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Mineralocorticoid Effects of 9α-Fluorodeoxycorticosterone in Adrenalectomized Rats.* (25726)

C. M. KAGAWA AND ROBERT S. JACOBS, JR.
Division of Biological Research, G. D. Searle and Co., Chicago

A large number of 9α-halogenated derivatives of hydrocortisone, corticosterone, 11β-hydroxyprogesterone and 11β,17α-dihydroxyprogesterone and of corresponding 11-oxo steroids have been prepared from 11α-hydroxy intermediates(1,2). In addition to glucocorticoid effects, many of these compounds have shown high levels of mineralocorticoid and survival activity(2-7). So far, however, no biological data seem to be available for 9α-halogenated steroids lacking oxygen functions at carbon 11. From this standpoint, our tests with 9α-fluorodeoxycorticosterone acetate for mineralocorticoid effects in rats are believed to be of interest. The steroid was synthesized directly from corticosterone acetate by Bergstrom and Dodson(8).

Materials and methods. Male Sprague-Dawley rats (150-200 g) fed Purina Chow for about a week were adrenalectomized and maintained with sucrose cubes and tap water overnight. Approximately 24 hours following operation, the animals were injected subcutaneously with deoxycorticosterone acetate (DCA) or with various doses of 9α-fluorodeoxycorticosterone acetate (9α-F-DCA) in Mazola oil. Brisk heating readily dissolved 9α-F-DCA in oil. All animals received in addition a subcutaneous injection of 2.5 ml isotonic saline. Four-hour samples of urine were collected in metabolism cages and analyzed for Na and K content. The DCA-like property of 9α-F-DCA was evaluated by its influence on Na excretion and the ratio of Na/K. After preliminary tests, 9α-F-DCA and DCA were tested simultaneously in a 4-point comparative assay with 14 rats/point, and relative potencies were calculated by the method of Irwin as discussed by Pugsley(9). All measurements of urinary electrolytes have been converted to logarithms. Repeated standardization tests have revealed that this adjustment gives statistically suitable responses, with relatively constant standard error values over a wide dosage range of DCA. Analyses for urinary Na and K were done on Beckman flame photometer.

Results. Data from several initial tests with 9α-F-DCA are summarized in Table I, together with those of 65 untreated animals for convenient reference. DCA as a reference standard at 10 μg/rat in each test produced Na retention and a reduction of ratio of Na/K. A small K excretion accompanied these urinary changes. In these data, 9α-F-DCA produced greater effects on electrolytes over the range of 1-500 μg/rat than did the standard dose of DCA. However, 9α-halo steroid was less effective at dose of 0.3 μg than 10 μg of DCA, to suggest a potency of at least 10 but less than about 30 times rela-

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TABLE 1. Effects of Graded Amounts of 9α-F-DCA on Urinary Excretion of Na and K in Adrenalectomized Rats.

Treatment		Mean log response		
Compound	μg/rat	Na	K	(Na × 10)/K
		.78	.45	1.34
DCA	10	.44	.51	.93
9α-F-DCA	500	.28	.69	.59
"	50	.06	.49	.56
DCA	10	.46	.59	.88
9α-F-DCA	4	.32	.46	.86
"	1	.33	.64	.69
DCA	10	.36	.56	.80
9α-F-DCA	.3	.62	.68	.94
"	.1	.77	.51	1.25

Na and K = log meq/l after dilution of sample/rat/4 hr to 25 ml with water; pooled s = ± 0.22, ± 0.21 and ± 0.20 for Na, K and (Na × 10)/K, respectively; least significant difference (P = 0.05) relative to untreated controls (65 animals) = −0.14 for Na, +0.13 for K and −0.12 for Na/K response; 8-10 animals/treatment.

tive to DCA. Results of simultaneous assay with 2 doses each of DCA and 9α-F-DCA are shown in Table II. Using Na excretion as index of DCA-like activity, a relative potency of 12 was calculated for 9α-F-DCA, with 95% limits of 6.1 to 23.7. No consistent pattern of effects on K excretion was seen with the compounds. Fig. 1 shows data on Na/K. A relative potency of 14.3 (95% limits = 9.1-22.5) was obtained using response index of Na/K for 9α-halo steroid. From these limits, it follows that potency of 9α-F-DCA is significantly greater than that of DCA, with $P < 0.05$. Parallel regression lines in the Figure were drawn in accordance with the pooled slope of DCA and 9α-F-DCA. A chi-square test for parallelism of dose-response curves indicated validity of the assay(9).

Discussion. Our study demonstrates that 9α-F-DCA is a potent mineralocorticoid. The steroid possesses 12 and 14.3 times activity of DCA by response indices of Na retention and reduction of Na/K, respectively. It appears to possess, therefore, an interesting level of activity as found in other mineralocorticoids such as aldosterone(10,11), 19-nor-deoxycorticosterone(12) and various synthetic 9α-halo corticosteroids(2,3,5,11,13,14). Its closest structural relative in the series of 9α-halo steroids can be considered to be 9α-fluorocorticosterone acetate, which possesses 26.2 times the potency of DCA (95% confidence interval = 10.9-66.0) according to Na retention tests of Fried and Borman in rats(2,5). Other data on life maintenance activity in adrenalectomized animals also suggest a strong DCA-like property for this steroid(4,6). These data show that Na retaining activity may be increased by 2.2-fold with introduction of oxygen function at carbon 11 of 9α-F-DCA (potency ratio = 26.2/12 for 9α-fluorocorticosterone acetate/9α-F-DCA). However, the potency of 9α-fluorocorticosterone acetate is not significantly greater, statistically, than that of 12 reported above for 9α-F-DCA.† It would thus appear from progression of mineralocorticoid activities, from DCA, 9α-F-DCA to 9α-fluorocorticosterone acetate, that 9α-halogenation is more influential in increasing activity than 11β-hydroxylation.

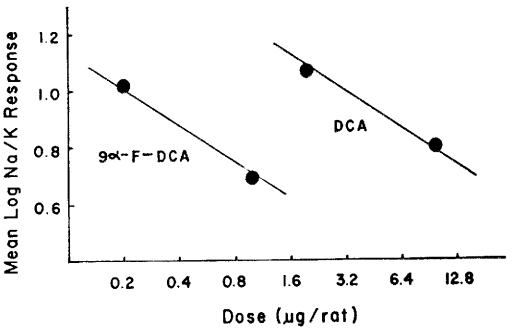

FIG. 1. Relative potencies of DCA and 9α-F-DCA as determined by ability to reduce Na/K response in adrenalectomized rats; potency of 9α-F-DCA = 14.3 (95% limits = 9.1-22.5) relative to DCA; composite slope for the 2 steroids = 0.42/10-fold increment of log dose; 14 rats/treatment.

TABLE II. Results of Assay for Mineralocorticoid Activity with 2 Dosage Levels of DCA and 9α-F-DCA in Adrenalectomized Rats.

Treatment		Mean log response	
Compound	μg/rat	Na	K
DCA	2	.71	.65
	10	.41	.63
9α-F-DCA	.2	.64	.63
	1.0	.41	.72

Potency = 12.0 (95% limits = 6.1-23.7) for Na response relative to DCA; Na and K = log meq/l after dilution of sample/rat/4 hr to 25 ml; 14 rats/treatment.

† A t value of 1.4 corresponding to P = 0.15 was computed with data for 9α-fluorocorticosterone acetate(2,5) and for 9α-F-DCA from our study.

Fried and Borman speculated on possible role of 9α-halo modifications in glucocorticoids (2). Data were presented to demonstrate parallelism between glucocorticoid activity and electronegativity, or ability of 9α-halo substituents to strengthen acidity of adjacent 11β-hydroxy radicals in steroids. However, that 9α-F-DCA is 12-14 times as potent as DCA would suggest other factors besides the influence of electronegativity must account for increased mineralocorticoid effects with 9α-halogenation.

Summary. The mineralocorticoid effects of 9α-fluorodeoxycorticosterone acetate were investigated in adrenalectomized rats. A quantitative 4-point assay gave potency estimates of 12 and 14.3 for the steroid relative to DCA, as judged by reduction of urinary Na excretion and the ratio of Na K, respectively. Thus, addition of a 9α-fluoro radical can increase mineralocorticoid activity in a steroid lacking oxygen function at carbon 11. These and other published data suggest that 9α-halogenation plays a singularly important part in high DCA-like activity of various 9α-halo corticosteroids.

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Intestinal Absorption of a Modified Heparin.* (25727)

B. SALAFSKY AND T. A. LOOMIS

Dept. of Pharmacology, School of Medicine, University of Washington, Seattle

Schanker *et al.*(1) and Hogben *et al.*(2) have shown that intestinal absorption of many drugs is in part dependent on the state of ionization of the drugs. Drugs which are weak organic acids or bases were more completely absorbed from the gastro-intestinal tract in pH ranges which favor the unionized radical. Loomis(3) has shown that sodium heparin is absorbed from the duodenal lumen of the dog at pH 4 but not at higher pH. Warner(4) believes that glucuronic acid carboxyl groups of chondroitin sulfate protonize between pH 5 and 2 thereby reducing ionization of the complex. A similar situation may exist for glucuronic acid carboxyl groups of heparin. If ionization is an important factor in regulating absorption from the intestine, then suitable modification of ionizing potentialities of heparin may enhance absorption in the physiological pH range. The current study is an attempt to accomplish this by esterification of heparin. A modified heparin has been prepared and evidence presented indicating that it is partially methylated and has undergone partial desulfation. It retains its original anticoagulant action and significant absorption

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