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19-NORANDROST-4-ENE-3, 17-DIONE AMPLIFIES THE ACTION OF ALDOSTERONE ONLY IN SODIUM-LOADED CONDITIONS: EVIDENCE FOR A NEW CLASS OF AMPLIFIERS OF ALDOSTERONE

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SUMMARY

The amplification effect of 19-norandrost-4-ene-3, 17-dione (19-nor-A-dione) on aldosterone in normal and sodium-loaded conditions was evaluated using adrenalectomized rats fed a normal or high sodium diet. The administration of 19-nor-A-dione in normal or sodium-loaded conditions did not cause any significant change in urinary Na/K ratio. The simultaneous administration of subthreshold doses of aldosterone and 19-nor-A-dione in normal conditions also did not cause any significant change in urinary Na/K ratio. However, the simultaneous administration of subthreshold doses of aldosterone and 19-nor-A-dione in sodium-loaded conditions caused a significant decrease in urinary Na/K ratio. The decrease in urinary Na/K ratio was caused by a decrease in urinary Na excretion. These results demonstrate that 19-nor-A-dione, which did not amplify the action of aldosterone in normal conditions, amplified the action of subthreshold doses of aldosterone in sodium-loaded conditions. 19-Nor-A-dione is considered to be an amplifier of aldosterone which works only in sodium-loaded conditions.

INTRODUCTION

Aldosterone is a potent mineralocorticoid (1). As amplifiers of the action of aldosterone, 16α, 18-dihydroxy-11-deoxycorticosterone (2), 5α-dihydrocortisol (3) and 19-hydroxyandrostenedione (4) have been reported. In the present study, we evaluated the problem of whether 19-norandrost-4-ene-3, 17-dione (19-nor-A-dione) amplified the action of aldosterone. 19-Nor-A-dione did not amplify the action of aldosterone in normal conditions. However, it amplified the action of subthreshold doses of aldosterone after sodium-loading. The present paper describes a new amplifier of aldosterone which works only in sodium-loaded conditions.

MATERIALS AND METHODS

Materials

Aldosterone (11β, 21-dihydroxy-18-oxopregn-4-ene-3, 20-dione) was obtained from Makor Chemicals Ltd., Jerusalem, Israel and 19-nor-A-dione

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was obtained from Steraloids, Inc., Wilton, U.S.A. Male Sprague-Dawley rats were purchased from Clea Japan Inc., Tokyo, Japan. A normal sodium diet (NaCl 0.3 %) and a high sodium diet (NaCl 5 %) for rats were also purchased from Clea Japan Inc.

Mineralocorticoid bioassay

Mineralocorticoid bioassays were performed as described previously (4-6). The method, in brief, is as follows: male Sprague-Dawley rats weighing 110-130 g were fed a normal sodium diet or a high sodium diet for 14 days. The rats were then adrenalectomized under sodium pentobarbital anesthesia and fasted overnight. The following day, 3 ml of normal saline along with aldosterone alone, 19-nor-A-dione alone or a combination of aldosterone plus 19-nor-A-dione dissolved in 0.5 ml of 10 % ethanol were injected into 5 rats intraperitoneally. For control, 3 ml of normal saline along with 0.5 ml of 10 % ethanol were injected into 5 rats. The rats were sacrificed 3 hours later and the urine was aspirated from bladders. The concentrations of Na and K of the urine were measured with a flame photometer. Urinary Na/K ratio was calculated from the concentrations of Na and K and was used as the index of mineralocorticoid activity; the greater the mineralocorticoid activity, the lower the Na/K ratio. Urinary Na and K excretion was calculated from the concentrations of Na and K and urine volume. The index of precision of the bioassay ranged between 0.2 and 0.3.

Dose-response curves of mineralocorticoid activity of aldosterone and 19-nor-A-dione in normal and sodium-loaded conditions are shown in Figure 1.

Aldosterone showed mineralocorticoid activity in normal and sodium-loaded conditions. The mineralocorticoid activity of aldosterone in sodium-loaded conditions was less potent than that in normal conditions. The minimum detectable amount of aldosterone was 0.0625 μg/rat in normal conditions and 0.20 μg/rat in sodium-loaded conditions. 19-Nor-A-dione did not show mineralocorticoid activity in doses up to 600 μg in normal or sodium-loaded conditions.

RESULTS

To examine the amplification of the action of aldosterone by 19-nor-A-dione in normal conditions, graded doses (0.025-0.25 μg) of aldosterone were injected into rats fed a normal sodium diet either alone or simulta-

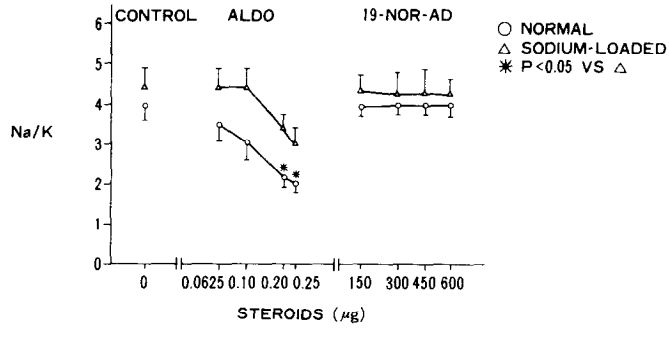


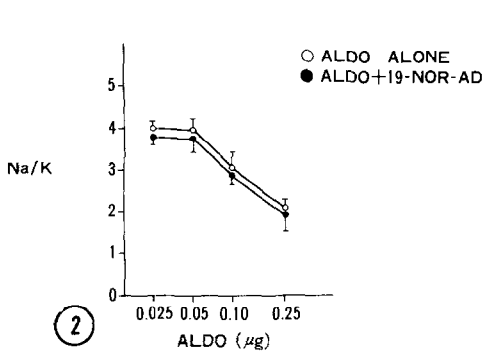
Figure 1. Dose-response curves of mineralocorticoid activity of aldosterone and 19-nor-A-dione in normal and sodium-loaded conditions. The mean ± SE of urinary Na/K ratio is shown. ALDO, aldosterone; 19-NOR-AD, 19-nor-A-dione.

neously with 300 μg 19-nor-A-dione and the urinary Na/K ratio was evaluated (Figure 2).

Urinary Na/K ratio of rats given a combination of graded doses (0.025-0.25 μg) of aldosterone plus 300 μg 19-nor-A-dione was not significantly different from that of rats given graded doses (0.025-0.25 μg) of aldosterone alone.

To examine the amplification of the action of aldosterone by 19-nor-A-dione in sodium-loaded conditions, graded doses (0.025-0.25 μg) of aldosterone were injected into rats fed a high sodium diet either alone or simultaneously with 300 μg 19-nor-A-dione and the urinary Na/K ratio was evaluated (Figure 3).

Urinary Na/K ratio of rats given a combination of 0.025, 0.05 or 0.25 μg aldosterone plus 300 μg 19-nor-A-dione was not significantly different from that of rats given 0.025, 0.05 or 0.25 μg aldosterone alone. However, urinary Na/K ratio of rats given a combination of 0.10 μg aldosterone plus 300 μg 19-nor-A-dione was significantly lower than that of rats given 0.10 μg aldosterone alone.



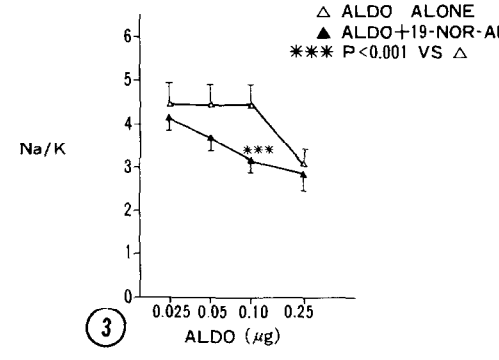


Figure 2. Urinary Na/K ratio of rats given graded doses (0.025-0.25 μg) of aldosterone alone and a combination of graded doses of aldosterone plus 300 μg 19-nor-A-dione in normal conditions. The mean ± SE of urinary Na/K ratio is shown. Abbreviations are the same as in Figure 1.

Figure 3. Urinary Na/K ratio of rats given graded doses (0.025-0.25 μg) of aldosterone alone and a combination of graded doses of aldosterone plus 300 μg 19-nor-A-dione in sodium-loaded conditions. The mean ± SE of urinary Na/K ratio is shown. Abbreviations are the same as in Figure 1.

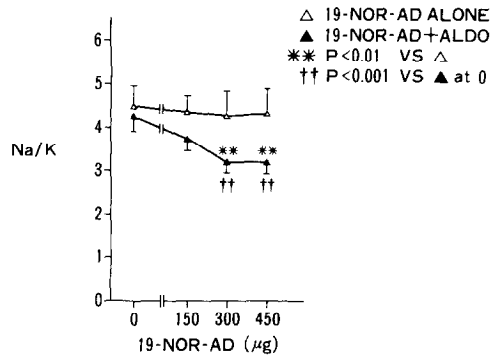


Figure 4. Urinary Na/K ratio of rats given graded doses (150-450 μg) of 19-nor-A-dione alone and a combination of graded doses of 19-nor-A-dione plus 0.10 μg aldosterone in sodium-loaded conditions. The mean ± SE of urinary Na/K ratio is shown. Abbreviations are the same as in Figure 1.

Urinary Na/K ratio of rats given a combination of 0.10 μg aldosterone plus 300 μg 19-nor-A-dione was approximately equivalent to that of rats given 0.25 μg aldosterone alone. Therefore, a 2.5 fold increase in mineralocorticoid activity of 0.10 μg aldosterone was obtained by the simultaneous administration of 0.10 μg aldosterone and 300 μg 19-nor-A-dione.

To examine the dose dependence of the amplification effect of 19-nor-A-dione on aldosterone in sodium-loaded conditions, graded doses (150-450 μg) of 19-nor-A-dione were injected into rats fed a high sodium diet simultaneously with 0.10 μg aldosterone and the urinary Na/K ratio was evaluated (Figure 4).

Urinary Na/K ratio of rats given a combination of 0.10 μg aldosterone plus 150 μg 19-nor-A-dione was not significantly different from that of rats given 0.10 μg aldosterone alone. In contrast, urinary Na/K ratio of rats given a combination of 0.10 μg aldosterone plus 300-450 μg 19-nor-A-dione was significantly lower than that of rats given 0.10 μg aldosterone alone and that of rats given 300-450 μg 19-nor-A-dione alone.

To examine whether the decrease in urinary Na/K ratio was caused by a decrease in urinary Na excretion or an increase in K excretion, urinary

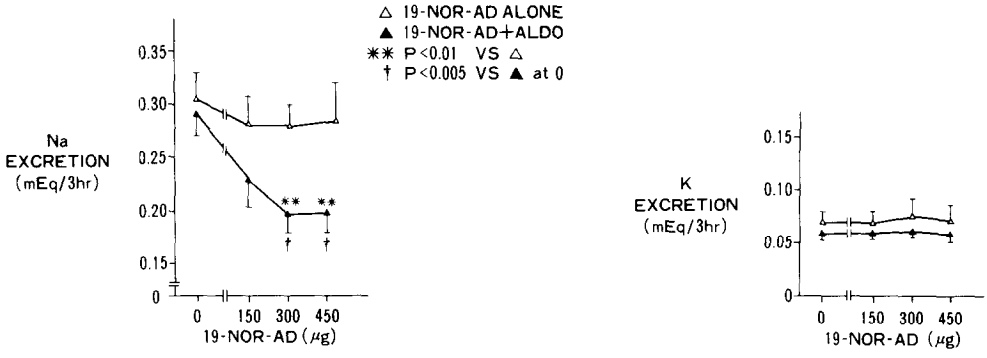


Figure 5. Urinary Na and K excretion of rats given graded doses (150-450 μg) of 19-nor-A-dione alone and a combination of graded doses of 19-nor-A-dione plus 0.10 μg aldosterone in sodium-loaded conditions. The mean ± SE of urinary Na and K excretion is shown. Abbreviations are the same as in Figure 1.

Na and K excretion of rats given a combination of 0.10 μg aldosterone plus 300-450 μg 19-nor-A-dione was evaluated (Figure 5).

Urinary Na excretion of rats given a combination of 0.10 μg aldosterone plus 300-450 μg 19-nor-A-dione was significantly lower than that of rats given 0.10 μg aldosterone alone and that of rats given 300-450 μg 19-nor-A-dione alone. In contrast, urinary K excretion of rats given a combination of 0.10 μg aldosterone plus 300-450 μg 19-nor-A-dione was not significantly different from that of rats given 0.10 μg aldosterone alone.

DISCUSSION

In the present study, the administration of 19-nor-A-dione in normal or sodium-loaded conditions did not cause any significant change in urinary Na/K ratio. The simultaneous administration of subthreshold doses of aldosterone and 19-nor-A-dione in normal conditions also did not cause any significant change in urinary Na/K ratio. However, the simultaneous administration of subthreshold doses of aldosterone and 19-nor-A-dione in sodium-loaded conditions caused a significant decrease in urinary Na/K ratio. The decrease in urinary Na/K ratio was caused by a decrease in urinary Na excretion. These results demonstrate that 19-nor-A-dione,

which did not amplify the action of aldosterone in normal conditions, amplified the action of subthreshold doses of aldosterone in sodium-loaded conditions. 19-Nor-A-dione is an amplifier of aldosterone which works only in sodium-loaded conditions.

Although several amplifiers of aldosterone which work in normal conditions have been reported (2-4), amplifiers which do not work in normal conditions and work only in sodium-loaded conditions have not yet been described. 19-Nor-A-dione is considered to belong to a new class of amplifiers of aldosterone.

The mechanism by which 19-nor-A-dione amplifies the action of aldosterone in sodium-loaded conditions is not clear at the present time. The details should be evaluated further.

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