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Inhibition of Furosemide-Induced Kaliuresis in the Rat by Trilostane, an Inhibitor of Adrenal Steroidogenesis (41961)

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Abstract. In rats treated with furosemide, urinary losses of water, sodium and potassium were accompanied by increased circulating levels of aldosterone. Trilostane, an inhibitor of adrenal 3β-hydroxysteroid dehydrogenase activity, prevented furosemide-induced hyperaldosteronism which resulted in a partial inhibition of diuretic-induced kaliuresis without a change in sodium and water excretion. Spironolactone, an antagonist of mineralocorticoid action with inherent diuretic activity, produced qualitatively similar effects to those of trilostane on urinary electrolyte excretion in furosemide-treated intact rats. However, mineralocorticoid-induced potassium loss in adrenalectomized rats was not altered by trilostane but was prevented by spironolactone reflecting the direct effect of spironolactone on the kidney. In addition, furosemide-induced kaliuresis in adrenalectomized rats was not prevented by trilostane. Therefore, although both trilostane and spironolactone reduce diuretic-induced potassium loss, spironolactone acts by competing with aldosterone for the mineralocorticoid receptor while trilostane appears to act exclusively by preventing secondary hyperaldosteronism.

The use of furosemide (1, 2) and the thiazide diuretics (3) results in a loss of potassium as well as sodium and water. In addition, treatment with these diuretics causes an increase in circulating aldosterone levels (3–7). Presumably, the secondary hyperaldosteronism is a consequence of the fluid and sodium loss and, at least in part, accounts for the excessive potassium loss.

Spironolactone, acting as a competitive antagonist of aldosterone at the distal tubules, prevents the sodium for potassium exchange and, thus, is used to prevent or reverse thiazide-induced hypokalemia (8, 9). We have reported that trilostane [(4α,5α,17β)-4,5-epoxy-3,17-dihydroandrost-2-ene-2-carbonitrile], an inhibitor of 3β-hydroxysteroid dehydrogenase activity that is devoid of hormone agonist or antagonist activities, inhibited the production of aldosterone measured 4 hr after treatment in rats fed a low-sodium diet and treated with ACTH (10). The onset of the action of trilostane would be expected to be rapid since circulating levels of trilostane were maximal one-half hour after oral administration (11). The following studies were undertaken to determine the acute effect of trilostane on the circulating aldosterone levels and the kaliuresis which occurs following treatment with diuretics.

Materials and Methods. *Animal experiments.* Male Sprague–Dawley rats weighing approximately 200 g were housed with a 12-hr light/dark cycle and were fed Wayne Lab-Blox. Adrenalectomized rats were maintained on 0.9% saline drinking water and were placed on test 2 days following surgery. The animals in each test were fasted overnight and were then placed in individual metabolism cages equipped for urine collection.

Furosemide (Lasix, Hoechst-Roussel Pharmaceuticals Inc., Somerville, N.J.), spironolactone (Aldactone, Searle and Co., San Juan, Puerto Rico), and trilostane were suspended in 1% gum tragacanth (gum tragacanth:water, 1:99) and were administered orally at doses of 12.5 to 50 mg/kg as indicated in the individual studies in a volume of 5 ml/kg body weight (bw). Spironolactone or trilostane was given to rats (n = 12/group) at t = 0, 2, and 4 hr and furosemide was given at t = 2, 4, and 6 hr; Krebs–Ringer phosphate buffer, 50 ml/kg bw, was given at t = 2 hr and urine was collected from the second to seventh hour. In two experiments, at the completion of the 5-hr urine collection period, the rats (n = 12/group) were anesthetized with sodium pentobarbital and blood samples were obtained by aortic puncture.

In one of the studies using adrenalecto-

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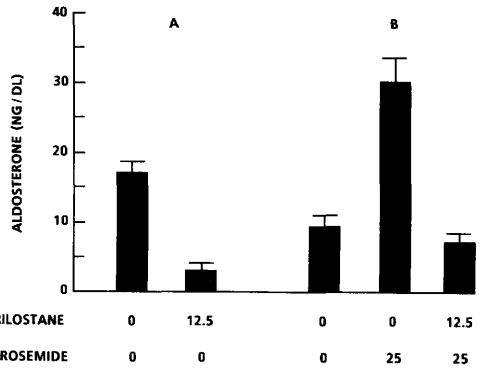

FIG. 1. Plasma levels of aldosterone in rats. The doses of trilostane and furosemide in mg/kg are indicated. The values are means ± SEM of six samples paired from 12 rats/group. Comparison for trilostane alone relative to vehicle and for trilostane in combination with furosemide relative to furosemide alone is statistically significant, $P < 0.01$.

mized rats, deoxycorticosterone acetate (Sigma Chemical Co., St. Louis, Mo.) was prepared as a solution in ethanol:cottonseed oil (1:9) and was administered sc in volume of 1 ml/kg bw at $t = 0$, 2, and 4 hr. Trilostane and spironolactone were administered ($n = 8$/group) as described above and isotonic saline, 50 ml/kg bw, also was administered at $t = 0$ hr; urine was collected for the following 24 hr.

Biochemical determinations. Urinary sodium and potassium were measured by flame photometry. Plasma aldosterone levels were determined by radioimmunoassay using antiserum obtained from New England Nuclear Corporation (Boston, Mass.) following purification of plasma by column chromatography (12, 13).

Results. Circulating levels of aldosterone in normal male rats treated with trilostane at a dose of 12.5 mg/kg were significantly lower than those in vehicle-treated control rats (Fig. 1A). In a similar study (Fig. 1B), the circulating aldosterone levels were significantly higher in the rats treated with furosemide at 25 mg/kg than they were in the vehicle-treated control rats while concomitant treatment with trilostane prevented the increase in aldosterone produced by the diuretic.

As shown in Table I, trilostane alone at a dose of 25 mg/kg had no significant effect on urine volume or sodium excretion but reduced potassium excretion. Furosemide alone at a dose of 25 mg/kg had a significant diuretic effect accompanied by marked losses in both sodium and potassium. When administered with furosemide, trilostane reduced furosemide-induced kaliuresis by 56% and the conservation of potassium was accomplished without significantly altering the

TABLE I. INHIBITION OF FUROSEMIDE-INDUCED KALIURESIS BY EITHER TRILOSTANE OR SPIRONOLACTONE

Treatment[a]	Urine (5-hr collection)		
	Volume (ml)	Sodium (Total meq)	Potassium (Total meq)
Vehicle	4.0 ± 0.2[b]	0.11 ± 0.01	0.12 ± 0.02
Trilostane	3.9 ± 0.4	0.14 ± 0.03	0.05 ± 0.01[c]
Furosemide	12.7 ± 0.4[d]	1.18 ± 0.05[d]	0.37 ± 0.02[d]
Furosemide + trilostane	11.7 ± 0.4	0.99 ± 0.06	0.21 ± 0.02[e]
Vehicle	3.9 ± 0.4	0.21 ± 0.03	0.12 ± 0.01
Spironolactone	7.0 ± 0.7[d]	0.74 ± 0.07	0.08 ± 0.01[d]
Furosemide	15.7 ± 1.4[d]	1.55 ± 0.16[d]	0.44 ± 0.04[d]
Furosemide + spironolactone	15.2 ± 0.7	1.73 ± 0.10	0.22 ± 0.02[e]

[a] Trilostane and furosemide were each administered orally at a dose of 25 mg/kg × 3 and spironolactone was administered orally at a dose of 50 mg/kg × 3.

[b] Mean ± SEM of 12 rats/group.

[c] Significantly different from the mean of the group treated with vehicle alone, $P < 0.01$.

[d] Significantly different from the mean of the group treated with vehicle alone, $P < 0.001$.

[e] Significantly different from the mean of the group treated with vehicle alone and the group treated with furosemide alone, $P < 0.01$.

TABLE II. MODIFICATION OF DOCA-INDUCED CHANGES IN URINARY ELECTROLYTE EXCRETION IN ADRENALECTOMIZED RATS BY SPIRONOLACTONE

Treatment[a]	Urine (5-hr collection) Volume (ml)	Sodium (Total meq)	Potassium (Total meq)
Vehicle	13.4 ± 0.9[b]	2.43 ± 0.27	0.45 ± 0.10
Spironolactone (po)	12.8 ± 1.0	2.90 ± 0.17	0.50 ± 0.05
Trilostane (po)	12.4 ± 0.9	2.73 ± 0.18	0.29 ± 0.03
DOCA (sc)	6.3 ± 0.7[c]	1.14 ± 0.14[c]	0.75 ± 0.03[c]
DOCA + spironolactone	11.4 ± 0.8[d]	2.49 ± 0.13[d]	0.54 ± 0.06[d]
DOCA + trilostane	7.1 ± 0.7	1.34 ± 0.16	0.62 ± 0.04

[a] Trilostane and spironolactone were each administered at a dose of 50 mg/kg × 3 and DOCA was administered subcutaneously at a dose of 1 mg/kg × 3.
[b] Mean ± SEM of 8 rats/group.
[c] Significantly different from the mean of the group treated with vehicle alone, $P < 0.01$.
[d] Significantly different from the mean of the group treated with DOCA alone, $P < 0.001$.

water and sodium loss. In contrast, spironolactone, when administered by itself at a dose of 50 mg/kg, produced modest losses in sodium and water and caused retention of potassium. Combined treatment with spironolactone and furosemide produced results similar to those obtained with the trilostane–furosemide combination, i.e., a retention of potassium without altering the furosemide-induced effects on sodium and water loss. With both potassium sparing agents, the prevention of potassium loss was partial; i.e., urine potassium levels were significantly lower than those seen in the group treated with furosemide but significantly higher than values in the control group.

Treatment of adrenalectomized rats with either trilostane or spironolactone at 50 mg/kg did not alter urine volume or the urinary electrolyte excretion pattern. However, in rats treated with deoxycorticosterone acetate (DOCA), the urine volume and sodium excretion were significantly less than that of vehicle-treated control rats while potassium excretion was significantly higher. Administration of spironolactone to the DOCA-treated adrenalectomized rats completely reversed the DOCA-induced effects on urine volume and electrolytes while administration of trilostane did not modify the DOCA-induced effects (Table II).

In adrenalectomized rats, furosemide had a direct kaliuretic effect at 25 mg/kg in the absence of endogenous mineralocorticoid (Table III) which was not prevented by trilostane.

Discussion. Water and sodium loss are known to stimulate aldosterone production. In the present studies, as well as those of others (14), furosemide treatment caused elevated levels of plasma aldosterone in rats. The change in mineralocorticoid levels has been attributed to the stimulation of the renin–angiotensin system as a consequence of the loss in sodium and decrease in extracellular fluid volume produced by the diuretic (4, 5).

Potassium loss occurs as a direct effect of the diuretic and as a result of secondary aldosteronism. In intact rats, trilostane did not completely prevent the kaliuretic effects

TABLE III. LACK OF EFFECT OF TRILOSTANE ON FUROSEMIDE-INDUCED KALURESIS IN ADRENALECTOMIZED RATS

Treatment[a]	Urine (5-hr collection) Volume (ml)	Sodium (total meq)	Potassium (total meq)
Vehicle	10 ± 0.4[b]	0.78 ± 0.03	0.13 ± 0.01
Furosemide	18 ± 1.0[c]	1.98 ± 0.09[c]	0.29 ± 0.03[c]
Trilostane	8.4 ± 0.6	0.75 ± 0.07	0.11 ± 0.01
Furosemide + trilostane	17 ± 0.8	1.92 ± 0.11	0.25 ± 0.02

[a] Trilostane and furosemide were each administered orally at a dose of 25 mg/kg × 3.
[b] Mean ± SEM of 8 rats/group.
[c] Significantly different from the mean of the group treated with vehicle alone, $P < 0.01$.

of furosemide suggesting that a portion of the furosemide-induced kaliuresis is independent of mineralocorticoid activity. In support of a direct kaliuretic effect of furosemide is the experiment with adrenalectomized rats which demonstrated that furosemide alone had a direct kaliuretic effect and that the effect was not altered by treatment with trilostane. In intact rats, the increased mineralocorticoid production and kaliuresis produced by furosemide can be at least partially inhibited by the concomitant administration of trilostane but trilostane did not alter the diuresis or natruresis associated with furosemide treatment.

Spironolactone has direct diuretic activity in man [e.g., (9)] and the diuretic effect in the rat is shown by the increased urine volume and natruresis noted in the present studies (Table I). In contrast, trilostane alone had no diuretic effects, however, both compounds when administered alone produced a modest reduction in kaliuresis.

The kaliuresis induced by diuretics is of major significance since hypokalemia is the most common side effect seen with diuretic therapy (15), and almost always occurs with diuretic treatment of edematous states such as congestive heart failure or cirrhosis of the liver (16). The use of spironolactone, a competitive antagonist of aldosterone at the distal tubule mineralocorticoid receptors, is recognized as being effective in conserving potassium. The present studies indicate that inhibition of aldosterone production by trilostane provides another means of preventing or reversing diuretic-induced kaliuresis.

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