means and that an anticholinergic drug can increase appreciably the bioavailability of such a substance.

REFERENCES

(1) G. Levy and B. K. Rao, *J. Pharm. Sci.*, **61**, 279(1972).
(2) G. Levy, M. Gibaldi, and J. A. Procknal, *ibid.*, **61**, 798 (1972).
(3) W. J. Jusko, G. Levy, S. J. Yaffe, and J. E. Allen, *Amer. J. Dis. Child.*, **121**, 48(1971).
(4) G. Levy and R. R. Hewitt, *Amer. J. Clin. Nutr.*, **24**, 401 (1971).
(5) G. M. McLeod, A. B. French, C. J. Good, and F. S. Wright, *J. Lab. Clin. Med.*, **71**, 192(1968).
(6) U. Stave, *Z. Kinderheilk.*, **77**, 554(1956).
(7) D. L. Miller and H. P. Schedl, *Gastroenterology*, **62**, 48 (1972).
(8) H. Kunze and W. Vogt, *Naunyn-Schmiedebergs Arch. Pharmakol. Exp. Pathol.*, **256**, 139(1967).
(9) H. Kunze, *ibid.*, **259**, 260(1968).
(10) R. C. Heading, J. Nimmo, P. Tothill, and L. F. Prescott, *Gastroenterology*, **62**, 761(1972).

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Effect of Various Steroids and ACTH on Plasma Levels of Zoxazolamine and Dicumarol

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Abstract □ Comparative experiments were performed on rats given pregnenolone-16α-carbonitrile, estradiol, progesterone, triamcinolone, hydroxydione, or ACTH (corticotropin) to study correlations between the *in vivo* conditioning effects upon resistance to zoxazolamine and dicumarol and the plasma concentrations of these drugs. The conditioners were classified as catatoxic when a decrease in toxicity was associated with increased blood clearance of the drug, and they were classified as syntoxic when *in vivo* protection was accomplished without a simultaneous fall in the plasma drug level. With these criteria, pregnenolone-16α-carbonitrile was markedly protective and catatoxic whereas triamcinolone and ACTH were moderately protective and syntoxic against zoxazolamine. The remaining steroids elicited very mild changes in zoxazolamine paralysis and blood clearance. Of all the conditioners tested, only estradiol protected against dicumarol intoxication and accelerated plasma clearance of this drug. Hence, only estradiol exhibited a manifest catatoxic effect against this anticoagulant.

Keyphrases □ Steroids (pregnenolone-16α-carbonitrile, estradiol, progesterone, triamcinolone, and hydroxydione)—effect on plasma levels of zoxazolamine and dicumarol □ ACTH—effect on plasma levels of zoxazolamine and dicumarol □ Zoxazolamine, plasma levels—effect of various steroids and ACTH, resistance–plasma concentration correlations □ Dicumarol, plasma levels—effect of various steroids and ACTH, resistance–plasma concentration correlations

It is well known that certain pharmacological agents, including steroidal hormones, can reduce the biological half-life of others by the induction of drug-metabolizing enzymes (1). During the past few decades, numerous observations have confirmed that, in addition to their classic hormonal functions as regulators of reproduction and general metabolism, steroids also play a decisive role in determining the resistance of the body against the most varied types of injury (2). These adaptive steroids can be classified according to their mechanism of action into two main groups: (*a*) "syntoxic" steroids, which improve host-tissue tolerance by permitting coexistence with the substrate (*e.g.*, by suppressing nonspecific inflammatory or allergic reactions against it); and (*b*) "catatoxic" steroids, which enhance the detoxication of endogenous and exogenous toxicants *via* induction, activation, decreased degradation of drug-metabolizing enzymes, and/or accelerated substrate elimination from the body. To obtain the best catatoxic effect, the steroids are usually administered 2–3 days before the toxicant. However, recent findings (3) indicate that even posttreatment facilitates protection against certain intoxications.

Systematic studies (2, 4) in these laboratories revealed that, among more than 1200 steroids tested, pregnenolone-16α-carbonitrile and its close derivatives exert the greatest prophylactic effect *in vivo*. Biochemical investigations (5) on rats showed that pregnenolone-16α-carbonitrile stimulates catatoxic mechanisms. On the other hand, steroids such as estradiol protect *in vivo* only against a limited number of drugs, and it is not known whether their actions are syntoxic or catatoxic (6, 7).

In view of these observations, an attempt was made to investigate the correlation between the *in vivo* effects of steroids and the plasma concentrations of zoxazolamine and dicumarol after treatment with pregnenolone-16α-carbonitrile, estradiol, triamcinolone, progesterone, hydroxydione, or ACTH. The steroids were selected on the basis that the syntoxic effects are virtually limited to glucocorticoids, whereas the catatoxic properties appear to be independent of any other known steroid

Table I—Effect of Various Steroids and ACTH upon Plasma Level of Zoxazolamine

Conditioner	Plasma Concentration of Zoxazolamine, mcg./ml.			Paralysis Time, min.	
	Conditioned[a] Animals	Control I[b]	Control II[a]	Conditioned Animals	Control II
Pregnenolone-16α-carbonitrile	26.5 ± 0.7 (18)[c]	41.4 ± 1.3 ***[d] (16)	28.1 ± 2.0 NS (5)	19 ± 1 ***	115 ± 17
Estradiol	25.3 ± 0.7 (15)	30.5 ± 1.1 *** (14)	24.7 ± 1.8 NS (7)	166 ± 10 NS	203 ± 29
Progesterone	22.0 ± 1 (16)	27.5 ± 0.7 *** (15)	25.1 ± 1.6 NS (4)	123 ± 15 NS	100 ± 3
Triamcinolone	35.0 ± 0.9 (20)	31.1 ± 1.4 * (16)	26.2 ± 1.1 *** (9)	89 ± 8 ***	130 ± 6
Hydroxydione	28.7 ± 0.7 (17)	33.0 ± 1.6 * (18)	26.4 ± 1.2 NS (6)	188 ± 9 *	252 ± 24
ACTH	32.6 ± 0.9 (17)	34.8 ± 0.8 NS (19)	27.0 ● 0.8 *** (10)	111 ± 8 ***	160 ± 10

[a] Killed when the righting reflex was regained. [b] Killed when the conditioned group regained the righting reflex. [c] Figures in parentheses indicate number of animals. [d] The plasma levels of zoxazolamine in Controls I and II are compared with the plasma concentrations of the drug in the conditioned rats: * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.005$, and NS = not significant.

hormone action. ACTH protects against several toxic compounds (8); hence, it was used in this experimental series to stimulate endogenous glucocorticoid secretion.

EXPERIMENTAL

Two series of experiments were performed on female ARS/Sprague–Dawley rats[1], averaging 100 g. (range 90–110 g.) and maintained *ad libitum* on laboratory food[2] and tap water.

In the zoxazolamine series, the animals were divided into three groups, of which the first and second received 1 ml. of water twice daily *per os*. The rats of the remaining group were given pregnenolone-16α-carbonitrile[3] (3β-hydroxy-20-oxo-5-pregnene-16α-carbonitrile), estradiol[4], progesterone[4], hydroxydione hemisuccinate[5], triamcinolone[6] (10 mg. in 1 ml. water twice daily *per os*), or ACTH[7] (5 I.U. in 0.2 ml. water three times daily subcutaneously) throughout the experiment. Zoxazolamine[8] (10 mg./100 g. body weight in 1 ml. water) was administered intraperitoneally to all groups on the 4th day, 1 hr. after the last pretreatment. Blood samples were taken from control (Group I) and conditioned (Group III) animals when the latter regained the righting reflex. Samples were removed from Group II when their righting reflex reappeared.

In the dicumarol series, the animals were divided into four groups, of which the first two received 1 ml. of water twice daily *per os*. The remaining two groups were given the conditioners as described. Only triamcinolone was administered at the individual dose of 1 mg. because of its toxicity at a higher dose level during 4 days of pretreatment plus 5 days of treatment. Dicumarol[9] (13 mg./100 g. body weight in 1 ml. water *per os*) was given once daily to all groups beginning on the 4th day, always 1 hr. after the first daily dose of the conditioners. Blood samples were taken from Groups I and III on the 7th day, 5 hr. after dicumarol treatment. Mortality was recorded in the remaining two groups on the 9th day.

Zoxazolamine was extracted from plasma into ethylene dichloride and from the organic solvent into hydrochloric acid. The plasma concentration of the drug was measured spectrophotometrically at 278 nm., where it exhibits a pronounced absorption maximum (9). Dicumarol was extracted from acidified plasma into heptane and from heptane into a sodium hydroxide solution. The drug concentration was determined spectrophotometrically at 315 nm. (10). All measurements were performed with a spectrophotometer[10]. Drug-free plasma from conditioned and nonconditioned animals was used for preparing standards and blanks.

[1] Madison, Wis.
[2] Purina Laboratory Chow, Ralston Purina Co. of Canada.
[3] The Upjohn Co.
[4] Roussel Corp.
[5] Pfizer Laboratories.
[6] E. R. Squibb & Sons.
[7] N. V. Organon.
[8] K & K Laboratories.
[9] Abbott Laboratories.
[10] Unicam SP 8000.

RESULTS AND DISCUSSION

These experiments were performed to establish whether the protection offered by a steroid or ACTH is due to a catatoxic or syntoxic effect, *i.e.*, to the enhanced elimination of, or increased tolerance to, zoxazolamine and dicumarol.

The plasma levels of zoxazolamine were compared in control and conditioned animals when the latter regained the righting reflex as well as when paralysis disappeared in both groups.

The following changes in plasma drug concentrations were regarded as characteristic of protection through catatoxic or syntoxic mechanisms:

1. The zoxazolamine concentration in the blood of conditioned animals was lower than that of the controls at the time when the former regained the righting reflex; it corresponded to the level in control Group II when paralysis disappeared in these rats.

This characteristic catatoxic pattern was clearly exhibited by pregnenolone-16α-carbonitrile. The duration of zoxazolamine-induced paralysis was shortened from 115 to 20 min. and the plasma drug concentration decreased from 41 to 26 mcg./ml. as compared with the controls when the righting reflex reappeared in the former. A similar concentration (28 mcg./ml.) was noted in the controls that were sacrificed when they regained the righting reflex.

2. If a steroid acts syntoxically, the plasma levels of zoxazolamine should be higher in the pretreated rats than in control Group II when paralysis disappears in both groups. On the other hand, the blood levels of the drug should be the same in both the conditioned and control Group I when blood samples are taken from the conditioned rats after they regain the righting reflex.

Triamcinolone and ACTH reduced zoxazolamine paralysis from 130 to 89 and from 160 to 111 min., respectively. This reduction took place although the plasma levels of the drug in the conditioned rats were significantly higher than in the controls when both groups regained the righting reflex (Table I).

When paralysis disappeared, the plasma concentrations of zoxazolamine in the estradiol- or hydroxydione-treated rats were slightly lower than in the controls killed at the same time. However, the plasma drug levels in the conditioned groups were not significantly different from those in the controls sacrificed when they regained the righting reflex. Since paralysis was slightly reduced, these two steroids seem to have a mild catatoxic effect.

The action of progesterone on the plasma concentration of zoxazolamine was comparable to that of estradiol and hydroxydione. Although paralysis was prolonged by this steroid, the apparent difference was not significant. This discrepancy may be attributed to the anesthetic properties of progesterone, administered 1 hr. before zoxazolamine.

The plasma level of dicumarol was influenced by the steroids in three different ways (Table II). The *in vivo* protection offered by estradiol was associated with a significant decrease in the plasma drug concentration. Triamcinolone and progesterone, on the other hand, markedly elevated the plasma level of dicumarol as compared with the concentration of the drug in nonconditioned rats. The increased plasma drug concentration after pretreatment (*e.g.*, with triamcinolone or progesterone) might have been due to inhibition

Table II—Effect of Various Steroids and ACTH upon Plasma Level of Dicumarol

Conditioner	Plasma Concentration of Dicumarol[a], mcg./ml.		Mortality[b]	
	Conditioned Animals	Control	Conditioned Animals	Control
Pregnenolone-16α-carbonitrile	19.4 ± 1.5 NS (17)[c]	18.6 ± 2.2 (17)	9/10 NS	7/9[e]
Estradiol	13.7 ± 1.5 ***[d] (18)	26.6 ± 2 (18)	1/10 ***	7/9[e]
Progesterone	43.3 ± 2.9 *** (16)	31.1 ± 2.3 (11)	9/10 NS	9/10
Triamcinolone	55.8 ± 4.1 ** (6)	19.3 ± 2.6 (7)	10/10 NS	7/9[e]
Hydroxydione	26.3 ± 3.3 NS (15)	20.6 ± 3.3 (11)	12/15 NS	11/15
ACTH	29.9 ± 2.8 NS (14)	25.1 ± 2.8 (17)	6/9 NS	8/10

[a] Determined on the 7th day, 5 hr. after dicumarol treatment. [b] Recorded on the 9th day. [c] Figures in parentheses indicate number of animals. [d] ** = $p < 0.01$, *** = $p < 0.005$, and NS = not significant. [e] *Cf. Reference 5.*

of the hepatic microsomal enzyme system responsible for the metabolism of these steroids (1, 11, 12). The mortality rates were comparable in the control and pretreated groups. Pregnenolone-16α-carbonitrile, hydroxydione, and ACTH, which also did not offer *in vivo* protection, did not markedly influence the plasma concentration of dicumarol. Hence, only estradiol demonstrated a significant catatoxic effect against dicumarol intoxication.

The results of these investigations indicate that steroids can offer protection against drugs through syntoxic or catatoxic mechanisms. The catatoxic effect is not dependent upon any particular hormonal activity whereas the syntoxic action is limited to triamcinolone, a glucocorticoid, and to ACTH, a stimulator of endogenous glucocorticoid secretion. Most of the catatoxic actions of steroids, particularly those of pregnenolone-16α-carbonitrile, are mediated through enhanced hepatic microsomal enzyme activity. Since zoxazolamine is a substrate of this system, there is little doubt that the blood levels are lowered and paralysis time reduced through increased hydroxylation of this drug (13). However, pregnenolone-16α-carbonitrile does not act against dicumarol, which is known to be detoxified through microsomal hydroxylation (14).

The catatoxic effect of estradiol against dicumarol is uncommon; it could be attributed to the fact that this steroid induces drug-metabolizing enzymes in female but not in male rats (15–18). The influence of triamcinolone and ACTH on zoxazolamine paralysis is even more difficult to explain. It may be due to reduced receptor sensitivity, altered drug distribution in the body, decreased substrate excretion, or interactions at receptor sites. Future work will have to clarify whether these factors play decisive roles in diminishing zoxazolamine paralysis.

REFERENCES

(1) A. H. Conney, *Pharmacol. Rev.*, **19**, 317(1967).

(2) H. Selye, "Hormones and Resistance," Springer-Verlag, Heidelberg, West Germany, 1971.

(3) H. Selye, *Experientia*, **27**, 1445(1971).

(4) H. Selye, *J. Pharm. Sci.*, **60**, 1(1971).

(5) B. Solymoss, J. Werringloer, and S. Toth, *Steroids*, **17**, 427 (1971).

(6) H. Selye, *Horm. Behav.*, **2**, 337(1972).

(7) H. Selye and S. Szabo, *Vox. Sang.*, **22**, 229(1972).

(8) S. Szabo, P. Kourounakis, J. Werringloer, and H. Selye, *Ann. ACFAS*, **38**, 57(1971).

(9) J. J. Burns, T. F. Yü, L. Berger, and A. B. Gutman, *Amer. J. Med.*, **25**, 401(1958).

(10) J. Axelrod, J. R. Cooper, and B. B. Brodie, *Proc. Soc. Exp. Biol. Med.*, **70**, 693(1949).

(11) L. F. Soyka and R. J. Long, *Fed. Proc.*, **30**, 281(1971).

(12) G. J. Mannering, in "Handbook of Experimental Pharmacology," part 2, B. B. Brodie and J. R. Gillette, Eds., Springer-Verlag, Heidelberg, West Germany, 1971, p. 452.

(13) A. Trevor, in "Fundamentals of Drug Metabolism and Drug Disposition," B. N. La Du, H. G. Mandel, and E. L. Way, Eds., Williams & Wilkins, Baltimore, Md., 1971, p. 591.

(14) J. J. Burns, in *ibid.*, p. 340.

(15) K. P. DuBois and F. Kinoshita, *Arch. Int. Pharmacodyn. Ther.*, **156**, 418(1965).

(16) G. P. Quinn, J. Axelrod, and B. B. Brodie, *Biochem. Pharmacol.*, **1**, 152(1958).

(17) J. K. Inscoe and J. Axelrod, *J. Pharmacol. Exp. Ther.*, **129**, 128(1960).

(18) W. Klinger, A. Neugebauer, F. K. Splinter, K. H. Chemnitius, D. Baner, R. Ernst, and E. Karge, *Biochem. Pharmacol.*, **19**, 2677(1970).

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