Effects of 2-Methylation on Glucocorticoid Activity of Various C-21 Steroids. (22929)

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The wide use of steroids as therapeutic agents has led to attempts to increase or change the biological activity by chemical modification. This has been particularly true of the adrenal glucocorticoids since hydrocortisone was found to be extremely beneficial in the treatment of inflammatory diseases. Introduction of a fluorine atom at the C-9 position of several C-21 steroids produces striking enhancement of biological effectiveness(1,2,3). Unsaturation at the C-1–C-2 bond increases the glucocorticoid activity of hydrocortisone and cortisone(4). More recently it has been shown that the combination of 9α-fluorine and 1,2-unsaturation in the same molecule further increases the glucocorticoid activity of hydrocortisone(5,6). A methyl group at the C-2 of hydrocortisone or 9α-fluorohydrocortisone molecule is accompanied by a pronounced increase in biological activity of either compound(7,9). Investigation into the effects of 2-methylation on the activity of other 11-oxygenated C-21 steroids structurally related to hydrocortisone has been carried out. This report presents results of glycogen deposition assays and anti-inflammatory tests on a number of steroids in this series. The work has uncovered some interesting correlations between structure and biological activity.

Methods. Animals. All animals were male rats obtained from the Upjohn colony (Sprague-Dawley ancestry). They were adrenalectomized at body weight of 140-160 g and were maintained on stock diet (Archer dog pellets) and 1% sodium chloride as drinking fluid. *Steroids.* The steroids* were administered as suspension in an aqueous system of 0.5% carboxymethyl-cellulose, 0.4% Tween 80, 1.5% benzyl alcohol, and 0.9% sodium chloride. Suspension was accomplished by grinding crystals and vehicle together in ground glass tissue homogenizer. *Glycogen deposition test.* Liver glycogens were determined by the anthrone method previously described by Stafford *et al.*(5) on tissues removed 7 hours following a single subcutaneous injection of the compounds. *Anti-inflammatory test.* The test used was previously described by Dulin(6) and depends on the ability of 7 daily injections of a compound to inhibit granuloma formation around subcutaneously implanted cotton pellets. *Experimental design.* The unknown compounds were tested simultaneously with hydrocortisone which was used as a standard and assigned a potency value of 1. When the unknowns were tested at two or more dose levels in parallel with hydrocortisone, potency ratios were determined by the method of Irwin(8). In those assays in which one dose of the unknown was tested against 2 or more doses of standard, the potencies of the unknowns were determined by graphically plotting the hydrocortisone dose-response curve and correlating the dose of standard required to produce a response equal to that produced by the test dose of the unknown. A description of the experimental design of the assays is shown in Table I.

Results. 2-Methylation of hydrocortisone (I) and of 9α-fluorohydrocortisone (II, III) increased both anti-inflammatory and glycogen deposition activity of the parent steroids. In the case of 11β, 17α-dihydroxyprogesterone (IV), although the biological activity of the parent compound was barely demonstrable at the doses used, the 2-methylated compound (V) was 30% of hydrocortisone as an anti-inflammatory agent and 110% on the glycogen test. The 2-methylated derivative (XXIII) of 9α-fluoro-11β, 17α-dihydroxyprogesterone (XXIV) was more potent on the anti-inflammatory test than the parent compound (250% and 67% as active as hydrocortisone respectively). It is further seen

* The compounds used were generously supplied by the Chemistry Department, The Upjohn Co.

TABLE I. Description of Experimental Design of Glycogen Deposition and Anti-Inflammatory Tests Which Are Presented in Table II.

Test	Description of test: No. doses of standard	No. doses of unknown	No. rats per group	Compounds tested by each design*
Glycogen deposition	2	2	4–5	I, XIII
	3	3	4–6	II, IV, VI, VIII
	2	3	5–10	IX, XVI, XV, XVIII
	3	8	5–10	III
	2	1	5	V, VII, X, XI
	3	1	6–10	XII, XIV, XVII
Anti-inflammatory	2	2	7–10	I, II, III, V, XV, XVII, XVIII, XXI, XXII
	2	1	7–16	IV, VIII, IX, X, XI, XII, XIII, XIV, XIX, XX, XXIII
	1	1	6	XXIV

* Compound numbers and names are shown in Table II.

that 2-methyl-Δ-6-hydrocortisone (VII) was 270% as effective as hydrocortisone on the glycogen test while its nonmethylated parent (VI) was only 100% of hydrocortisone.

In the case of cortisone (VIII), 2-methylation (IX) reduced the anti-inflammatory and glycogen depositing activities. A similar relationship was found to exist between the 2-methyl analogue of 9α-fluorocortisone (XVI) and the parent compound. This is shown by the fact that the glycogen activity of the methyl derivative is only 40% of hydrocortisone while activity of fluorocortisone is reported(3) to be much greater than that of hydrocortisone.

The activities of 11-ketoprogesterone (XII) and 11β-hydroxyprogesterone (XIV) were only very slight by both tests and this was also true of the 2-methyl analogues (XIII and XV). The activities of these compounds were not significantly enhanced by 2-methyl substitution. It could not be determined whether there was an actual decrease in activity induced by 2-methylation since the parent compounds possessed very slight activity.

Compound A or 11-dehydrocorticosterone (X) and its 2-methyl analogue (XI) are related to one another much as cortisone and its analogue. In this case the parent compound was 20% of hydrocortisone on the anti-inflammatory test while the 2-methyl analogue was less than 10% as potent as hydrocortisone. In the glycogen deposition test compound A was 40% as active as hydrocortisone while the 2-methyl analogue was less than 10%. Introduction of a 2-methyl group had no significant effect on the anti-inflammatory or glycogen deposition activities of corticosterone (XVII, XVIII), 9α-fluorocorticosterone (XIX, XX), 11β-hydroxy-progesterone (XIV, XV), or 9α-fluoro-11β-hydroxyprogesterone (XXI, XXII).

Discussion. It is apparent from these results (Table II) that 2-methylation does not universally increase anti-inflammatory and liver glycogen deposition activity of C-21 steroids. Of the steroids studied, the only cases where 2-methylation unequivocally increased the activity were in hydrocortisone, 9α-fluorohydrocortisone, 11β, 17α-dihydroxyprogesterone, 9α-fluoro-11β, 17α-dihydroxyprogesterone, and Δ-6-hydrocortisone. In contrast, 2-methylation produced striking decreases in anti-inflammatory and liver glycogen deposition effects of cortisone, fluorocortisone and dehydrocorticosterone. In the case of 11-ketoprogesterone no depression of activity was seen since the parent compound was essentially inactive on the above tests. In the cases of corticosterone, fluorocorticosterone, 11β-hydroxyprogesterone, and fluoro-11β-hydroxyprogesterone, 2-methylation did not alter the activities in either direction.

The augmentation of anti-inflammatory and liver glycogen deposition activities seen with 2-methylation is a different phenomenon than that obtained with 9α-halogenation. In

TABLE II. Summary of Results of 2-Methylation on Glucocorticoid and Anti-Inflammatory Activity of Various C-21 Steroids.

Parent compound	No.	Activity (× F) Glycogen	Anti-inflammatory	2-Methyl analog No.	Activity (× F) Glycogen	Anti-inflammatory
Hydrocortisone		1	1	I	5.8	3.5
9α-Fluorohydrocortisone acetate	II	12.6	7	III	38	9
11β,17α-Dihydroxyprogesterone	IV	.1	.05	V	1.1	.3
6-Dehydrohydrocortisone	VI	1		VII	2.7	
9α-Fluoro-11β,17α-dihydroxyprogesterone	XXIII		.7	XXIV		2.5
Cortisone	VIII	.6	.4	IX	<.4	<.07
9α-Fluorocortisone acetate		6*		XVI	.4	
11-Dehydrocorticosterone acetate	X	.4	.2	XI	<.1	<.1
11-Ketoprogesterone	XII	<.4	<.07	XIII	<.3	<.1
11β-Hydroxyprogesterone	XIV	<.4	<.07	XV	10	<.1
Corticosterone acetate	XVII	.8	.3	XVIII	.5	.3
9α-Fluorocorticosterone acetate	XIX		3.5	XX		2.4
9α-Fluoro-11β-hydroxyprogesterone	XXI		.2	XXII		.2

* Fried, J., *Annals N. Y. Acad. Sci.*, 1955, v61, 573.

all cases of published data on 9α-halogenation of corticoids, the analogues are more potent than the parent compounds(1,2,3). The changes in activity accompanying 2-methylation of C-21 steroids described here may not be seen in other biological activities than the two reported since preliminary data on these compounds indicate that different structure-function relationships appear to occur in the case of salt retention and pituitary ACTH inhibition.

The relationships described in this series of compounds show the danger of predicting biological activity for analogues of compounds of known activity. The experience with the potency augmenting effects of 9α-halogenation and 1,2-unsaturation of cortisone and hydrocortisone would have led to the prediction that since 2-methylhydrocortisone was more active than hydrocortisone, the same relationship would hold between 2-methylcortisone and cortisone. Such a prediction would obviously have been wrong.

Summary. The anti-inflammatory and glycogen deposition activities of some C-21 steroids and their 2-methyl analogues have been described. The 2-methyl analogues were more potent than the parent compound in the cases of hydrocortisone, 9α-fluorohydrocortisone, 6-dehydrohydrocortisone, 11β, 17α-dihydroxyprogesterone and 9α-fluoro-11β, 17α-dihydroxyprogesterone. In the cases of 11β-hydroxyprogesterone, corticosterone, 9α-fluorocorticosterone, 9α-fluoro-11β-hydroxyprogesterone and 11-ketoprogesterone the 2-methyl did not potentiate the activity. The 2-methyl analogues of cortisone, 9α-fluorocortisone and 11-dehydrocorticosterone were less effective than the parent steroid.

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