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THE INFLUENCE OF TETRACOSACTIDE AND ADRENAL STEROIDS ON RENAL KALLIKREIN ACTIVITY AND URINARY KALLIKREIN EXCRETION IN RATS

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Abstract—The effect of adrenal steroids (mineralo- and glucocorticoids) as well as that of the adrenocorticotrophic peptide tetracosactide (β^{1-24} corticotropin) on the renal kallikrein activity and on the urinary kallikrein excretion of rats was investigated. After the animals had been adapted to metabolic cages, they were injected with deoxycorticosterone acetate (15 mg/kg day), corticosterone (40 mg/kg day), both steroids combined or the vehicle (sesame oil). Additional groups of rats received tetracosactide (0.05, 0.1 or 0.2 mg/day) or the vehicle (100 μl of 38×10^{-3} M $ZnCl_2$).

After four days of treatment the urinary kallikrein excretion was higher in deoxycorticosterone-treated rats than in their controls. This increase was prevented when corticosterone was administered simultaneously. The renal kallikrein activity of corticosterone as well as that of deoxycorticosterone plus corticosterone-treated rats was subnormal. A dose-related reduction of both the renal kallikrein activity and the urinary kallikrein excretion was observed 2 days after starting the tetracosactide administration. It may be concluded that a stimulation of the endogenous release of glucocorticoids in the rat reduces the renal kallikrein activity and that glucocorticoids can prevent the stimulating effect of mineralocorticoids.

It has been repeatedly shown that mineralocorticoid administration causes a rise of the renal kallikrein activity and of the urinary kallikrein excretion [1–6]. Manoeuvres which stimulate the endogenous release of aldosterone, such as low sodium or high potassium diet, also lead to an enhancement of renal and urinary kallikrein activity [7–10]. However, some exceptions have been reported (see ref. 11 for references). For instance, in spite of the secondary aldosteronism the renal kallikrein activity and the urinary kallikrein excretion are subnormal in rats with renal hypertension [12–14]. This suggests the existence of a factor or factors which interfere with the kallikrein stimulating effect of the mineralocorticoids.

We have previously shown that corticosterone, the main glucocorticoid of the rat, and tetracosactide (β^{1-24} corticotropin) reduce the urinary excretion of kallikrein in that species [4, 15]. To test whether corticosterone could counteract the kallikrein stimulating effect of deoxycorticosterone, both steroids were administered simultaneously. The influence of various doses of tetracosactid on both renal kallikrein activity and urinary kallikrein excretion was also investigated.

MATERIALS AND METHODS

Male Wistar rats (Dr Karl Thomae GmbH, 7950 Biberach, F.R.G.) having a body weight of about 250 g were used.

Corticosterone and deoxycorticosterone administration

Seven animals were allotted to each of four groups. Deoxycorticosterone acetate (15 mg/kg body wt day) was injected subcutaneously (s.c.) in 0.2 ml sesame oil once daily to the animals of the first group. The rats of the second group were injected twice daily with corticosterone (40 mg/kg body wt day, s.c.). Both deoxycorticosterone and corticosterone (same doses as above) were given to the rats of the third group. The fourth group of animals served as control. These animals received the vehicle (0.2 ml sesame oil/day, s.c.).

Urine was collected at room temperature in plastic containers while the rats were kept in stainless steel metabolic cages in a temperature, light and humidity (22°, 12 hr cycle, 50%) controlled room. Two 24-hr urine collections were performed starting on the fourth day of treatment. Then the rats were anesthetized with sodium pentobarbital (40–50 mg/kg body wt, i.p.) and the kidneys were excised. Urine and kidneys were kept frozen (−20°) until assayed.

Tetracosactide administration

Four groups of seven rats each were used. The rats of the control group were injected once daily s.c. with 100 μl of the vehicle, which contained 5.2 mg/ml zinc chloride. The rats of the other three groups received β^{1-24} corticotropin (Cortrophin S-Depot, Organon, Munich, F.R.G.) in a dose of 0.05, 0.1 and 0.2 mg/day, s.c. respectively. Urine was collected as described above, for two 24-hr periods, two days after initiating the treatment. The kidneys were excised under pentobarbital anesthesia (dose as above) at the end of the experiment.

The renal kallikrein activity was measured, after the kidneys had been homogenized and the enzyme solubilized with deoxycholate, using the synthetic

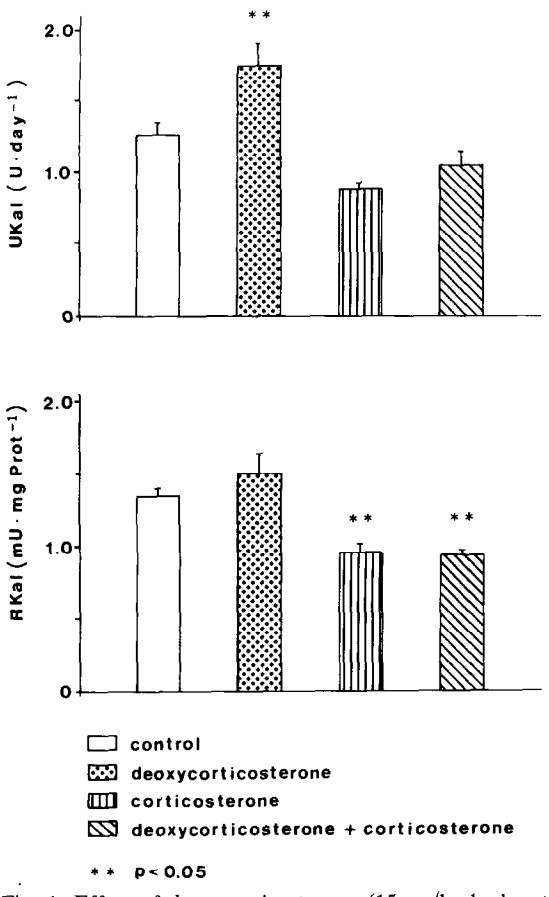

Fig. 1. Effect of deoxycorticosterone (15 mg/kg body wt day, s.c.), corticosterone (40 mg/kg body wt day, s.c.) or both combined on daily urinary kallikrein excretion (UKal) and on renal kallikrein activity (RKal) in rats. One Unit is the amount of enzyme capable of hydrolysing 1 μmole of the substrate per minute.

tripeptide substrate D-val-leu-arg-paranitroanilide as previously described [16]. Urinary kallikrein was measured with the same substrate [17, 18]. Protein in kidney homogenate supernatant was estimated by the method of Lowry *et al.* [19]. Since no differences between the first and the second urine collections were detected, only the data from the last day are reported. The results are expressed as means + standard error of the mean. The analysis of variance was used to assess the effect of treatments. The significance of the differences was calculated with the Scheffe's test.

RESULTS

Deoxycorticosterone treatment caused a rise of the urinary kallikrein excretion. This enhancement was prevented when, concomitant to deoxycorticosterone, the rats also received corticosterone. Although the kallikrein excretion of the rats treated with corticosterone alone (Fig. 1, upper panel) was lower than control this difference did not reach statistical significance. The slight enhancement of the renal kallikrein activity found in deoxycorticosterone-treated rats was also insignificant. On the other hand, administration of corticosterone reduced the renal kallikrein activity (Fig. 1, lower panel), so that deoxycorticosterone-treated rats had a higher kallikrein activity in their kidneys than rats treated with both steroids combined ($P < 0.01$).

Deoxycorticosterone-treated rats, with or without addition of corticosterone, had a higher urine flow than controls. The animals which were treated with both steroids combined ate less food than those of the control group (Table 1). Both kidneys from rats which received deoxycorticosterone were slightly heavier (1.99 ± 0.02 g) than those from controls (1.88 ± 0.07 g) whereas those from rats given corticosterone were slightly lighter (1.78 ± 0.02 g). Thus, a significant difference between deoxycorticosterone- and corticosterone-treated rats was detected ($P < 0.05$). The animals treated with both steroids had a total kidney weight of 1.88 ± 0.04 g. Although the blood pressure of corticosterone-treated animals (with or without deoxycorticosterone) rose, the difference did not reach statistical significance (Table 1).

Although significantly less urinary kallikrein excretion was only observed in the rats receiving the highest dose of tetracosactide, it is apparent that the excretion of the enzyme is reduced in a dose-dependent manner by the peptide (Fig. 2, upper panel).

Table 1. Effect of deoxycorticosterone and corticosterone

	Control	DOCA	B	DOCA + B
Food intake (g/day)	23.6 + 0.4	25.3 + 0.6	22.0 + 1.0	20.3 + 0.6*
Water intake (ml/day)	39.2 + 2.1	40.9 + 2.1	34.8 + 2.9	42.7 + 1.9
Urine flow (ml/day)	14.7 + 1.3	23.3 + 1.7*	14.6 + 1.4	28.5 + 2.2†
Blood pressure (mmHg)	117 + 4	115 + 5	132 + 8	127 + 5

Control: sesame oil (0.1 ml/day); DOCA: deoxycorticosterone acetate (15 mg/kg body wt day); B: corticosterone (40 mg/kg body wt day); DOCA + B: deoxycorticosterone and corticosterone combined (same dose as above). * $P < 0.05$ vs control; † $P < 0.01$ vs control.

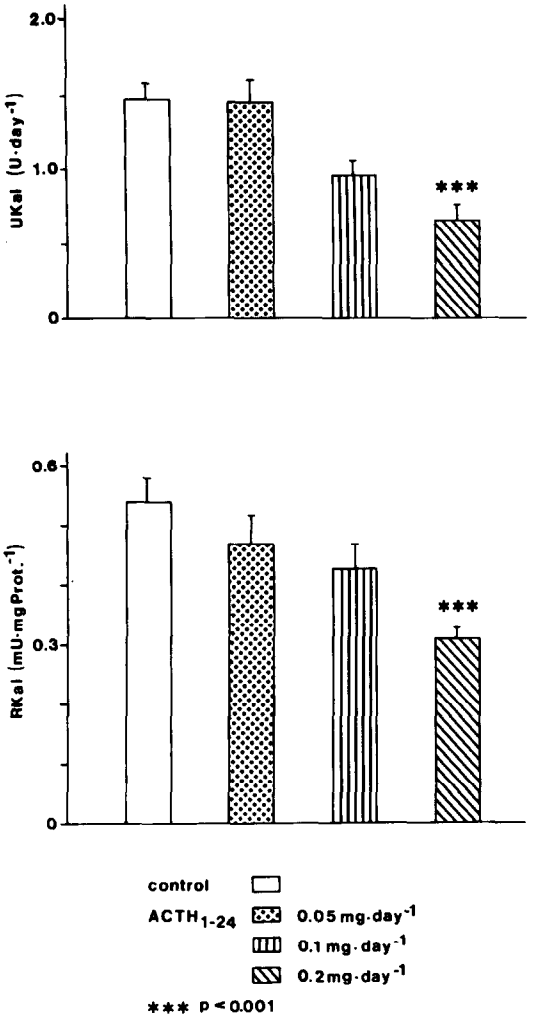

Fig. 2. Effect of tetracosactide (β^{1-24} corticotropin) on urinary kallikrein excretion (UKal) and on renal kallikrein activity (RKal) in rats. Units as defined in Fig. 1.

Similarly, the renal kallikrein activity was progressively lowered by increasing dose of β^{1-24} corticotropin. This reduction attained significance with the 0.2 mg/day dose (Fig. 2, lower panel).

No influence of tetracosactide on water intake and urine flow was detected. Although blood pressure showed a tendency to rise in β^{1-24} corticotropin-treated rats, this difference did not reach significance (Table 2). The total kidney weight rose from 1.82 ± 0.04 g in controls to 2.00 ± 0.04 g in rats which received 0.2 mg/day β^{1-24} corticotropin ($P < 0.05$). A total kidney weight of 1.83 ± 0.05 g was recorded in rats treated with 0.05 mg/day tetracosactide, and one of 1.87 ± 0.06 g in animals which received 0.1 mg/day of the peptide.

DISCUSSION

The observation that a rise of the plasma mineralocorticoid concentration, whether due to administration of an exogenous steroid or to a physiologic or pathologic enhancement of the endogenous aldosterone release, is accompanied by an increased renal kallikrein activity and an enhanced urinary kallikrein excretion [1–6, 20–22], prompted the suggestion that the mineralocorticoids are the natural regulators of the kallikrein activity. However, in some conditions a dissociation between the renal kallikrein activity and the aldosterone concentration has been reported [12, 20, 23–26]. We have previously shown that administration of corticosterone, the main glucocorticoid of the rat, reduces the kallikrein excretion in this animal [4]. A similar effect has been found after administration of a synthetic glucocorticoid to rats [27, 28]. Glucocorticoids could reduce kallikrein activity by acting at one of three different levels: (1) pre-renal, by altering the factors which regulate kallikrein synthesis (i.e. by reducing aldosterone secretion rate); (2) renal, by altering pre-kallikrein synthesis or the activation of pre-kallikrein to kallikrein; (3) post-renal, by enhancing kallikrein deactivation either through enzymatic degradation or through enhanced inhibitor concentration.

It is yet unclear if only one or more of these possible mechanisms is responsible for glucocorticoid-dependent kallikrein reduction. Although the aldosterone secretion rate is indeed lowered by corticosterone [4], this alone cannot explain the kallikrein lowering effect of this steroid. The reduction of renal kallikrein activity in the group of rats which in addition to deoxycorticosterone also

Table 2. Effect of tetracosactide

	Control	0.05 (mg/day)	0.1 (mg/day)	0.2 (mg/day)
Food intake (g/day)	17.8 + 1.1	21.8 + 0.8	18.7 + 1.5	16.4 + 0.7*
Water intake (ml/day)	32.1 + 1.2	37.4 + 1.9	37.5 + 3.5	35.2 + 3.0
Urine flow (ml/day)	16.2 + 1.0	19.7 + 1.8	22.5 + 2.4	24.9 + 3.2
Blood pressure (mmHg)	126 + 4	138 + 4	137 + 3	143 + 7

Control: 0.52 mg $ZnCl_2$, s.c.; * $P < 0.05$ vs tetracosactide 0.05 mg/day.

received corticosterone, indicates that the glucocorticoid exerts its inhibiting effect in spite of an increased level of mineralocorticoids.

There is some evidence supporting a renal site of action. It has been proposed that glucocorticoids may prevent the conversion of pre-kallikrein to kallikrein by releasing an inhibitor of a pre-kallikrein activator. This proposal is based on the observation that dexamethasone increases trypsin activatable kallikrein (= pre-kallikrein) in the basolateral membrane-enriched fraction of the rat kidney [29]. This effect of the glucocorticoid is distinct from that of reduced mineralocorticoid activity. In adrenalectomized rats the activatable enzyme was depressed.

Alternatively, the activity (and release) of renal kallikrein may depend on the effects of mineralocorticoids on distal tubule electrolyte transport. Corticosterone could functionally oppose these effects and thus, indirectly, reduce kallikrein activity.

Some evidence for an increased kallikrein deactivation after glucocorticoid administration has also been advanced. The excretion of proteins which bind and inhibit renal kallikrein rose during dexamethasone treatment to rats [28].

Thus, it appears likely that the kallikrein reduction produced by glucocorticoids depends on three different effects: (1) reduction of the aldosterone secretion rate; (2) reduction of pre-kallikrein activation; and (3) enhancement of renal kallikrein inhibitors.

The finding that corticosterone prevents the kallikrein-stimulating effect of deoxycorticosterone suggests that glucocorticoid overproduction may be responsible for the occasional dissociation between plasma aldosterone concentration and renal kallikrein activity. This may be only true if endogenous release of glucocorticoids would have the same effect. In a previous report we have shown that tetracosactide at a dose of 0.5 mg/kg day, in spite of stimulating aldosterone release, reduces the renal kallikrein activity and the urinary kallikrein excretion [15]. The present report confirms and extends those findings. The inhibiting effect of the adrenocorticotrophic peptide is dose-related.

Thus, it may be concluded that, in the rat, both endogenous release and exogenous administration of glucocorticoids reduce the renal kallikrein activity. It is somehow contradictory that Fejes-Tóth and Náray-Fejes-Tóth did not find a further reduction of urinary kallikrein excretion when they administered corticosterone to adrenalectomized rats [30]. Perhaps this is due to the dose used. Our corticosterone dose, and also the effective tetracosactide dose, increased the daily urinary corticosterone excretion by 20 times [15]. The dose used by Fejes-Tóth and Náray-Fejes-Tóth, being 40 times lower than ours, probably did not increase the plasma corticosterone levels sufficiently to inhibit kallikrein.

The renal kallikrein activity of the control group in the tetracosactide experiment was somewhat lower than that of the control group in the steroid experiment, and also lower than previously reported data in normal rats [16]. This might be due to the injection of zinc, which could mimic the kallikrein-reducing effect of cadmium [31]. Zinc belongs to the same group in the periodic chart of the elements as cadmium. The lower renal kallikrein activity of controls perhaps masks a greater reducing effect of β^{1-24} corticotropin on tissue enzyme activity.

Apparently, the effect of the adrenocorticotrophic hormone on the renal kallikrein activity in humans is different from that in rats. Two groups of investigators have reported a stimulating effect [32, 33], which appears to correlate with a more than 10-fold increase in plasma deoxycorticosterone concentration [32]. It remains unknown whether cortisol reduces the renal kallikrein activity in man. The lower kallikrein excretion of essential hypertensive patients, however, does not seem to be due to a glucocorticoid excess [26].

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