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Blockade of Adrenal Catecholamine Release by Chlordimeform and its Metabolites

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Chlordimeform (N'-(4-chloro-o-tolyl)-N, N-dimethylformamidine) (CDM) is a relatively new pesticide which is said to modify insect behavior by "interfering with amine-mediated control of nervous and endocrine systems in a variety of ways" (MATSUMURA and BEEMAN 1976). Multiple mechanisms of the pesticidal effect and of the toxic effects in mamals have been postulated (HOLLINGWORTH et al. 1979). Since solution of liposoluble agents in membranes can disrupt cellular secretory processes (SEEMAN 1972), it seemed that the lipid soluble formamidines might act in part by modifying secretory mechanisms. We therefore tested the effects of CDM and some of its metabolites (KNOWLES 1978) (certain of which are more toxic to rats and mice than the parent compound) in the isolated bovine adrenal which is a reliable system for evaluating effects of agents on neuroendocrine secretion. An effort was made to correlate effectiveness in isolated adrenals with solubility in polar and nonpolar solvents.

METHODS

Fresh bovine adrenals were obtained from a local abattoir and used within 1 hour post mortem. Glands were perfused intravenously using a multichannel metering pump (Harvard Apparatus Co., Millis, MA) with oxygenated Tris buffered Lockes solution (1.1 mM Ca^{++}, 37° C) at a flow rate of 10 ml/min (BOROWITZ 1971). Adrenals were allowed to equilibrate to the Tris Lockes solution at pH 7.4 for 45 minutes prior to any treatment.

Paired glands from the same animals were used throughout this study. One of each pair was exposed to CDM or one of its metabolites, while the other gland was used as an untreated control. The blocking actions of these agents were tested against acetylcholine (100 ug/ml, for 5 min) and KCl (56 mM, for 5 min). Amounts of NaCl were adjusted in the perfusion medium of the high potassium solutions to maintain isotonicity. The method of Von Euler and Hamberg (1949) was used to estimate catecholamines in aliquots of the perfusates. Resting secretion was determined just prior to evoked release and all values for stimulated secretion are increases over resting levels.

Chlordimeform-HCl (m.p. 223-225° C) (CDM-HCl), demethylchlordimeform-HCl (m.p. 189-191° C) (DCDM-HCl) and didemethylchlordimeform-HCl (DDCDM-HCl (m.p. 192-196° C) were synthesized by reacting 4-chloro-2-methylaniline with the appropriate formamide in the

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presence of phosphorous oxychloride (BREDERECK et al. 1959). Tetrahydrofuran was used as the medium. Reactants were mixed portion wise at 5° C followed by gradual warming to 45° C. N-Formyl-o-toluidide (NFT) (m.p. 118-120° C) was isolated as a secondary product from the above reactions or by allowing 4-chloro-1-methyl-aniline to react with formic acid in boiling benzene. The water formed in the latter reaction was distilled off azeotropically. Chemical identification of the prepared compounds was determined using micro and spectral analysis and GLC-mass spectrometry. Octonal/water partition coefficients of the formamidines were determined at 23° C.

All formamidine compounds were employed in a concentration of 3×10^{-4} M. Alcohol, 0.25% was used to aid in the solution of DCDM-HC1, DDCDM-HC1 and NFT but was unnecessary for solution of CDM-HC1. An equivalent amount of alcohol was included when appropriate in control glands.

Both the physiological neuromediator, acetylcholine and high potassium concentrations evoke adrenal catecholamine secretion in a manner dependent on extracellular calcium. These agents have been used to evaluate the inhibitory actions of liposoluble substances on calcium-dependent secretory processes (GOTHERT et al. 1976, HOLMES and SCHNEIDER 1973) and both were employed for this purpose in the present study.

The blocking effect of DCDM was also tested against calcium itself as an agonist. Glands were perfused with calcium-free Lockes solution for 15 minutes, then isotonic Lockes solution containing 20 mM Ca^{++} (HOLMES and SCHNEIDER 1973) was perfused through the treated and paired control glands for 5 minutes.

Blocking agents were present in the medium 5 minutes before and during stimulation of the adrenals in all experiments.

RESULTS

Solubility of Chlordimeform and its Metabolites: Table 1 shows the octonal/water partition coefficients of CDM, DCDM, DDCDM and NFT. Octanol/water partition coefficients of these substances are approximately the same except for CDM which has an exceptionally high coefficient.

TABLE 1

OCTANOL/WATER PARTITION COEFFICIENT OF CHLORDIMEFORM AND ITS METABOLITES

CDM	19.0
DCDM	4.26
DDCDM	5.67
NFT	6.1

Inhibition of Adrenal Catecholamine Secretion by Chlordimeform and its Metabolites: Inhibition of acetylcholine or high potassium-induced adrenal catecholamine release by CDM and its metabolites is shown in Table 2. It is evident that the effect of acetylcholine is inhibited to a greater extent than that of high potassium by these agents. Blockade of the action of acetycholine is most pronounced in the case of the unsubstituted amine, DDCDM. Addition of methyl groups or conversion of the amine to an aldehyde decreases effectiveness against acetylcholine-induced adrenal catecholamine secretion.

TABLE 2

INHIBITORY EFFECTS OF CHLORDIMEFORM AND ITS METABOLITES ON CATECHOLAMINE RELEASE FROM ISOLATED BOVINE ADRENALS

	RESPONSE TO ACETYLCHOLINE 100 ug/ml	RESPONSE TO 56 mM KCL
	$\frac{\text{Treated}}{\text{Control}} \times 100$	$\frac{\text{Treated}}{\text{Control}} \times 100$
CDM-HCl	25.6 ± 10.5	93.8 ± 24.5
DCDM-HCl	16.2 ± 8.7	50.6 ± 19.5
DDCDM-HCl	5.8 ± 2.3	71.3 ± 13.1
NFT	32.5 ± 7.7	64.2 ± 16.2

Means ± S. E. are given for four pairs of glands for each treatment. One of each pair served as control and was exposed to acetylcholine or high potassium only. The other gland of each pair was treated with CDM or one of the metabolites (0.3 mM) for five minutes before and during stimulation. Means ± S. E. of the control responses to acetylcholine and potassium are 110.5 ± 19.6 and 195.4 ± 22.4 ug/5 minutes respectively. Inhibition of acetycholine-induced adrenal catecholamine release by CDM and its metabolites is significantly greater than inhibition of K^+-induced catecholamine release by these agents ($p < 0.01$ by students t-test). The demethylated products are significantly ($p < 0.05$ by students t-test) better inhibitors of the action of acetycholine in adrenal medulla than NFT.

Inhibition of Ca^{++}-induced adrenal catecholamine release by DCDM is shown in Figure 1. Degree of inhibition of this response by DCDM is intermediate between degree of inhibition of the effect of acetylcholine and that of high potassium.

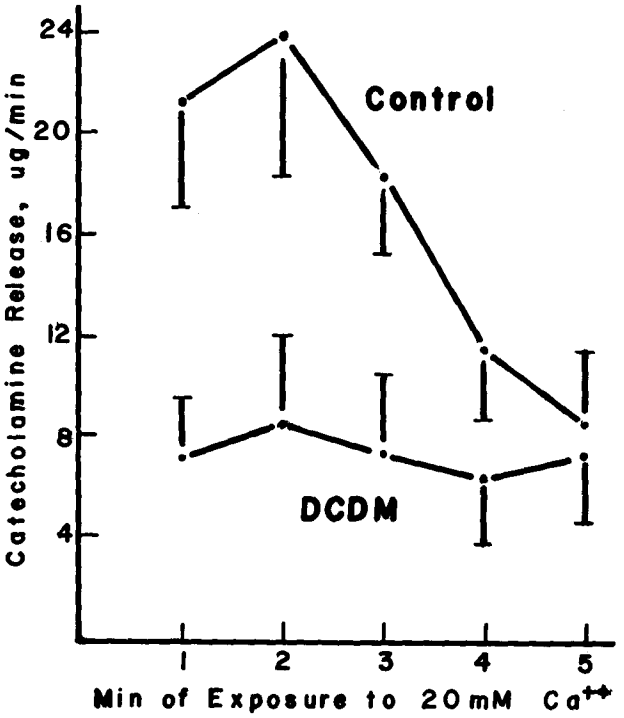

FIGURE 1
Blockade of Calcium-Induced Adrenal Catecholamine Release by Demethylchlordimeform

Bovine adrenals were perfused for 15 minutes with calcium-free Lockes solution and then exposed for 5 minutes to isotonic 20 mM calcium-containing Lockes solution. The DCDM (0.3 mM) was present 5 minutes before and during exposure to high calcium. Means ± S.E. are given for 4 pairs of glands.

DISCUSSION

CDM has a much higher octanol/water partition coefficient than any of the metabolites used in this study and yet it is no more effective than the metabolites against acetylcholine or high potassium in adrenal medulla. Simple solution of the formamidines in plasma membranes to modify secretion mechanisms therefore probably does not explain their biological actions. The parent compound CDM, however, is much more effective as a pesticide than the metabolites. Against black cut worm ova for example, the LC_{50} of CDM is

one third that of the N-demethyl analog (HOLLINGWORTH 1976). Pesticidal potency of CDM and its analoges probably relates to the octanol/water partition coefficients of these substances. The more lipid soluble CDM is no doubt more easily absorbed into the animal organism.

Toxic symptoms after i.p. injection in rats are the same for CDM and its demethylated metabolites (marked hyperexcitability with exaggerated responses to external stimuli, rapid running and intense escape behavior, later prostration with hyperextension of hind legs occurred) (BENEZET et al. 1978). Toxic doses of NFT however produce a depressant effect in which the animals are unresponsive to external stimuli. Thus, mechanisms of toxicity of CDM and its demethylated metabolites are similar but differ from those of NFT.

NFT contrasted with the other agents used in the present study in that it was significantly less effective against acetylcholine-induced catecholamine release and also in that the effectiveness against acetycholine was relatively low compared to that against a high potassium stimulus.

The lethality of CDM in rats is probably due to its local anesthetic-like actions with consequent depressor effects on the cardiovascular system (LUND et al. 1978). PFISTER et al. 1978), noted that the gross toxic symptoms of CDM in mice resemble those observed following systemic local anesthetic overdose and suggest that the toxic action of CDM involves a systemic local anesthetic effect. Blockade of adrenal catecholamine release by CDM and its analogs supports the above hypothesis since local anesthetics are known to inhibit adrenomedullary secretion (RUBIN and MIELE 1968). The parallelism between effectiveness of CDM and its analogs against the stimulant effect of acetylcholine in adrenal medulla and the LD_{50}'s of these substances in rats and mice (BENEZET 1978) is striking.

DCDM was quite effective in antagonizing calcium-induced adrenomedullary secretion in the present study. Treatment of rats with CDM causes elevation of brain serotonin and norepinephrine levels (BEEMAN and MATSUMURA 1976) which could be explained in part by blockade of secretion mechanisms. The CDM-related abnormal behavior of pest organisms leading to decreased feeding, etc. may also involve disruption of calcium metabolism. Blockade of calcium influx into muscle and secretory tissues may be a fundamental mechanism by which formamidines exert their effects as pesticides as well as their toxic actions in mammals.

CONCLUSIONS

The pesticide chlordimeform and certain of its metabolites inhibit acetylcholine-evoked catecholamine release from isolated bovine adrenals. Effectiveness in isolated adrenals parallels toxicity in rats and mice. Since local anesthetics block adrenomedullary secretion and since a systemic local anesthetic effect has been implicated in the toxicity of chlordimeform, the results of

this study support the hypothesis that a local anesthetic action is important in the systemic toxicity of chlordimeform and certain of its metabolites.

Demethylchlordimeform strongly inhibits calcium-evoked secretion from isolated adrenals which suggests that blockade of calcium influx may be an important aspect of the toxicology of formamidine pesticides.

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