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## SHORT COMMUNICATION

# EFFECT OF 6-DEHYDRO-DOCA AND 6-DEHYDRO-9α-FLUOROCORTISOL ACETATE ON THE EXCRETION OF SODIUM AND POTASSIUM IN THE RAT

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**Summary**—Deoxycorticosterone acetate (DOCA) and 9α-fluorocortisol acetate (9α-F-Cac) can be modified by the introduction of a double bond at carbons 6 and 7 (6-dehydro-derivatives). Such a modification markedly changes the effect of the steroids on urinary excretion of $Na^+$ and $K^+$. Since 6–7 reduction of DOCA and 9α-F-Cac substantially reduces affinity for Type II receptors but not Type I receptors, 6-dehydro-derivatives will thus bind preferentially to receptors influencing the retention of sodium (the "mineralocorticoid" or Type I receptor), and compete with mineralocorticoids for such receptors. We interpret the increase in both natriuresis and kaliuresis when mineralocorticoids and their dehydro-derivatives are administered together as evidence for a Type II receptor mediation of these ion fluxes.

In previous work we have shown that a slight molecular modification of DOCA, that of introducing a double bond at carbons 6 and 7 to form the 6-dehydro-derivative, results in a loss of mineralocorticoid agonist activity in the rat. Injected into the caudal vein with aldosterone, DOCA or 9α-fluorocortisol, 6-dehydro-DOCA is a mineralocorticoid antagonist in terms of urinary $Na^+/K^+$ ratio [1].

The present paper is an approach to the mechanism of that antagonistic effect by estimating the excretion of sodium and potassium in rat urine, using a method described elsewhere [1, 2]. 6-Dehydro-DOCA (6-DOCA) and 6-dehydro-9α-fluorocortisol-21-acetate (6-9α-F-Cac) were prepared by dehydrogenation of DOCA and 9α-fluorocortisol acetate with chloranil, following the method described by Agnello and Laubach[3].

Figures 1, 2 and 3 show the results, expressed in percent of increase or decrease of excretion compared with the values obtained before injection. DOCA (70 μg/100 g rat) induces a well characterized sodium retention with no effect on potassium excretion; the same amount of 6-DOCA shows a very much smaller effect on sodium excretion (Fig. 1). Larger doses of 6-DOCA (210 μg/100 g rat) similarly induce a decrease in sodium excretion without any action on potassium (Fig. 2). No additional effect is found with doses of 6-DOCA above 210 μg/100 g rat.

In contrast, aldosterone (10 μg/100 g rat) both decreases urinary excretion of sodium (only at 5 h 00) and increases potassium excretion, a well described phenomenon (Fig. 2). The two compounds together does not affect the natriuresis after 2 h 30. From 2 h 30 to 5 h, however, sodium retention is lower than when aldosterone and 6-DOCA are administered separately ($P = 0.05$).

Under the same conditions an injection of 30 μg/100 g rat of 9α-F-Cac induces retention of sodium and kaliuresis as does aldosterone (Fig. 3). The injection of 30 μg of 6-9α-F-Cac gives only a decrease in sodium excretion; potassium excretion is not significantly changed. A mixture

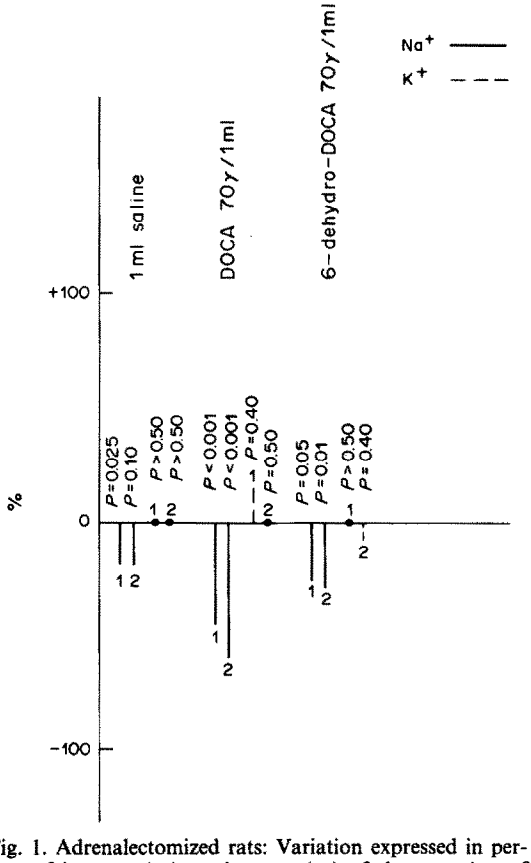


Fig. 1. Adrenalectomized rats: Variation expressed in percent of increase (+) or decrease (−) of the excretion of sodium and potassium found 2 h 30 and 5 h after injection compared with the values obtained before injection. $n = 30$ for each experiment. $P$ gives the probability of a $t$-test comparing results before and at 2 h 30 and 5 h after injection.

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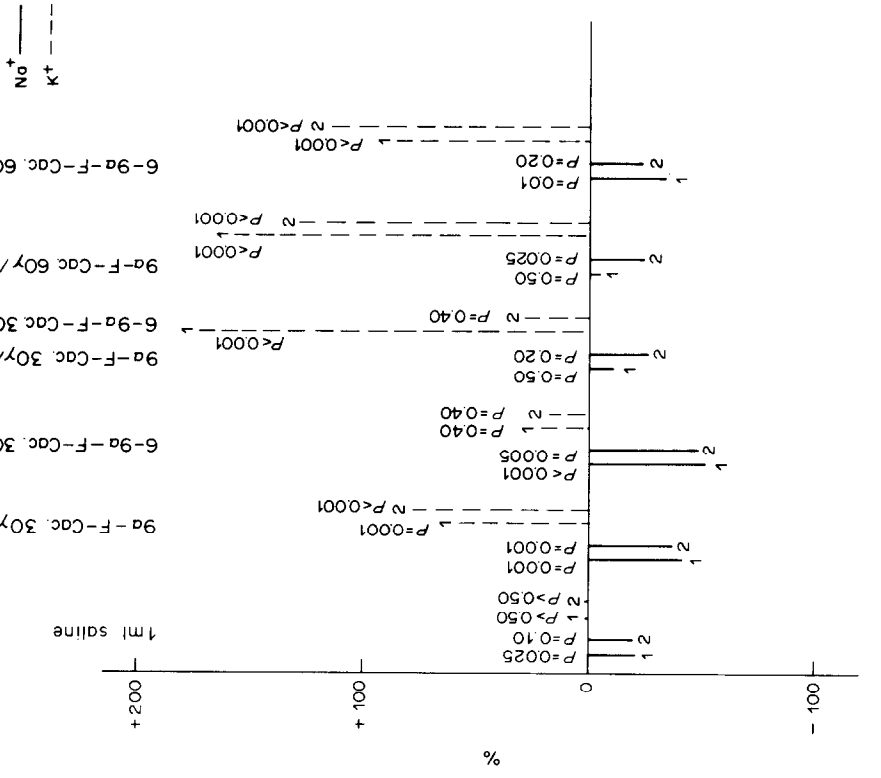
Na+
K+
6-9a-F-Cac. 60y/1ml
9a-F-Cac. 60y/1ml
6-9a-F-Cac. 30y/1ml
9a-F-Cac. 30y/1ml+
6-9a-F-Cac. 30y/1ml
9a-F-Cac. 30y/1ml
1ml saline
+200
+100
0
-100
%

Fig. 3.

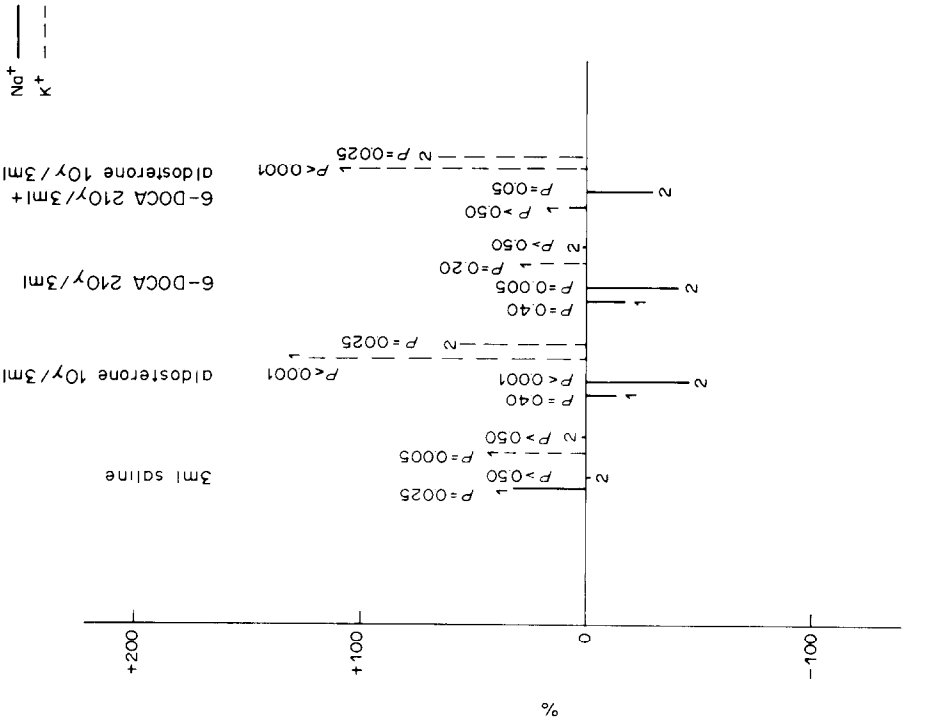
Na+
K+
6-DOCA 210y/3ml+
aldosterone 10y/3ml
6-DOCA 210y/3ml
aldosterone 10y/3ml
3ml saline
+200
+100
0
-100
%

Fig. 2.

Table 1. Rat renal glucocorticoid receptor competition assay results

| Steroid | Cytoplasmic DM-receptor | |
|---|---|---|
| | Concentration for 50% saturation | Relative binding affinity (%) |
| Dexamethasone | $6.0 \times 10^{-9}$ M | 100 |
| DOCA | $4.2 \times 10^{-8}$ M | 14 |
| 6-DOCA | $5.4 \times 10^{-7}$ M | 1.11 |
| 9α-F-Cac | $1.8 \times 10^{-8}$ M | 33 |
| 6-9α-F-Cac | $4.8 \times 10^{-8}$ M | 12.5 |

of 30 μg of each compound, however, is followed by no significant retention of sodium, and the kaliuretic effects is greater than that induced by the 9α-F-Cac alone. Larger amounts (60 μg) of both 9α-F-Cac and 6-9α-F-Cac produce an effect similar to that found with the mixture of 30 μg of each compound; i.e. sodium retention is lower than that induced by 30 μg of either steroid injected separetely.

These data suggest an hypothesis based on the previous suggestion that the so called Type I or "mineralocorticoid receptor" is involved in $Na^+$ retention but not in $K^+$ excretion [4, 5]. 9α-F-Cac binds both to receptors affecting sodium retention and receptors affecting kaliuresis (possibly the Type II or "glucocorticoid" receptor). As the dose increases the Type I receptors would be saturated; as the steroid levels rise further, it binds to the Type II receptors, inducing an increase in natriuresis. Since kaliuresis also increases under these same conditions, we suggest that the receptor controlling the increased excretion of sodium is identical to that inducing an increase in kaliuresis, and may be the glucocorticoid or Type II receptor.

6-DOCA and 6-9α-F-Cac (at lower doses) seem to bind preferentially to receptors controlling sodium retention (Type I receptors). They saturate these receptors and compete at this level with aldosterone and 9α-F-Cac, but do not affect the binding of the mineralocorticoid agonists to the receptors inducing an increase of natriuresis and kaliuresis. At higher concentrations, however, 6-9α-F-Cac induces an increase of kaliuresis. This suggests that it binds to some extent to the Type II receptors, and indicates that 6-9α-F-Cac may be less active, but not totally inactive, in terms of kaliuresis.

To further explore this hypothesis we determined the affinity of DOCA, 6-DOCA, 9α-F-Cac and 6-9α-F-Cac for Type II receptors using the classic [$^3$H]dexamethasone ([$^3$H]DM) binding/competition assay. Kidneys of adrenalectomized Wistar rats were perfused with iced isotonic solution via a catheter in the abdominal aorta. Kidneys were then removed, minced and homogenized in fresh TEG buffer (20 mM Tris, 1.5 mM EDTA, 1 mM $MgCl_2$, 10% glycerol adjusted to pH 7.4 at 20°C with acetic acid). Homogenates were centrifuged for 50 min at 4°C with $6 \times 10^{-9}$ M [$^3$H]DM (40 Ci Mm NEN) either alone or in the presence of 1–3-fold non radioactive DM or 3–100 fold competitors in a total volume of 500 μl. Incubation was followed by charcoal separation previously described [6]. Values shown in Table 1 represent the mean of four different preparations. In every experiment each data point was performed in duplicate. Non-specific binding was defined as [$^3$H]DM bound in the presence of $3 \times 10^{-6}$ M unlabelled DM. Specific binding was calculated by subtracting non-specific binding from total binding. Cytosol proteins were determined by the method of Lowry[7]. The protein concentration was 6 mg/ml.

Table 1 shows that 6-DOCA has ~8% the affinity of DOCA for Type II receptors, and 6-9α-F-Cac ~37% the affinity of 9α-F-Cac.

Since the affinities of DOCA and 6-DOCA on the one hand, and of 9α-F-Cac and 6-9α-F-Cac on the other, for Type I receptors are almost identical [1] [8], we conclude that the $C_6C_7$ reduction of DOCA and 9α-F-Cac preferentially reduces affinity for Type II receptors. That reduced affinity is very likely responsible for the changed ionic flux patterns induced by the 6-dehydro-derivatives. 6-DOCA binds very poorly to Type II receptors and is principally a mineralocorticoid agonist. 6-9α-F-Cac, in contrast, probably retains some glucocorticoid agonist activity, in the import of its moderate affinity for Type II receptors and its kaliuretic effect at higher concentrations.

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