measured by its appearance in the medium (6).

These findings tend to support the contention (6) that tissue kallikrein might be an aldosterone-induced protein. This suggestion has subsequently been discussed by others (7–9). Indeed, the daily administration of pharmacologic amounts of aldosterone over a period of 1–2 weeks has been shown to increase kidney kallikrein concentration in the rat (10, 11). However, the suggestion has also been disputed by some studies which have failed to demonstrate such an effect (12–14).

The effects of aldosterone on sodium transport are evident within 1–3 hr of administration of the hormone (15), and previous reports have described increased synthesis of proteins within this period (16–18). If increased synthesis of kallikrein could be demonstrated during this time, the suggestion that it has a role as a mediator of the physiological action of mineralocorticoids on tubular electrolyte transport would be strengthened. If no such increase can be shown, however, the idea would be seriously weakened. Accordingly, we have measured both kallikrein concentration and the rate of kallikrein synthesis in the kidney and submaxillary gland of the rat after a single, physiological dose of aldosterone, and within the time period during which an effect on renal electrolyte transport could be demonstrated.

Materials and Methods. Male Sprague–Dawley rats, 250–350 g, were obtained from a local supplier. They were housed in a light-controlled (12-hr light/12-hr dark), constant temperature (25°C) room, and allowed free access to tap water and normal lab chow (0.18 meq Na^+/g; Wayne Pet Food Division, Chicago, Ill.). When adrenalectomized animals were used, they were either purchased as such from Sprague–Dawley (Madison, Wis.), or bilateral adrenalectomies were performed locally under sodium pentobarbital anesthesia. The adrenalectomized animals were then housed and fed in the same way as the normal animals, but were given a saline solution to drink. When only tissue

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kallikrein concentrations were measured, 1% saline was given for 10–14 days prior to the experiments. When the rate of tissue kallikrein synthesis was determined, 0.45% saline was given for only 3 days after adrenalectomy and then replaced with distilled water. Two days later, or a total of 5 days after the adrenalectomy, the labeling experiments were performed. This period of steroid depletion was used to maximize any aldosterone-induced effect subsequent to the known increase in aldosterone receptor binding which occurs after adrenalectomy (19). Lack of supplemental sodium was to avoid the kallikrein-repressing effect sometimes seen during high salt intake (20).

D-Aldosterone (Sigma Chemical Co., St. Louis, Mo.) was dissolved at 1 mg/ml in ethanol. Just prior to use, it was diluted 1:100 into 0.15 *M* NaCl, then injected intraperitoneally at 1.6 μg aldosterone/100 g body wt. For experiments not involving labeling, the animals were killed 3 hr later. In labeling experiments, 2 hr after aldosterone administration, short-term labeling was carried out as previously described (3) to determine the rate of incorporation of radioactive amino acid into tissue kallikrein. [^{35}S]Methionine (sp act ~ 400 Ci/mmole; New England Nuclear Corp., Boston, Mass.) was diluted with 0.15 *M* NaCl to a volume of 0.2–0.3 ml and injected intraperitoneally at a dose of 0.8 mCi/kg body wt. The animals were killed 20 min after injection of the radioactive label.

Tissue preparation has been described in detail (3), and consists of deoxycholate solubilization of kallikrein in tissue homogenates followed by immunoprecipitation of kallikrein with a polyclonal antiserum directed against rat urinary kallikrein. The immunoprecipitates are washed, dissolved with 0.1 *N* NaOH and incorporated radioactivity determined by liquid scintillation spectrometry. To determine the incorporation of radioactivity into total protein, trichloroacetic acid precipitates were also prepared. The ratio of kallikrein-specific radioactivity in the immunoprecipitate to total incorporated radioactivity in the trichloroacetic acid precipitate was used as the indicator of the rate of kallikrein synthesis (3).

Immunoreactive kallikrein in tissue homogenates was determined using the radioimmunoassay of Shimamoto *et al.* (21), and protein concentrations were measured by the method of Lowry *et al.* (22), using bovine serum albumin as standard. Kallikrein activity, measured by its kinin-forming activity with purified bovine low molecular weight kininogen as substrate, was measured as described by Shimamoto *et al.* (23).

Results and Discussion. The dose of aldosterone required to bring about a half-maximal change in urinary electrolyte excretion has been reported to be 0.35–0.45 μg/100 g body wt (17, 24). To be within the physiological range, and yet be close to the top of the dose–response curve, we have used an aldosterone dose of 1.6 μg/100 g body wt. This dose was given to five adrenalectomized rats maintained on the same fluid regimen as that used for the kallikrein synthesis experiments. It changed the Na^+/K^+ ratio in urine collected for 4 hr after the dose from 0.267 ± 0.077 to 0.055 ± 0.023 ($P < 0.02$). The ratio did not change in the five vehicle-injected animals.

When this dose was administered to either adrenalectomized or sham-operated rats, no significant differences in renal cortical kallikrein concentrations were found between the aldosterone and vehicle-treated groups within 3 hr of hormone administration, as seen in Table I. Neither immunoreactive kallikrein content nor total kininogenase activity of the tissue changed in response to aldosterone.

A change in the rate of kallikrein synthesis, though, due to the administration of aldosterone could be compensated for by changes in the rate of secretion or degradation of the enzyme. In this event, the measurement of an amount of kallikrein at any given time might not be different from control, and only a measure of the rate of synthesis itself would reveal an effect. For this reason the rate of kallikrein synthesis was measured 2 hr after the administration of aldosterone or vehicle. Synthesis was measured in the entire kidney as well as the submaxillary gland.

To optimize the conditions for seeing an aldosterone effect, the animals were adrenalectomized 5 days before the experiments. They drank 0.45% saline for 3 days, but over the last 2 days drank distilled water, with the normal rat chow diet throughout.

The results in Table II demonstrate that

TABLE I. RENAL CORTICAL KALLIKREIN AFTER ADMINISTRATION OF ALDOSTERONE

	Kininogenase activity[a]		Immunoreactive kallikrein[b]	
	Aldosterone	Vehicle	Aldosterone	Vehicle
Adx	3.82 ± 0.36[c]	4.19 ± 0.48	25.7 ± 1.6[c]	29.0 ± 2.2
Sham	5.24 ± 0.51[c]	5.66 ± 0.43	33.8 ± 2.1[c]	39.1 ± 1.5

[a] Expressed as pg kinin produced/μg protein/20 min.
[b] Expressed as ng kallikrein/mg protein.
[c] Not significantly different from vehicle group with nine animals in each group.

this dose of aldosterone does not affect the rate of kallikrein synthesis in either tissue, and again, the immunoreactive kallikrein content of the tissues is unchanged. The incorporation of the label into total protein is likewise not affected, but this is to be expected, as the number of aldosterone-induced proteins is only a small fraction of the total.

It is well established that exogenously administered aldosterone can increase kidney kallikrein in the rat. Nasjletti *et al.* (10), for example, showed that subcutaneous injection of 250 μg aldosterone per day for 14 consecutive days caused a significant increase in urinary kallikrein excretion. Nishimura *et al.* (11) likewise demonstrated that 250 μg aldosterone, injected subcutaneously for 6 days, would increase the kallikrein content of kidney tissue. It is important, however, to distinguish between true aldosterone-induced proteins, and proteins which might be synthesized secondarily, in response to the physiological changes brought about by aldosterone. Aldosterone has been shown to act by increasing the transcription of specific messenger RNAs (25). The translation of these messengers into proteins follows, and, within 3 hr of the administration of the hormone, changes in the sodium transporting capabilities of target epithelia result that are thought to depend upon the expression of the biological activities of these proteins (26). If tissue kallikrein were one of these proteins, its direct participation in the mechanism of sodium transport would be strongly indicated, and the identification of the still unknown functional role of this enzyme would be facilitated.

On the other hand, since effects of aldosterone on the production of the enzyme cannot be demonstrated within 3 hr, we must conclude that kallikrein's presumed role in ion transport (27) is manifested in some other

TABLE II. RATE OF KALLIKREIN SYNTHESIS AFTER ADMINISTRATION OF ALDOSTERONE TO ADRENALECTOMIZED RATS

	Kidney		Submaxillary gland	
	Aldosterone	Vehicle	Aldosterone	Vehicle
(1) ng kallikrein/ mg protein	35.1 ± 2.6 (n = 8)	34.2 ± 2.7 (n = 7)	(187 ± 18) × 10^3 (n = 8)	(183 ± 14) × 10^3 (n = 7)
(2) Kallikrein cpm/ mg protein	10.1 ± 0.7 (n = 7)	12.6 ± 1.6 (n = 6)	486 ± 64 (n = 8)	383 ± 49 (n = 6)
(3) Total protein cpm/ mg protein	6231 ± 391 (n = 8)	7068 ± 444 (n = 6)	4194 ± 586 (n = 8)	4090 ± 679 (n = 6)
10^3 × Ratio[a] (2)/(3)	1.62 ± 0.1 (n = 7)	1.78 ± 0.21 (n = 6)	122 ± 12 (n = 8)	101 ± 13 (n = 6)

[a] Rate of kallikrein synthesis relative to total protein synthesis. There are no significant differences between aldosterone and vehicle-treated groups.

way, and the increases in kallikrein concentrations observed previously should be considered as secondary to the effects of aldosterone on sodium transport or even other cellular processes. For example, chronic administration of aldosterone can increase extracellular volume, cause alkalosis, and increase urinary calcium and magnesium excretion (28). Increases in kallikrein occurring over a period of days could therefore be a response to one or more of these phenomena, and still be considered as being influenced by aldosterone, though not necessarily induced by it.

Our results are entirely consistent with a recent report that tested the effects of DOCA on kallikrein in the rabbit nephron (29). That study showed that chronic treatment with DOCA increased the kininogenase activity of kallikrein, localized by their method to the connecting tubule segment of the nephron. Acute administration to adrenalectomized rabbits, however, had no effect on kininogenase activity, measured 3 hr after DOCA injection.

A related issue is raised by further consideration of Table I. This table shows that an acute dose of aldosterone does not change the tissue content of kallikrein, as illustrated by there being no significant differences when comparing an aldosterone-treated group to a vehicle-treated group. But if the values for an adrenalectomized group are compared to those of the corresponding sham-operated group, it can be seen that adrenalectomy significantly reduces kallikrein content ($P < 0.05$ for kininogenase activity, $P < 0.01$ for immunoreactive kallikrein), as previously observed (4). Whether this reduction is due to a chronic lack of aldosterone, or to some other phenomenon related to the many physiological changes that occur after adrenalectomy, is unknown.

In summary, there was no increase in the kallikrein content of the rat kidney cortex in adrenalectomized or sham-operated animals when examined 3 hr after a physiological dose of aldosterone. Furthermore, the same aldosterone dose does not affect the rate of kallikrein synthesis, either in the kidney or the submaxillary gland, even though effects on urinary electrolyte composition can be demonstrated. We think that this rules out the possibility that tissue kallikrein is a protein induced by aldosterone to function in acute sodium retention by the renal tubule, although there is little question that it is somehow involved in some secondary response (3), at least in the presence of excess hormone. Tissue kallikrein seems likely to be importantly involved in epithelial ion transport by virtue of producing kinin, now known as a potent stimulus to epithelial chloride secretion (29, 30), but its involvement in aldosterone-regulated sodium reabsorption remains unclear.

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