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EFFECTS OF TREATMENT WITH MINERALOCORTICOIDS ON LATE STEPS OF ALDOSTERONE BIOSYNTHESIS IN THE RAT*

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SUMMARY

Treatment of rats with either 9 α-fluorocortisol or deoxycorticosterone trimethyl acetate for two weeks resulted in a marked decrease in the conversion of tritiated corticosterone and deoxycorticosterone to 18-hydroxycorticosterone and aldosterone and, to a lesser extent, also in the conversion of added deoxycorticosterone and 11-deoxycortisol to 11 β-hydroxylated derivatives by incubated capsular adrenal tissue ('zona glomerulosa'). It did not affect the rate of steroid 18- and 11 β-hydroxylations by the decapsulated portions ('zona fasciculata-reticularis') of the glands. When incubated with serotonin, capsular adrenals of mineralocorticoid-treated rats produced less aldosterone but more deoxycorticosterone from endogenous precursors than capsular adrenals of control animals. Such effects of mineralocorticoids were not observed in animals kept on a sodium-deficient diet. However, in rats kept on a potassium-deficient diet, the administration of either mineralocorticoid induced an additional decrease in the capsular adrenal conversion of tritiated corticosterone to 18-hydroxycorticosterone and aldosterone. According to these observations, mineralocorticoids decrease the activity of two enzymes involved in the late stages of aldosterone biosynthesis, i.e. zona glomerulosa 18-hydroxylase and 11 β-hydroxylase, by a yet unknown mechanism, which depends on an unrestricted sodium intake. Similar effects had been previously induced in rats by sodium loading or potassium restriction.

INTRODUCTION

Long-term experimental manipulations of the rat which are known to suppress the secretion of aldosterone *in vivo*, e.g. sodium loading or potassium restriction, also lead to a diminished aldosterone output by adrenal tissue incubated *in vitro* [1, 2]. Similarly, the prolonged treatment with 9α-fluorocortisol, a potent mineralocorticoid, caused a decreased aldosterone production by quartered adrenal glands incubated with or without stimulating agents such as serotonin, potassium ions or ACTH [3]. A marked decrease in the *in vitro* aldosterone output of adrenal tissue was also induced by the treatment of rats with aldosterone or deoxycorticosterone [4]. These experiments gave no information on the biosynthetic site of the mineralocorticoid-induced impairment of aldosterone production. According to a considerable body of evidence, specific alterations in the activity of one or more of the enzymes involved in late stages of aldosterone biosynthesis, i.e. the conversion of deoxycorticosterone to aldosterone could play an important role in the adaptation of aldosterone production to changes in sodium and potassium balance in the rat, the dog and perhaps also the sheep [5–12]. Because of their known actions on the body sodium and potassium balance, mineralocorticoids would also be expected to have a selective suppressive effect on the final steps of aldosterone biosynthesis. The following experiments were carried out in order to test this hypothesis.

MATERIALS AND METHODS

Rats and diets. Groups of 12 pure-bred male Osborne–Mendel rats (Zbz: CARA), weighing between 170 and 240 g (mean 224 g) were kept on semisynthetic diets and treated with steroids for periods of two weeks before the *in vitro* assay. The diets, with slight modifications, were made up to the specifications of Hartroft and Eisenstein [13] and had the following sodium and potassium contents:

'complete': Na^+ 230 mmol/kg, K^+ 230 mmol/kg
'sodium-deficient': Na^+ 5 mmol/kg, K^+ 230 mmol/kg
'potassium-deficient': Na^+ 230 mmol/kg, K^+ 0.7 mmol/kg

Steroid medication. 9α-Fluorocortisol (Merck) was added in a concentration of 2 mg/l. (2 mg of the steroid dissolved in 2 ml of 96% ethanol, added to a litre of water) to the drinking fluid, which was offered to the rats for two weeks *ad libitum*. This resulted in the following mean daily consumptions of the steroid: complete diet 0.29 mg/kg, sodium-deficient diet 0.23 mg/kg, potassium-deficient diet 0.24 mg/kg. Ethanol (96%, 2 ml/l.) was added to the drinking fluid of the control animals. A dose of 10 mg per rat (average 44 mg/kg) of deoxycorticosterone trimethyl acetate (Percorten-M®, Ciba) was subcutaneously injected in the form of a microcrystal suspension on

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days 1 and 8 of the dietary treatment. These animals received demineralized water as drinking fluid.

Incubations. Groups of 12 rats were decapitated. The excised adrenals were bisected and decapsulated by the method of Giroud *et al.* [14]. The capsular and the decapsulated portions, respectively, were evenly distributed into 4 homogenous tissue pools [15]. The average mass of tissue pool per incubation flask was 8.6 mg (range: 6.7–11.1 mg) and 60 mg (range: 54–67 mg) for capsular and decapsulated adrenals, respectively.

In conversion experiments, 12 capsular or decapsulated hemi-adrenals were incubated for 120 min without pre-incubation in 6 ml of modified Krebs–Ringer bicarbonate buffer containing 3.6 mmol potassium and 2 g glucose per litre. Labelled steroid substrates were added in 0.06 ml of ethanol. Incubation was carried out for 2 h at 37°C in an atmosphere of 95% O_2 and 5% CO_2.

In studies of endogenous corticosteroid output, capsular adrenals were preincubated for 30 min in buffer only. The final incubation was carried out for 120 min in 6 ml of fresh buffer with or without serotonin (5-hydroxytryptamine creatinine sulphate, 1.6×10^{-5} M). Decapsulated adrenals were incubated for 120 min without preincubation in 6 ml of buffer with or without ACTH (Cortrophine®, Organon, 5 IU per flask).

*Labelled steroids**. [4-^{14}C]-Corticosterone (56.7 Ci/mol), [4-^{14}C]-aldosterone (56.7 Ci/mol), [4-^{14}C]-cortisol (10 Ci/mol) and [1,2-^{3}H]-corticosterone (40 Ci/mmol) were purchased from the Radiochemical Centre (Amersham, England); [4-^{14}C]-deoxycorticosterone (54.3 Ci/mol), [1,2-^{3}H]-deoxycorticosterone (40 Ci/mmol) and [1,2-^{3}H]-11-deoxycortisol (54.6 Ci/mmol) from the New England Nuclear Corporation. These substances were purified by paper chromatography [9, 10]. [4-^{14}C]18-hydroxycorticosterone was prepared by incubation of [4-^{14}C]corticosterone with capsular rat adrenals, [4-^{14}C]18-hydroxydeoxycorticosterone by incubation of [4-^{14}C]deoxycorticosterone with decapsulated adrenals [9].

Steroid analysis. Tritiated aldosterone, corticosterone, cortisol, 18-hydroxycorticosterone and 18-hydroxydeoxycorticosterone were measured by previously described and evaluated double isotope dilution procedures [9, 10]. Unlabelled aldosterone, corticosterone and deoxycorticosterone were determined by modifications [16, 17] of the double isotope dilution derivative assay of Kliman and Peterson[18, 19].

Serum electrolytes. Blood was obtained by aortic punction under ether anaesthesia from separate rats between 9 and 10 a.m. Sodium and potassium concentrations in the serum were measured by flame spectrophotometry using an internal lithium standard.

RESULTS

18-Hydroxylation and 18-hydroxydehydrogenation. In rats kept on a complete diet, the treatment with 9α-fluorocortisol resulted in a significant decrease in the capsular adrenal conversion of tritiated corticosterone to aldosterone and 18-hydroxycorticosterone to 29 and 25% of the respective control values (Fig. 1). Injection of deoxycorticosterone trimethyl acetate caused an even greater reduction in these conversion rates to 5 and 7%, respectively. No such effects were observed in the animals kept on the sodium-deficient diet. In rats kept on the potassium-deficient diet, the treatment with either mineralocorticoid led to a significant additional suppression of the capsular adrenal conversion of tritiated corticosterone to aldosterone. A similar mean decrease in the conversion to 18-hydroxycorticosterone was however statistically not significant.

The time course of the suppression of the capsular adrenal conversions of added corticosterone to 18-hydroxycorticosterone and aldosterone due to the treatment of rats on a complete diet with deoxycorticosterone trimethyl acetate is shown in Fig. 2. The conversion to 18-hydroxycorticosterone significantly decreased to 48% of the mean control value within 2 days after the first injection of the mineralocorticoid, whereas the fall in the conversion to aldosterone became significant within four days. The conversions remained constant between days 5 and 8, but dropped further during the second week of the experiment following the second injection of the steroid.

The treatment of sodium-replete rats with the two mineralocorticoids was also followed by marked decreases in the capsular adrenal conversion of tritiated deoxycorticosterone to aldosterone (−95%) and to 18-hydroxycorticosterone (−88 and −85%, Fig. 3). Only 9α-fluorocortisol induced a 50% decrease in the capsular adrenal conversion of deoxycorticosterone to 18-hydroxydeoxycorticosterone, which was however not significant. The treatment with either mineralocorticoid did not affect the steroid 18-hydroxylation in the zona fasciculata reticularis according to the unaltered conversion of tritiated deoxycorticosterone to 18-hydroxydeoxycorticosterone and 18-hydroxycorticosterone in decapsulated adrenals.

11β-Hydroxylation. The capsular adrenal conversion of tritiated deoxycorticosterone to three 11β-hydroxylated derivatives, i.e. corticosterone, 18-hydroxycorticosterone and aldosterone, was decreased to 21% of the control value following two weeks of 9α-fluorocortisol intake and to 44% of the control value following the treatment with deoxycorticosterone trimethyl acetate (Table 1). The capsular adrenal conversions

* The following trivial names and abbreviations are used in this report: aldosterone = ALDO = 11β,21-dihydroxy-3,20-dioxo-4-pregnene-18-al; corticosterone = B = 11β, 21-dihydroxy-4-pregnene-3,20-dione; cortisol = F = 11β, 17,21-trihydroxy-4-pregnene-3,20-dione; deoxycorticosterone = DOC = 21-hydroxy-4-pregnene-3,20-dione; 11-deoxycortisol = S = 17,21-dihydroxy-4-pregnene-3,20-dione; 18-hydroxycorticosterone = 18-OH-B = 11β,18,21-trihydroxy-4-pregnene-3,20-dione; 18-hydroxydeoxycorticosterone = 18-OH-DOC = 18,21-dihydroxy-4-pregnene-3,20-dione.

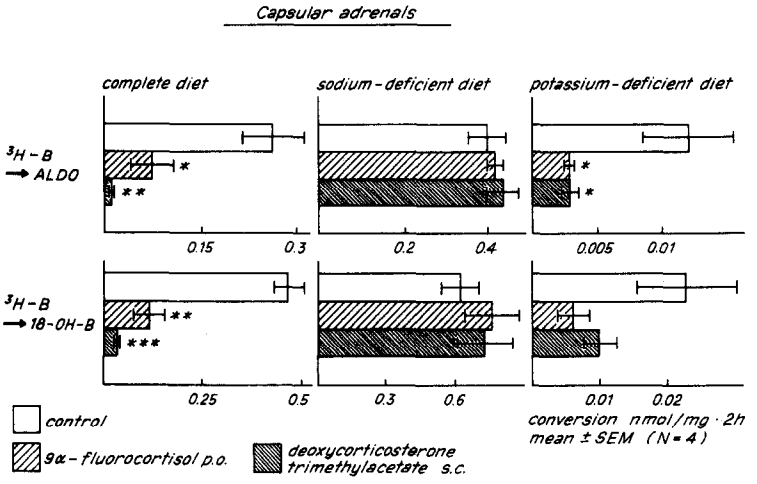

Fig. 1. Effects of two weeks of treatment of rats kept on different diets with 9α-fluorocortisol or deoxycorticosterone trimethyl acetate on the conversion of tritiated corticosterone ([^{3}H]-B, 300 nmol/flask) to aldosterone (ALDO) and 18-hydroxycorticosterone (18-OH-B) by incubated capsular adrenals. Mean values of two experiments ± standard error of the mean. Asterisks denote the statistical significance of the difference from the respective control values as calculated by *t* test: * $p < 0.05$; ** $p < 0.01$: *** $p < 0.001$.

of tritiated 11-deoxycortisol to cortisol were also suppressed to 45 and 52%, respectively, of the mean control value. These parameters of 11β-hydroxylase activity remained essentially unaltered in the decapsulated portions of the glands.

Capsular adrenal output of endogenous steroids. The capsular adrenal production of endogenous aldosterone was markedly diminished in the tissue of mineralocorticoid-treated rats under basal conditions of incubation as well as under stimulation by serotonin added *in vitro* (Fig. 4). The aldosterone production

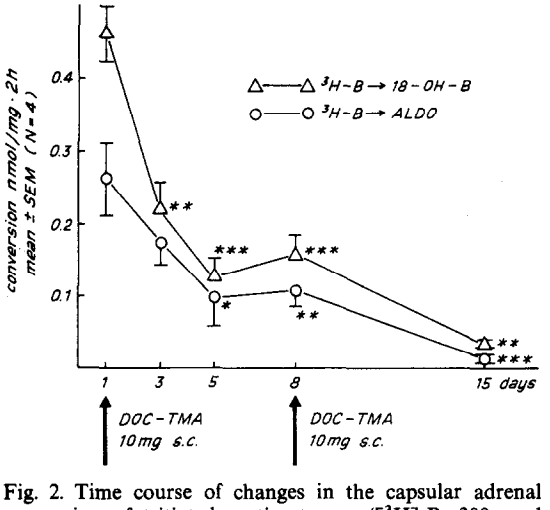

Fig. 2. Time course of changes in the capsular adrenal conversion of tritiated corticosterone ([^{3}H]-B, 300 nmol per flask) to aldosterone (ALDO) and 18-hydroxycorticosterone (18-OH-B) due to treatment of rats kept on a complete diet with deoxycorticosterone trimethyl acetate (DOC-TMA). Mean values of two experiments ± standard error of the mean. Asterisks near day-3, day-5 and day-8 values denote the statistical significance of differences from day-1 values; asterisks near the day-15 values refer to differences from day-8 values as calculated by *t* tests. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

by capsular adrenals of deoxycorticosterone-treated rats did not increase in response to serotonin. However, in this tissue as well as in capsular adrenals of 9α-fluorocortisol-treated rats, serotonin elicited a markedly enhanced response in deoxycorticosterone output.

By contrast, both mineralocorticoids induced decreases in the capsular adrenal corticosterone output; however, they were small and with one exception statistically not significant.

Adrenal weight and corticosterone production by decapsulated adrenals. Treatment with 9α-fluorocortisol but not with deoxycorticosterone trimethyl acetate resulted in a marginal (−7%) but statistically significant decrease in the relative adrenal weight (Table 2). The output of endogenous corticosterone by decapsulated adrenals incubated with and without ACTH was not altered as a consequence of the administration of either mineralocorticoid.

Serum electrolytes. In rats kept on the complete diet, the serum sodium and potassium concentrations were not significantly affected by the treatment with deoxycorticosterone trimethyl acetate, but there was a small increase in the serum sodium and a small decrease in the serum potassium in response to the treatment with 9α-fluorocortisol (Table 3). No significant changes in the serum sodium and potassium concentrations were induced by either mineralocorticoid in rats kept on the sodium-deficient or the potassium-deficient diet.

DISCUSSION

Negative feedback control is an important principle in the physiological regulation of the secretory activity of most endocrine glands. There is good evidence that negative feedback loops are also involved in the complex control system maintaining an adequate

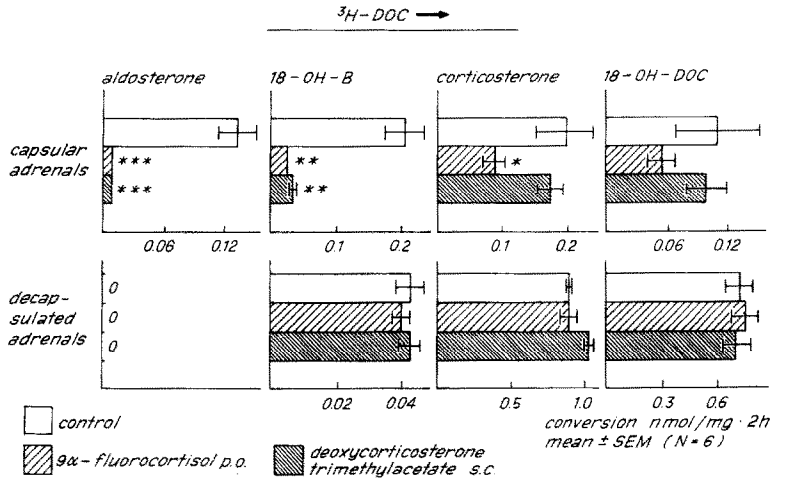

Fig. 3. Effect of two weeks of treatment of rats (complete diet) with 9 α-fluorocortisol or deoxycorticosterone trimethyl acetate on the conversion of tritiated deoxycorticosterone (^{3}H-DOC, 300 nmol per flask) to aldosterone, 18-hydroxycorticosterone (18-OH-B), corticosterone and 18-hydroxydeoxycorticosterone (18-OH-DOC). Mean values of 3 experiments ± standard error of the mean. Asterisks denote the statistical significance of the difference from the respective control values as calculated by *t* tests: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

aldosterone secretion in the mammalian organism. Thus, the aldosterone secretory activity of the human adrenal cortex is temporarily suppressed after the removal of an aldosterone-producing adenoma [20, 21], when pathological amounts of other mineralocorticoids are produced by the zona fasciculata [22–25] or when high doses of an exogenous mineralocorticoid are administered [26, 27]. Similarly, in rats the administration of a mineralocorticoid such as deoxycorticosterone leads to a decreased aldosterone secretion *in vivo* [28, 29] and a decreased plasma aldosterone concentration [30]. If we only consider the efferent axis of aldosterone control, a mineralocorticoid-induced decrease in aldosterone production could be due to either lowered blood levels of circulating aldosterone stimulators, a diminished mass of aldosterone-producing cells, a generally suppressed or qualitatively altered steroidogenic response of the zona glomerulosa or a combination of these phenomena. According to Greep and Deane [31], the prolonged treatment of rats with deoxycorticosterone resulted in marked histochemical changes but not in a significant reduction in the width of the zona glomerulosa. Although it is possible that a reduction in the mass and overall steroidogenic capacity of the zona glomerulosa could have contributed to the decreased aldosterone output and the diminished conversion of added precursor steroids to aldosterone and 18-hydroxycorticosterone by quartered or capsular adrenals of mineralocorticoid-treated rats observed in earlier [3, 4] and the present experiments, they could not have accounted for the strikingly increased output of endogenous deoxycorticosterone in response to added serotonin. This agent is known to act exclusively on the zona glomerulosa cells [15, 22] and to stimulate aldosterone production by primarily enhancing an early biosynthetic step, i.e. the conversion of cholesterol to pregnenolone [33]. Its decreased aldosterone-stimulating but increased deoxycorticosterone-stimulating effect upon zona glomerulosae cells of mineralocorticoid-treated animals indicates that the aldosterone biosynthetic pathway

Table 1. Effect of treatment with mineralocorticoids on the conversion of tritiated deoxycorticosterone ([^{3}H]-DOC, 300 nmol/flask) to 11β-hydroxylated products (corticosterone, 18-hydroxycorticosterone, aldosterone) and of tritiated 11-deoxycortisol ([^{3}H]-S) to cortisol by adrenal tissue in nmol per mg tissue

	Conversion of [^{3}H]-DOC to 11β-OH-steroids ($N = 4$)		Conversion of [^{3}H]-S to cortisol ($N = 6$)	
Treatment	capsular adrenals	decapsulated adrenals	capsular adrenals	decapsulated adrenals
(Control)	0.48 ± 0.16*	0.95 ± 0.06	0.31 ± 0.09	1.03 ± 0.43
9α-Fluorocortisol	0.10 ± 0.04 ($p < 0.005$)†	0.95 ± 0.12	0.14 ± 0.12 ($p < 0.05$)	0.96 ± 0.40 (n.s.)‡
Deoxycorticosterone trimethyl acetate	0.21 ± 0.05 ($p < 0.02$)	1.09 ± 0.05 ($p < 0.01$)	0.16 ± 0.10 ($p < 0.05$)	1.08 ± 0.47 (n.s.)

* Mean values of 2 and 3 experiments, respectively.
† *p* values of significance were calculated by *t* tests and refer to differences from control values.
‡ n.s.: not significant at the $p = 0.05$ level.

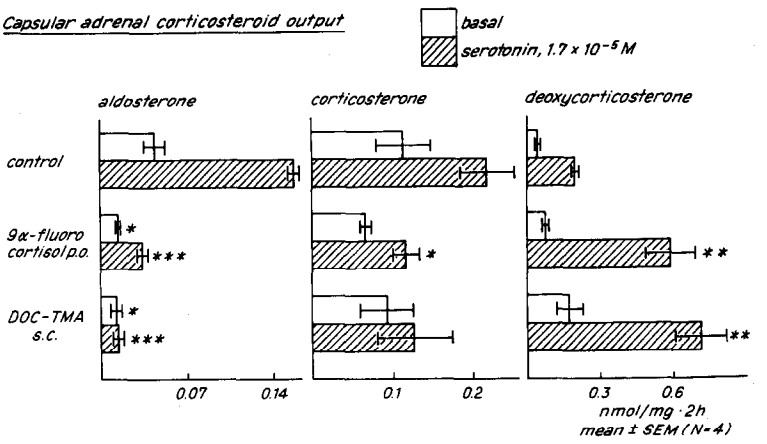

Fig. 4. Effects of two weeks of treatment of rats (complete diet) with 9α-fluorocortisol or deoxycorticosterone trimethyl acetate (DOC-TMA) on the output of endogenous corticosteroids by capsular adrenals incubated with or without serotonin. Mean values of two experiments ± standard error of the mean. Asterisks denote the statistical significance of the difference from the respective control value as calculated by t tests: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

was intact in the early stages up to the formation of deoxycorticosterone but impaired in the late steps involved in the conversion of this intermediary product to aldosterone.

According to the results of our conversion experiments, mineralocorticoid treatment led to a decreased activity of two of the three enzymes necessary for the formation of aldosterone from deoxycorticosterone, i.e. zona glomerulosa 18-hydroxylase and, to a lesser extent, zona glomerulosa 11β-hydroxylase. The present experiments gave no information on a possible effect of mineralocorticoids on the activity of the enzyme involved in the ultimate step of aldosterone biosynthesis, i.e. 18-hydroxydehydrogenase. Alterations in the conversion of tritiated corticosterone or deoxycorticosterone to aldosterone were generally paralleled by proportional alterations in the conversion to 18-hydroxycorticosterone. Small differences observed in the fractional 18-hydroxydehydrogenation [9] were neither consistent nor statistically significant.

Whereas the treatment with 9α-fluorocortisol and the treatment with deoxycorticosterone trimethyl acetate resulted in identical changes in corticosteroid biosynthesis in other respects, the former steroid had a lesser effect on the 18-hydroxylation of added corticosterone and a more marked suppressive effect on the 11β-hydroxylation of tritiated deoxycorticosterone. These small differences may be due to the fact that 9α-fluorocortisol is also a potent glucocorticoid [34, 35]. According to Haack *et al.* [36], its effects on sodium, potassium and water homeostasis are partially due to its 'glucocorticoid' activity. However, it is unlikely that it affected aldosterone biosynthesis by suppressing the secretion of ACTH. Hypophysectomy of rats was not followed by any decrease in the apparent zona glomerulosa 18-hydroxylase activity [37]. At the dosage used in our experiments, 9α-fluorocortisol had only a very small effect on the adrenal weight and did not influence the output of corticosterone by the zona fasciculata-reticularis neither under basal conditions of incubation nor under stimulation by added ACTH. According to these parameters, deoxycorticosterone did not display the ACTH-suppressing activity it had shown in the study of Kraulis *et al.* [38].

The two mineralocorticoids had no effect on aldosterone biosynthesis in rats kept on a sodium-deficient diet in accordance with previous observations made in rats [3, 4] and sheep [39]. This does not

Table 2. Effect of two weeks of treatment with 9α-fluorocortisol or deoxycorticosterone trimethyl acetate (DOC-TMA) on the relative adrenal weight and on corticosterone output† by decapsulated adrenals incubated with or without ACTH‡

Treatment	N	Relative adrenal weight mg/100 g	N	Corticosterone output by decapsulated adrenals Addition to incubation medium —	ACTH
(Control)	15	11.33 ± 0.82§	14	20.23 ± 4.67	60.79 ± 12.26
9α-Fluorocortisol	16	10.49 ± 1.06 (0.025)‖	16	17.97 ± 4.67 (n.s.)	66.20 ± 10.84 (n.s.)
DOC-TMA	14	11.21 ± 1.08 (n.s.)¶	10	17.67 ± 6.40 (n.s.)	58.55 ± 8.96 (n.s.)

* mg adrenal tissue/100 g body weight; based on tissue pool weights determined at the end of incubation.
† μg/100 mg/2 h.
‡ 5 IU/flask.
§ Mean values ± S.D.
‖ In brackets p values of significance referring to differences from control values.
¶ n.s. not significant at the $p = 0.1$ level.

Table 3. Effects of two weeks of treatment with 9α-fluorocortisol (9α-FF) or deoxycorticosterone trimethyl acetate (DOC-TMA) on serum sodium and potassium concentrations of rats kept on different diets

Diet	Treatment	N	Serum electrolytes Sodium	Potassium
Complete	(control)	16	132.5 ± 4.4*	4.43 ± 0.41
	9α-FF	16	137.1 ± 4.6 (0.01)†	3.97 ± 0.33 (0.005)
	DOC-TMA	16	134.8 ± 2.5 (n.s.)‡	4.20 ± 0.44 (n.s.)
Sodium-deficient	(control)	6	135.5 ± 2.3	4.33 ± 0.38
	9α-FF	6	134.8 ± 3.7 (n.s.)	4.70 ± 0.49 (n.s.)
	DOC-TMA	6	133.6 ± 1.8 (n.s.)	4.55 ± 0.65 (n.s.)
Potassium-deficient	(control)	4	140.3 ± 5.1	2.78 ± 0.32
	9α-FF	4	133.5 ± 5.2 (n.s.)	2.34 ± 0.19 (n.s.)
	DOC-TMA	4	135.8 ± 1.5 (n.s.)	2.38 ± 0.48 (n.s.)

* Mean values in mmol/l ± standard deviation.
† In brackets p values of significance calculated by t tests and referring to differences from control values.
‡ n.s.: not significant at the $p = 0.1$ level).

necessarily mean that sodium retention is an essential element in the chain of events by which mineralocorticoids suppress aldosterone production. Perhaps the doses of the mineralocorticoids administered were small in relation to the amounts of aldosterone secreted during sodium deficiency. It is also possible that, as long as the organism is in sodium deficit, aldosterone biosynthesis is stimulated irrespectively of other suppressive mechanisms. Thus, sodium restriction prevented a decrease in zona glomerulosa 18-hydroxylase activity due to a simultaneous potassium restriction [40].

Increases in aldosterone secretion in response to sodium deficiency are generally assumed to be mediated by the renin–angiotensin system. However, there is only scarce information available about possible effects of angiotensin II on the final steps of aldosterone biosynthesis [1]. The plasma renin activity of dogs [41] and rats [42, 43] was suppressed by the administration of deoxycorticosterone, but only when the animals were sodium replete. This may depend on the dosage and form of administration, since the kidney renin content of rats kept on a sodium-deficient diet was suppressed by daily injections of deoxycorticosterone acetate but not by daily injections of deoxycorticosterone trimethyl acetate [44].

There was an additional decrease in the capsular adrenal conversion of tritiated corticosterone to aldosterone and 18-hydroxycorticosterone during the second week of the treatment with deoxycorticosterone trimethyl acetate, i.e. at a time when the animals would have been expected to be escaping from the sodium-retaining action of the mineralocorticoid but still be losing potassium [45]. Perhaps the continuing decrease in the zona glomerulosa 18-hydroxylase activity resulted from a mineralocorticoid-induced potassium loss. At the end of 2 weeks of treatment there was, however, only a small decrease in the serum potassium concentration in animals receiving 9α-fluorocortisol and an unchanged serum potassium in animals receiving deoxycorticosterone. The potassium losses must therefore have been small or compensated by a shift of potassium from the intracellular to the extracellular compartment.

In conclusion, the present studies have shown that a moderate dose of exogenous mineralocorticoid has a marked and relatively specific effect on the activity of two enzymes involved in the final steps of aldosterone biosynthesis. This indicates that alterations in late steps of aldosterone biosynthesis could play a role in the long- and intermediate-term fedback regulation of aldosterone secretion.

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