Archives of Environmental Health: An International Journal

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/vzeh20

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Hans Selye MD, PhD, DSc [a]

[a] Montreal

Published online: 03 May 2013.

To cite this article: Hans Selye MD, PhD, DSc (1970) Resistance to Various Pesticide, Archives of Environmental Health: An International Journal, 21:6, 706-710, DOI: 10.1080/00039896.1970.10667323

To link to this article: http://dx.doi.org/10.1080/00039896.1970.10667323

Original Articles

Resistance to Various Pesticide

Hans Selye, MD, PhD, DSc, Montreal

In the rat the toxic actions of various pesticides such as ethion, dioxathion (Navadel), EPN, azinphosmethyl, and parathion are markedly, and often totally, inhibited by such typical catatoxic steroids as ethylestrenol, SC-11927, spironolactone, and norbolethone. To a lesser extent this is also true of oxandrolone, prednisolone, and progesterone, which also proved to be somewhat less potent in detoxicating other substrates. Triamcinolone, desoxycorticosterone, hydroxydione sodium succinate, estradiol, and thyroxine, previously shown to be essentially devoid of any catatoxic activity, also failed to offer consistent protection against the pesticides just mentioned. DDT, physostigmine sulfate and pralidoxime chloride, tested for comparative purposes, proved to be much more resistant against detoxication by even the most powerful catatoxic steroids. Many pesticides are highly amenable to detoxication by potent catatoxic steroids. This detoxicating effect is independent of the classic hormonal properties of the steroids examined.

THAT resistance to various pesticides may be greatly altered by the induction of hepatic microsomal drug-metabolizing enzymes is so well known, that the problem need not be discussed here in detail. Recently, DuBois, and Kinoshita[1] examined the effect of phenobarbital pretreatment upon the toxicity of 15 cholinergic organic phosphate pesticides in rats and mice. They found that this enzyme-inducing barbiturate either decreased or had no effect upon the toxicity of most of these compounds in either species. There was but one exception, octamethyl pyrophosphoramide (OMPA), whose toxicity was actually increased, presumably by the enhanced production of a metabolite even more toxic than OMPA itself.

Since certain hormones are likewise known to affect the toxicity of pesticides, it was decided to reexamine this problem in the light of recent observations on the induction of resistance by "catatoxic steroids."[2]

It has long been known that adrenalectomy decreases resistance to most toxic agents whereas substitution therapy with corticoids restores it to normal. This protection is effected mainly by combatting stress to which the organism is particularly sensitive in the absence of the adrenal cortex. Yet overdosage with corticoids is singularly ineffective in raising the nonspecific resistance of intact animals above normal, presumably because a near optimal corticoid supply is assured by the physiologic activity of the adrenal cortex.[3] Only against inflammatory irritants and a few other agents, such as bacterial endotoxins[3,4] is it possible to raise resistance far above normal by treatment with glucocorticoids.

However, several reports from this laboratory have shown that certain steroids (not necessarily endowed with corticoid potency) can protect the intact rat against various

Submitted for publication March 23, 1970; accepted April 15.

From the Institut de Médecine et de Chirurgie expérimentales, Université de Montréal, Montreal.

Reprint requests to Professor and Director, Institut de Médecine et de Chirurgie expérimentales, Université de Montréal, PO Box 6128, Montreal (Dr. Selye).

nduced by Catatoxic Steroids

types of severe intoxications. For example, these catatoxic steroids (from the Greek word *kata* which means down, against)[2] can induce resistance against steroid anesthesia,[5] picrotoxin[6] or pentylenetetrazol convulsions,[7] the calcinosis elicited by vitamin-D compounds,[8-10] digitoxin[11] or colchicine[12] poisoning, the hypnotic action of pentobarbital and methyprylon,[13] the adrenal necrosis produced by 7,12-dimethylbenz(a) anthracene,[14] and the perforating jejunal ulcers with peritonitis elicited in the rat by indomethacin overdosage.[15] Probably many, if not all, of these protective effects are due to the induction by catatoxic steroids of hepatic microsomal drug-metabolizing enzymes.[14,15]

Materials and Methods

More than 1,000 female Sprague Dawley rats, with an initial body weight of 100 gm (range 90 to 110 gm), were maintained exclusively on laboratory chow (Purina) and tap water, divided into 104 groups of 9 to 15 rats (8 series of 13 groups each) and treated as outlined in Table 1. To obtain the best prophylactic effect it is important to allow a few days of pretreatment with catatoxic steroids; hence, all animals received the toxic compounds once on the fourth day after initiation of steroid treatment, except for DDT, parathion and pralidoxime chloride of which a second dose was administered on the fifth day.

In view of the anticholinergic properties of some of the pesticides tested, physostigmine sulfate (a parasympathomimetic anticholinesterase agent widely used in pharmacology) and pralidoxime chloride (a reactivator of cholinesterase) were also included in our series. The dosages (per 100 gm of body weight) of the toxic agents used were:

Ethion [Ethyl methylene phosphorodithioate]; 0.5 ml of a 1.2% solution in corn oil by stomach tube once.

Dioxathion (Navadel) [2,3-*p*-Dioxanedithiol S,S-bis(O,O-diethyl phosphorodithioate]; 4 mg in 1 ml corn oil by stomach tube once.

EPN [Phenylphosphonothioic acid O-ethyl O-p-nitrophenyl ester]; 0.7 mg in 0.2 ml DMSO administered intraperitoneally once.

Azinophosmethyl [*O,O*-dimethyl-*S*-(4-oxobenzotriazino-3-methyl) phosphorodithioate; 1 mg in 0.2 ml propylene glycol administered subcutaneously once.

Parathion [O,O-diethyl O-nitrophenyl phosphorothioate]; 1 mg in 0.5 ml DMSO administered intraperitoneally once daily.

DDT [1,1,1-Trichloro-2,2-bis(*p*-chlorophenyl)ethane]; 25 mg in 1 ml corn oil by stomach tube once daily.

Physostigmine sulfate; 1 mg in 1 ml water by mouth once.

Pralidoxime chloride [2-Formyl-1-methylpyridinium chloride oxime]; 16 mg in 0.2 ml water administered subcutaneously once daily.

The following steroids were tested for possible protective effects: Ethylestrenol, SC-11927 [potassium 3-(3-oxo-9α-fluoro-11β,17β-dihydroxy-4-androstene-17α-yl)], spironolactone, norbolethone, oxandrolone, prednisolone acetate, progesterone, triamcinolone, desoxycorticosterone acetate, hydroxydione sodium succinate, and estradiol.

These steroids were administered by stomach tube at the dose of 10 mg in 1 ml of water twice daily; only in the case of triamcinolone and estradiol did we have to reduce the dose to 1 mg per day in order to avoid extreme loss of body weight. In each series one group of controls received 1 ml of water twice daily by stomach tube.

Thyroxine is known to influence resistance to various drugs and often antagonizes the beneficial effect of catatoxic steroids. Hence, in each

Effect of Various Steroids and of Thyroxine on

Toxicants	Ethion		Dioxathion		EPN		Azinphosmethyl	
	Prostration (4 hr)	Mortality (24 hr)	Prostration (5 hr)	Mortality (24 hr)	Prostration (1 hr)	Mortality (24 hr)	Prostration (2 hr)	Mortality (24 hr)
None	10/10	7/10	3/10	9/10	14/15	9/15	8/10	0/10
Ethylestrenol	0/9 V	0/9 V	0/10 N	0/10 V	0/15 V	0/15 V	0/10 V	0/10 N
SC-11927	0/10 V	0/10 V	0/10 N	0/10 V	0/15 V	0/15 V	0/10 V	0/10 N
Spironolactone	0/10 V	0/10 V	0/10 N	0/10 V	1/15 V	0/15 V	0/10 V	0/10 N
Norbolethone	0/10 V	0/10 V	0/10 N	0/10 V	0/15 V	0/15 V	0/10 V	0/10 N
Oxandrolone	1/9 V	0/9 V	0/10 N	0/10 V	0/15 V	0/15 V	0/10 V	0/10 N
Prednisolone	0/10 V	1/10 H	0/9 N	1/9 V	8/15 S	6/15 N	3/10 S	0/10 N
Progesterone	3/10 V	0/10 V	5/10 N	4/10 S	9/15 S	4/15 N	8/10 N	0/10 N
Triamcinolone	10/10 N	10/10 N	10/10 **V**	10/10 N	12/15 N	7/15 N	10/10 N	1/10 N
DOC	9/10 N	7/10 N	4/9 N	1/9 V	12/15 N	1/15 V	8/10 N	0/10 N
Hydroxydione sodium succinate	7/10 N	6/10 N	2/5 N	2/5 N	4/15 V	2/15 S	4/5 N	0/5 N
Estradiol	4/10 H	6/10 N	5/15 N	4/15 V	8/15 S	0/15 V	5/10 N	0/10 N
Thyroxine	10/10 N	10/10 N	10/10 **V**	10/10 N	15/15 N	15/15 **H**	10/10 N	5/10 **S**

* In each case the hours enclosed in parentheses refer to the time elapsed between the administration of the toxic substance and the reading of prostration and mortality as indicated in the text. N indicates not significant; S, significant ($P<0.05$); H, highly significant ($P<0.01$); V, very highly significant ($P<0.005$). Letters in lightface type indicate the degree of significance of the apparent increase in resistance as compared to the corresponding untreated controls; letters in boldface type refer to apparent decreases in resistance. Numbers left of virgule indicate pretreatment resistance values, and those to the right, resistance treatment values.

series an additional group received the sodium salt of this hormone (British Drug Houses) at the dose of 0.2 mg in 0.2 ml of water administered subcutaneously once daily. Administration of thyroxine, as that of all steroids, began four days prior to treatment with the toxic compounds and continued until the termination of the experiments.

The Table lists the mortality rates (dead per total number of animals per group) at the end of the experiment and the severity of prostration (positive per total number of animals per group) at the height of the immediate drug effect (the exact time is indicated in the Table). Prostration was guaged in terms of an arbitrary scale in which 0 indicates no lesion; 1, just detectable; 2, moderate; and 3, most pronounced clinical evidence of depression and stupor as described elsewhere.[12] However, for statistical evaluation we recognized only two grades: minor and sometimes dubious degrees of prostration (between 0 and 1 in our scale) were rated as negative, all others as positive. These data as well as the mortality rates were then arranged in a 2 × 2 contingency table and their statistical significance computed by the "Exact Probability Test" of Fisher and Yates.[16,17]

Results

Most of the findings summarized in the Table are self-explanatory and require little comment. It is clear that the typical catatoxic steroids (those that were previously shown to be most effective in increasing resistance against various other substrates), namely, ethylestrenol, SC-11927, spironolactone, and norbolethone, were also most potent in preventing prostration and mortality, or both, following intoxication with ethion, dioxathion, EPN, azinphosmethyl, and parathion. As shown by earlier work, oxandrolone, prednisolone, and progesterone also raise resistance against certain substrates, but to a lesser extent. Accordingly, in the present series these last mentioned three steroids proved to be somewhat less effective in combatting the toxicity of several of the pesticides just mentioned. Finally, with very few exceptions, triamcinolone, desoxycorticosterone (DOC), hydroxydione sodium succinate, estradiol, and thyroxine, which have shown no catatoxic activity in earlier tests, were also essentially inactive against the toxic substances of the present series. Triamcinolone actually aggravated the toxicity of dioxathion, estradiol that of parathion, and thyroxine that of dioxathion, EPN, parathion, and physostigmine sulfate. The apparent increase in resistance against dioxathion and EPN following DOC or estradiol treatment, as well as the diminished toxicity of EPN following administration of hydroxydione sodium succinate, may be due to specific drug antagonisms or other incidental circumstances, but it would be premature to speculate upon their mechanisms.

Resistance to Pesticides, Physostigmine, and Pralidoxime*

Parathion		DDT		Physostigmine Sulfate		Pralidoxime Chloride	
Prostration (3 hr)	Mortality (72 hr)	Prostration (24 hr)	Mortality (48 hr)	Prostration (1 hr)	Mortality (24 hr)	Prostration (30 min)	Mortality (72 hr)
11/15	4/15	8/10	5/10	6/10	2/10	11/13	7/13
1/15 V	0/15 S	5/10 N	1/10 N	0/10 H	0/10 N	3/8 S	0/8 S
0/10 V	0/10 N	8/10 N	5/10 N	0/10 H	0/10 N	7/9 N	5/9 N
0/15 V	0/15 S	10/10 N	6/10 N	1/10 S	0/10 N	8/10 N	6/10 N
1/10 V	0/10 N	10/10 N	7/10 N	3/10 N	1/10 N	6/10 N	2/10 N
7/10 N	3/10 N	9/10 N	4/10 N	4/10 N	1/10 N	6/10 N	3/10 N
3/10 S	7/10 **S**	9/10 N	5/10 N	1/10 S	1/10 N	0/10 V	0/10 H
4/10 N	3/10 N	8/9 N	7/9 N	5/10 N	1/10 N	8/10 N	4/10 N
11/15 N	10/15 **S**	10/10 N	10/10 **S**	5/10 N	3/10 N	2/5 N	0/5 N
7/10 N	4/10 N	7/9 N	5/9 N	3/10 N	2/10 N	8/10 N	1/10 S
3/10 S	1/10 N	5/5 N	1/5 N	5/9 N	1/9 N	8/10 N	6/10 N
10/10 N	10/10 **V**	15/15 N	11/15 N	10/10 **S**	8/10 **S**	3/9 S	0/9 S
15/15 S	15/15 **V**	15/15 N	14/15 **S**	10/10 **S**	9/10 **V**	4/9 N	1/9 N

Interestingly, DDT clearly differs from all the other pesticides just mentioned in that, under our experimental conditions, its toxicity is not significantly affected even by the most potent catatoxic steroids. This fact has been confirmed by numerous experiments not mentioned here in detail. In similarly conducted ancillary experiments, phenobarbital (6 mg administered subcutaneously in 1 ml water, given in the same manner as the catatoxic steroids) offered highly significant protection ($P < 0.005$) against DDT intoxication, hence, in this respect, phenobarbital is a far superior prophylactic against DDT than are even the most potent of our catatoxic steroids.

Physostigmine sulfate and pralidoxime chloride, which were added merely for comparative purposes, behaved quite differently from the majority of the anticholinesterase pesticides; resistance to these compounds could not be comparably raised by any of the typical catatoxic steroids. However, curiously, prednisolone offered highly significant protection against physostigmine sulfate.

Comment

Although a great deal of work has been done on the effect of various drugs upon the detoxication of pesticides, comparatively little attention was devoted to hormones and hormone-like substances in this connection. Durham et al[18,19] noted that diethylstilbestrol increases the storage of DDT (and its metabolite DDE) in the fat of male rats, whereas testosterone decreases these values in female rats. They concluded that "an endocrine mechanism may account for the sex differences in this regard." It has also been observed that male rats are more resistant than female rats to parathion poisoning; testosterone increases the resistance of female rats, whereas estrone diminishes that of male rats.[20] As judged by the experiments described in this communication, the sex hormone effects of the steroids have nothing to do with their influence upon pesticide detoxication. The latter action appears to be related only to catatoxic potency, as manifested also with respect to numerous other toxic compounds.[5-15]

On the other hand, in view of the widespread use of pesticides, and their important role as pollutants in our environment, it is noteworthy that (with the exception of DDT) all of those tested here are effectively detoxicated by certain steroids. Indeed in many instances the toxicity of these compounds is totally abolished by all of the most potent catatoxic compounds of our series. This is not true of physostigmine sulfate, an anticholinesterase widely used in pharmacology, nor of pralidoxime chloride, a potent antidote of anticholinesterases.

Here, as in all the earlier work on the effect of steroids upon other toxic substances, the catatoxic effect appeared to be independent of the classic hormonal actions of the compounds used. Ethylestrenol and norbolethone are anabolics devoid of antimineralocorticoid properties, whereas SC-11927 and spironolactone are potent antagonists of mineralocorticoids but possess no anabolic properties. In fact, spironolactone is virtually devoid of all classic hormonal actions and yet possesses powerful detoxicating properties. Prednisolone and triamcinolone are both highly potent glucocorticoids essentially devoid of other known hormone effects, yet the former does, while the latter does not protect against most of the substrates tested here or in our previously cited studies. This difference is not due to the fact that, in the present series, prednisolone was given at a higher dose than triamcinolone, because from the point of view of their typical glucocorticoid effect these doses were essentially comparable. Since triamcinolone greatly diminishes resistance to spontaneous infections, it cannot be administered at the 10 mg dose level at which prednisolone was tested. However, complementary experiments have shown that in rats simultaneously treated with terramycin, triamcinolone is tolerated at the 10 mg dose level, yet even then it fails to exhibit catatoxic properties similar to those of the same amount of prednisolone.

This work was supported by grants from the Ministère de la Santé, Québec, and by the Medical Research Council of Canada, block term grant MT-1829.

The following companies supplied the compounds used in these experiments: Ayerst, McKenna & Harrison Ltd., pralidoxime chloride; Ciba Co., Ltd., desoxycorticosterone acetate; G. D. Searle & Co., SC-11927, spironolactone, and oxandrolone; Lederle Laboratories, triamcinolone; Organon Inc., ethylestrenol; Pfizer (Canada) Ltd., hydroxydione sodium succinate; Roussel (Canada) Ltd., prednisolone acetate and estradiol; Schering Corporation Ltd., progesterone; and Wyeth Laboratories Inc., norbolethone.

Nonproprietary and Trade Names of Drugs

Ethylestrenol—*Maxibolin.*
Norbolethone—*Genabol.*
Triamcinolone—*Aristocort Tablets, Kenacort.*
Hydroxydione sodium succinate—*Viadril.*
Pralidoxime chloride—*Protapam Iodide.*
Pentobarbital—*Nembutal.*

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