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Research Article

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DISSOCIATION OF CORTICOTROPIN-SUPPRESSING ACTIVITY FROM THE EOSINOPENIC AND HYPERGLYCEMIC ACTIVITIES OF CORTICOSTEROID ANALOGUES *

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Previous studies have indicated close correlation between the adrenocorticotropin (ACTH)-suppressing activity of corticosteroids and their effectiveness in inducing decreases in circulating eosinophils and increases in blood glucose. Heretofore, any structural modification of steroid which has resulted in altered potency with respect to one of these activities has resulted in equivalent alteration of potency with respect to the others (1, 2). The present study is concerned with three synthetic analogues of cortisol which exhibit dissociation of these properties when administered to human subjects. Chemically, all three compounds differ from cortisol (hydrocortisone) in that they lack hydroxyl groups at the carbon-21 position. Biologically, all three have been found to be effective ACTH-suppressing agents with little or no eosinopenic or hyperglycemic activity.

METHODS

The following steroids (with convenient abbreviations) were assayed in normal human subjects: *1*) 9α-fluoro-11β,17α-dihydroxy-$\Delta^{1,4}$-pregnene-3,20-dione (21-desoxy-ΔFF); *2*) 9α-21-difluoro,11β,17α-dihydroxy-Δ^{4}-pregnene-3,20-dione (difluoro); *3*) 6α-methyl-9α-fluoro-11β,17α-dihydroxy-$\Delta^{1,4}$-pregnene-3,20-dione-21-mesylate (mesylate); and *4*) 9α-fluoro-11β,17α,21-trihydroxy-$\Delta^{1,4}$-pregnene-3,20-dione (ΔFF). In each assay ΔFF served as a biological standard.

Assays of ACTH-suppressing potency. ACTH-suppressing potency was judged from percentage decreases in urinary 17-hydroxycorticosteroids which occurred in response to steroids administered orally every 6 hours for a total of 48 hours. Tests were carried out at weekly intervals with each of 4 subjects receiving graded doses of each compound on a random schedule. Daily urinary 17-hydroxycorticosteroids were determined by a modification of the method of Silber and Porter (3). Completeness of urine collection was checked by creatinine determinations performed by the method of Folin as described by Hawk, Oser, and Summerson (4).

Assays of eosinopenic potency. Circulating eosinophils were counted at 9 a.m., 1 p.m., and 4 p.m. Steroids were administered orally immediately after the 9 a.m. determination. The decrease in circulating eosinophils was calculated for each of the two later determinations and expressed as a percentage of the initial count. The mean of these two percentages was taken as the index of eosinopenic potency. At least 4 subjects received graded doses of each test substance.

TABLE I

*Urinary 17-hydroxycorticosteroids (in mg per 24 hrs) in four subjects receiving graded doses of ΔFF and carbon-21-modified steroids**

		Subject							
		W		C		H		S	
Steroid	Dosage	Cont.	Rx	Cont.	Rx	Cont.	Rx	Cont.	Rx
	mg								
ΔFF	0.5	10.1	8.8	4.8	3.0	8.6	6.5		
	1.0	13.3	8.9	6.5	3.8	6.6	3.9	6.9	3.9
	2.0	9.3	4.2	5.7	1.6	8.1	2.8	8.8	2.8
	4.0	10.1	1.7	6.1	1.1	8.5	1.0	6.8	1.2
U 19,871 Mesylate	32	9.4	5.8	4.9	3.5	8.2	7.2	8.8	4.6
	48					7.6	4.3		
	64	12.0	5.2	5.8	4.0			5.4	2.7
	96	10.5	3.9			7.3	5.3	6.2	3.6
	192	11.0	1.0	5.0	0.6	8.0	1.8	6.6	1.1
U 6,942 21-Desoxy-ΔFF	4	11.0	8.0	4.9	4.5	8.9	5.6	5.4	3.3
	10	11.9	8.8	6.6	4.2	8.9	5.2	7.3	3.9
	20	11.5	5.9	4.9	2.4	9.0	4.5	8.6	2.6
	40	10.5	3.1	4.4	1.9	8.3	2.5	7.6	0.8
U 7,686 Difluoro	40	11.5	5.5	4.6	2.5	7.3	3.1	6.5	4.6
	80	11.6	4.0	5.6	1.0	9.5	3.0	7.5	2.7
	160	12.1	1.2						

* Each "control" value represents the mean of determinations carried out on the two days immediately preceding treatment. Each "Rx" value represents the determination carried out on the second day of treatment. Total dosage (8 times the individual 6 hourly dosage) is represented. Abbreviations: ΔFF = 9α-fluoro-11β, 17α, 21-trihydroxy-$\Delta^{1,4}$-pregnene-3,20-dione; mesylate = 6α-methyl-9α-fluoro-11β,17α-dihydroxy-$\Delta^{1,4}$-pregnene-3,20-dione-21-mesylate; 21-desoxy-ΔFF = 9α-fluoro-11β, 17α-dihydroxy-$\Delta^{1,4}$-pregnene-3, 20-dione; and difluoro = 9α-21-difluoro, 11β, 17α-dihydroxy-Δ^{4}-pregnene-3, 20-dione.

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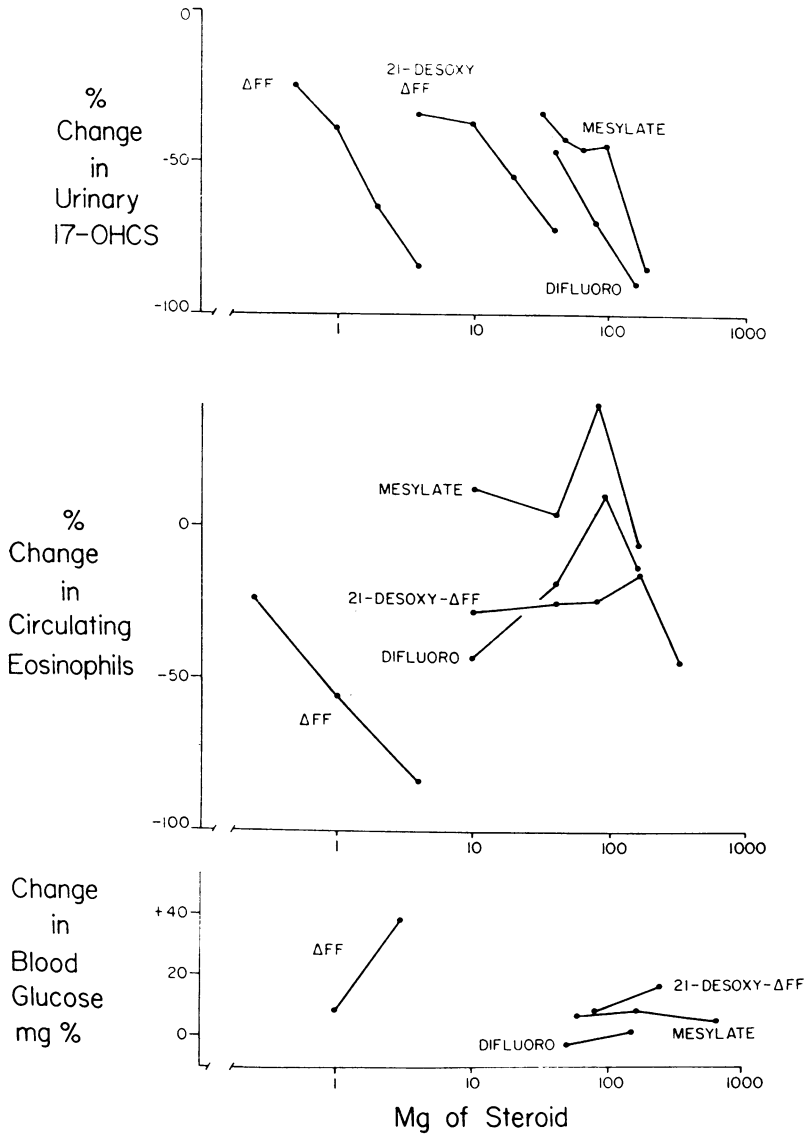

FIG. 1. EFFECTS OF GRADED DOSES OF FOUR STEROIDS UPON URINARY 17-HYDROXYCORTICOSTEROIDS, CIRCULATING EOSINOPHILS, AND BLOOD GLUCOSE. Each point represents the mean change from control observations (represented as zero on the ordinates). For example, in the hyperglycemia assay the value plotted represents the difference between the 1-hour postinfusion blood glucose concentration obtained on the day of steroid treatment and the 1-hour post-infusion blood glucose obtained on days when no steroid was given. Dosage is represented on a logarithmic scale.

Assays of hyperglycemic potency. Estimates of hyperglycemic potency were based on a modification of the method of West (2). Each steroid was assayed at 2 or more dosage levels in each of 4 or more subjects. Individual tests were performed at intervals of 3 or 4 days. Subjects were fasted from 9 p.m. of the evening before the test. The test steroid was administered orally at 12:30 a.m. A fasting blood glucose specimen was obtained at 8:30 a.m. Twenty-five g of dextrose was then given intravenously (as 250 ml of 10% dextrose in water) over an 11- to 14-minute period. A postinfusion blood specimen was obtained exactly one hour after the beginning of the infusion. Blood glucose determinations were performed in duplicate by a modification of the methods of Nelson (5) and Somogyi (6). In agreement with the observations of West, it has been the experience

TABLE II

*Numbers of eosinophils per millimeter³ of blood of subjects receiving graded doses of ΔFF and carbon 21-modified steroids**

		Subject													
		E		T		K		P		C		D		W	
Steroid	Dosage	Cont.	Rx	Cont.	Rx	Cont.	Rx	Cont.	Rx	Cont.	Rx	Cont.	Rx	Cont.	Rx
	mg														
None	0	56	76	92	83	94	61	192	176	120	104	146	123	141	160
ΔFF	0.25	92	92	101	71	144	63	175	125	100	78	150	120	118	100
	1.0	87	46	93	46	104	28	218	107	153	69	178	53	263	138
	4.0			68	12					106	13				
Mesylate	10	91	105			143	154	62	124					144	111
	40					110	80	160	156					90	128
	80	91	100			116	115	208	211					62	206
	160	81	76			106	69	153	108					103	151
21-Desoxy-ΔFF	10			149	122	134	52	266	220	143	120				
	40	81	69	93	84	125	56	243	134	119	114				
	80	62	77			107	46	170	89			134	113	168	118
	160							168	82					93	112
	320					100	15	193	129			125	52	138	137
Difluoro	10					243	155			93	46				
	40					146	110			81	69				
	80							234	180					103	148
	160					103	59	159	140					151	143

* Each "control" value indicates the eosinophil count immediately before treatment. Each "Rx" value represents the average of the eosinophil counts at 4 and 7 hours after treatment. Abbreviations as in Table I.

of this laboratory that the hyperglycemic effect of a glucocorticoid is more readily demonstrable 1 hour after the rapid intravenous infusion of a glucose load than it is under fasting conditions. Consequently, in this study the 1 hour postinfusion blood glucose concentration was employed in calculating the hyperglycemic potencies of the various steroids.

RESULTS

Mean responses to graded doses of the various steroids are depicted graphically in Figure 1. Individual responses in the ACTH-suppression assay are shown in Table I, in the eosinopenia assay in Table II, and in the hyperglycemia assay in Table III.

ACTH-suppression assays (Figure 1; Table I)

The standard, ΔFF, was progressively more effective in suppressing ACTH as dosage increased from 0.06 to 0.5 mg every 6 hours. At somewhat

TABLE III

*Blood glucose, in mg per 100 ml, of five subjects receiving graded doses of ΔFF and carbon 21-modified steroids**

		Subject									
		J.Y.		E.S.		J.J.		N.N.		F.D.	
Steroid	Dosage	FBS	1 Hr	FBS	1 Hr	FBS	1 Hr	FBS	1 Hr	FBS	1 Hr
	mg										
None	0	81	45	75	52	73	71	80	59	82	71
ΔFF	1	82	50	88	74	80	83	87	53	81	85
	3	97	102	101	95	88	111	96	78	104	97
Mesylate	60	80	63	78	61	80	67	90	70	81	71
	160	79	69	85	62	76	72	88	64	87	77
	640	83	54	82	54			87	62	84	77
21-Desoxy-ΔFF	80	80	60	77	64	77	67	86	68		
	240	80	71	83	60	85	89	98	73		
Difluoro	50	77	41	78	46	79	73	90	54		
	150	73	51	81	56	73	75	74	58		

* "FBS" indicates values obtained prior to glucose administration. "1 Hr" indicates values obtained 1 hour after the rapid intravenous infusion of 25 g of glucose. Steroids were given at 12:30 a.m., glucose at 8:30 a.m. Abbreviations as in Table I.

TABLE IV

*Estimates of potency (relative to ΔFF) and dissociation ratios in ACTH-suppression and eosinopenia assays**

Steroid	ACTH suppression		Eosinopenia			Dissociation ratio	
	Potency (all doses)	Fiducial limits	Dose	Potency	Fiducial limits		Fiducial limits
		95%	mg		95%		95%
21-Desoxy-ΔFF	1/11	1/8 to 1/16	10	1/36	1/9 to 1/142	3	0.8 to 13
			40	1/183	1/100 to 1/335	16	8 to 31
			80	1/409	1/186 to 1/897	36	15 to 84
			160	1/916	1/266 to 1/3,160	81	23 to 289
			320	1/2,050	1/1,720 to 1/2,450	181	30 to 1,081
Difluoro	1/34	1/26 to 1/44	10	1/296	5/1 to 1/665,000	9	0.0 to 19,770
			40	1/801	1/24 to 1/27,100	24	0.7 to 807
			80	1/1,320	1/303 to 1/5,720	39	9 to 172
			160	1/2,170	1/717 to 1/6,540	64	21 to 199
Mesylate	1/55	1/40 to 1/74	10	1/537	1/12 to 1/24,600	10	0.2 to 453
			40	1/1,680	1/154 to 1/18,400	31	3 to 341
			80	1/2,970	1/113 to 1/77,800	54	2 to 1,440
			160	1/5,250	1/49 to 1/563,000	96	0.9 to 10,300

* Dissociation ratio = potency in ACTH-suppresion assay ÷ potency in eosinopenia assay. Abbreviations as in Table I.

larger doses the carbon-21-modified steroids were also effective in suppressing ACTH, as judged by decreases in urinary 17-hydroxycorticosteroids. 21-Desoxy-ΔFF was effective in doses of 0.5 to 5.0 mg every 6 hours. The potency [1] of 21-desoxy-ΔFF relative to the ΔFF standard was 1/11 (Table IV). Difluoro was effective in doses of 5 to 20 mg every 6 hours, and was calculated to be 1/34 as potent as ΔFF. Mesylate was effective in doses of 4 to 24 mg every 6 hours, and was calculated to be 1/55 as potent as ΔFF.

Ancillary studies, performed to test the possibilities that the carbon-21-modified steroids would lower the urinary 17-hydroxycorticosteroids either by altering the extra-adrenal metabolism of cortisol or by inhibiting adrenocortical biosynthetic mechanisms directly, yielded negative answers (Figure 2). Similar results were obtained with all three of the carbon-21-modified steroids. Not only were urinary 17-hydroxycorticosteroids de-

[1] Estimates of relative potency, unless otherwise specified, were calculated according to Finney (7).

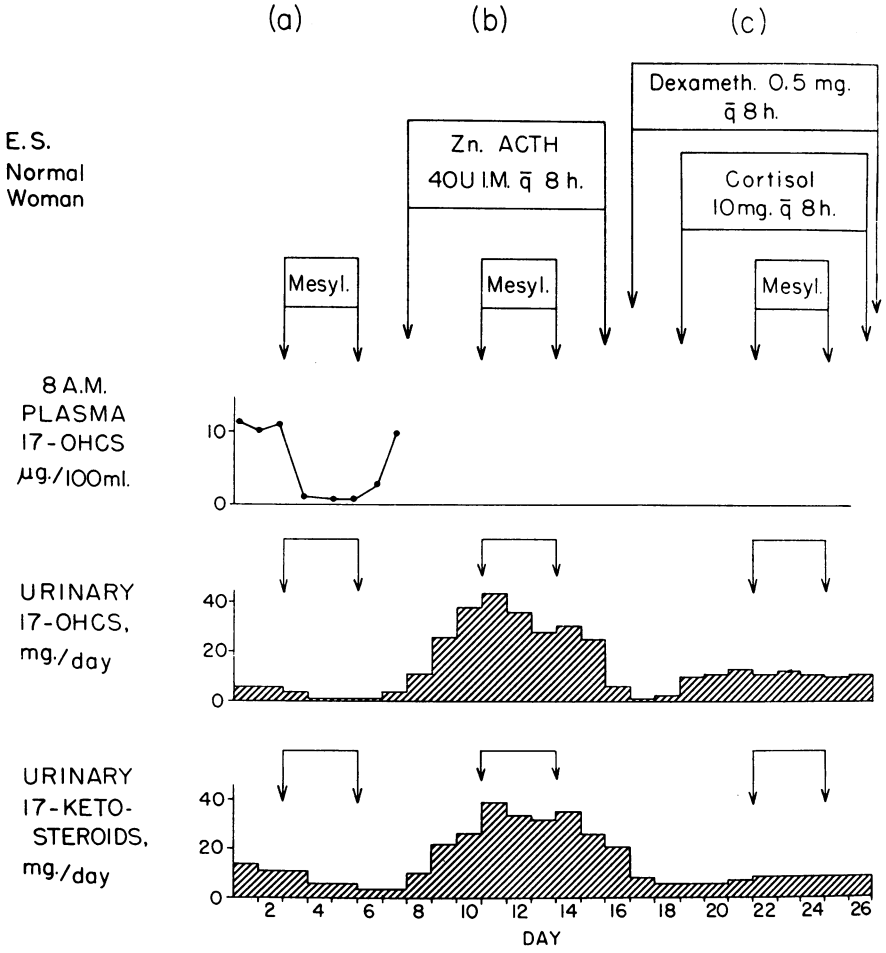

FIG. 2. PLASMA AND URINARY STEROID LEVELS OF A NORMAL SUBJECT DURING A THREE-PART STUDY. First mesylate alone was given in oral dosage of 60 mg every 8 hours. Next zinc ACTH was given for 8 days; mesylate treatment was superimposed (in the same dosage as before) for 3 days. Finally, dexamethasone and cortisol were given in constant dosage and again mesylate treatment was superimposed for 3 days.

creased during treatment with the carbon-21-modified steroids, but plasma 17-hydroxycorticosteroids and urinary 17-ketosteroids were also decreased. When the carbon-21-modified steroids were administered along with exogenous ACTH, no decrease in urinary steroids was observed, supporting the view that the carbon-21-modified steroids act by suppressing the secretion of ACTH rather than by inhibiting adrenocortical biosynthetic mechanisms directly. When the carbon-21-modified steroids were administered along with constant doses of exogenous cortisol (either to Addisonian patients or to normal subjects whose endogenous 17-hydroxycorticosteroids had been reduced to negligible levels by treatment with small doses of dexamethasone), no decrease in urinary 17-hydroxycorticosteroids was observed.

Eosinopenia assays (Figure 1; Tables II and IV)

The standard, ΔFF, was progressively more effective in decreasing the number of circulating eosinophils as dosage was increased from 0.25 to 4.0 mg. In contrast, the carbon-21-modified steroids failed to cause significant depression of eosinophils in any dosage employed. Slight eosinopenic activity could not, however, be excluded by examination of the available data. Therefore, calculation of the admittedly equivocal eosinopenic potency was attempted. The usual assumption

of parallelism between the dose response curves of the standard and unknown steroids was clearly not valid. For this reason the method of Cornfield (8) was employed in calculating eosinopenic potencies of the carbon-21-modified steroids relative to that of ΔFF. This method makes no assumption of parallelism and accepts, instead, the possibility that the relative potencies of two compounds may vary with dosage.

Thus, when 21-desoxy-ΔFF was tested at a dosage of 10 mg, its eosinopenic potency was 1/36 that of ΔFF, but when tested at a dosage of 320 mg, its potency was only 1/2,050 that of ΔFF. Similarly, when difluoro was tested at a dosage of 10 mg, its eosinopenic potency was 1/296 that of ΔFF, but when tested at a dosage of 160 mg, its potency was only 1/2,170 that of ΔFF. When mesylate was tested at a dosage of 10 mg, its eosinopenic potency was 1/537 that of ΔFF, and when tested at a dosage of 160 mg, its potency was only 1/5,250 that of ΔFF. It thus appeared that the larger the dose of 21-carbon-modified steroid, the less was its eosinopenic potency relative to that of the standard compound, ΔFF. This diminution of ratio of potency with increasing dosage may be more apparent than real. It is possible that the carbon-21-modified steroids are devoid of eosinopenic effect regardless of dosage. If this is true, then the true ratio of potency should be 0 at all dosage levels. Because of the inherent variability of the biological assay, however, it is never possible to say with certainty that a steroid is absolutely devoid of eosinopenic activity. It has been necessary to use increasingly large doses of the carbon-21-modified steroids, therefore, in order to establish conclusively that their eosinopenic potencies (relative to the standard) are virtually infinitesimal.

Hyperglycemia assays (Figure 1; Tables III and V)

The standard, ΔFF, was increasingly effective in impairing glucose tolerance as dosage of the steroid was increased from 1 to 3 mg. In contrast, the carbon-21-modified steroids failed to cause significant impairment of glucose tolerance in any dosage employed. Once again, however, a slight degree of hyperglycemic activity could not be excluded. Therefore, as in the case of eosino-

TABLE V

*Estimates of potency (relative to ΔFF) and dissociation ratios in ACTH-suppression and hyperglycemia assays**

Steroid	ACTH suppression		Hyperglycemia			Dissociation ratio	Fiducial limits
	Potency (all doses)	Fiducial limits	Dose	Potency	Fiducial limits		
		95%	mg		95%		95%
21-Desoxy-ΔFF	1/11	1/8 to 1/16	80	1/86	1/12 to 1/588	8	1.1 to 53
			240	1/194	1/27 to 1/1,418	17	2 to 127
Difluoro	1/34	1/26 to 1/44	50	1/82	1/29 to 1/236	2	0.8 to 7
			150	1/193	1/87 to 1/427	6	3 to 13
Mesylate	1/55	1/40 to 1/74	60	1/75	1/34 to 1/165	1.4	0.6 to 3
			160	1/206	1/104 to 1/411	4	1.8 to 8
			640	1/866	1/471 to 1/1,590	16	8 to 31

* Dissociation ratio = potency in ACTH-suppression assay ÷ potency in hyperglycemia assay. Abbreviations as in Table I.

penic activity, an attempt was made to calculate hyperglycemic potencies by the method of Cornfield. Once again, it was found that the relative potencies of the unknowns, as compared with the ΔFF standard, varied depending upon dosage. Thus, when 21-desoxy-ΔFF was tested at a dosage of 80 mg, its hyperglycemic potency was 1/86 that of ΔFF, but when tested at a dosage of 240 mg, its potency was only 1/194 that of ΔFF. When difluoro was tested at dosage of 50 mg, its apparent hyperglycemic potency was 1/82 that of ΔFF; when tested at the 150 mg dosage level, its potency was only 1/193 that of ΔFF. Similarly, when mesylate was tested at the 60 mg dosage level, it had 1/75 the potency of ΔFF, but when tested at the 640 mg level, it was only 1/866 as potent as the standard. Again, insofar as hyperglycemic activity was concerned, the larger the dose of carbon-21-modified steroid the less was its apparent potency relative to that of ΔFF.

Dissociation of ratios of potency

ACTH-suppressing vs eosinopenic potencies (Table IV). The estimate of the potency of 21-desoxy-ΔFF relative to ΔFF in the ACTH-suppression assay was 1/11. The estimate of the potency of 21-desoxy-ΔFF relative to ΔFF in the eosinopenia assay was 1/36 at the lowest dose and 1/2,050 at the highest dose. The disparity between estimates of potencies for the two types of assay we refer to as "dissociation" of corticoid properties. As a measure of the disparity we have used the ratio of the estimates, which we refer to as the "dissociation ratio." For example, at the 10 mg dose level, 1/11 ÷ 1/36 gives a dissociation ratio of 3.3. The dissociation ratio ranged from 3 at the 10 mg dose level to 181 at the 320 mg dose level. The 95% fiducial limits [2] on the ratio were 0.8 to 13 at the 10 mg dose level, but at each of the 40, 80, 160, and 320 mg dose levels, the lower fiducial limits excluded unity. At these last four dose levels, the probability that the ACTH-suppressing potency exceeded that of the eosinopenic potency by a factor of at least eight was greater than 0.95; the probability that the dissociation ratio was greater than unity was greater than 0.999. This is taken to mean that, although 21-desoxy-ΔFF is only slightly less effective than ΔFF as an ACTH-suppressing agent, it is far less effective than ΔFF as an eosinopenic agent.

Similar dissociation of ACTH-suppressing activity from eosinopenic activity was observed with the other carbon-21-modified steroids. The estimate of the potency of the difluoro compound relative to ΔFF in the ACTH-suppression assay was 1/34. In the eosinopenia assay, the estimate was 1/1,320 at the 80 mg dose level and 1/2,170 at the 160 mg dose level. At these dose levels, the probability that the dissociation ratio exceeded nine was greater than 0.95, indicating extensive dissociation of biological properties.

Mesylate, in the ACTH-suppression assay, had a potency relative to ΔFF of 1/55. The potency in the eosinopenia assay was 1/1,680 at the 40 mg dose level, 1/2,970 at the 80 mg dose level and 1/5,250 at the 160 mg dose level. The probability that the dissociation ratios exceeded two at the 40 and 80 mg levels was greater than 0.95.

ACTH-suppressing vs hyperglycemic potencies (Table V). The estimate of potency of 21-desoxy-ΔFF relative to ΔFF in the ACTH-suppression assay was 1/11. In the hyperglycemia assay, the estimate of potency of 21-desoxy-ΔFF relative to ΔFF was 1/86 at the low dose and only 1/194 at the high dose. At both dose levels, the probability that the dissociation ratio exceeded unity was greater than 0.95.

The difluoro compound was observed to have 1/34 the ACTH-suppressing potency of ΔFF, but in the hyperglycemia assay the estimate of the potency difluoro to ΔFF was only 1/82 at the 50 mg dose and 1/193 at the 150 mg dose. At the 150 mg dose, the probability that the dissociation ratio exceeded three was 0.95.

Mesylate was 1/55 as potent as ΔFF in the ACTH-suppression assay but only 1/206 as potent as ΔFF in the hyperglycemia assay at the 160 mg dose level and 1/866 as potent as ΔFF at the 640 mg dose level. At both dose levels, the probability that the dissociation ratio exceeded unity was greater than 0.95, and at the highest dose level it was greater than 0.999.

[2] Calculated from the antilog of the difference in log potency ± a multiple of the standard error of the difference in log potency. The multiple is obtained from Table V_1 of Fisher and Yates (9). For degrees of freedom not covered in that table, the multiple was obtained from an approximation given by Cochran and Cox (10).

DISCUSSION

There have been no previous reports of quantitative assays in human subjects documenting a significant degree of dissociation of the ACTH-suppressing activity from the eosinopenic and hyperglycemic activities of corticosteroid derivatives. There have been several reports indicating that these activities are closely correlated for most corticosteroid derivatives. The results of the present study indicate that steroids which suppress ACTH need not be potent eosinopenic or hyperglycemic agents.

In studies of the clinical pharmacology of new steroids, it is important that a variety of effects be tested in order to demonstrate selective attenuation or enhancement of certain properties. The principle that the electrolyte-regulating activities of corticosteroids can be dissociated to varying degrees from other activties has gained general acceptance. It has been more difficult to establish the principle that significant degrees of dissociation among the various organic-metabolism-regulating activities of corticosteroids can be achieved.

On the basis of the present study alone, one can say little about the precise relationship between chemical structure and biological function of steroids. It is of interest, however, that the compounds which were demonstrated to have little eosinopenic and hyperglycemic activity while retaining potent ACTH-suppressing activity were steroids which lacked the 21-hydroxyl group. It is thus suggested that the 21-hydroxyl group may be a more important determinant in the mechanism through which steroids exert their eosinopenic and hyperglycemic effects than it is in the mechanism through which they exert their ACTH-suppressing effect.

SUMMARY

Although the ACTH-suppressing, eosinopenic, and hyperglycemic activities of corticosteroids have previously been considered to be inseparable, three synthetic cortisol analogues have been found to exhibit significant degrees of dissociation among these biological activities. These three compounds were *1*) 9α-fluoro-11β,17α-dihydroxy-$\Delta^{1,4}$-pregnene-3,20-dione; *2*) 9α,21-difluoro-11β,17α-dihydroxy-Δ^{4}-pregnene-3,20-dione; and *3*) 6α-methyl-9α-fluoro-11β,17-dihydroxy-$\Delta^{1,4}$-pregnene-3,20-dione-21-mesylate. When compared with 9α-fluoro-11β,17α,21-trihydroxy-$\Delta^{1,4}$-pregnene-3,-20-dione as a biological standard, all three of the above steroids were found to be somewhat less effective as ACTH-suppressing agents, but almost totally ineffective as eosinopenic and hyperglycemic agents. The degree of dissociation of ACTH-suppressing activity from eosinopenic and hyperglycemic activities became greater as dosage was increased and was statistically significant at the higher dosage levels for each of the three steroids.

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REFERENCES

1. Liddle, G. W., and M. Fox. Structure-function relationships of antiinflammatory steroids *in* Inflammation and Diseases of Connective Tissue, L. C. Mills and J. H. Moyer, Eds. Philadelphia, Saunders, 1961, p. 302.
2. West, K. M. Relative eosinopenic and hyperglycemic potencies of glucocorticoids in man. Metabolism 1958, **7**, 441.
3. Liddle, G. W., J. E. Richard, and R. E. Peterson. An improved method for assaying the steroidogenic potency of ACTH. Endocrinology 1955, **57**, 594.
4. Hawk, P. B., B. L. Oser, and W. H. Summerson. Practical Physiological Chemistry, 13th ed. New York, Blakiston, 1954, p. 899.
5. Nelson, N. A photometric adaptation of the Somogyi method for the determination of glucose. J. biol. Chem. 1944, **153**, 375.
6. Somogyi, M. A new reagent for the determination of sugars. J. biol. Chem. 1945, **160**, 61.
7. Finney, D. J. Statistical Method in Biological Assay. New York, Hafner, ch. 4 and 10, 1952.
8. Cornfield, J. Potency estimation for comparative assays. Science. In press.
9. Fisher, R. A., and F. Yates. Statistical Tables. New York, Hafner, 1948.
10. Cochran, W. G., and G. M. Cox. Experimental Designs, 2nd ed. New York, J. Wiley & Sons, 1957.