Biochemical Pharmacology, Vol 23, pp 2083–2094 Pergamon Press, 1974 Printed in Great Britain

COMPARATIVE STUDIES ON THE EFFECT OF ADRENOCORTICOTROPHIC HORMONE (ACTH) AND PREGNENOLONE-16α-CARBONITRILE (PCN) UPON DRUG RESPONSE AND DISTRIBUTION IN RATS

SANDOR SZABO,* HANS SELYE, PANAGIOTIS KOUROUNAKIS and YVETTE TACHÉ

Institut de médecine et de chirurgie expérimentales, Université de Montréal, Montreal 101, Que., Canada

(*Received* 31 *March* 1973; *accepted* 9 *November* 1973)

Abstract—The effects of pretreatment with pregnenolone-16α-carbonitrile (PCN) or crystalline adrenocorticotrophic hormone (ACTH) and depot ACTH were investigated in female rats given picrotoxin, nikethamide, succinylcholine, strychnine, ethylmorphine, dioxathion, acetanilide, aniline, *N*-methylaniline, pancuronium, allopurinol, methyprylon, barbital, cyclobarbital, hexobarbital, phenobarbital, zoxazolamine, mephenesin, carisoprodol, sodium aurothiomalate or *N*-carbamoylarsanilic acid. Both ACTH and PCN offered significant protection against most of the drugs, but nikethamide and dioxathion toxicity was diminished solely by PCN, whereas that of aniline, *N*-methylaniline, barbital and phenobarbital was decreased by ACTH alone. Time–sequence studies revealed that a single injection of depot ACTH, or PCN gavage, 24 hr prior to zoxazolamine, significantly shortened paralysis. However, a few days of pretreatment with PCN were needed for maximal protection. ACTH, unlike the steroid, reduced zoxazolamine paralysis even in the presence of diethylaminoethyl-2,2-diphenylvalerate (SKF 525-A), a microsomal enzyme inhibitor. Protection by ACTH was not associated with decreased concentrations of zoxazolamine in blood, brain and muscle (syntoxic action). In contrast, PCN lowered the drug level, probably through increased biotransformation and/or excretion (catatoxic action). These findings furnish additional support to the view that catatoxic steroids mostly operate via the induction of drug-metabolizing enzymes in hepatic microsomes, while syntoxic agents augment resistance, probably through altered drug distribution, interactions at receptor sites or decreased receptor sensitivity.

DURING the last few decades, it has become evident that adrenocorticotrophic hormone (ACTH) plays an important role not only in non-specific stress reactions, but also in modifying specific responses to drugs.[1,2] In rats, ACTH and corticosterone (i.v.), like stress, diminish zoxazolamine paralysis as well as hexobarbital, pentobarbital and meprobamate anesthesia.[3–5] The reduction of barbiturate anesthesia by ACTH and corticosterone[6] is accompanied by a fall in barbiturate levels in the brain, as compared with non-pretreated animals.[7] Stress decreases the concentration of hexobarbital, pentobarbital and meprobamate in the blood of rats.[5] In animals given ACTH or formaldehyde, the 9000 *g* fraction of the liver does not markedly alter the degree of biotransformation of hexobarbital, aminopyrine, aniline and zoxazolamine.[8] Modification of the drug response by stress depends upon the length of exposure to the stressor(s), the strain of rats used, etc.[9,10]

* Present address: Department of Pathology, Peter Bent Brigham Hospital, Harvard Medical School, Boston, Mass. 02115, U.S.A. The experiments were performed during a Fellowship from the Medical Research Council of Canada.

Most of these experiments were performed on intact animals. Hence, it is not clear whether ACTH and stress act directly or through the adrenals. According to our earlier results,[2,11] the effect of crystalline ACTH upon the drug response is mediated through the adrenals, although protection is not associated with decreased levels of the toxicants in plasma.[2,12] Thus, ACTH and glucocorticoids (e.g. triamcinolone) act "syntoxically," in that hormone-pretreated rats tolerate high blood concentrations of drugs without accelerated blood clearance. Pretreatment with certain steroids (e.g. PCN, spironolactone, ethylestrenol, cyproterone, etc.) protects rats against intoxications caused by digitoxin, indomethacin, parathion, hexobarbital, progesterone and zoxazolamine.[1] The action of these "catatoxic" steroids is mostly associated with increased drug biotransformation, due to induction of microsomal drug-metabolizing enzymes in the liver,[13–15] and with decreased levels of the toxicants in plasma.[12] Some extramicrosomal enzymes, such as phosphoprotein phosphatase, are also induced by catatoxic compounds.[16,17] The effect of the steroids does not depend upon the pituitary because prophylaxis can be achieved even in hypophysectomized rats,[18] which, like intact controls, respond with smooth-surfaced endoplasmic reticulum (SER) proliferation in hepatocytes.[19,20]

Systematic studies[1] have revealed that, among more than 1200 synthetic or naturally occurring steroids, PCN possesses the greatest catatoxic activity *in vivo*. Since ACTH stimulates the secretion of endogenous corticoids, we began a detailed investigation on the effects of the adrenocorticotrophic hormone and PCN upon drug responses and distribution in rats.

MATERIALS AND METHODS

Animals. Throughout our experiments, we used female Sprague–Dawley rats [ARS (Madison, Wis., U.S.A.) or CBF (Canadian Breeding Farms & Laboratories Ltd., St. Constant, Que., Canada)], averaging 100 g and maintained *ad lib.* on Purina Lab Chow (Ralston Purina Co. of Canada) and tap water. The rats were divided into groups (each containing at least 10 animals), and treated as indicated in the tables.

First experiment. ACTH and PCN were tested for protection *in vivo* against a series of drugs. Unless otherwise stated, crystalline porcine ACTH [Cortrophin Powder (Organon) or Porcine ACTH (Ferring)] was injected at 25 i.u. in 0·2 ml distilled water, thrice daily, s.c., on the first, second and third days, and once on the fourth day, 1 hr before the toxicants. This technical arrangement was recently found to be most advantageous for toxicity studies in rats.[2] Depot ACTH [Synacthen Depot (Ciba)] was given at the dose of 5 i.u. (50 μg or 0·05 ml of the original suspension), once s.c., 24 hr before the drugs. 3β-Hydroxy-20-oxo-5-pregnene-16α-carbonitrile (PCN, Upjohn) was administered in the amount of 1 mg in 1 ml distilled water (homogenized with a trace of Tween 80), twice daily, p.o., on the first, second and third days, and once on the fourth day, 1 hr before the following toxicants*:

Picrotoxin (British Drug Houses), 430 μg (with ACTH) to CBF rats or 350 μg (with PCN) to ARS rats in 0·2 ml distilled water, s.c., on the fourth day. The severity of the convulsions was estimated in a scale of 0–3,[1] 1 hr after the injection. Mortality was listed on the fifth day.

* The toxicants were always administered per 100 g body weight

Nikethamide (Ciba), 35 mg in 0·2 ml water, s.c., on the fourth day. Dyskinesia was assessed 3 hr later in a scale of 0–3.[1] Mortality was recorded on the fifth day.

Succinylcholine Cl (K & K Laboratories), 200 μg in 0·2 ml water, s.c., on the fourth day. The severity of the convulsions was estimated 15 min after the injection. Mortality was listed on the fifth day.

Strychnine HCl (British Drug Houses), 150 μg in 0·2 ml water, s.c., on the fourth day. The severity of the convulsions was registered 30 min later, and mortality recorded on the fifth day.

Ethylmorphine HCl (May & Baker), 20 mg in 0·2 ml water, s.c., on the fourth day. Dyskinesia was estimated 2 hr later, and mortality recorded on the fifth day.

Dioxathion (Hercule), 4 mg in 1 ml corn oil, p.o., on the fourth day. Dyskinesia was listed 5 hr after treatment. Mortality was registered on the sixth day.

Acetanilide (K & K Laboratories), 40 mg in 0·1 ml dimethylsulfoxide (DMSO), i.p., on the fourth day. In rats (CBF) given ACTH, the depth of coma (scale 0–3) was estimated 2 hr after acetanilide treatment, whereas in the PCN group (ARS) it was recorded 3 hr later.

Aniline HCl (Baker), 55·66 mg (equivalent of 40 mg aniline) in 1 ml water, i.p., on the fourth day. Dyskinesia was listed 4 hr after treatment, and mortality registered on the sixth day.

N-*Methylaniline* (Eastman), 45 mg in 1 ml water, i.p., on the fourth day. Dyskinesia was estimated after 2 hr, and mortality recorded on the sixth day.

Methyprylon (Hoffman-La Roche), 20 mg in 0·2 ml water, s.c., on the fourth day. Sleeping time was determined in min on this same day.

Barbital (Brickman), 20 mg in 2 ml water, i.p., on the fourth day. Sleeping time was assessed immediately.

Cyclobarbital (Winthrop), 7·5 mg in 2 ml water, i.p., on the fourth day. Sleeping time was recorded immediately.

Hexobarbital sodium (Winthrop), 7·5 mg in 1 ml water, i.p., on the fourth day. Sleeping time was determined on this same day.

Phenobarbital (British Drug Houses), 15 mg in 1 ml water, i.p., on the fourth day. Sleeping time was recorded immediately.

Zoxazolamine (K & K Laboratories), 10 mg in 1 ml water (homogenized with a trace of Tween 80), i.p., on the fourth day. Paralysis time was estimated immediately.

Mephenesin (Squibb), 25 mg in 1 ml water, i.p., on the fourth day. Paralysis time was measured immediately.

Carisoprodol (Schering), 30 mg in 1 ml water (homogenized with a trace of Tween 80), i.p., on the fourth day. Paralysis time was recorded on this same day.

Pancuronium Br (Organon), 60 μg in 0·2 ml water, s.c., on the fourth day. Dyskinesia was assessed 30 min after the injection, and mortality registered on the fifth day.

Allopurinol (Burroughs Wellcome), 30 mg in 0·4 ml water (homogenized with a trace of Tween 80), s.c., once daily from the fourth day. Renal weight and kidney changes were recorded on the fourteenth day.

Sodium aurothiomalate (Poulenc), 2·5 mg in 1 ml water, i.v., on the fourth day. Corticomedullary nephrocalcinosis was estimated soon after the animals died (at least 2 days after treatment), or on the seventh day when mortality was listed.

N-*Carbamoylarsanilic acid* (Lilly), 40 mg in 1 ml water, s.c., on the fourth day. Neurolathyrism (ECC syndrome: excitation, choreiform and circling movements)[21] and

corticomedullary nephrocalcinosis were assessed on the sixth day. Mortality was registered on the ninth day.

Statistical evaluation. All the results (except those measured by time) were expressed in an arbitrary scale of 0–3: 0 and 1 (dubious changes) were rated as negative, all others as positive. These data (postitive/total) as well as the mortality rates were then arranged in a 2 × 2 contingency table and their statistical significance was computed by the "Exact Probability Test" of Fisher and Yates.[22,23] The significance of paralysis and sleeping time was calculated by Student's *t*-test. The differences between the non-pretreated and pretreated groups are expressed as follows: * = $P < 0{\cdot}05$, ** = $P < 0{\cdot}01$, *** = $P < 0{\cdot}005$, NS = not significant. (Underlined symbols in the tables indicate aggravation of drug toxicity.) These figures have been further simplified to express the results of the screening studies *in vivo*,[24] as follows: 0 = NS, 1 = $P < 0{\cdot}05$, 2 = $P < 0{\cdot}01$, 3 = $P < 0{\cdot}005$, −1 = $P < \underline{0{\cdot}05}$ (aggravation). For example, in Table 1, paralysis time in the zoxazolamine controls was 179 ± 11 min, whereas after a single dose of depot ACTH (+ zoxazolamine) it was 70 ± 25 min, the statistical significance of this difference being 3 ($P < 0{\cdot}005$) = ***. Whenever the mortality rates were also taken into consideration, the figures in the same table represented the arithmetic mean of significance of mortality and the clinical changes. Thus, when protection by depot ACTH against picrotoxin convulsions was 3, and mortality 1, the mean would be 2.

Second experiment. Five i.u. (50 μg = 0·05 ml) of depot ACTH was injected, s.c., at various time intervals, as indicated in Table 2. Zoxazolamine was given, i.p., on the fourth day at 10 mg in 1 ml water (homogenized with a trace of Tween 80).

Third experiment. One mg of PCN was administered, p.o., in 1 ml water (homogenized with a trace of Tween 80), at stages indicated in Table 2. Zoxazolamine was injected as in the second experiment.

Fourth experiment. Depot ACTH (s.c., once on the third day) and PCN (p.o., twice daily on the first, second and third days in 1 ml water homogenized with a trace of Tween 80) were administered at different dose levels, as indicated in Fig. 1. Zoxazolamine was injected as described earlier.

Fifth experiment. Five i.u. (50 μg = 0·05 ml) of depot ACTH and 1 mg of PCN (in 1 ml water homogenized with a trace of Tween 80) were administered 24 hr before zoxazolamine. To avoid interference with the latter, diethylaminoethyl-2,2-diphenylvalerate (SKF 525-A, Smith, Kline & French) was given, p.o., at the 5- or 8-mg dose level in 1 ml water, 1 hr before zoxazolamine (i.p., as in the second experiment).

Sixth experiment. Depot ACTH was injected as described in the previous experiment. PCN (1 mg in 1 ml water homogenized with a trace of Tween 80) was given, p.o., twice daily on the first, second and third days. Zoxazolamine was administered on the fourth day, as in the second experiment. The rats were decapitated on this same day, as follows: "Control 1" and pretreated groups (ACTH or PCN), when the pharmacologic response disappeared in the latter; "control 2," when the righting reflex reappeared spontaneously. Blood was then collected, and the brain (severed just above the first cervical vertebra) removed. Muscle specimens (biceps femoris, quadratus femoris and rectus abdominis) were placed in 1:2 volumes of ice-cold 0·9% NaCl, and homogenized. Zoxazolamine was extracted from the plasma and whole organ homogenates into ethylene dichloride, and from the organic solvent into hydrochloric acid. The drug concentrations were measured at 278 nm in a UNICAM

SP 8000 spectrophotometer.[25] Drug-free plasma, brain and muscle from pretreated and non-pretreated rats were used for preparing standards and blanks. The values were statistically calculated by Student's *t*-test.

RESULTS

First experiment (Table 1). ACTH significantly diminished the effects of picrotoxin, acetanilide, aniline HCl, *N*-methylaniline, barbital, zoxazolamine, carisoprodol and allopurinol. The toxicity of methyprylon, cyclobarbital and pancuronium bromide

TABLE 1. EFFECT OF ACTH AND PCN ON DRUG ACTIVITY*

Substrate	Conditioner		
	Crystalline ACTH	Depot ACTH	PCN
Picrotoxin	1·5	2	3
Nikethamide	0	0	1·5
Succinylcholine Cl	0	0	−1
Strychnine HCl	0·5	0	3
Ethylmorphine HCl	0·5	0	2
Dioxathion	0	0	2·5
Acetanilide	1	1	1
Aniline HCl	1	2	0
N-Methylaniline	0·5	2	0
Methyprylon	0	2	3
Barbital	3	1	0
Cyclobarbital	0	1	3
Hexobarbital	0	0	1
Phenobarbital	1	0	0
Zoxazolamine	2	3	3
Mephenesin	0	0	3
Carisoprodol	3	2	3
Pancuronium Br	0	1·5	0·5
Allopurinol	1·5	1·5	1 5
Sodium aurothiomalate	0·5	0·5	0
N-Carbamoylarsanilic acid	0·5		0

* PCN and crystalline ACTH were given daily for 3 days (and once on the fourth day), while depot ACTH was administered once 24 hr before the substrates. The results are presented on the basis of the statistical significance of the apparent differences between the control and the pretreated groups. These figures represent the arithmetic mean of the significance of the clinical changes and the mortality rates (if the latter were listed), e.g. if $P < 0{\cdot}005 = 3$ for the former and $P < 0{\cdot}05 = 1$ for the latter, the mean is 2. The minus sign indicates aggravation of toxicity.

was reduced only by depot ACTH, whereas that of phenobarbital was decreased solely by crystalline ACTH. The latter slightly influenced the effects of strychnine HCl, ethylmorphine HCl and sodium aurothiomalate. PCN diminished the toxicity of all the drugs except aniline, *N*-methylaniline, barbital, phenobarbital, sodium aurothiomalate and *N*-carbamoylarsanilic acid; it slightly aggravated succinylcholine poisoning.

Second experiment (Table 2). Depot ACTH, injected daily for 2 or 3 days, markedly reduced zoxazolamine paralysis, although adrenal necroses were seen in a few rats. Even a single injection (24 or 48 hr before zoxazolamine) diminished paralysis. However, depot ACTH was ineffective when given 4 hr before the toxicant.

TABLE 2. EFFECT OF DEPOT ACTH AND PCN ON ZOXAZOLAMINE PARALYSIS

Group	Pretreatment* (days)	Paralysis time† (min)	
		ACTH pretreatment	PCN pretreatment
1	None	174 ± 8	148 ± 24
2	First, second and third	78 ± 9‡	54 ± 10‡
3	Second and third	84 ± 12‡	85 ± 13§
4	First	161 ± 20 NS	188 ± 2 NS
5	Second	104 ± 9‡	179 ± 22 NS
6	Third (− 24 hr)	93 ± 8‡	87 ± 13§
7	Fourth (− 4 hr)	174 ± 20 NS	231 ± 14‖

* Depot ACTH (5 i.u. = 50 μg) was injected s.c., whereas PCN (1 mg) was administered p.o., at the time intervals listed under "Pretreatment." Zoxazolamine (10 mg/100 g body weight) was given, i.p., as described in Materials and Methods (second experiment).

† NS = not significant. The underlined symbol indicates aggravation of paralysis.

‡ $P < 0.005$. § $P < 0.05$. ‖ $P < 0.01$.

Third experiment (Table 2). Two or 3 days of pretreatment with PCN shortened zoxazolamine paralysis, as did a single dose, administered 24 hr before the drug.

Fourth experiment (Fig. 1). This experiment demonstrates the dose-dependent action of ACTH and PCN against zoxazolamine. Paralysis time was markedly diminished by 50 μg ACTH, with the curve stabilizing between 0·1 and 1 mg. On the other hand, while PCN first significantly inhibited zoxazolamine toxicity only at the 0·1- and 1-mg dose levels, the over-all reduction of paralysis was far greater than that elicited by ACTH. PCN offered maximal protection at 5 and 10 mg.

Fifth experiment (Table 3). The influence of SKF 525-A upon conditioning with ACTH and PCN is shown in this experiment. At the 5-mg dose level, this hepatic microsomal enzyme inhibitor did not significantly modify paralysis in itself. However, when given with ACTH, unlike with PCN, it markedly reduced the effect of zoxazolamine as compared to absolute controls and with the group given SKF 525-A

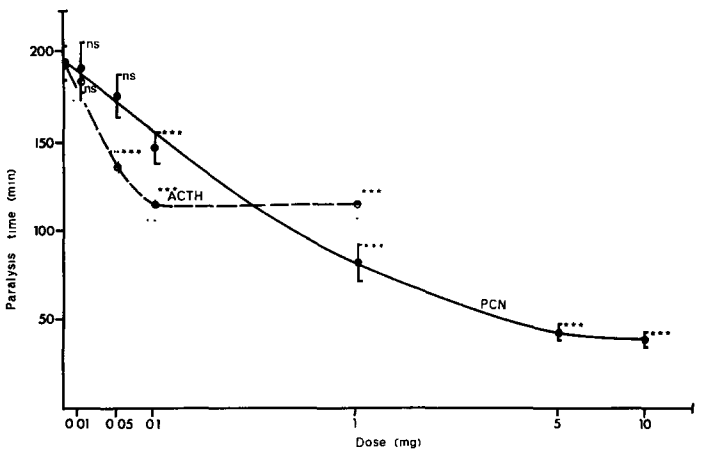

FIG. 1. Dose–response curve showing the effect of ACTH and PCN on zoxazolamine paralysis (mean ± S. E.), expressed on semilogarithmic paper: * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.005$; NS = not significant.

TABLE 3. EFFECT OF DEPOT ACTH, PCN AND SKF 525-A ON ZOXAZOLAMINE PARALYSIS

Group	Pretreatment*	Paralysis time† (min) (Expt. 1)	Paralysis time† (min) (Expt. 2)
1	None	162 ± 11	154 ± 14
2	ACTH	70 ± 25‡	108 ± 8§
3	PCN	82 ± 12‡	91 ± 14‡
		SKF 525-A (5 mg)	SKF 525-A (8 mg)
4	SKF 525-A	203 ± 19 NS	234 ± 18‡
5	SKF 525-A + ACTH	119 ± 17‖(‡)	156 ± 17 NS(‡)
6	SKF 525-A + PCN	236 ± 18‡ (NS)	197 ± 22 NS (NS)

* Depot ACTH (5 i.u. = 50 μg) was injected s.c., whereas PCN (1 mg) was administered p.o., 24 hr before zoxazolamine. SKF 525-A (5 or 8 mg) was given. p.o., 1 hr before the substrate. Consult Materials and Methods (second experiment) for details of treatment with zoxazolamine (10 mg/100 g body weight, i.p.).

† NS = not significant, as compared to group 1 Symbols/abbreviations in parentheses are compared with group 4. The underlined symbol indicates aggravation of paralysis.

‡ $P < 0.005$. § $P < 0.01$. ‖ $P < 0.05$.

alone. At 8 mg, SKF 525-A in itself considerably prolonged paralysis, while ACTH + SKF 525-A significantly shortened it. PCN had no effect in the presence of the enzyme inhibitor. As seen in other investigations (not reported here), it can even aggravate zoxazolamine paralysis.

Sixth experiment (Figs. 2 and 3). In this experiment, the influence of depot ACTH and PCN on the distribution of zoxazolamine is shown. There were no significant differences in the plasma and brain concentrations of zoxazolamine in ACTH-treated and in untreated rats (unrecovered, still paralyzed controls) killed when the pharmacologic response disappeared in the former. However, these drug concentrations were markedly reduced in animals sacrificed when the righting reflex was spontaneously regained (recovered, no longer paralyzed controls). There were highly significant differences between the paralyzed controls and PCN-pretreated rats, and almost no change between the nonparalyzed (recovered) controls and the PCN group.

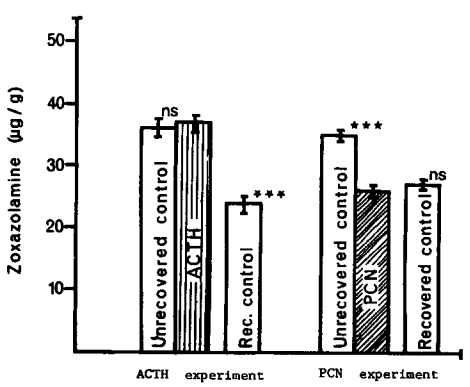

FIG. 2. Effect of ACTH and PCN on zoxazolamine concentrations in rat plasma (mean ± S. E.): * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.005$; NS = not significant. The values of the ACTH and PCN groups are compared with those of the respective recovered and unrecovered controls.

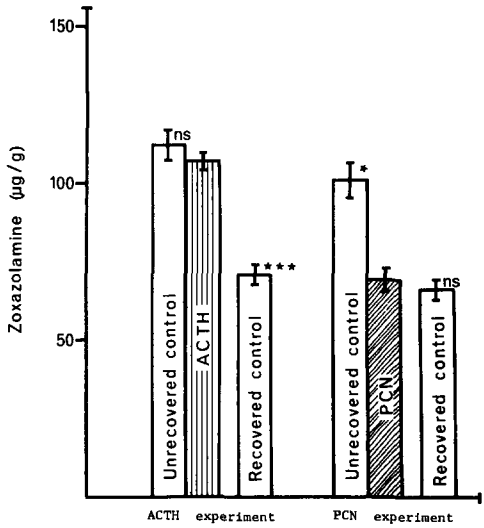

FIG. 3. Effect of ACTH and PCN on zoxazolamine concentrations in the rat brain (mean ± S.E.):* = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.005$; NS = not significant. The values of the ACTH and PCN groups are compared with those of the respective recovered and unrecovered controls.

The zoxazolamine concentrations in the muscles of depot ACTH-treated rats and paralyzed controls were not markedly different; both of these were considerably higher than in the nonparalyzed controls (Fig. 4). On the other hand, the drug levels were significantly lower in rats given PCN when compared with both controls.

DISCUSSION

The results presented here indicate that there is no major difference in the influence of crystalline and depot ACTH upon drug responses. While at least 3–4 days of pretreatment with large doses of crystalline ACTH (5 or 25 i.u. thrice daily) are needed for protection,[2] similar effects can be obtained with a single injection of depot ACTH (5 i.u. or 500 µg/kg), given 24 hr before the toxicant. Additional experiments (not

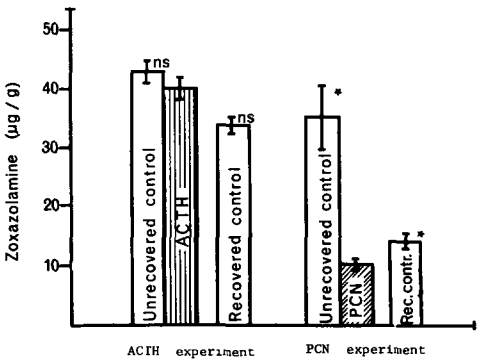

FIG. 4. Effect of ACTH and PCN on zoxazolamine concentrations in the rat muscle (mean ± S. E.): * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.005$; NS = not significant. The values of the ACTH and PCN groups are compared with those of the respective recovered and unrecovered controls.

reported here) have shown that even smaller amounts of depot ACTH (1 i.u.) can significantly reduce zoxazolamine paralysis. Barthe and Desaulles[26] found that the plasma corticosterone concentrations in rats given 300 μg/kg of depot ACTH, s.c., reach a peak between 16 and 24 hr. Thus, the protection offered by ACTH against drug overdosage coincides with maximal corticosterone secretion. As previously reported,[2] we concur with Chatterjee and Harper[27] in that corticosterone and some glucocorticoids are not the only steroids secreted under the influence of ACTH. Other corticoids liberated by the adrenals might play an important role in the effect of the adrenocorticotrophic hormone on drug response, as on interruption of implantation and gestation.[27]

The duration of pretreatment with ACTH or PCN and the frequency of the treatments are of considerable importance. There must be an interval of at least 24 hr between the injection of a single dose of ACTH or PCN and the toxicant. Four days of pretreatment are needed for maximal protection, but almost equally significant prophylaxis can be achieved with a single dose or daily pretreatments. Based on earlier observations,[1,24,28] in most cases, we gave PCN as a pretreatment for 3 days to obtain an optimal effect. On the other hand, we mostly administered a single dose of depot ACTH since, in preliminary experiments, repeated injections occasionally caused adrenal necroses. This effect seems to be restricted to the highly potent depot derivative. Large amounts of crystalline ACTH (50 i.u. daily) are essentially protective against the adrenal apoplexy and mortality produced by acrylonitrile.[29] Our results demonstrate the dose-dependent actions of ACTH and PCN, the latter being the more active of the two, against zoxazolamine paralysis. It appears that ACTH exerts a maximal effect at 1 mg, and PCN at 5 mg.

The actions of the other drugs tested were sometimes differently influenced by ACTH and PCN. For example, the toxicity of nikethamide and dioxathion was diminished solely by PCN, whereas that of aniline, *N*-methylaniline, barbital and phenobarbital was prevented by ACTH alone. Curiously, while ACTH did not modify succinylcholine poisoning, PCN slightly aggravated it. Even when the former offered protection (e.g. against strychnine, ethylmorphine and cyclobarbital), the steroid usually proved to be more potent in this respect.

It appears that prophylaxis by ACTH or PCN is not associated with any particular pharmacologic action of the drugs tested. This is well known in the case of PCN[1,24] but, with ACTH, no major comparative studies have been performed. We had previously assumed that ACTH pretreatment reduced the effects of drugs possessing CNS-depressing properties mostly through the "psychostimulatory" actions of corticoids.[30] The present study suggests that this became less probable, since even the excitation and mortality caused by picrotoxin were markedly reduced and the toxicity of strychnine and *N*-carbamoylarsanilic acid was slightly diminished under the influence of ACTH. It was also believed that ACTH could decrease the actions of drugs that are not metabolized, or transformed very slowly (e.g., barbital and phenobarbital). Now, it appears that mostly the crystalline adrenocorticotrophic hormone protects against these agents. It was even generally thought that ACTH reduces the effects of centrally acting muscle relaxants. Such a generalization is no longer tenable, because mephenesin toxicity is not modified by this hormone. Nephropathies caused by allopurinol, sodium aurothiomalate or *N*-carbamoylarsanilic acid seem to be another common target for protection by ACTH. Moreover, some

glucocorticoids, like ACTH, reduce the effects of overdosage with the uricosuric agents, zoxazolamine and allopurinol.[12,31] It remains to be determined whether certain gluco- or mineralo-corticoids, and glucomineralocorticoids, have an effect similar to that of ACTH upon drug responses.

ACTH and PCN most probably act through different mechanisms. PCN pretreatment is known to induce synthesis of hepatic microsomal drug-metabolizing enzymes,[14] and to increase cytochrome P-450.[32–34] This seems to agree with our present findings in that the effects of PCN are completely abolished by prior administration of SKF 525-A. On the other hand, ACTH shortens zoxazolamine paralysis even in the presence of the enzyme inhibitor. Both ACTH and PCN alter drug responses in hypophysectomized rats[18] (Szabo *et al.*, manuscript in preparation), but only the action of the former depends upon the adrenals.[2,35] The reduction of hexobarbital anesthesia by acute stress is blocked by SKF 525–A,[3] which shows the importance of timing and the difference in the effects of stress and pure ACTH upon drug responses. Unlike ACTH, PCN (given 4 hr before zoxazolamine) prolonged paralysis time. This might be explained by competition between the substrate (zoxazolamine) and the conditioner (PCN) for drug-metabolizing enzymes of hepatic microsomes. Since prolongation of the effect of zoxazolamine did not occur after ACTH treatment, it would seem that only PCN acts through microsomal enzyme induction.

The drug distribution studies clearly demonstrate the respective syntoxic and catatoxic actions of the two conditioners. The plasma levels of zoxazolamine were very similar in the depot ACTH-pretreated rats and the paralyzed controls killed when the righting reflex was regained by the former. On the other hand, the second control group (sacrificed at spontaneous recovery) had significantly lower plasma concentrations of the drug. In other words, the ACTH-treated rats tolerated high amounts of zoxazolamine without showing any marked clinical signs of poisoning (syntoxic action). This is in agreement with our earlier findings on the effects of crystalline ACTH.[2,12] In contrast, PCN significantly lowered the plasma level of zoxazolamine, as compared with controls that were killed when the steroid-pretreated rats regained the righting reflex; the drug concentration in the blood of the PCN group was almost similar to that found in control samples taken when the animals recovered spontaneously. Thus, PCN acted catatoxically, since the reduction of paralysis *in vivo* was associated with decreased plasma levels of zoxazolamine. Our results are in agreement with those of Buchel,[36] who found that pretreatment with chlordane, a microsomal enzyme inducer, markedly diminished the plasma and brain concentrations of zoxazolamine.

After ACTH or PCN pretreatment, the changes in the levels of zoxazolamine in brain and muscle were similar to those in plasma. This demonstrates that ACTH offers protection despite high amounts of the drug in blood, muscle and brain (syntoxic action). Our findings are in agreement with the observations of Kato and Gillette[8] in that ACTH does not markedly alter drug metabolism in the liver. Prophylaxis is probably due to interactions at receptor sites, diminished receptor sensitivity or altered production of endogenous protective substances. However, as more detailed studies[37] on drug distribution have revealed, ACTH does not seem to influence the permeability of the blood–brain barrier. The beneficial effect of PCN appears to be more clear-cut. There were signs of decreased drug activity because

the concentrations of zoxazolamine in plasma, muscle and brain were reduced (catatoxic action). A similar relationship was established recently between pretreatment with ACTH or PCN and methyprylon plasma levels.[38] This might have been due to hepatic microsomal drug-metabolizing enzyme induction,[14,34] increased substrate excretion, or some other mechanism(s). Recently, Fuller *et al.*[39] noted that cold (4° for 4 days) decreased zoxazolamine concentrations in plasma and brain, and increased hexobarbital and meprobamate metabolism in hepatic microsomes. These data again suggest that it is hazardous to identify the effects of stress with that of ACTH in studies on drug response and metabolism. It is clear, however, that ACTH and PCN exert different actions on the distribution of zoxazolamine. It can also be assumed that they modify drug activity in two or more relatively independent ways. More recent preliminary data[40,41] indicate that only PCN, and not ACTH, enhances the metabolism of zoxazolamine by the 9000 *g* liver supernatant fraction. These results, with those on the biliary and urinary excretion of zoxazolamine, will be the subject of another report.

Acknowledgements—This work was supported in part by the Medical Research Council of Canada (Block Term Grant MT-1829), the Ministère des Affaires Sociales, Quebec, and Succession J. A. DeSève. The authors thank Mr. O. Da Silva for helping with the preparation of this manuscript. The technical assistance of D Daboval. M Girard and J Lombarbo is gratefully acknowledged The authors also thank the various companies listed in the section on Materials and Methods for their kind donations

REFERENCES

1. H. SELYE, *Hormones and Resistance*, p. 1140. Springer, New York (1971).
2. S. SZABO, P. KOUROUNAKIS and H. SELYE, *Can. J. Physiol. Pharmac.* **51**, 169 (1973).
3. B. D. RUPE, W F. BOUSQUET and T. S. MIYA, *Science, N.Y.* **141**, 1186 (1963).
4. W. F. BOUSQUET, B. D. RUPE and T. S. MIYA, *J. Pharmac. exp. Ther.* **147**, 376 (1965).
5. C. W. DRIEVER, W. F. BOUSQUET and T S. MIYA, *Int. J. Neuropharmac.* **5**, 199 (1966).
6. C. A. WINTER and L. FLATAKER, *J. Pharmac exp. Ther* **105**, 358 (1952).
7. A. KOMIYA and K. SHIBATA, *J. Pharmac. exp. Ther.* **117**, 98 (1956).
8 R. KATO and J. R. GILLETTE, *J. Pharmac. exp. Ther.* **150**, 285 (1965).
9. R. E. STITZEL and J S. MCCARTHY, *Biochem. Pharmac* **20**, 2085 (1971).
10. L. BUCHEL, M. PRIOUS-GUYONNEAU, L. LIBLAU AND M MURAWSKY, *Therapie* **27**, 609 (1972).
11. S SZABO, P. KOUROUNAKIS, J. WERRINGLOER and H. SELYE, *Ann. ACFAS* **38**, 57 (1971).
12. P. KOUROUNAKIS, S. SZABO, J. WERRINGLOER and H. SELYE, *J. pharm. Sci.* **62**, 690 (1973).
13. K. KOVACS, J. A BLASCHECK and C. GARDELL, *Z. ges exp. Med.* **152**, 104 (1970).
14. B SOLYMOSS, J. WERRINGLOER and S. TOTH, *Steroids* **17**, 427 (1971).
15. B. D. GARG, K. KOVACS, J. A. BLASCHECK and H. SELYE, *Folia endocr.* **23**, 357 (1970).
16. S. SZABO, H. SELYE, I. JAPUNDZIC, J. MIMIC-OKA and M. JAPUNDZIC, *Fedn Proc.* **31**, 271 (1972).
17. I. JAPUNDZIC, J. MIMIC-OKA, M. JAPUNDZIC and S. SZABO, *Eur. J. Biochem.* **28**, 475 (1972).
18. S. SZABO, K. KOVACS, B. D GARG, J. D. KHANDEKAR and H. SELYE, *Proc. Can Fedn. biol. Soc.* **14**, 135 (1971).
19. B. D. GARG, S. SZABO, J. D. KHANDEKAR and K KOVACS, *Naunyn–Schmiedebergs Arch. exp. Path. Pharmak* **269**, 7 (1971).
20. S. SZABO, K. KOVACS, B. D. GARG, B. TUCHWEBER and G. LAZAR, *Hormone Metab. Res.* **5**, 109 (1973).
21. H. SELYE, *Revue can. Biol.* **16**, 1 (1957)
22 D. J. FINNEY, *Biometrika* **35**, 145 (1948).
23. S. SIEGEL, *Nonparametric Statistics for the Behavioral Sciences*, p. 270. McGraw-Hill, New York (1956).
24. H. SELYE, *J. pharm. Sci.* **60**, 1 (1971).
25. J. J. BURNS, T F. YU, L. BERGER and A B. GUTMAN, *Am. J Med.* **25**, 401 (1958).
26. P. L. BARTHE and P. A. DESAULLES, *Hormones* **2**, 327 (1971).
27. A. CHATTERJEE and M. J. K. HARPER, *Endocrinology* **87**, 966 (1970).
28. A. H. CONNEY, *Pharmac. Rev.* **19**, 317 (1967).
29. S. SZABO and H. SELYE, *Fedn Proc.* **30**, 307 (1971).
30. D. M. WOODBURY, *Pharmac Rev.* **10**, 275 (1958).
31. H. SELYE, *Acta endocr.* (Kbh.) **69**, 347 (1972).

32. J. WERRINGLOER, *Physiologist* **14,** 252 (1971).
33. J. WERRINGLOER, *Fedn Proc.* **31,** 641 (1972).
34. A. Y. H. LU, A. SOMOGYI, S. WEST, R. KUNTZMAN and A. H. CONNEY, *Archs Biochem. Biophys.* **152,** 457 (1972).
35. B. D. GARG, S. SZABO, B. TUCHWEBER and K. KOVACS, *Anat. Rec.* **175,** 326 (1973).
36. L. BUCHEL, *Archs Sci. physiol.* **25,** 19 (1971).
37. P. KOUROUNAKIS, S. SZABO and H. SELYE, *J. pharm. Sci.* **62,** 1946 (1973).
38. P. KOUROUNAKIS, S. SZABO and H. SELYE, *J. Pharm. Pharmac.* **25,** 670 (1973).
39. G. C. FULLER, W. F. BOUSQUET and T. S. MIYA, *Toxic. appl. Pharmac.* **23,** 10 (1972).
40. S. SZABO, P. KOUROUNAKIS and H. SELYE, *Proc. Can. Fedn. biol. Soc.* **16,** 69 (1973).
41. P. KOUROUNAKIS, S. SZABO and H. SELYE, *Proc. Thirty-third Int. Cong. Pharm. Sci.*, Stockholm, September 3–7, 1973.