Effect of Various Steroids and ACTH on Distribution of Zoxazolamine in Rats

P. KOUROUNAKIS[▲], S. SZABO*, and H. SELYE

Abstract □ A study was performed on rats given pregnenolone-16α-carbonitrile (I), triamcinolone, fludrocortisone acetate, corticosterone, estradiol, or ACTH to investigate correlations between the *in vivo* effects upon zoxazolamine paralysis and the concentrations of this drug in plasma, brain, liver, kidney, heart, spleen, muscle, and fat. Pretreatment with the steroids or ACTH altered the organ concentrations of zoxazolamine but not the relationships between the interorgan concentrations. Protection by I was associated with a simultaneous reduction of zoxazolamine in plasma, brain, and most of the other organs (catatoxic mechanism) as compared with controls killed when paralysis disappeared in the pretreated group. The protective actions of ACTH and triamcinolone were often accompanied by high concentrations of zoxazolamine in plasma and most of the organs (syntoxic mechanism) when compared with controls sacrificed at spontaneous termination of the pharmacological response. Fludrocortisone seemed to protect through both phenomena. Corticosterone and estradiol were ineffective under the same conditions, eliciting no changes in the plasma clearance or organ concentrations of the drug.

Keyphrases □ Steroids (pregnenolone-16α-carbonitrile, triamcinolone, fludrocortisone acetate, corticosterone, and estradiol)—effect on plasma and organ concentrations of zoxazolamine □ ACTH—effect on plasma and organ concentrations of zoxazolamine □ Zoxazolamine, plasma concentrations—effect of steroids and ACTH, resistance–plasma concentration correlations

Numerous observations (1–3) have confirmed that steroids and certain other pharmacological agents play a decisive role in altering drug activity, thereby influencing the resistance of the body against the most varied types of injury. These adaptive steroids have been classified according to their mechanism of protective action into: (*a*) syntoxic steroids, which improve host tissue tolerance by permitting coexistence with the toxic agent (*e.g.*, by suppressing nonspecific inflammatory or allergic reactions against it); and (*b*) catatoxic steroids, which enhance the detoxication of endogenous or exogenous toxicants *via* induction, activation, decreased degradation of drug-metabolizing enzymes, and/or accelerated substrate elimination. Of the many steroids studied, it has been found that pregnenolone-16α-carbonitrile (I) exerts the greatest protective effect *in vivo*. This compound is a known hepatic microsomal enzyme inducer (4, 5). Biochemical studies have shown that protection offered by catatoxic steroids (*e.g.*, I and spironolactone) is usually accompanied by a significant reduction of drug concentrations in plasma (6, 7). To obtain the best catatoxic effect, the steroids have to be administered at least 3 days before the toxicant. However, in some cases, even posttreatment offers significant protection (8).

Recent studies (6, 7) showed that the protective effect of I and spironolactone against zoxazolamine and methyprylon can be considered as catatoxic and that of triamcinolone and ACTH can be considered as syntoxic. On the basis of these findings, it was established that these phenomena are characterized by the following relationships:

catatoxic	$C_{cd} < C_1$	$C_{cd} \simeq C_2$
syntoxic	$C_{cd} \simeq C_1$	$C_{cd} > C_2$

where:

C_{cd} = plasma concentration of the drug in pretreated animals at the termination of the pharmacological response

C_1 = plasma concentration of the drug in not yet recovered controls killed when the pharmacological response has disappeared in the pretreated group

C_2 = plasma concentration in recovered controls sacrificed at the termination of the pharmacological response

In previous experiments (6), estradiol was found to be very active against dicumarol. Although unusual, this is not an altogether surprising observation since estradiol can affect the response to other drugs (9–12). The protection offered by syntoxic compounds (*e.g.*, triamcinolone and ACTH) is difficult to explain, although altered drug distribution may be involved. The concentrations of zoxazolamine in some organs and the correlation between the plasma and brain concentrations of this drug were reported by others (13, 14). Changes in distribution of zoxazolamine in the central nervous system (CNS) and plasma as a function of time or chlordane pretreatment were also studied (15). However, it appears that there have been no investigations on the concentrations of this drug in other organs and on the effect, if any, of steroid pretreatment.

In addition to experiments on possible changes in the organ concentrations, it seemed of interest to determine whether: (*a*) the two adaptive steroid groups also produce characteristic alterations in drug distribution; (*b*) changes in the drug plasma concentrations are reflected in the brain, liver, kidney, heart, muscle, and fat; and (*c*) the characteristic relationships for syntoxic and catatoxic steroids, based on plasma concentrations, are applicable to drug concentrations in the parameters just mentioned.

EXPERIMENTAL

Female Sprague-Dawley rats[1], averaging 100 g. (range of 90–110 g.), were used and maintained on laboratory food[2] and tap water *ad libitum*. The animals were divided into three groups. The controls (Groups 1 and 2) were given 1 ml. of water twice daily *per os* for 3 days and once (1 hr. before zoxazolamine) on the 4th day. The rats of the remaining group received I[3] (3β-hydroxy-20-oxo-5-

[1] Canadian Breeding Farms & Laboratories Ltd., St. Constant, Quebec, Canada.
[2] Purina laboratory chow, Ralston Purina Co. of Canada.
[3] The Upjohn Co.

Table I—Effect of Various Steroids and ACTH on the Distribution of Zoxazolamine

Pretreatment	Group	Zoxazolamine Concentration[a], mcg./g. Liver	Brain	Kidney	Muscle	Adipose Tissue	Heart	Spleen	Plasma
	Unrecovered control[b]	144.08 ± 6.5*** (13)[c]	98.23 ± 6.9*** (11)	115.86 ± 1.9*** (8)	34.74 ± 6.0** (6)	88.98 ± 5.7*** (10)	92.90 ± 3.1*** (8)	47.00 ± 6.7 NS (6)	35.44 ± 1.1*** (8)
I	Pretreated[d]	87.42 ± 8.4 (13)	63.57 ± 4.8 (9)	73.75 ± 2.6 (8)	11.76 ± 2.3 (6)	45.08 ± 4.9 (8)	59.12 ± 2.9 (8)	39.33 ± 4.9 (8)	26.07 ± 1.4 (6)
	Recovered control[d]	97.91 ± 2.6 NS (11)	69.79 ± 3.5 NS (10)	85.90 ± 2.7* (6)	28.83 ± 2.8*** (8)	81.35 ± 7.7** (8)	63.68 ± 2.9 NS (6)	42.17 ± 6.1 NS (6)	27.34 ± 0.7 NS (6)
	Unrecovered control[b]	130.91 ± 9.7*** (11)	91.12 ± 4.1 NS (10)	100.07 ± 2.2 NS (6)	31.08 ± 2.1 NS (8)	81.61 ± 4.1 NS (10)	84.95 ± 3.9 NS (8)	45.00 ± 4.3 NS (10)	28.80 ± 1.3* (12)
Triamcinolone	Pretreated[d]	88.13 ± 4.8 (12)	85.36 ± 4.2 (12)	98.73 ± 4.6 (6)	31.68 ± 0.9 (6)	79.13 ± 2.4 (10)	73.36 ± 4.1 (10)	42.50 ± 4.2 (6)	32.90 ± 1.7 (12)
	Recovered control[d]	103.32 ± 6.9 NS (11)	72.14 ± 3.6* (10)	88.02 ± 4.2 NS (6)	24.16 ± 2.3* (6)	79.47 ± 5.5 NS (8)	72.72 ± 1.4 NS (6)	35.50 ± 4.7 NS (6)	24.70 ± 1.0** (12)
	Unrecovered control[b]	117.35 ± 3.4* (8)	105.44 ± 6.6 NS (6)	81.57 ± 6.2 NS (8)	38.88 ± 4.0 NS (6)	116.17 ± 8.8 NS (6)	62.31 ± 1.8 NS (6)	50.70 ± 3.3 NS (8)	23.03 ± 1.3 NS (7)
Corticosterone	Pretreated[d]	102.92 ± 3.4 (8)	114.41 ± 7.5 (7)	70.88 ± 0.9 (8)	28.64 ± 1.1 (8)	97.01 ± 4.9 (8)	62.63 ± 2.7 (8)	46.59 ± 3.5 (8)	23.09 ± 0.7 (7)
	Recovered control[d]	94.00 ± 3.0 NS (6)	100.96 ± 3.8 NS (6)	70.46 ± 4.2 NS (6)	23.07 ± 2.1 NS (6)	105.63 ± 5.3 NS (6)	59.55 ± 4.8 NS (8)	40.95 ± 4.0 NS (6)	22.64 ± 1.1 NS (7)
	Unrecovered control[b]	190.68 ± 4.1*** (8)	150.59 ± 3.6*** (8)	90.80 ± 2.9*** (8)	42.16 ± 2.2* (8)	146.49 ± 7.5*** (8)	59.28 ± 3.1 NS (6)	56.00 ± 2.0*** (8)	26.55 ± 1.4* (7)
Fludrocortisone	Pretreated[d]	121.77 ± 4.0 (7)	101.33 ± 2.3 (7)	70.09 ± 3.5 (8)	31.96 ± 3.2 (8)	87.83 ± 4.4 (8)	57.16 ± 2.5 (6)	48.64 ± 2.5 (8)	23.10 ± 1.3 (7)
	Recovered control[d]	137.11 ± 3.9* (6)	93.88 ± 3.1 NS (6)	60.27 ± 0.3* (6)	34.26 ± 2.0 NS (6)	94.15 ± 4.1 NS (6)	54.36 ± 2.0 NS (6)	44.85 ± 4.4 NS (6)	22.64 ± 1.1 NS (6)
	Unrecovered control[b]	102.56 ± 5.0 NS (8)	116.37 ± 4.5 NS (8)	70.22 ± 2.7 NS (8)	30.59 ± 2.7 NS (8)	88.78 ± 6.4 NS (8)	63.61 ± 0.6 NS (8)	44.32 ± 2.7 NS (8)	28.30 ± 1.3 NS (6)
Estradiol	Pretreated[d]	98.39 ± 3.3 (8)	108.67 ± 6.3 (7)	63.10 ± 6.7 (8)	27.82 ± 5.7 (6)	88.86 ± 5.1 (8)	66.76 ± 4.4 (8)	36.55 ± 3.2 (8)	26.65 ± 1.4 (7)
	Recovered control[d]	87.94 ± 4.3 NS (6)	107.54 ± 5.0 NS (6)	60.04 ± 2.1 NS (6)	23.73 ± 1.8 NS (6)	81.99 ± 4.5 NS (6)	61.73 ± 2.9 NS (6)	40.72 ± 7.1 NS (6)	27.23 ± 1.7 NS (8)
	Unrecovered control[b]	141.31 ± 9.7** (7)	111.94 ± 7.5 NS (7)	117.48 ± 11.3 NS (8)	42.54 ± 2.9 NS (8)	106.01 ± 6.5 NS (8)	78.18 ± 7.9 NS (6)	52.84 ± 2.5 NS (8)	36.93 ± 2.3 NS (7)
ACTH	Pretreated[d]	105.51 ± 3.5 (7)	106.71 ± 4.3 (7)	98.54 ± 1.9 (8)	39.87 ± 2.6 (6)	94.70 ± 3.9 (8)	71.32 ± 5.7 (6)	49.53 ± 5.4 (8)	37.33 ± 2.1 (7)
	Recovered control[d]	92.09 ± 2.6** (6)	71.42 ± 5.2*** (6)	69.60 ± 1.0*** (6)	33.86 ± 2.3 NS (6)	62.98 ± 6.5* (6)	56.14 ± 2.9 NS (6)	39.16 ± 2.9 NS (6)	23.51 ± 1.6*** (6)

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

pregnene-16α-carbonitrile), triamcinolone[4], corticosterone[5], fludrocortisone (9α-fluorocortisol) acetate[3], estradiol[6] (1 mg. in 1 ml. water, homogenized with a trace of polysorbate 80, twice daily *per os* for 3 days and once on the 4th day), or depot ACTH[7] (5 I.U. = 50 mcg., subcutaneously) 24 hr. before the toxicant. Zoxazolamine[8] (10 mg./100 g. body weight in 1 ml. water, homogenized with a few drops of polysorbate 80) was administered intraperitoneally to all rats on the 4th day.

Blood samples were collected from the unrecovered control and pretreated animals when the latter regained the righting reflex and from the second control group at spontaneous recovery. Specimens were immediately taken from the liver (left lateral lobe), brain (severed just above the first cervical vertebra), kidney, heart (blood was drained by pressing the organ immediately after removal), spleen, muscle (biceps femoris, quadratus femoris, and rectus abdominis), and adipose tissues (subcutaneous, mesenteral, and perirenal). These specimens were then placed in an ice-cold 0.9% NaCl solution (ratio 1:2) and homogenized.

Zoxazolamine was extracted from plasma, organ, muscle, or adipose tissue homogenates into ethylene dichloride and from the organic solvent into hydrochloric acid. The drug concentrations were measured spectrophotometrically at 278 nm., the λ_{max} of zoxazolamine (13). Drug-free plasma from pretreated and unpretreated animals was used for preparing standards and blanks.

RESULTS AND DISCUSSION

Table I shows that the zoxazolamine concentrations decreased approximately in the following order: liver, heart, spleen, muscle,

[4] E. R. Squibb & Sons.
[5] Merck Sharp & Dohme.
[6] Roussel Corp.
[7] Ciba.
[8] K & K Laboratories.

Table II—Effect of Various Steroids and ACTH on Zoxazolamine-Induced Paralysis

Pretreatment	Zoxazolamine Paralysis[a], Duration in min. Control	Pretreated
I	149 ± 11 (10)[b]	44 ± 3*** (10)
Triamcinolone	164 ± 15 (12)	116 ± 9** (12)
Corticosterone	138 ± 12 (7)	143 ± 12 NS (8)
Fludrocortisone	148 ± 12 (8)	93 ± 12** (7)
Estradiol	143 ± 12 (6)	141 ± 18 NS (8)
ACTH	156 ± 6 (6)	80 ± 6*** (7)

[a] ** = $p < 0.01$, *** = $p < 0.005$, and NS = $p > 0.05$. [b] Figures in parentheses indicate number of animals.

and plasma. Neither the steroids, nor ACTH, nor time had any influence on these parameters. Essentially similar drug concentrations were found in the brain, fat, and kidney; these concentrations lay between those of the heart and liver. The results on the zoxazolamine concentrations in plasma and brain are in agreement with other comparative studies (14–16). The changes in drug concentrations in the spleen were not significantly altered by time or pretreatment.

In I- or fludrocortisone-pretreated rats, the concentrations of zoxazolamine in most organs were greatly reduced as compared with unrecovered controls (sacrificed when the righting reflex was regained in the former); they were similar to those in the second control group (killed at spontaneous recovery). There were considerable differences between the drug concentrations in the organs of the two controls. In the estradiol series, the differences between the zoxazolamine concentrations in the controls were not significant. This was expected because the duration of paralysis was similar in both groups (Table II).

The relationship for catatoxic activity is obviously common to drug concentrations in the plasma, brain, liver, heart, muscle, and spleen of I- or fludrocortisone-pretreated rats. Fludrocortisone was the only agent that caused a significant reduction of zoxazolamine in the spleen as compared with concentrations of the drug in the unrecovered controls.

The zoxazolamine concentrations in the various organs of the ACTH- or triamcinolone-pretreated animals were similar to those in the paralyzed controls; however, the pretreated groups showed lower drug concentrations in the liver, which could possibly be ascribed to the increase of hepatic glycogen. The ACTH- or triamcinolone-pretreated rats generally had higher drug concentrations in all organs than the recovered controls. The zoxazolamine concentrations were significantly increased in the plasma of the triamcinolone group as well as in the liver, brain, kidney, fat, and plasma of the ACTH-pretreated animals. Some changes in the other parameters were evident, but the differences were not significant. These findings show that the changes characteristic of syntoxic activity (of ACTH) manifest themselves not only in plasma drug concentrations but also in most of the organs examined. In triamcinolone-pretreated rats, there was a tendency for this relationship to be fulfilled. However, of all the parameters, only the plasma concentrations clearly indicated a syntoxic phenomenon.

The zoxazolamine concentrations in the organs of the corticosterone- or estradiol-pretreated animals usually lay between those of the corresponding two control groups, but the differences were not significant.

The brain: plasma concentrations of zoxazolamine were generally the same in pretreated and unpretreated rats (Table III), but there was a significant difference between the fludrocortisone group and the corresponding controls. This could indicate that the steroids and ACTH, unlike fludrocortisone, did not modify the permeability of the blood–brain barrier.

Generally, the liver:plasma concentrations of zoxazolamine varied in the pretreated and unpretreated groups. This may have been due to intense biotransformation of the drug in the liver and/or to increased glycogen in certain cases. As seen in Table III, the only exceptions were estradiol-pretreated animals and their corresponding controls, which showed none of these characteristics. Differences between the liver weights of pretreated and unpretreated rats could not have accounted for the variations in the hepatic concentrations of zoxazolamine.

Previous studies (17–19) established that the metabolic degradation of zoxazolamine is increased after induction of hepatic microsomal enzymes. In I-pretreated rats, the general decline of zoxazolamine concentrations in various organs after microsomal enzyme induction (4, 5) does not seem to alter drug distribution significantly in the body. The decrease of zoxazolamine paralysis (Table II) is well correlated with reduced drug concentrations in the brain (Table I). On the other hand, the protection offered by ACTH and triamcinolone can be considered as syntoxic since the plasma and brain concentrations of zoxazolamine are higher in pretreated animals than in recovered controls (killed at the same clinical stage). The organism is rendered less sensitive to the toxicant through a mechanism that has not yet been clarified (*e.g.*, interactions at receptor sites). Here the possibility of alterations in drug distribution may be excluded, as the findings indicate.

The *in vivo* protection offered by fludrocortisone (Table II) seems to be due to more than merely increased drug metabolism through hepatic microsomal enzyme induction. This is evident from the present results (Table I) and from *in vitro* studies on liver homogenates (20). The syntoxic effect of fludrocortisone is indicated by the seemingly reduced permeability of the blood–brain barrier. It is suggested that fludrocortisone has a dual action (catatoxic and syntoxic), because the mild fall in the plasma concentration of zoxazolamine in itself could not account for marked *in vivo* protection.

Under the experimental conditions, corticosterone and estradiol were neither catatoxic nor syntoxic, judging from the unaltered drug concentrations in the various organs. The failure of corticosterone to offer protection was previously demonstrated in methyprylon-intoxicated rats (7).

The present results indicate that the various steroids and ACTH generally modify the organ concentrations of zoxazolamine but not its organ distribution. Thus, the syntoxic effects of triamcinolone and ACTH (6, 7, 21) are not mediated through altered drug distribution. Fludrocortisone appears to be catatoxic (*e.g.*, increased zoxazolamine biodegradation) and syntoxic (*e.g.*, reduced permeability of the blood–brain barrier).

Finally, the effects of catatoxic and syntoxic steroids upon zoxazolamine concentrations in plasma are generally associated with altered drug concentrations in various organs. The latter changes

Table III—Effect of Various Steroids and ACTH on the Brain:Plasma and Liver:Plasma Ratios of Zoxazolamine

Organ	Group	Pretreatment: I	Triamcinolone	Corticosterone	Fludrocortisone	Estradiol	ACTH
Brain	Unrecovered control[a]	2.9	3.2	4.6	5.7	4.1	3.0
	Pretreated[b]	2.7	2.8	5.0	4.4	4.1	2.9
Liver	Unrecovered control[a]	4.1	4.9	5.1	7.2	3.6	3.9
	Pretreated[b]	3.3	2.9	4.5	5.3	3.7	2.8

[a] Killed when the pretreated group regained the righting reflex. [b] Killed when the righting reflex was regained.

follow the patterns demonstrated for catatoxic and syntoxic phenomena in plasma (6, 7).

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Drug Absorption Kinetics in Goldfish

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Abstract □ A model is presented for relating turnover time in goldfish to the concentration of drug in the bathing solution. The model, based on passive transport and the existence of a critical concentration of drug within the fish being required for turnover, was applied to the dose–response data of eight homologous esters of *p*-aminobenzoic acid. Absorption rate constants, relative membrane water partition coefficients, and critical concentrations were evaluated for the methyl through hexyl esters. Heptyl aminobenzoate was only effective in saturated solution. No turnover could be produced by the octyl ester which has a solubility below the critical concentration required for activity.

Keyphrases □ Goldfish—drug absorption kinetics, model and equations relating turnover time and alkyl *p*-aminobenzoate concentration □ Drug absorption kinetics—relationship between turnover time (goldfish) and drug concentration (alkyl *p*-aminobenzoates), model, equations □ *p*-Aminobenzoic acid esters—model and equations relating drug concentration and goldfish turnover time □ Dose–response data—alkyl *p*-aminobenzoate-induced turnover of goldfish

The relationship between the concentration of drug in a bulk solution containing a goldfish and the turnover time of the fish has been studied in several laboratories (1–12) over the past few years. Levy and Gucinski (1) developed a model which relates turnover time to the passive transport rate of the drug across the appropriate membrane of the fish. Their model, while frequently useful, does not account for the commonly observed threshold concentrations of drug, *i.e.*, the minimum concentration producing turnover. Nightingale and Gibaldi (8) recently extended the Levy–Gucinski model to cover situations where a measurable threshold concentration exists. Unfortunately, their derivation appears to predict a dependency on fish volume and on bathing solution volume. This report presents an alternative derivation which overcomes certain difficulties of previous mathematical models.

The equations developed will be used to interpret the dose–response data obtained for several normal alkyl esters of *p*-aminobenzoic acid.

THEORETICAL

In the course of these experiments and in the work of previous investigators, a linear relationship was observed between drug concentration and goldfish turnover time. The linearity and intercept of the reciprocal turnover time–concentration data can be explained by a model based on the following assumptions:

1. Absorption of the drug is passive and is, therefore, a reversible and unsaturable process.
2. Permeability characteristics of the membrane are unchanged during the experiment and unaffected by drug concentration.
3. Drug metabolism or active excretion is negligible during the experiment. There is no development of drug tolerance.