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AMPLIFICATION OF THE ACTION OF SUBTHRESHOLD DOSES OF ALDOSTERONE BY 19-HYDROXYANDROST-4-ENE-3, 17-DIONE

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SUMMARY

Mineralocorticoid activity of 19-hydroxyandrost-4-ene-3, 17-dione was evaluated by mineralocorticoid bioassays using adrenalectomized rats. 19-Hydroxyandrost-4-ene-3, 17-dione was devoid of mineralocorticoid activity. However, it caused a significant decrease in urinary Na/K ratio and Na excretion and a significant increase in urinary K excretion when it was administered simultaneously with subthreshold doses of aldosterone. The results demonstrate that 19-hydroxyandrost-4-ene-3, 17-dione amplified the action of subthreshold doses of aldosterone.

INTRODUCTION

Aldosterone is known to be a potent mineralocorticoid (1). Progesterone (2-4), 5α-dihydro-11-deoxycorticosterone (5) and spironolactone (6, 7) have been reported as the inhibitors of aldosterone. In contrast, 16α, 18-dihydroxy-11-deoxycorticosterone (8) and 5α-dihydrocortisol (9) have been reported to amplify the action of aldosterone. In the present study, we evaluated mineralocorticoid activity of 19-hydroxyandrost-4-ene-3, 17-dione by bioassay using adrenalectomized rats and found that 19-hydroxyandrost-4-ene-3, 17-dione was devoid of mineralocorticoid activity. However, it amplified the action of subthreshold doses of aldosterone. Although the amplifying action for aldosterone has been rather doubtful for a long time (10), the present paper clearly demonstrates the amplification of the action of aldosterone by other steroid hormone.

MATERIALS AND METHODS

Materials

19-Hydroxyandrost-4-ene-3, 17-dione (19-OH-A-dione) was kindly supplied by Dr. D.N. Kirk of the Steroid Reference Collection, London, Eng-

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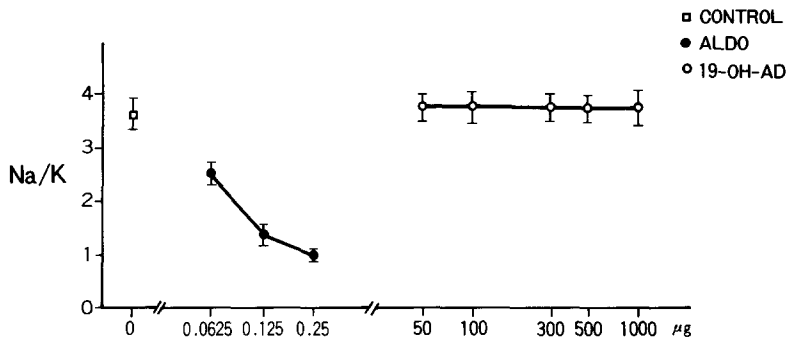


Figure 1. Dose-response curves of mineralocorticoid activity of aldosterone and 19-OH-A-dione. ALDO, aldosterone; 19-OH-AD, 19-OH-A-dione. The mean ± SE of urinary Na/K ratio is shown.

land. Aldosterone (11β, 21-dihydroxy-18-oxopregn-4-ene-3, 20-dione) was obtained from Makor Chemicals Ltd., Jerusalem, Israel. Male Sprague-Dawley rats weighing 110-130g were purchased from Clea Japan Inc., Tokyo, Japan.

Mineralocorticoid bioassay

Mineralocorticoid bioassays were performed as described previously (11-14). The method, in brief, is as follows: male rats were bilaterally adrenalectomized under sodium-pentobarbital anesthesia and then fasted overnight. The following day, 3ml normal saline along with aldosterone alone, 19-OH-A-dione alone and a combination of aldosterone plus 19-OH-A-dione dissolved in 0.5 ml of 10% ethanol were injected into 5 rats intraperitoneally. For control, 3ml of normal saline along with 0.5ml of 10% ethanol were injected into 5 rats. The rats were sacrificed 3 hours later and the urine was aspirated from the bladders. The concentration of Na and K of the urine was measured with a flame photometer. Urinary Na/K ratio was calculated from the concentration of Na and K and was used as the index of mineralocorticoid activity. Urinary Na and K excretion was calculated from the concentration of Na and K and urine volume. The dose-response curves of mineralocorticoid activity of aldosterone and 19-OH-A-dione are shown in Figure 1. Aldosterone showed mineralocorticoid activity. In contrast, 19-OH-A-dione did not show mineralocorticoid activity in doses up to 1000 μg/rat. The minimum detectable amount of aldosterone was 0.0625 μg/rat. The amount of aldosterone which showed the maximum mineralocorticoid activity was 0.25 μg/rat. The index of precision for the bioassay ranged between 0.2 and 0.3.

RESULTS

To examine the amplification effect of 19-OH-A-dione on subthreshold doses of aldosterone, 0.05 μg aldosterone alone, 50-300 μg 19-OH-A-dione alone and a combination of 0.05 μg aldosterone plus 50-300 μg 19-OH-A-dione were injected into rats and urinary Na/K ratio was evaluated (Figure 2).

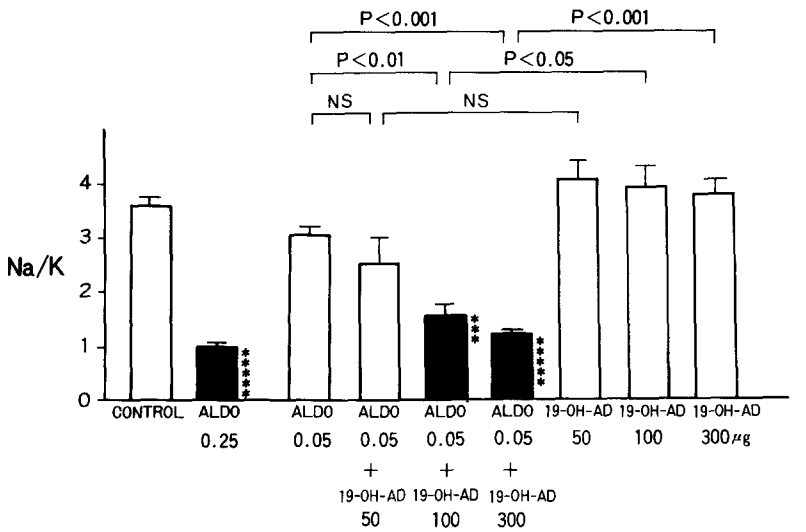


Figure 2. Urinary Na/K ratio of control rats and rats given 0.05 μg aldosterone alone, 50 -300 μg 19-OH-A-dione alone and a combination of 0.05 μg aldosterone plus 50-300 μg 19-OH-A-dione. Urinary Na/K ratio of rats given 0.25 μg aldosterone alone is also shown for comparison. Each bar shows the mean ± SE. Black bars are significantly lower than control (Student t test, ***P<0.01, *****P<0.001). Abbreviations are the same as in Figure 1.

The administration of 0.05 μg aldosterone alone or 50, 100 or 300 μg 19-OH-A-dione alone did not cause any significant change in urinary Na/K ratio. The simultaneous administration of 0.05 μg aldosterone and 50 μg 19-OH-A-dione did not cause any significant change in urinary Na/K ratio. However, the simultaneous administration of 0.05 μg aldosterone and 100 or 300 μg 19-OH-A-dione caused a significant decrease in urinary Na/K ratio. Urinary Na/K ratio of rats given a combination of 0.05 μg aldosterone plus 100 or 300 μg 19-OH-A-dione was significantly lower than that of rats given 0.05 μg aldosterone alone and 100 or 300 μg 19-OH-A-dione alone.

Urinary Na/K ratio of rats given a combination of 0.05 μg aldosterone plus 100 μg 19-OH-A-dione was approximately equivalent to that of rats given 0.10 μg aldosterone alone and urinary Na/K ratio of rats given a combination of 0.05 μg aldosterone plus 300 μg 19-OH-A-dione was approx-

Table 1

	Control	ALDO 0.25μg	ALDO 0.05μg	ALDO 0.05μg plus 19-OH-AD 50μg	ALDO 0.05μg plus 19-OH-AD 100μg	ALDO 0.05μg plus 19-OH-AD 300μg	19-OH-AD 50μg	19-OH-AD 100μg	19-OH-AD 300μg
Na Concentration (mEq/ℓ)	207±9	128±4	192±13	157±7	135±5	133±3	192±13	194±6	218±8
K Concentration (mEq/ℓ)	60±3	130±4	62±3	75±9	116±8	112±3	65±17	52±5	61±4
Urine Volume (mℓ/3hr)	0.8±0.04	0.4±0.04	0.9±0.01	0.9±0.02	0.7±0.01	0.4±0.01	0.9±0.02	0.8±0.01	0.8±0.08

Urinary Na and K concentration and urine volume of control rats and rats given 0.05 μg aldosterone alone, 50-300 μg 19-OH-A-dione alone and a combination of 0.05 μg aldosterone plus 50-300 μg 19-OH-A-dione. Urinary Na and K concentration and urine volume of rats given 0.25 μg aldosterone alone are also shown for comparison. The mean ± SE of each value is shown. Abbreviations are the same as in Figure 1.

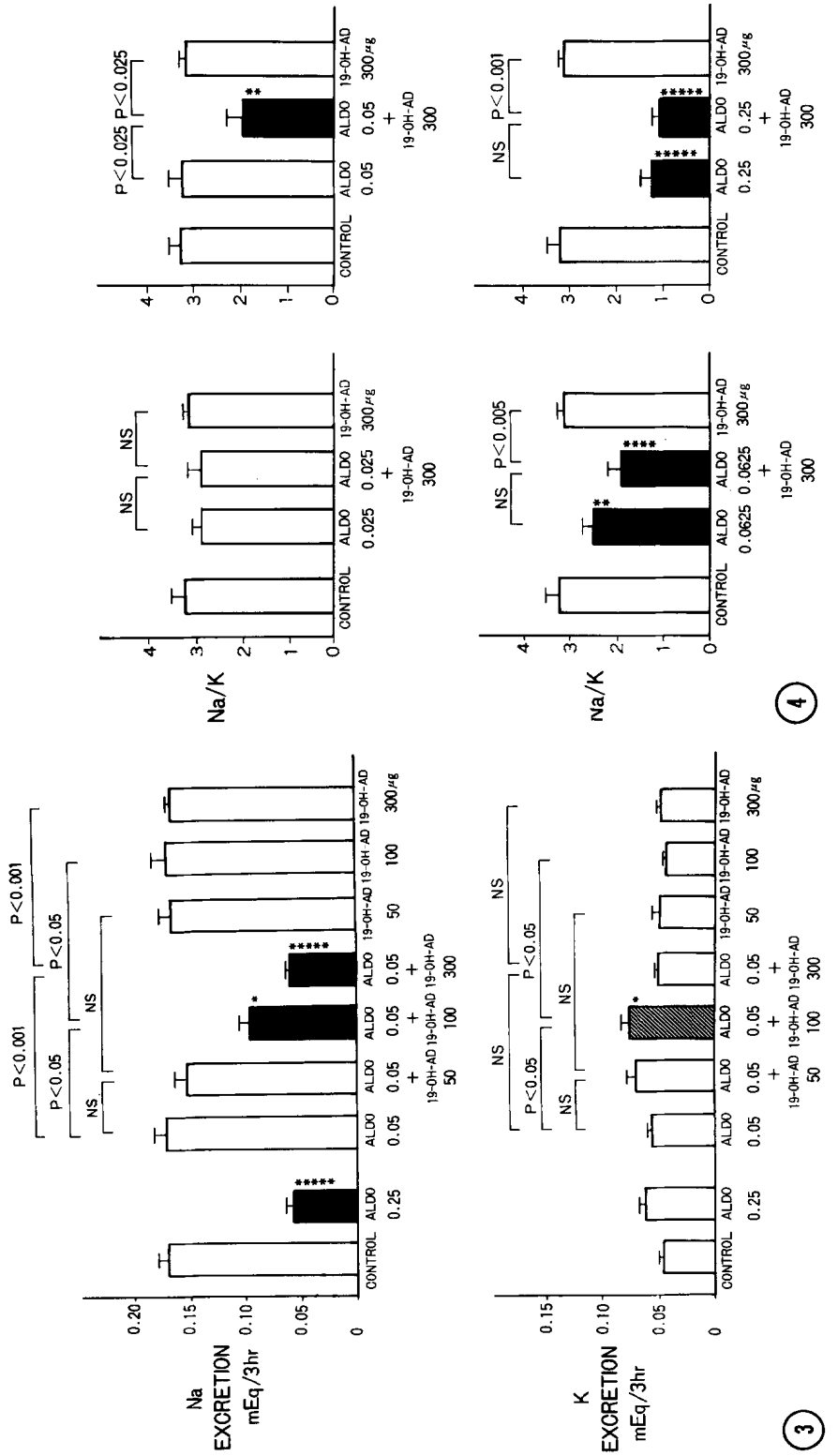
imately equivalent to that of rats given 0.15 μg aldosterone alone. Therefore, a 2-3 fold increase in mineralocorticoid activity of 0.05 μg aldosterone was obtained by the simultaneous administration of 0.05 μg aldosterone and 100-300 μg 19-OH-A-dione.

Urinary Na and K excretion of rats given 0.05 μg aldosterone alone, 50, 100 or 300 μg 19-OH-A-dione alone and a combination of 0.05 μg aldosterone plus 50, 100 or 300 μg 19-OH-A-dione was calculated from the concentration of Na and K and urine volume (Table 1) and is shown in Figure 3.

Urinary Na and K excretion of rats given 0.05 μg aldosterone alone, 50, 100 or 300 μg 19-OH-A-dione alone and a combination of 0.05 μg aldosterone plus 50 μg 19-OH-A-dione was not significantly different from that of control rats. In contrast, urinary Na excretion of rats given a combination of 0.05 μg aldosterone plus 100 or 300 μg 19-OH-A-dione was significantly lower than that of control rats and rats given 0.05 μg aldosterone alone and 100 or 300 μg 19-OH-A-dione alone. Urinary K excretion of rats given a combination of 0.05 μg aldosterone plus 100 μg 19-OH-A-dione was significantly higher than that of control rats and rats given 0.05 μg aldosterone alone and 100 μg 19-OH-A-dione alone although urinary K excretion of rats given a combination of 0.05 μg aldosterone plus 300 μg 19-OH-A-dione was not significantly different from that of control rats.

To examine further the amplification effect of 19-OH-A-dione on aldosterone, graded doses (0.025-0.25 μg) of aldosterone were injected into rats simultaneously with 300 μg 19-OH-A-dione and urinary Na/K ratio was evaluated (Figure 4).

The administration of 0.025 or 0.05 μg aldosterone alone did not cause any significant change in urinary Na/K ratio and the administration of 0.0625 or 0.25 μg aldosterone alone caused a significant decrease in urinary Na/K ratio. The administration of 300 μg 19-OH-A-


Na EXCRETION mEq/3hr
K EXCRETION mEq/3hr
Na/K
CONTROL
ALDO
19-OH-AD
19-OH-AD 50
19-OH-AD 100
19-OH-AD 300
P<0.001
P<0.05
NS
P<0.025
P<0.005
P<0.001
0.25
0.05
0.025
0.0625
0.25
300μg
3
4

dione alone did not cause any significant change in urinary Na/K ratio. The simultaneous administration of 0.025 μg aldosterone and 300 μg 19-OH-A-dione did not cause any significant change in urinary Na/K ratio. In contrast, the simultaneous administration of 0.05, 0.0625 or 0.25 μg aldosterone and 300 μg 19-OH-A-dione caused a significant decrease in urinary Na/K ratio. Urinary Na/K ratio of rats given a combination of 0.05 μg aldosterone plus 300 μg 19-OH-A-dione was significantly lower than that of rats given 0.05 μg aldosterone alone and 300 μg 19-OH-A-dione alone again. However, urinary Na/K ratio of rats given a combination of 0.0625 or 0.25 μg aldosterone plus 300 μg 19-OH-A-dione was not significantly different from that of rats given 0.0625 or 0.25 μg aldosterone alone.

DISCUSSION

In the present study, the administration of 19-OH-A-dione alone did not cause any significant change in urinary Na/K ratio and Na and K excretion. However, the administration of a combination of 19-OH-A-dione plus subthreshold doses of aldosterone caused a significant decrease in urinary Na/K ratio and Na excretion and a significant increase in urinary K excretion. The results clearly demonstrate that 19-OH-A-dione amplified the action of subthreshold doses of aldosterone.

Figure 3. Urinary Na and K excretion of control rats and rats given 0.05 μg aldosterone alone, 50-300 μg 19-OH-A-dione alone and a combination of 0.05 μg aldosterone plus 50-300 μg 19-OH-A-dione. Urinary Na and K excretion of rats given 0.25 μg aldosterone alone is also shown for comparison. Each bar shows the mean ± SE. Black bars are significantly lower than control and Shaded bars are significantly higher than control (Student t test, *P<0.05, *****P<0.001). Abbreviations are the same as in Figure 1.

Figure 4. Urinary Na/K ratio of control rats and rats given graded doses (0.025-0.25 μg) of aldosterone alone, 300 μg 19-OH-A-dione alone and a combination of graded doses (0.025-0.25 μg) of aldosterone plus 300 μg 19-OH-A-dione. Each bar shows the mean ± SE. Black bars are significantly lower than control (Student t test, **P<0.025, ****P<0.005, *****P<0.001). Abbreviations are the same as in Figure 1.

According to current concepts of the mechanism of physiological action of mineralocorticoids, the initial event in the intracellular action is considered to involve binding of the steroid to stereospecific cytoplasmic receptors in renal tubular cells. The steroid-receptor complex is then activated and transferred to the nucleus where it attaches to chromatin acceptor sites and initiates DNA-dependent RNA synthesis (15). Progesterone (16) and spironolactone (15, 17) which are known as the antagonists of mineralocorticoid have been demonstrated to inhibit the binding of aldosterone to the renal cytoplasmic receptors and to diminish the generation of nuclear aldosterone complexes capable of attaching to chromatin. Whether or not 19-OH-A-dione potentiates the binding of aldosterone to the renal cytoplasmic receptors and increases the generation of nuclear aldosterone complexes is not clear at the present time. The problem should be evaluated further to elucidate the mechanism of the amplification effect of 19-OH-A-dione on aldosterone.

19-OH-A-dione has been reported to be formed during the conversion of androst-4-ene-3, 17-dione to estrogen in microsomes of the human term placenta (18). Whether or not endogenous 19-OH-A-dione is ever present in quantities sufficient to exert important effects in the regulation of blood pressure and the pathogenesis of hypertension, especially low-renin essential hypertension (19) is the problem further to be evaluated.

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