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EFFECT OF FLUOROSTEROIDS ON DRUG RESPONSE AND METABOLISM

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Abstract—In female rats, zoxazolamine paralysis was significantly reduced by pretreatment with pregnenolone-16α-carbonitrile (PCN), spironolactone, dexamethasone acetate, betamethasone acetate, 9α-fluoro-11β,21-dihydroxy-16α,17α-dimethyl-1,4-pregnadiene-3,20-dione, 6α-fluoro-9α-chloro-11β-acetoxy-21-valeryloxy-16α-methyl-1,4-pregnadiene-3,20-dione, 9α-fluorocortisol acetate, triamcinolone or adrenocorticotropic hormone (ACTH). With the exception of triamcinolone and ACTH, the protective effects of these compounds were associated with decreased drug concentrations in plasma at the end of the pharmacologic response, compared with controls killed at the same time. The drug levels were found to be lowered via hepatic drug-metabolizing enzyme induction. All the fluorosteroids exerted glucocorticoid activity. Thus, PCN and spironolactone protect via increased drug metabolism, triamcinolone and ACTH via decreased organ sensitivity, and the remaining fluorosteroids via both prophylactic mechanisms.

It has been established that steroids occupy an important position among various factors that alter the body's response to drugs through different biochemical processes [1, 2]. Thus, many steroids decrease an agent's pharmacologic and toxic effects via biotransformation. This type of protection, called catatoxic, is associated with diminished drug concentrations in plasma [3, 4]. However, there are certain steroids which considerably reduce the toxic manifestations of many drugs without a concomitant fall in the blood level of the drugs. Here, a syntoxic mechanism renders the body less sensitive to the pathogen by increasing tissue tolerance.

In our previous reports [5, 6], certain fluorosteroids were shown to protect experimental animals against various intoxications but the mode of their prophylactic action was not clearly demonstrated. We, therefore, undertook an investigation into the underlying mechanism and correlated our findings with the other pharmacologic properties of the fluorosteroids (e.g. glucocorticoid activity). At the same time, the influence of these steroids upon the body's response to zoxazolamine was compared with that of typical catatoxic [e.g. pregnenolone-16α-carbonitrile (PCN) and spironolactone] and syntoxic [e.g. triamcinolone and adrenocorticotropic hormone (ACTH)] agents.

MATERIALS AND METHODS

Female Charles River CD® rats (Canadian Breeding Farms & Laboratories Ltd., St. Constant, Quebec), weighing 90–110 g, were maintained *ad lib.* on Purina laboratory chow (J. Mondou Inc., Montreal, Quebec) and tap water.

In the *first experiment*, the animals were pretreated twice daily p.o. for 3 days with 1 mg PCN (Upjohn), spironolactone (Searle), dexamethasone acetate (Merck), betamethasone acetate (Schering), 9α-fluoro-11-β,21-dihydroxy-16α,17α-dimethyl-1,4-pregnadiene-3,20 dione (Organon), 6α-fluoro-9α-chloro-11β-acetoxy-21-valeryloxy-16α-methyl-1,4-pregnadiene-3,20-dione (Schering), 9α-fluorocortisol acetate (Upjohn) and triamcinolone (Pfizer) in 1 ml water (as micronized suspensions with a trace of Tween 80), or once s.c. on day 3 with 5 I.U. of ACTH [Synacthen Depot (Ciba)]. Zoxazolamine (K & K Laboratories) was administered i.p. on day 4 at a dose level of 10 mg/100 g body wt, 18 hr after the last steroid pretreatments or 24 hr after the ACTH injection. Two control groups (given water plus Tween 80) were used for comparison with each of the steroid- or ACTH-pretreated groups. The latter and one set of controls (recovered) were killed at the end of the pharmacologic response (i.e. when the righting reflex was regained), while the other (unrecovered) was sacrificed when the pretreated animals recovered spontaneously.

Drug-free plasma from pretreated and non-pretreated rats was used for preparing standards and blanks. The zoxazolamine concentrations in plasma were determined by the method of Burns *et al.* [7].

In the *second experiment*, 20 μmoles of the above-mentioned steroids or 5 I.U. of ACTH was administered as described in the first experiment, and the rats were killed by decapitation 18 hr after the last treatment. The livers were immediately removed, weighed and washed in an ice-cold 1·15% KCl solution. Samples were taken and processed at 0–4°, after which 1 g liver was homogenized in 3 ml isotonic KCl containing 0·02 M Tris-HCl (pH 7·4). The homogenate was centrifuged at 9000 *g* for 20 min, and the 9000 *g* supernatant fraction was used in a study on zoxazolamine and ethylmorphine metabolism. Zoxazolamine ring oxidation was determined by the method of Juchau *et al.* [8], and ethylmorphine *N*-demethylation was assessed [9] with the Nash [10]

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reaction by estimating the amount of formaldehyde formed.

The incubation medium for the assays contained: 0·02 M Tris-HCl buffer (pH 7·4), 5 mM $MgCl_2$, 5 mM glucose 6-phosphate, 0·4 mM NADP, 1·2 μM zoxazolamine or 3 μM ethylmorphine, and postmitochondrial supernatant corresponding to 0·220 and 0·083 g liver for zoxazolamine and ethylmorphine, respectively; the total incubation volume was 3 ml. The duration of incubation with zoxazolamine and ethylmorphine was 30 and 10 min respectively. The method of Lowry *et al.* [11] was used for the protein determinations.

In the *third experiment*, the glucocorticoid activity of the steroids was assessed by the criteria of thymus involution and hepatic glycogen deposition [12]. Liver glycogen was determined by the phenol sulfuric acid method of Montgomery [13]. The steroids were given at a dose level of 0·1 mg, once daily p.o. for 3 days.

RESULTS

Pretreatment with all the test compounds considerably reduced zoxazolamine paralysis time in the following descending order of potency: dexamethasone, PCN, betamethasone, 9α-fluoro-11β,21-dihydroxy-16α,17α-dimethyl-1,4-pregnadiene-3,20-dione, ACTH, 9α-fluorocortisol acetate, 6α-fluoro-9α-chloro-11β-acetoxy-21-valeryloxy-16α-methyl-1,4-pregnadiene-3,20-dione, triamcinolone and spironolactone (Table 1).

With the exception of rats given prior doses of triamcinolone or ACTH, the zoxazolamine levels in the plasma of pretreated animals were lower than those in the unrecovered controls. Moreover, the drug concentrations in the triamcinolone- or ACTH-pretreated groups were higher than in the recovered controls. Similar results were obtained with prior administration of 6α-fluoro-9α-chloro-11β-acetoxy-21-valeryloxy-16α-methyl-1,4-pregnadiene-3,20-dione, 9α-fluoro-11β,21-dihydroxy-16α,17α-dimethyl-1,4-pregnadiene-3,20-dione or betamethasone.

The metabolism *in vitro* of zoxazolamine by the liver was altered by pretreatment in the following order of activity: dexamethasone, betamethasone, 6α-fluoro-9α-chloro-11β-acetoxy-21-valeryloxy-16α-methyl-1,4-pregnadiene-3,20-dione, 9α-fluoro-11β,21-dihydroxy-16α,17α-dimethyl-1,4-pregnadiene-3,20-dione, PCN, spironolactone, 9α-fluorocortisol acetate, ACTH and triamcinolone (Table 2). Based on the results in μmoles/g of liver, however, the effects of the protective agents would be expressed as percentages (control 0 per cent) as follows: dexamethasone (206 per cent); PCN (189 per cent); 9α-fluoro-11β,21-dihydroxy-16α, 17α-dimethyl-1,4-pregnadiene-3,20-dione (184 per cent); betamethasone (180 per cent); 6α-fluoro-9α-chloro-11β-acetoxy-21-valeryloxy-16α-methyl-1,4-pregnadiene-3,20-dione (176 per cent); spironolactone (140 per cent); 9α-fluorocortisol acetate (32 per cent); ACTH (17 per cent) and triamcinolone (9 per cent). The same order applies more or less to ethylmorphine *N*-demethylation (Table 2).

The test compounds exhibited diminishing glucocorticoid activity in the following order: dexamethasone, betamethasone, 9α-fluoro-11β,21-dihydroxy-16α,17α-dimethyl-1,4-pregnadiene-3,20-dione, triamcinolone, 9α-fluorocortisol acetate, 6α-fluoro-9α-chloro-11β-acetoxy-21-valeryloxy-16α-methyl-1,4-pregnadiene-3,20-dione and PCN (Table 3).

Table 1. Effect of various steroids and ACTH on zoxazolamine concentrations and paralysis time

Pretreatment	Zoxazolamine concentrations in plasma (μg/ml)			Paralysis time (min)		Reduction of paralysis (%)
	Pretreated*	Unrecovered control†	Recovered control*	Pretreated	Control	
Pregnenolone-16α-carbonitrile	26·13 ± 1·22 (7)‡	36·09 ± 1·14§, ¶ (9)	27·51 ± 0·59 NS• (7)	45 ± 4§	150 ± 10	70
Spironolactone	23·00 ± 1·50 (7)	26·37 ± 0·87** (10)	20·64 ± 1·72 NS (8)	78 ± 3**	104 ± 8	25
Dexamethasone acetate	18·89 ± 1·06 (9)	28·03 ± 1·66§ (10)	17·58 ± 0·70 NS (10)	31 ± 2§	128 ± 7	76
Betamethasone acetate	31·09 ± 1·69 (10)	40·22 ± 0·68§ (8)	28·05 ± 0·23†† (10)	127 ± 3§	292 ± 17	60
9α-Fluoro-11β,21-dihydroxy-16α,17α-dimethyl-1,4-pregnadiene-3,20-dione	25·34 ± 0·84 (15)	29·96 ± 0·96§ (14)	19·59 ± 0·69§ (13)	65 ± 8§	132 ± 14	51
6α-Fluoro-9α-chloro-11β-acetoxy-21-valeryloxy-16α-methyl-1,4-pregnadiene-3,20-dione	34·93 ± 0·51 (8)	38·23 ± 0·99 NS (8)	26·45 ± 1·79** (8)	72 ± 9**	104 ± 8	31
9α-Fluorocortisol acetate	23·10 ± 1·30 (7)	26·55 ± 1·40** (7)	22·64 ± 1·10 NS (7)	93 ± 12††	148 ± 12	37
Triamcinolone	31·35 ± 2·12 (7)	27·19 ± 1·20 NS (7)	23·55 ± 0·90§ (7)	115 ± 9**	160 ± 15	28
ACTH	37·33 ± 2·10 (7)	36·93 ± 2·30 NS (7)	23·51 ± 1·60§ (6)	81 ± 5§	156 ± 5	48

* Killed when the righting reflex was regained ("recovered control").

† Killed when the pretreated groups regained the righting reflex ("unrecovered control").

‡ Figures in parentheses indicate number of animals.

§ $P < 0·005$.

¶ Plasma levels of zoxazolamine in the recovered and unrecovered controls are compared with the plasma concentrations of the drug in the pretreated rats.

• NS = not significant.

** $P < 0·05$.

†† $P < 0·01$.

Table 2. Effect of steroids and ACTH on the metabolism *in vitro* of zoxazolamine and ethylmorphine

Pretreatment	No. of animals	Zoxazolamine metabolism: Rate (μmoles/g/hr)*	Zoxazolamine metabolism: Increase (%)	Ethylmorphine metabolism: Rate (HCHO/μmoles/g/hr)*	Ethylmorphine metabolism: Increase (%)	Protein: Concn (mg/g)	Protein: Increase (%)
Pregnenolone-16α-carbonitrile	6	42·8 ± 1·6†	166	388·4 ± 22·7†	437	104·2 ± 1·2‡	7
	4	(16·0 ± 1·4)§		(72·4 ± 2·3)		(97·4 ± 2·0)	
Spironolactone	5	45·7 ± 0·3†	130	203·7 ± 13·0†	132	99·7 ± 1·2 NS¶	2
	6	(19·8 ± 0·6)		(87·7 ± 11·3)		(97·4 ± 2·0)	
Dexamethasone acetate	6	65·3 ± 3·9†	213	483·0 ± 20·6†	603	82·8 ± 1·3 NS	2
	4	(20·9 ± 2·0)		(68·6 ± 2·3)		(84·3 ± 1·3)	
Betamethasone acetate	5	73·0 ± 1·5†	198	422·5 ± 28·6†	262	79·4 ± 2·4	−6
	5	(28·3 ± 2·1)		(116·8 ± 13·2)		(84·3 ± 1·3) NS	
9α-Fluoro-11β,21-dihydroxy-16α,17α-dimethyl-1,4-pregnadiene-3,20-dione	6	52·3 ± 1·8†	176	307·9 ± 21·9†	325	90·4 ± 2·7 NS	−8
	6	(18·9 ± 2·0)		(72·4 ± 2·3)		(98·4 ± 2·9)‡	
6α-Fluoro-9α-chloro-11β-acetoxy-21-valeryloxy-16α-methyl-1,4-pregnadiene-3,20-dione	6	54·7 ± 3·0†	188	259·3 ± 8·7†	258	84·1 + 3·1‡	−15
	6	(19·0 ± 2·1)		(72·4 ± 2·3)		(98·4 ± 2·9)	
9α-Fluorocortisol acetate	6	24·8 ± 1·1†	54	180·7 ± 17·0†	131	77·7 ± 1·6‡	−8
	4	(16·0 ± 1·4)		(78·0 ± 0·6)		(84·3 ± 1·4)	
Triamcinolone	5	28·3 ± 2·1 NS	15	151·2 ± 13·3 NS	30	84·8 ± 1·7	1
	4	(24·5 ± 2·2)		(116·8 ± 13·1)		(84·1 ± 1·4) NS	
ACTH	16	30·9 ± 1·4 NS	19	132·3 ± 9·1 NS	−20	82·7 ± 1·7	−4
	7	(25·8 ± 1·7)		(166·0 ± 10·9)		(86·0 ± 2·0) NS	

* Grams of liver protein.
† $P < 0{\cdot}005$.
‡ $P < 0{\cdot}05$.
§ Figures in brackets represent control values.
¶ NS = not significant.

DISCUSSION

Pretreatment with all the compounds tested more or less markedly reduced zoxazolamine paralysis time (Table 1). This is in agreement with earlier studies [1, 3–5, 14, 15] on some of these agents. The greatest protection was offered by PCN and dexamethasone, followed by betamethasone, 9α-fluoro-11β,21-dihydroxy-16α,17α-dimethyl-1,4-pregnadiene-3,20-dione and ACTH; the remaining compounds provided moderate protection. In most cases, this prophylaxis was correlated with decreased zoxazolamine concentrations in the plasma of pretreated animals (killed when paralysis disappeared) compared to unrecovered controls (sacrificed at the same time). Furthermore, Table 1 indicates that the plasma levels of zoxazolamine in PCN- or spironolactone-pretreated rats are similar to those in the recovered controls, which were killed upon regaining the righting reflex. However, spironolactone lowers the drug concentrations in plasma less significantly than does PCN as measured directly and shown by the degree of protection offered. Earlier, similar differences in potency were observed with other substrates [1, 4] as well as in the effects upon the mixed-function oxygenase system (e.g. cytochrome P-450 and cytochrome *c* reductase) [16] of pretreated rats, indicating important divergences in the detoxication mechanism of the two steroids. Furthermore, although female rats were used in this study, it should be mentioned that great sex differences in the induction of microsomal enzymes have been reported after spironolactone and, to a lesser extent, after PCN treatment. These differences have been attributed to impairment of testosterone biosynthesis [17, 18]. The protection offered by triamcinolone or ACTH was not associated with reduced drug concentrations in plasma. In particular, here, the plasma levels of zoxazolamine at the end of the pharmacologic response were the same as those in the unrecovered controls but much higher than in the recovered control group.

These findings confirm our earlier results [3, 4], which suggest characteristic differences between the

Table 3. Effect of various steroids on hepatic glycogen and thymus weight

Pretreatment	No. of animals	Hepatic glycogen (mg/g)	Thymus weight (mg/100 g body wt)
Water	14	0·59 ± 0·10	389 ± 21
Dexamethasone acetate	7	61·99 ± 4·10*	80 ± 5*
Betamethasone acetate	7	30·81 ± 1·00*	130 ± 4*
9α-Fluoro-11β,21-dihydroxy-16α,17α-dimethyl-1,4-pregnadiene-3,20-dione	7	22·40 ± 1·00*	113 ± 9*
Triamcinolone	7	20·52 ± 1·80*	330 ± 20*
9α-Fluorocortisol acetate	6	11·53 ± 0·83*	106 ± 9*
6α-Fluoro-9α-chloro-11β-acetoxy-21-valeryloxy-16α-methyl-1,4-pregnadiene-3,20-dione	7	10·10 ± 0·01*	295 ± 25†
Pregnenolone-16α-carbonitrile	7	0·46 ± 0·02*	474 ± 30 NS‡

* $P < 0{\cdot}005$.
† $P < 0{\cdot}01$.
‡ NS = not significant (compared with the group given water).

two protective mechanisms [1, 4, 5, 19], attributed mainly to: (1) hepatic drug-metabolizing enzyme induction (i.e. a more rapid inactivation of drugs by pretreatment with PCN, spironolactone, etc.), or (2) increased organ tolerance without enhanced drug degradation (e.g. by prior administration of triamcinolone or ACTH). These differences are also indicated by our experiments *in vitro* on zoxazolamine and ethylmorphine metabolism by the 9000 *g* liver supernatant fraction (Table 2). Our results clearly show that PCN and spironolactone greatly increase zoxazolamine hydroxylation and ethylmorphine *N*-demethylation by the liver. Triamcinolone and ACTH, on the other hand, demonstrated no significant effect on the metabolism *in vitro* of either drug. This fact suggests that their protective action is achieved by an increase of organ tolerance to the drugs and not by drug-metabolizing enzyme induction. Table 2 shows that there are differences in the effects of the steroids on zoxazolamine and ethylmorphine metabolism, the biotransformation of zoxazolamine being two to three times higher than that of ethylmorphine; spironolactone and ACTH did not exhibit such differences. The fluorosteroids present an interesting case, lying between the two types of protective actions. The zoxazolamine levels in the plasma of pretreated rats tend to be lower than in the unrecovered and higher than in the recovered controls. The fluorosteroids also stimulate both zoxazolamine hydroxylation and ethylmorphine *N*-demethylation *in vitro*, thus appearing to act via both mechanisms, although in some instances one is more pronounced than the other. For example, dexamethasone was more catatoxic than the other fluorosteroids, while betamethasone and 9α-fluoro-11β,21-dihydroxy-16α,17α-dimethyl-1,4-pregnadiene-3,20-dione demonstrated prominent syntoxic and catatoxic characters.

Table 2 also shows that there is little effect on the protein content of the postmitochondrial liver fraction (9000 *g* supernatant). In the PCN-treated animals, the slight increase in protein becomes significant since it is accompanied by a simultaneous decrease of hepatic glycogen (Table 3) [20], while the mild reduction of protein after administration of most of the glucocorticoids could be attributed to augmented glycogen deposition.

Expressed in μmoles/g of liver protein, PCN seems to be less active than the fluorosteroids in inducing zoxazolamine metabolism. However, if expressed per g of liver tissue, it is second on the list as regards potency. Fluorosteroid pretreatment increases hepatic glycogen and consequently reduces protein, whereas prior administration of PCN has an inverse effect [20]. However, it cannot be ruled out that the activity of liver protein (lower after PCN than after fluorosteroid pretreatment) or the presence of glycogen may play a role in zoxazolamine metabolism. Furthermore, an element that may be important in considerations of the body's drug response is the fact that fluorosteroids provide readily available sources of energy (e.g. carbohydrates, free fatty acids) for adaptive work, while also inducing drug-metabolizing enzymes.

Table 3 shows a classification of the glucocorticoids in decreasing order of glucocorticoid activity. Spironolactone, known to be devoid of glucocorticoid properties [21–23], and ACTH, which could not be tested with the method used (adrenalectomized rats), have not been included. Dexamethasone was the most potent glucocorticoid in this series. PCN, which was being tested for the first time to determine its glucocorticoid activity, was found to have none and, in fact, demonstrated weak antiglucocorticoid properties; the hepatic glycogen depletion noted after PCN treatment has already been reported [20].

Tables 1 and 2, in conjunction with Table 3, indicate that the protective effects of the fluorosteroids (reduction of zoxazolamine paralysis), though mediated via both mechanisms, depend more intimately upon the catatoxic character of the test compounds. As this decreases, their prophylactic activity *in vivo* declines but is not closely correlated with glucocorticoid potency.

This dual protective action has also been suspected for 9α-fluorocortisol acetate [4, 5]. The proposed relationships [4, 5, 14] between drug concentrations in the plasma of pretreated animals and controls that are characteristic of these mechanisms can now be completed as follows:

Mechanism I (catatoxic) $C_d < C_1$, $C_d \simeq C_2$
Mechanism II (syntoxic) $C_d \simeq C_1$, $C_d > C_2$
Dual action $C_1 > C_d > C_2$.

[Drug concentrations in: (1) C_d = pretreated (conditioned) rats killed at the endpoint of the pharmacologic response; (2) C_1 = unrecovered controls sacrificed when the drug effect has vanished in the pretreated group; and (3) C_2 = spontaneously recovered controls.]

The present experiments confirm the view [1, 20, 24, 25] that mechanism I is independent of any other known hormonal and/or pharmacologic activities of the test compounds, whereas mechanism II is related to their glucocorticoid properties. Moreover, many fluorosteroids protect pretreated animals against zoxazolamine intoxication via both mechanisms. This overlap is not uncommon in biologic processes (e.g. between gluco- and mineralocorticoids); yet, distinction between the two phenomena is justified because usually individual steroids predominantly elicit either action although the manifestation of one does not exclude the other.

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