SHORT COMMUNICATION

Dexamethasone, aldosterone and kallikrein release by isolated rat kidney

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Summary

1. The effect of D-aldosterone and dexamethasone on kallikrein release was studied in the isolated rat kidney perfused with modified Krebs–Henseleit buffer.

2. Animals received D-aldosterone or dexamethasone (10 μg/100 g body weight) daily for 3 days. Their isolated kidneys were then perfused with the same steroids at rates of 2.0–2.5 μg/min for 30 min.

3. Dexamethasone increased kallikrein release significantly (P < 0.001) both in urine and in venous outflow.

4. D-Aldosterone had no significant effect on kallikrein release, either in urine or in venous outflow.

Key words: D-aldosterone, bradykinin radioimmunoassay, dexamethasone, kallikrein release, perfused kidney, renal kallikrein–kinin system.

Introduction

The isolated kidney perfused in optimal conditions appears to be a suitable preparation to investigate the effect of different humoral agents and drugs on the renal kallikrein–kinin system [1, 2]. It has been shown that factors such as frusemide [2] or sodium overloading [3], which increase urinary kallikrein excretion in man [4], dogs [5] and rats [6], cause a similar response on the isolated rat kidney.

According to several reports, aldosterone could have a major regulatory influence on renal kallikrein [7–9]. Furthermore Margolius, Chao & Kaisu [10], using a suspension of renal cortical cells, showed that aldosterone increases kallikrein excretion, whereas spironolactone decreases it. They suggested that kallikrein could be an aldosterone-induced protein.

Corticosteroids may be important in the regulation of kidney humoral factors involved in blood pressure homeostasis. Hence, it was considered of interest to investigate the release of kallikrein by isolated kidney under the direct action of either D-aldosterone or dexamethasone. The latter was selected because studies on normal humans [11] and rats (H. R. Croxatto, personal communication) have demonstrated that this synthetic steroid, without a sodium-retaining effect, induced a considerable increase in urinary kallikrein excretion.

Methods

Experimental

Male Wistar–Holtzman rats (280–380 g) maintained on a regular diet were anaesthetized with pentobarbital sodium (50 mg/kg intravenously). The right kidneys were perfused with a blood-free defined medium by a procedure described previously [1, 12].

Before perfusion of the kidney the animals were treated for 3 days according to the following scheme. (1) Control group: 10 rats were injected with the solvent alone. (2) Aldosterone group: six rats received D-aldosterone, 10 μg/100 g body weight, intraperitoneally. (3) Dexamethasone group: 11 rats received dexamethasone, 10 μg/100 g body weight, intraperitoneally.

The animals were kept in individual cages and the right kidneys were prepared for perfusion on day 3, 2 h after the last injection. Heparin
(50–100 units, intravenously) and mannitol (50 mmol/100 g body weight) were given 30 min before perfusion was started. The fluid was delivered by pulsatile flow through a peristaltic pump (Harvard model 1003) and continuously filtered through an in-line Millipore filter (3 μm pore size). Perfusion pressure (100–120 mmHg) was recorded by a Statham transducer connected to a Grass polygraph and the flow rate (20–25 ml/min) was measured at regular intervals. Urine (10–14 μl/min) was collected in Eppendorf tubes of known weight.

After 20–30 min, when perfusion pressure, flow rate and diuresis had stabilized, the fluid was replaced by an identical medium containing either D-aldosterone (10 pg/dl) or dexamethasone (10 μg/dl). The perfusion rate was kept at 20–25 ml/min so that the dose rate for both steroids varied between 2.0 and 2.5 μg/min. At the end of the perfusion period the total dose administered to the kidney was 60–75 μg of either D-aldosterone or dexamethasone.

Thereafter a single-pass perfusion system was used. Samples of urine and of the perfusate were collected, at 15 and 30 min, for kallikrein determination.

**Kallikrein determination**

The activities of the enzymes in venous outflow and urine samples were measured by two methods: (1) bioassay as described by Croxatto & Noé [13] with isolated rat uterus; (2) radioimmunoassay [14] with 125I-labelled tyrosine-8-bradykinin (New England Nuclear) and synthetic bradykinin (Sandoz) as standard.

Kininogen were generated by incubating either urine (5–10 μl) or the perfusate (0.5–1.0 ml) with dog plasma kininogen [15] in phosphate buffer (0.1 mol/l, pH 8.5) for 15 min at 37°C, in the presence of kininase inhibitors (0.3 mmol of 1,10-phenanthroline and 30 mmol of disodium EDTA). The enzymatic reaction was stopped with ethanol (96%) and the kinins were isolated [14].

Kallikrein release, either in venous outflow or in urine, was expressed as ng of bradykinin/min of perfusion.

**Results**

A good correlation \( r = 0.084, P < 0.001 \) was found between bioassay and radioimmunoassay measurements of kallikrein. The values obtained by radioimmunoassay are reported here therefore.

Control and experimental data are presented in

![Fig. 1](image-url)

**Discussion**

The increase of kallikrein excretion produced by dexamethasone demonstrated a direct effect of this steroid on the kidney. Our results are in keeping with observations in normal human subjects, where a single injection of the synthetic glucocorticoid enhances considerably urinary kallikrein excretion [11]. However, the lack of effect of aldosterone does not agree with other findings previously reported [10]. Most of the evidence as to the effect of mineralocorticoids is indirect; it is based on observations made in patients with hyperaldosteronism or on the effect of drugs such as fluorocortisone and spironolactone. In the case of hyperaldosteronism, other factors such as sodium retention, volume expansion or the effect of other corticoids cannot be discounted as a cause of kallikrein increase.

The stimulating effect of aldosterone on isolated renal cortical cells [10] was obtained by using...
Aldosterone and dexamethasone on kallikrein release

extremely high hormone doses. R. Arriagada (personal communication), using D-aldosterone (1–10 µg/rat), was unable to observe any change in urinary kallikrein. Under the same experimental conditions, dexamethasone (5–10 µg/rat) produced a significant increase in the kallikrein excretory rate (H. R. Croxatto, unpublished work).

Despite these contradictory results, corticoids appear to be involved in blood pressure homeostasis; Hoefnagels, Drayer, Hofman, Kloppenborg & Bennraad [16] demonstrated that dexamethasone could normalize blood pressure in hypertensive patients showing signs of adrenal hyperfunction. The stimulatory effect of dexamethasone on the kallikrein–kinin system may partially explain the hypotensive effects in these patients.

Furthermore the high level of renal kallikrein released by the perfused kidneys might account for the occurrence of glandular kallikrein in blood plasma in normal rats [17].

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References


