THE EFFECT OF CORTICOSTEROID ANALOGUES ON THE THYMUS GLAND OF THE IMMATURE RAT

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The thymolytic activity of hydrocortisone (cortisol) was increased approximately 9-fold by the introduction of a fluorine atom at either the 6α- or the 9α-position. The substitution of a methyl group in the α-position at carbons 2, 6 or 16, with one exception, enhanced the ability of the corticosteroid to involute the thymus gland 3- to 4-fold. The only exception was noted when a methyl group at carbon 2 failed to increase the potency of fludrocortisone (9α-fluorohydrocortisone acetate). The formation of a double bond at the 5,6-position of the steroid molecule consistently decreased the thymolytic activity. Although the addition of an hydroxyl group in the α-position at carbon 16 decreased the relative potency of the corticosteroids, the formation of the 16α, 17α-acetonide derivative markedly enhanced it.

The involution of the thymus gland of the immature rat which occurs as a result of the parenteral administration of an adrenal corticosteroid has been shown to be associated with the following molecular configuration: a ketone group at carbon 3, a double bond at the 4,5 position, an oxygen function at carbon 11 and an α-ketol side chain at carbon 17[1]. In addition, it was found that various alterations of the corticosteroid molecule, such as the addition of an α-hydroxyl at carbon 17, a double bond at the 1,2 position, or a fluorine at the 9α-position, enhanced the thymolytic activity[2].

The present communication is a continuation of earlier studies[2], and is concerned with the effect of substitution at carbons 1, 2, 6, 9 and 16 of the corticosteroid molecule on thymic involution.

Experimental

Immature male or female albino rats, 25 to 30 days of age, were weighed and distributed at random in groups of equal numbers. The average body weight in each group was equalised by appropriate exchange of animals between groups. These rats were originally of a Wistar strain which has been inbred in the animal colony of the Food and Drug Laboratory since 1928.

The thymus involution assay procedure employed in this study was modified slightly from that described earlier[1,2]. The corticosteroids were administered on the basis of the initial body weight, mg. of steroid per 100 g. of weight, and either two or three unknown compounds were assayed against the reference standard. Usually the relative potency of one of the "unknowns" had been determined previously, and this served as a check on the reproducibility of the assay procedure. A "2 × 2" design was used when three unknowns were tested, and a "3 × 3" design when two unknowns were assayed against the same reference compound.

The log dose interval for the "2 × 2" design was 0·3010, while that for the "3 × 3" design was 0·1761. The steroids were dissolved in maize oil and injected subcutaneously in the back, 3 times daily for a period of 2 days.

The calculations for estimating the potency ratios and their confidence limits were made either by the procedure outlined by Bliss[3] or that by Gaddum[4]. If an observation appeared to be an outlier, a rejection criterion based on the range[5] was applied to the assay data. Tests for significance and homogeneity of the individual slopes between assays were employed in all of the experiments. In addition to these tests, linearity

TABLE I

EFFECT OF INTRODUCING A FLUORINE ATOM AT THE 6- AND 9-POSITIONS ON THE RELATIVE POTENCY OF THE CORTICOSTEROIDS

Compound	Relative potency (equimolar basis)	Confidence limits (P = 0·95)
Hydrocortisone	1·0	
6α-Fluorohydrocortisone	8·9	8·3–9·5
6α, 9α-Difluoroprednisolone	101·1	94·0–108·9
Hydrocortisone acetate	1·0	
9α-Fluorohydrocortisone acetate	8·8	8·0–9·7
9α-Fluoroprednisolone acetate	17·1	16·9–17·3
2α-Methylhydrocortisone	1·0	
2α-Methyl-9α-fluorohydrocortisone acetate	2·1	2·0–2·2

of the individual log dose response lines was checked in the "3 × 3" assay design. A common slope was used to calculate each potency ratio, and the error term was estimated to include the variation of the treatment means about the log dose response lines. Since all of the responses were included in the estimation of the error term, a higher degree of precision was obtained than would have been possible if each compound had been assayed individually against the reference standard. The index of precision, obtained by dividing the standard deviation of the assay (s) by the common slope (b) was consistently less than 0·10. Usually two or more estimates of the potency ratio for each corticosteroid analogue were combined by the method of Bliss[3]. Homogeneity of the individual potency ratios was checked by the χ^2 test before the potency ratios were combined to provide the weighted mean value.

Results and Discussion

The data in Table I show the effect of the thymolytic activity of a fluorine atom at the 6α- or 9α-position of the corticosteroid molecule. The potency values relative to hydrocortisone (cortisol) suggest that a fluorine at carbon 6 has approximately the same action as one at carbon 9. The combination of a double bond at the 1,2-position and a fluorine at carbon 9 increased the thymolytic activity of hydrocortisone 17-fold. When a second fluorine was added at carbon 6 of 9α-fluoroprednisolone (6α, 9α-difluoroprednisolone) the potency was found to be approximately 100

times that of hydrocortisone. However, this augmentation in activity attributable to the addition of fluorine appears to be modified by changes in the molecular configuration at carbons 1 and 2. Relative potency values reported previously revealed that prednisolone is 4 to 5 times more active than hydrocortisone[2]. Therefore, the relative potency value of 17·1 shown in Table I for 9α-fluoroprednisolone indicates that the fluorine at carbon 9 brought about a 4-fold increase in the ability of prednisolone to produce thymic involution. Also, the potency of 2α-methylhydrocortisone was doubled only by the introduction of a fluorine at the 9α-position. In contrast, the addition of an α-fluorine at carbon 9 of hydrocortisone acetate, a steroid without changes at carbons 1 and 2, produced a 9-fold increase in the thymolytic activity.

TABLE II

EFFECT OF A DOUBLE BOND AT THE 5,6-POSITION ON THE THYMOLYTIC ACTIVITY OF THE CORTICOSTEROIDS

Compound	Relative potency (equimolar basis)	Confidence limits (P = 0·95)
Cortisone acetate	1·0	
6-Dehydrocortisone acetate	0·93	0·88–0·98
Hydrocortisone acetate	1·0	
6-Dehydrohydrocortisone acetate	0·79	0·75–0·84
6-Dehydroprednisolone acetate	1·9	1·8–2·0
6-Dehydroprednisone acetate	1·2	1·1–1·3
9α-Fluoroprednisolone acetate	17·1	16·9–17·3
9α-Fluoro-6-dehydroprednisolone acetate	4·4	4·1–4·8
9α-Fluoro-6-dehydrocortisone acetate	1·9	1·7–2·1
1-Chloro-6-dehydroprednisone acetate	0·0*	

* Activity absent at total dose level of 2·3 mg./100 g. of rat.

The relative potency values given in Table II reveal that a double bond between carbons 5 and 6 consistently reduced the ability of the corticosteroids to cause involution of the thymus gland. In addition, the data suggest that the lowered biological activity attributable to the 6-dehydro configuration was more pronounced in the corticosteroids with a fluorine at the 9α-position. According to the results obtained in Table II, substitution of a chlorine atom at carbon 1 further decreased the thymolytic action because 1-chloro-6-dehydroprednisone acetate did not possess any demonstrable activity when administered to the rats at a total dose of 2·3 mg./100 g. of weight. Under similar circumstances, hydrocortisone, in a total dose of 0·3 to 0·4 mg./100 g. of weight, would produce a significant decrease ($P \leqslant 0{\cdot}05$) in the relative thymus weight.

The results in Table III illustrate the effect of methyl group substitution on the relative potency of various corticosteroids. The introduction of a methyl group at the 2α-position of hydrocortisone, or at the 6α-position of either prednisolone or triamcinolone brought about a 3- to 4-fold increase in the thymolytic activity. Reference to both Tables I and III shows that the addition of a methyl group at the 16α- or 16β-position of 9α-fluoroprednisolone increased the potency relative to hydrocortisone from 17·1 to 72·7 and 51·7 respectively. According to these data the

introduction of a methyl group at the 16α-position enhanced the activity approximately 4-fold. On the other hand, a β-methyl group at carbon 16 produced a 3-fold increase in the relative potency. However, the results in Table III also reveal that the addition of an α-methyl group did not augment the thymolytic activity in every instance, because 2α-methyl-9α-fluorohydrocortisone acetate was actually less active than 9α-fluorohydrocortisone acetate.

TABLE III

INFLUENCE OF METHYL GROUP SUBSTITUTION ON THE RELATIVE POTENCY OF THE CORTICOSTEROIDS

Compound	Relative potency (equimolar basis)	Confidence limits (P = 0·95)
Hydrocortisone	1·0	
2α-Methylhydrocortisone	4·4	4·2–4·7
6α-Methylprednisolone	15·2	14·4–16·1
6α-Methyltriamcinolone	9·0	8·1–10·0
9α-Fluoro-16α-methylprednisolone	72·7	67·9–77·9
9α-Fluoro-16β-methylprednisolone	51·7	46·6–57·3
Prednisolone	1·0	
6α-Methylprednisolone	3·8	3·5–4·1
9α-Fluorohydrocortisone acetate	1·0	
2α-Methyl-9α-fluorohydrocortisone acetate	0·86	0·80–0·93

TABLE IV

EFFECT OF AN HYDROXYL GROUP AT THE 16α-POSITION, AND OF 16α, 17α-ACETONIDE FORMATION ON THE THYMOLYTIC ACTIVITY OF THE CORTICOSTEROIDS

Compound	Relative potency (equimolar basis)	Confidence limits (P = 0·95)
Hydrocortisone	1·0	
16α-Hydroxyhydrocortisone	0·13	0·10–0·17
16α-Hydroxyprednisolone	1·1	0·94–1·3
9α-Fluoro-16α-hydroxyprednisolone (triamcinolone)	3·8	3·6–4·0
9α-Fluoroprednisolone-16α-21-diacetate	3·6	3·1–4·2
6α-Methyl-9α-fluoro-16α-hydroxyprednisolone	9·0	8·1–10·0
6α-Methyl-9α-fluoroprednisone-16α-21-diacetate	4·7	4·1–5·4
Triamcinolone acetonide*	76·6	61·2–96·0
6α-Methyltriamcinolone acetonide	115·5	102·9–129·7
6α-Methyltriamcinolone-21-acetate-acetonide	97·1	86·5–108·9

* 9α-Fluoro-16α, 17α-isopropylidenedioxyprednisolone.

The data in Table IV demonstrate that an α-hydroxyl group at carbon 16 depressed the action of the corticosteroids on the rat thymus. This observation is based on the results obtained with the 16α-hydroxy derivatives of hydrocortisone, prednisolone, and 9α-fluoroprednisolone. Apparently acetylation of the hydroxyl groups at carbons 16 and 21 have no significant effect on the potency of the 16-hydroxycorticosteroid. In contrast to the depression of the ability to involute the thymus gland of the rat brought about by 16-hydroxylation of the corticosteroid, formation of the 16α, 17α-isopropylidenedioxy(16α, 17α-acetonide) derivative markedly enhanced the thymolytic action of these compounds. The

degree of augmentation achieved by acetonide formation appeared to be dependent on other groups present on the corticosteroid molecule. For example, triamcinolone acetonide is approximately 20 times more active than the free alcohol or diacetate, while the 6α-methyltriamcinolone acetonide derivative showed only a 10- to 12-fold increase in relative potency when compared with the parent compound. It is interesting to note that 6α-methyl-9α-fluoroprednisone 16α, 21-diacetate is only one-half as active as 6α-methyl-9α, 16α-hydroxyprednisolone (6-methyltriamcinolone). This is somewhat unexpected because previous work[2] has shown that the thymolytic activity of prednisone is approximately 70 to 75 per cent of that of prednisolone. Perhaps, the presence of the other groups on the molecule has modified the effect of the hydroxyl group at carbon 11[2].

During the course of this discussion, no attempt has been made to compare the results obtained by the thymus involution assay procedure with those used by other investigators for estimating glucocorticoid-like activity. In many instances either the route of administration or the injection medium has differed from that employed in the assays reported in this paper. Earlier work[1,2], has shown that the injection medium influenced the relative potency of some of the corticosteroid analogues as measured by the thymus involution assay. Ideally the various assays for glucocorticoid-like activity should be carried out simultaneously in test animals from the same group, and using the same route of administration and injection medium for all of the tests.

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