

Influence of Steroids and Stress on Toxicity and Disposition of Tetraethylammonium Bromide

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Abstract □ In female rats, pretreatment with dexamethasone acetate or triamcinolone reduced the toxicity and plasma concentrations of tetraethylammonium bromide while increasing its level in urine. Pretreatment with corticosterone acetate or pregnenolone-16 α -carbonitrile shared none of these effects. Although starvation or restraint neither diminished the tetraethylammonium bromide concentrations in plasma nor accelerated its urinary excretion, its toxicity was diminished by the stress induced with spinal cord lesions, heat, cold, hydrocortisone, or reserpine as well as starvation or restraint. The protection offered against the toxicant by stress and by the potent glucocorticoids seemed to be mediated, at least partly, *via* different mechanisms. Stress-induced resistance to tetraethylammonium bromide could not be attributed to elevated plasma corticosterone levels, whereas glucocorticoid-induced resistance could be partially ascribed to increased urinary excretion of the toxicant.

Keyphrases □ Tetraethylammonium bromide—toxicity and urinary excretion, effect of stress and pretreatment with steroids, rats □ Toxicity—tetraethylammonium bromide, effect of stress and pretreatment with steroids, rats □ Excretion, urinary—tetraethylammonium bromide, effect of stress and pretreatment with steroids, rats □ Stress—effect on toxicity and urinary excretion of tetraethylammonium bromide, rats □ Steroids—pretreatment, effect on toxicity and urinary excretion of tetraethylammonium bromide, rats □ Ganglionic blocking agents—tetraethylammonium bromide, toxicity and urinary excretion, effect of stress and pretreatment with steroids, rats

When systemic adaptation is required, the body can respond *via* the nervous system, the reticuloendothelial-immunological-phagocytic system, and/or the hormonal mechanisms that significantly affect the organism's adaptive reactions, which may vary from nonspecific to highly specific (1, 2). Steroids, which are decisive factors in regulating stress, largely because of their influence upon enzyme-controlled responses (3, 4), can increase the body's resistance to many toxic agents (3, 5, 6). This effect is mediated mainly *via* heightened tissue tolerance for drugs (syntoxic mechanism) and/or enhanced drug disposition (catatoxic mechanism).

Well-documented investigations (7, 8) described the pharmacological and toxicological effects as well as the mechanisms of action of quaternary ammonium gan-

glionic blocking agents, *e.g.*, tetraethylammonium bromide (I). Earlier (9), stress and some glucocorticoids were shown to reduce the toxicity of some ganglionic blocking agents in rats. Pregnenolone-16 α -carbonitrile (II), a potent microsomal enzyme inducer, influences the metabolism and, consequently, the toxicity of several drugs (6). However, sensitization or desensitization of target tissues by various agents (*e.g.*, hormones and stress) is not necessarily dependent upon drug disposition and may alter resistance to drugs. Therefore, it appeared of interest to study the influence of these dissimilar agents on the action of I and to correlate the findings with the *in vivo* protection offered by them.

EXPERIMENTAL

Female Charles River CD rats¹, averaging 120 g and allowed food² and tap water *ad libitum*, were used.

First Experiment—The animals were divided into five groups and given either water or 20 μ moles of triamcinolone³, dexamethasone⁴, II⁵, or corticosterone⁶ orally twice daily for 3 days. Compound I (10 mg/100 g of body weight) was administered intraperitoneally to all animals on the 4th day, 18 hr after the last pretreatment. The effect of the steroids on I⁷ toxicity was gauged by dyskinesia (estimated 30 min after I injection) and the mortality rate (measured after 24 hr) (Table I).

Dyskinesia was expressed in terms of an arbitrary scale in which 0 = no change, 1 = just detectable drowsiness or tremor, 2 = severe drowsiness and tremor (the animal did not respond to pinching), and 3 = intense convulsions and/or loss of the righting reflex (3). However, for statistical evaluation, only two grades were recognized: minor and dubious degrees of dyskinesia (0 and 1 in this scale) were rated as negative, and all others were considered positive. These data and the mortality rates were then arranged in a 2 \times 2 contingency table, and their statistical significance was determined by the Exact Probability Test of Fisher and Yates (10, 11).

Second Experiment—The rats were divided into eight groups, of which one served as the control. The others were exposed to: (a) fasting (Group 2), *i.e.*, food and water deprivation for 48 hr; (b) spinal cord transection (Group 3) at the level of the seventh cervical vertebra, using thermocautery under ether anesthesia; (c) restraint (Group 4) on a board in a supine position for 47 hr; (d) cold (Group 5) at 12 $^{\circ}$; (e) heat (Group 6), *i.e.*, immersion in hot water of 55 $^{\circ}$ for 1 min under light ether anesthesia; (f) hydrocortisone (Group 7), 10 mg po twice daily for 2 days; or (g) reserpine (Group 8), 50 μ g/day sc for 2 days.

In addition, all groups except 1 were deprived of food and water for 48 hr, and all groups received 10 mg sc of I. Dyskinesia and mortality were estimated (Table II) as in the first experiment (Table I).

Third Experiment—The animals were grouped, stressed as in the second experiment, and killed 48 hr after application of the stressors. The thymus, adrenals, and spleen were then excised and weighed. The incidence of gastric ulcers was recorded, and blood was taken by decapitation for the determination of plasma corticosterone (Table II).

Fourth Experiment—Five groups of rats were pretreated as in

Table I—Effect of Steroids on Tetraethylammonium Bromide Toxicity in Rats

Pretreatment	Toxicity	
	Dyskinesia (Positive/Total)	Mortality (Dead/Total)
Water plus polysorbate	18/18	4/18
Triamcinolone	3/18 ^a	0/18 ^c
Dexamethasone acetate	9/18 ^a	0/18 ^c
Pregnenolone-16 α -carbonitrile	13/18 ^b	1/18 ^c
Corticosterone acetate	12/12 ^c	0/12 ^c

^a $p < 0.005$. ^b $p < 0.05$. ^c $p > 0.05$ (not significant).

¹ Canadian Breeding Farms & Laboratories Ltd., St. Constant, Quebec, Canada.

² Purina Lab Chow.

³ E. R. Squibb & Sons.

⁴ Schering Corp. Ltd.

⁵ The Upjohn Co.

⁶ Merck Sharp & Dohme.

⁷ Eastman Organic Chemicals.

Table II—Effect of Various Stressors on Thymus, Adrenal, and Spleen Weight, Gastric Ulcer Formation, Plasma Corticosterone Concentrations, and Tetraethylammonium Bromide Toxicity in Rats

Stressor	Thymus		Adrenals		Spleen		Plasma Corticosterone		Toxicity	
	Weight, mg/100	Decrease, %	Weight, mg	Increase, %	Weight, mg	Decrease, %	mg %	Increase, %	Dyskinesia (Positive/Total)	Mortality (Dead/Total)
Control	430 ± 21	—	14 ± 0.4	—	420 ± 20	—	7.8 ± 0.4	—	20/20	19/20
Fasting	310 ± 12 ^a	28	19 ± 1.0 ^a	36	300 ± 20 ^a	29	34.8 ± 6.5 ^a	446	4/20 ^a	0/20 ^a
Restraint	250 ± 24 ^a	42	19 ± 0.5 ^b	36	160 ± 10 ^a	38	48.8 ± 6.7 ^a	530	15/20 ^a	9/20 ^c
Spinal cord lesions	250 ± 20 ^a	42	22 ± 1.0 ^a	57	240 ± 10 ^c	43	69.4 ± 12.3 ^a	790	9/18 ^a	4/18 ^d
Heat	230 ± 19 ^a	47	25 ± 1.0 ^a	79	240 ± 20 ^d	43	46.2 ± 4.0 ^a	592	3/19 ^a	0/19 ^a
Cold	260 ± 13 ^a	41	26 ± 1.0 ^a	86	240 ± 20 ^d	43	58.8 ± 4.2 ^a	620	5/16 ^a	3/16 ^a
Hydrocortisone	140 ± 8 ^a	67	15 ± 0.5 ^b	7	220 ± 20 ^a	48	35.0 ± 7.3 ^d	449	2/20 ^a	2/20 ^a
Reserpine	230 ± 13 ^a	47	24 ± 0.5 ^a	71	280 ± 10 ^d	33	27.4 ± 4.4 ^a	250	9/20 ^a	2/20 ^a

^a*p* < 0.005. ^b*p* > 0.05 (not significant). ^c*p* < 0.05. ^d*p* < 0.01.

the first experiment. On the 4th day, 8 mg ip of I was administered; blood was collected 15 min later to measure the plasma I level (Table III).

Fifth Experiment—Three groups were used: one for control purposes, another for fasting, and the third for fasting plus restraint, as described in the second experiment. Compound I (8 mg ip) was given, and blood was taken 15 min later to measure the plasma I level (Table III).

Sixth Experiment—The rats were pretreated as in the fourth experiment and then given 6 mg ip of I. Urine was collected during the first 2 hr for I determination (12).

Seventh Experiment—The conditions of the fifth experiment were repeated. Compound I (6 mg ip) was administered, followed by urine collection for 2 hr to measure urinary I levels (12).

RESULTS

The stressor agents applied produced severe stress in all animals, as indicated by increased adrenal weight, decreased thymus and spleen weights, gastric ulcers, and high plasma corticosterone concentrations. These changes were statistically significant when compared with control values (Table II).

The dyskinesia and mortality elicited by I were markedly diminished in the stressed and triamcinolone- or dexamethasone-treated rats, while those given corticosterone or II showed only very moderate amelioration of I intoxication (Tables I and II). The plasma concentrations of I were significantly lower in the triamcinolone- and dexamethasone-treated animals and were relatively higher in the restrained rats; fasted and corticosterone- or II-treated animals exhibited no important alterations in this respect (Table III).

Urine flow was significantly influenced only in rats stressed by fasting or restraint. These two stressors as well as triamcinolone and dexamethasone, unlike II, caused significant body weight reductions. However, whereas II, fasting, and restraint did not increase the percentage of the total I dose excreted, treatment with the two potent glucocorticoids, triamcinolone and dexamethasone, did.

DISCUSSION

The nonspecific response of the body to any demand (stress) made by several agents is estimated by a number of parameters (thymus, spleen, adrenal, and body weight; gastric ulcers; and plasma corticosterone concentration), which can be employed with confidence (1, 4). With the use of these criteria, all applied stressors provoked a severe nonspecific reaction (Table II). The resistance of these animals to I intoxication was increased considerably, as judged by the diminution of dyskinesia and the mortality rate (Table II).

Like most quaternary ammonium salts, I is readily soluble in water. It is not metabolized in the body, and more than 95% of an injected dose is excreted through the kidney *via* glomerular filtration as well as through the proximal tubuli *via* an active transport mechanism (13, 14). Evidently, protection against I is not due to stimulation of its metabolism by stress *via* a regulatory function of the endocrine system, namely rapid induction of hepatic drug-metabolizing enzymes (2). This assumption was confirmed by a lack of any protective activity of II, a potent inducer of drug-metabolizing enzymes that has no other known pharmacological or hormonal properties (6, 15, 16). However, pretreatment with glucocorticoids offered significant protection against I intoxication (Table I). These findings could explain the prophylaxis provided by stress, characterized by high levels of circulating corticoids in the blood. However, triamcinolone and dexamethasone are more potent glucocorticoids than the natural corticosterone liberated during stress in rats (5). The protection offered by the glucocorticoids was well correlated with reduced I concentrations in plasma, whereas there was no such change after II treatment. This result could be partly attributed to a marked increase in I excretion (percentage of eliminated I being significantly greater than in the corresponding controls).

The situation was different in restrained or fasted rats, which exhibited no significant reduction of I concentrations in plasma. On the contrary, the plasma I concentrations were increased significantly in restrained animals. In neither case (restraint or fasting) was there a great alteration in the excretion pattern, although urine flow was diminished, a fact that could be attributed to the "total fasting" (food and water) of the rats. Steroid administration had no influence on

Table III—Effect of Steroid or Stress Conditioning on Tetraethylammonium Bromide Concentrations in Plasma, Its Urinary Excretion, and Urine Flow^a

Pretreatment	Tetraethylammonium Bromide Concentrations in Plasma		Urinary Excretion of Tetraethylammonium Bromide				
	$\mu\text{g/ml}$	Change, %	Urine Flow		Total Excretion		
			$\mu\text{l/min}$	Change, %	% of Administered Dose	Change, %	
Triamcinolone	(11) 24.7 \pm 1.6 ^b (8) [45.1 \pm 2.3]	-45	(13) 8.5 \pm 1.1 ^c (10) [11.2 \pm 1.4]	-24	86.6 \pm 2.2 ^d [74.4 \pm 3.5]		16
Dexamethasone acetate	(9) 19.4 \pm 0.7 ^b (11) [45.1 \pm 2.3]	-57	(10) 7.3 \pm 1.2 ^c (10) [6.2 \pm 0.8]	17	85.8 \pm 2.6 ^e [77.2 \pm 2.0]		11
Pregnenolone-16 α -carbonitrile	(13) 39.8 \pm 2.8 ^c (11) [45.1 \pm 2.3]	-12	(9) 11.8 \pm 1.1 ^c (10) [9.5 \pm 1.1]	23	62.1 \pm 7.2 ^c [71.5 \pm 7.6]		13
Fasting	(10) 39.0 \pm 2.2 ^c (7) [46.7 \pm 3.8]	-17	(10) 6.2 \pm 0.9 ^d (3) [10.6 \pm 1.0]	-42	89.0 \pm 2.0 ^c [87.0 \pm 5.0]		2
Fasting plus restraint	(10) 96.6 \pm 13.6 ^b (7) [46.7 \pm 3.8]	207	(6) 3.3 \pm 0.3 ^b (3) [10.6 \pm 1.0]	-69	82.0 \pm 3.0 ^c [87.0 \pm 5.0]		-6
Corticosterone acetate	(11) 45.3 \pm 3.6 ^c (9) [44.4 \pm 3.9]	2	—	—	—		—

^a Figures in parentheses indicate number of animals; figures in square brackets indicate values of the controls. ^b $p < 0.005$. ^c $p > 0.05$ (not significant). ^d $p < 0.01$. ^e $p < 0.05$.

urine flow; therefore, it appears that the protection offered by the glucocorticoids against I is partly due to increased urinary excretion involving an active transport mechanism (17). Glucocorticoid treatment also may help provide readily available sources of energy (carbohydrates and free fatty acids) for I clearance or other adaptive work (2, 6, 16); stressed animals, depending on their state of stress, are handicapped in this respect.

Exhaustion may have been responsible for the high I concentrations in the plasma of restrained rats. However, they became less sensitive to the toxicant, probably through changes *via* unknown mechanisms at the molecular level. In stressful situations, the body's response to drugs apparently is influenced by a constellation of factors including the duration of stress, the time of sacrifice after exposure to stress, the superimposed specific actions of the stressor, the stage of the general adaptation syndrome that the animal has reached at the time of sacrifice for the *in vitro* tests, and the time of administration of the toxicant for the *in vivo* study (2). Thus, although both glucocorticoids and stress increase the organism's resistance to I, this protection probably is mediated, at least partly, *via* different pathways. Exogenous corticosterone did not significantly heighten resistance to I and did not alter the drug concentrations in plasma, which are further indications that the protection offered by stress is not mediated by corticoids produced by the adrenals during stress.

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